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COVER PHOTO:
A schematic diagram of the Sec dependent protein translocation system [from The 2001 Merck Frosst Prize Award Address Mark Paetzel and Natalie C. J. Strynadka]

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CSBMCB President's Report

The bold step that your Society took to organize its own Annual Meeting has been a resounding success so far. The Alliston meeting succeeded by any measure. The science was exceptional, the venue was wonderful, the attendance was great, we didn't lose money (and may have made a bit) and we attracted new members to the Society. We owe a tremendous debt of gratitude to our three immediate past-presidents for their outstanding work in ensuring the success of the meeting. Peter Lewis organized the program and obtained many of the leaders in genomics, proteomics and bioinformatics to speak. Peter Davies did an incredible job obtaining contributions from a variety of sources to offset the costs of holding the meeting. Frances Sharom worked with Council member Reinhart Reithmeier on the local arrangements. She also arranged the highly successful Web registration process and upgraded the Web page, which served an effective advertising function.

Our next task is to host a successful Winternational Symposium March 21-24 in Banff, which will serve as this year's Annual Meeting. Council has decided that the organization of two meetings per year would be too large a burden for an organization of this size. The Winternational in Banff will be followed in 2003 by the International Congress in Toronto in July. In 2004, the Winternational will be held in Quebec. The 2005 meeting will be held in Central Canada, probably returning to Alliston.

The IUBMB Congress will be our overriding concern for the next year and a half. There is a strong team looking after the details. Joel Weiner chairs the Executive Committee, and Mike Walsh chairs the Scientific Program Committee. They will be calling upon members of the Society to contribute time and effort to help make the meeting a success. Please be generous in contributing to make this the best International Congress yet. We all owe a debt of gratitude to Peter Dolphin, who played a key role in securing the Congress for Canada. He will be missed by all of us.

Dr. Leon W. Browder
Department of
Biochemistry &
Molecular Biology
University of Calgary

As I write this report, the events of September 11 in New York and Washington are all too fresh in our minds. The future has become more unpredictable. As governments shift their priorities and the economy absorbs these events, we may find that our jobs as advocates for our disciplines become more difficult. But, as a community , we will meet the challenge. Trivial matters fade into insignificance, and family and friends become our comfort zone.

I would be happy to hear from you at any time. Your ideas and assistance for improving our effectiveness as a Society are always welcome. My email address is browder@calgary.ca.

Sincerely,
Leon Browder

Incoming Members of CSBMCB Executive Board 2001-2002

President of CSBMCB: Leon W. Browder

Leon Browder was born in Pueblo, Colorado, where he received his early education. While studying for his Bachelor's degree at the University of Colorado at Boulder, he became fascinated with embryonic development. He continued his studies of embryology at Louisiana State University in Baton Rouge, where he obtained his Master's degree. When in Baton Rouge, Leon learned the power of combining genetics with experimental manipulation of embryos for understanding developmental mechanisms. He studied pigment pattern mutants of the leopard frog, *Rana pipiens*, as a means for understanding development of the neural crest, which produces the pigment cells. After obtaining his Master's degree, Leon obtained his Ph.D. from the University of Minnesota in Minneapolis. He was fortunate to study in Minnesota under Nelson Spratt, one of the pioneers in experimental embryology. Although Spratt worked on chick development, he gave Leon freedom to work on any problem that fascinated him. Interestingly, the mutants that he had studied in Louisiana occur naturally in the frog populations in Minnesota. David Merrill, a geneticist at the University of Minnesota, was interested in using these mutants to monitor gene polymorphisms in natural populations. So, fates converged, and Leon focused on the roles that the expression of these genes in the neural crest played in producing pigment patterns.

After completing his Ph.D. in Minnesota, Leon returned to Boulder as a Postdoctoral Fellow in the newly-formed Institute for Developmental Biology. Within a short time, the institute became a part of the new Molecular, Cellular and Developmental Biology Department (MCDB), chaired by Keith Porter. This was an exciting time as developmental biology began emerging as a discipline from the fusion of embryology, genetics and molecular biology, and the MCDB was one of the foci of that movement. The MCDB has since emerged as one of the premier life science departments in North America. While in Boulder, Leon continued to study the pigment pattern mutants that he and David Merrill had discovered in Minnesota.

When Leon was completing his graduate studies in Minnesota, he attended the Annual Meeting of a new scientific society (the American Society for Cell Biology) in Houston. At that meeting, he met Bob Church, a native of Calgary, who had just completed his post-doc in Seattle and was looking for an academic position. This was a fateful encounter, because Bob and Leon crossed paths a couple of years later in Boulder. Bob had accepted a position in the Department of Biology in Calgary. Soon after this, Calgary established a Faculty of Medicine, and Bob was named Head of Biochemistry in the new faculty. He painted a picture of Calgary of rapid growth and strong support for research that lured Leon to Calgary as a replacement for Bob in the Department of Biology. So, Leon, his wife, Sandy, and two young daughters headed north.

After establishing his own laboratory in Calgary, Leon focussed his research on the interface between oogenesis and early embryonic development and switched to a different frog as a model system: *Xenopus laevis*. He was fascinated with the role that the maternal dowry of proteins and RNAs play in programming early development, and Xenopus was a more appropriate organism in which to study this phenomenon. Leon taught developmental biology at both the undergraduate and graduate levels and soon realized that there was no textbook that adequately covered the field. So, he decided to write one. As he says, it was naive to assume that he could do this. If he had thought about it rationally, he never would have undertaken such a massive task. The first edition of *Developmental Biology* was published in 1980. Three editions of the book have been published; the third edition was published with two co-authors in 1991. Leon insists that this will be the last edition. Between editions, Leon edited a seven-volume series of books entitled *Developmental Biology: A Comprehensive Synthesis.*

Leon was elected to the Board of Directors of the Canadian Society for Cell Biology in 1985 and later became President of the Society, which changed its name to the Canadian Society for Cellular and Molecular Biology. One of Leon's accom-

plishments while President was to establish the Winternational Symposium series, which will serve as this year's Annual Meeting of CSBMCB.

As President of the Canadian Society for Cellular and Molecular Biology, Leon was a member of the Board of the Canadian Federation of Biological Societies (CFBS). After serving this two-year term, he was elected to an additional three year term on the Board of CFBS. He was then elected President-elect of CFBS, serving one year in that post, followed by terms as President and Past-President. He also served a six-year term as a member of the Board of Directors of the International Society for Differentiation.

In an ironic twist of fate, Leon was named Head of the Department of Biochemistry & Molecular Biology in the Faculty of Medicine at the University of Calgary in January, 1999. This is the same department that Bob Church founded when he left the Department of Biology. Leon finds this new challenge to be invigorating, particularly at this particular time when governments are more cognizant of the value of investing in scientific research. There have been unprecedented opportunities to develop programs in genomics, proteomics and bioinformatics.

The move to the Faculty of Medicine has allowed Leon to pursue new research opportunities. He has adopted technology for making transgenic *Xenopus*, which provides new opportunities to do functional studies on genes in *Xenopus*.

Leon is humbled by the opportunity to serve as President of CSBMCB in the wake of so many outstanding predecessors.

Dr. David W. Andrews, Vice-president

I decided to become a scientist at a very young age. My parents tell me that at age 4 or 5 I used the money given to me to buy a cowboy hat to buy rocks and a rock identification kit at the ROM. Since that time my interests have shifted away from geology and over many areas of science. In high school in Ottawa, I was fortunate to have the chance to do an independent study project looking at learning schedules and biofeedback training of brainwave patterns in my fellow students. The staff at the high school were remarkably supportive and I wonder if in the present environment of cutbacks and larger classes if a student like me would receive similar encouragement today. I also received tremendous help from scientists at NRC, a PhD student in the psychology department at University of Ottawa and from Tom Mousseau, an inventor. It was Tom that built for me (for free) the one piece of specialized equipment that I could not borrow from NRC. He also spent time with me discussing the creative process and instilling me with a healthy distrust of authority. At NRC, I spent one of my most memorable afternoons measuring the brainwaves of a cellulose sponge as part of a control experiment to debunk a bad diagnostic that was starting to be used clinically. That was my first introduction to the idea there is bad science and that it is important to properly address bad science. It was all of these interactions in Ottawa that led me to study Biochemistry at University of Ottawa. Working in factories on assembly lines during the summers and part-time during the winter to earn the money I needed to attend university provided additional motivation. Although I know longer remember the answer, I do remember calculating how many transformers I laminated to pay for one year's tuition.

The undergraduate program in Biochemistry at the University of Ottawa was fairly small at that time. There were only 10 of us in fourth year. The education that I received was excellent and has served me well over the years. A couple of the level 4 courses were also my first exposure to alternative teaching techniques. I am happy to say that I use some of those techniques in my own classes today. During that time I also had my first serious flirtation with the business world. Starting during my second year, with running the music for Pub nights at the university, I ended up running my own stereo store in Bells Corners by fourth year. Although I became fascinated with the course of growing a business, my first love remained science. As a result, I handed the business over to some of the guys that I had been working with, bought a very expensive stereo for myself (it was my last chance at dealer cost), and moved to Toronto to start grad school at the Ontario Cancer Institute.

Graduate training during the early 1980s at the OCI was a truly rewarding experience. Many of my compatriots now have academic or professional appointments, a testament to the training and the exciting environment that we found ourselves in. I will always be thankful to Rick Miller for seeing my potential and recruiting me to the OCI. Under the guidance of Peter Ottensmeyer and as part of a great group of students in his laboratory, I finally

became a scientist. And after much personal tutelage from Peter I even learned to write a semi-coherent paper. Those were exciting times in the Ottensmeyer laboratory and at the OCI. The electron filter devised by Peter was being incorporated into the Zeiss 902 electron microscope. Next door to us the T-cell receptor was being cloned by Tak Mak's group and just down the hall P-glycoprotein was being identified in Vic Ling's laboratory. Similar exciting results were going on throughout the old Sherbrooke street hospital. The 'work hard - play hard' family atmosphere of the OCI at that time influenced me profoundly. The family atmosphere at the OCI is definitely one of the reasons that I was eager to collaborate with Brian Leber, a hematologist at McMaster, after I set up my own laboratory. It was also during my graduate training that I first became interested in protein targeting. During the latter part of my graduate studies I worked on the structure of Signal Recognition Particle and used the new filter to map the RNA within it. During this collaboration with Peter Walter in Gunter Blobel's laboratory, I also decided that it was essential that I learn the new techniques of molecular biology and to purify my own proteins. What better place to learn both than at University of California San Francisco?

Everything about San Francisco was ideal for me at that time. Working with Vishu Lingappa was immensely rewarding. He is someone that cares passionately about science and society and he is without doubt the smartest person I have ever met. He is also the most competitive! In Vishu's laboratory I learned not only molecular biology and protein purification, but also the cell biology of membranes, a unique philosophy of science and the real meaning of dedication. Happily, I was also able to continue collaborating with Peter Walter. During this period the main steps in the targeting of proteins to membranes were worked out and I became interested in how proteins are shuttled into the bilayer rather than transported across the membrane.

When I moved to McMaster, and set up my own laboratory I began working on the regulation of translation because I could do experiments with one power supply, two gel boxes and a rabbit.

With the support of the MRC (a scholarship and a grant) I soon returned to the study of the translocation machinery in general, and the assembly of the SRP receptor in particular. The environment at McMaster was very attractive at that time, due primarily to the other faculty members in the department. Rick Rachubinski was studying import into peroxisomes, Karl Freeman was looking at import and assembly of uncoupling protein in mitochondria and Hara Ghosh was examining targeting of membrane proteins to the nuclear membrane. The department has also consistently attracted some of the best graduate students in the country. I was soon collaborating with Rick on the import of dimmers into peroxisomes, with David Johnson on antigen transport by T AP proteins and Jan Huizinga on the identification of c-kit in pacemaker cells. Internationally I have collaborated for years with Art Johnson on the mechanism of integration of transmembrane proteins into lipid bilayers.

During the late 1980s in San Francisco I began to be interested in the targeting and membrane assembly of cytochrome b5, a protein that did not use any of the conventional machinery to insert into the bilayer. I soon realized that cytochrome b5 was not unique but that a similar protein had been identified in almost every field. Almost uniformly these proteins were identified, characterized as being 'similar to cytochrome b5' and forgotten. The epiphany for me was that it was not possible to explain the targeting of any of these proteins with spontaneous insertion into any bilayer, the mechanism usually invoked for cytochrome b5. Slowly, we began to appreciate that proteins with 'tail-anchors' or 'insertion-sequences' constituted a an important class of proteins that are targeted by a variety of mechanisms. I have been fortunate in receiving MRC Scientist, MRC Senior Scientist awards and most recently the Canada Research Chair in Membrane Biogenesis. These have been critically important in providing me the time and extra resources needed to take on the risky challenges of a new field.

My interest in these proteins led me to collaborate with Bill Trimble on the targeting of Vesicle Associated Membrane Proteins (V AMPs) and with Brian Leber on the newly discovered anti-apoptosis protein Bcl-2. The collaboration with Brian has been tremendous fun because neither one of us knew much about Bcl-2 or apoptosis (a new field at the time) but we were both enthusiastic. I have also had a tremendous collaborator on this project in Linda Penn. Together, with Brian Leber we published the first demonstration that Bcl-2 can function at either the endoplasmic reticulum or mitochondria, a result that continues to confound almost every model put forth for the function of Bcl-2. Collaboration with Linda also means that I

have found my way back to the OCI. Moreover, the translocation machinery has since been shown to contain two tail-anchored proteins. As a result these proteins are gradually taking over larger and larger areas of my laboratory.

Somehow, during all of this very exciting science, I once again became interested in industry. On my first sabbatical, I helped set up a biotech company that operates in Canada and the USA. Separately, we have also had patents granted in translational regulation and for a method that permits cell-free two hybrid analysis and in vitro peptide display. I find it very gratifying that both of these patents are now licensed by companies and being commercialized. Unfortunately neither company is Canadian.

Throughout all of my time at McMaster I have had the unbelievable good fortune to work with a stellar group of technicians, students, post-docs, staff and faculty. As a result we have been continuously funded by MRC and now CIHR. We have also had some pretty exciting times scientifically as well as personally (the wholehearted support of my family has been fantastic) and I am sure that the most exciting science is yet to come! I am really glad that I decided to return to Canada as it has been a great place to work and live.

Dr. Claude Lazure, Councillor

When I climbed the steps leading to the University of Montreal as an eighteen-year-old, I had truly no inkling of what was to come nor did I know what to expect. Man had walked on the moon but this, without the power of the PC that I am using now. The atom had his nucleus, electron and neutron but none of those bosons, quarks or muons. Environmental concerns, ecology or wildlife conservation were very much in the air. Scientists were still pondering the essence of the genetic code while, at the same time, trying to define the logic of life. Acronyms and coined words such as RIA, PCR, gene arrays, genomics, now common language to even first year science student did not exist. Penicillin and sulphonamides were seen as the drugs of all foes and there was not a hint of gene therapy, cloning and pharmaco-genomics. However, there was very much a world, a society and science to discover.

My start in research was quite esoteric, having been hired as a summer student at the Department of Chemistry to work on exotic praesodymium and europium containing chelates to be used as NMR displacement shift reagents. Not only was it quite special, it was also quite a resounding start as, after two days of synthesis and at the time to reintroduce air in the system, the main scientist urged me to turn around at the precise moment the whole apparatus went off in the air starting fire at five or six places including my open drawer. The lesson, which I learned immediately, is that if one is to survive in a research laboratory, one has to keep his eyes open and his mind well alert. In following years, I tried to do just that. Leaving aside these materials, I decided that at twenty-one years old, I was not ready to join the market place and hence entered in graduate research at University of Montreal. Interested more and more by molecules of life, I tackled for my master thesis the synthesis of an 18-residues peptide, namely beta-MSH, using the recently introduced solid-phase peptide synthesis technology of Dr. B. Merrifield. Amazingly, at that time, such a short peptide represented a formidable challenge not only due to its length but also due to the presence of numerous sensitive residues not appreciating the harshness of the chemical methods used at the time. This was indeed a very interesting endeavour as more often than not we had to prepare ourselves many protected amino acids using, for example, such gentle reagents as hydrazyne, HF and phosgene and/or develop new ones.

Dr. Claude Lazure, Councillor

Clearly, as one always tries to reach higher, going from peptides to proteins was both entirely logical and well in keeping with my training in synthesis except that this time, I got to dissect proteins to know what they were made of. Hence, I moved to the Department of Biochemistry at the University of Sherbrooke though my studies actually were much more focused on Immunology. Thus, not only was I to learn the intricacies of biochemistry (quite distinct in those days from chemistry), but also those of immunology. Doing so, I discovered also phylogeny, evolution and genetics, and this was truly a full serving as I had to pass the comprehensive pre-doctoral exam within a year of my arrival! I succeeded thanks to the very good jury I had (though 20 years later, I still remember

the questions!!!!) and hence I could concentrate on the job at hand, namely to characterise the proteins found in the plasma and urine of an extremely rare (that was the 11th case in the world) case of immunoglobulin μ-heavy-chain disease. In the years past in Sherbrooke, I learned a lot in many areas, but also that scientific research when confronted to the reality of sickness can be heart breaking. Indeed, the Biochemistry Department being part of the Hospital, I had the opportunity to meet the patient suffering from this disease who would unfortunately die during my studies. This was both a human and scientific experience, which would stay forever with me. Furthermore, I also learned that protein chemistry was hard work, especially when my mentor received boxes full of parts from the prototype sequenator from Illinois Electronics which he acquired through auctioning – and I had to put it together! Then, I learned that, in order to fully exploit a technique, one cannot simply use the instrument but best understand how it functions so as to be able to fully exploit it.

At the end of my Ph.D. molecular biology was slowly rising in the future but, having spent so much energy and efforts in protein chemistry, I decided to stay in that field and turn my attention to endocrinology. After meeting with an amazing group of scientists at the Institut de recherches cliniques de Montreal (IRCM), I came for what I thought a year or two with no idea that I would spend the next 20 years here. Those were exciting and stimulating times where I had access to excellent scientists, equipment and environment. It was the time where protein chemistry was leading the way bringing out novel sequences (such as ANF) and structures though the revolution brought about by molecular biology and cloning was closing in very fast indeed. Hence in 1990, I decided to at least get acquainted with this field by going on sabbatical leave at the University of Lund in Malmö (Sweden) with my little family. Much more so than with the techniques of molecular biology, I was impressed by the difference in scientific culture and the way research was conducted. Prior to my going to Sweden, I began my long-term association with granting agencies as a committee member; my first MRC chairman surely remembers the young guy coming in with his 17 grants to review in a box which collapsed right in front of him. I served on the MRC committee for four years and indeed I am happy to have done so, because I learned a lot in diverse areas, I met fantastic individuals and I got convinced of the reach and great qualities of Canadian science and scientists who must be aware that there was, is and will continue to be great science done in this country.

On my coming back, I became director of a laboratory called Neuropeptides Structure and Metabolism where uncovering a new sequence, amino acid by amino acid still excites me as much as it did years ago. Being an adjunct professor at McGill University as well as a professor at University of Montreal in the Department of Medicine enables me to be in contact with a lively and stimulating scientific community. It is also upon my return that I got to be associated more closely with the Fonds de la Recherche en Santé du Québec after having been an FRSQ research-fellow from 1982 to 1995, as a committee member. Reviewing proposals and grants is a hard and time consuming job but, having been supported through fellowships and research grants, I feel very strongly about my commitment and duty to serve on these committees such as MRC (now CIHR with all its changes), FRSQ and HSFC. I also feel the same way about my election to CSBMCB and my nomination as a Scientific Advisor at the FRSQ. I will be pleased to contribute and ensure that science will continue if not strive in coming years.

Indeed, it has been a long journey from these tentative first steps climbing the hill to University of Montreal but, as hard as it may have been, it was stimulating and pleasant. Not only have we gained an unparalleled knowledge in terms of science but also we experienced through the years the merit of collaboration and gained from people in all areas. Science, I must say, has brought me pain and pleasures, failures and successes, but is not that what life is all about? Lastly, between you and me, science has also given me friends and collaborators, but importantly enough, a chemist wife and two marvellous guys for my life to enjoy.

David Litchfield, Councillor

David Litchfield began his research training as an undergraduate student in the laboratory of Dr. Karl Freeman at McMaster University. Following completion of a B.Sc. in Chemistry and Biochemistry at McMaster University, David moved to the University of Western Ontario to pursue graduate training under the supervision of Dr. Eric Ball. David obtained his Ph.D. in 1987 and then moved to the laboratory of Dr. Edwin Krebs in the Howard Hughes Medical Institute at the University of

Washington to pursue postdoctoral training. Studies in the Krebs' laboratory were focused on an investigation of the protein kinase CK2 family of enzymes and laid the groundwork for future studies as an independent investigator. In 1991, David returned to Canada and established an independent research program at the Manitoba Institute of Cell Biology with an academic appointment as an Assistant Professor in the Department of Biochemistry and Molecular Biology at the University of Manitoba. In 1996, David returned to the University of Western Ontario as an Associate Professor in the Department of Biochemistry. The Litchfield laboratory is currently among the leaders in elucidating the role of the protein kinase CK2 family of enzymes in various aspects of cellular regulation. Dr. Litchfield is a past recipient of a Research Scientist Award from the National Cancer Institute of Canada as well as the Premier's Research Excellence Award from the Province of Ontario. The Litchfield laboratory currently derives operating funds from the National Cancer Institute of Canada and the Canadian Institutes of Health Research as well as funding from the Natural Sciences and Engineering Research Council of Canada for a collaborative project with the laboratories of Drs. Chris Brandt and Brian Shilton. In recent years, David has been a very active participant in the peer review process and has served on panels for the Canadian Institutes of Health Research, the Medical Research Council, the Heart and Stroke Foundation of Canada, the National Cancer Institute of Canada and the Leukemia Research Fund of Canada. In the most recent competitions, David was a member of the Cell Physiology Panel of the Canadian Institutes of Health Research and the chair of the Biomedical Research Personnel Awards Panel of the National Cancer Institute of Canada. Since establishing an independent research program, David has been a very active participant in many aspects of the training of graduate students. David is married with two young children, a son born in 1995 and a daughter born in 2000, and enjoys the outdoors and a weekly game of pickup hockey.

Dr. Joe Casey, Councillor

I was born in 1963 in Lansing, Michigan, while my father completed his Ph.D. in Psychology at Michigan State University. Having been born into a university environment may explain why to this day I am still at university. I emmigrated to Canada with my family in the heady days of the summer of 1967. As an immigrant I think I have developed a very strong appreciation for Canada and what it stands for.

David Litchfield, Councillor.

I spent my primary school days in Kingston, Ontario and went to high school in downtown Toronto. I loved chemistry even then and believed it was in my blood, since my maternal grandfather was a paint chemist. Before starting university I read a Scientific American article on biotechnology that convinced me that biochemistry was the future.

From 1983-1987 I studied biochemistry at Queen's University, Kingston. During those years I had many influences as a nascent biochemist. I worked two summers in the plant physiology laboratory of Ken Budd, Department of Biology. Those summers were a dream for me, since Ken gave me a huge amount of latitude for a summer student. I learned about biochemistry as a lifestyle by coming into the lab at all hours to take readings of cyanobacterial growth. I also got my first exposure to protein chemistry, doing some crude purifications of pyruvate dehydrogenase from cyanobacteria and characterizing its kinetic properties on a massive Cary 210 spectrophotometer. My last summer as an undergraduate was spent with John Elce, Department of Biochemistry. There I further honed my protein purification skills on calpain and learned a lot about immunochemistry. My first exposure to molecular biology was in the laboratory of Peter Davies, where I completed my B.Sc. thesis on antifreeze gene chromatin. At the Queen's Outdoors Club I also met my wife, Rachel Wevrick.

Dr. Joe Casey, Councillor

In 1987 with a fresh B.Sc., I knew I wanted to study protein chemistry. It was the advice of Allan Mak and Peter Davies that lead me to the Department of Biochemistry, University of Toronto, to work with Reinhart Reithmeier, and fateful advice

that was! Little did I know it was membrane proteins that were to be my focus, which it has stayed to this day. I spent five fantastic years working with Reinhart, a superbly generous and insightful supervisor. After trying my hand at just abut every protein chemical and biophysical technique that could be thrown at the erythrocyte membrane anion exchanger, Band 3, I decided that for postdoctoral work I would like to combine molecular biological approaches with protein chemical techniques.

So off I went to work with Ron Kopito at Stanford University. Ron had cloned the genes for all the family members of the anion exchanger family. I dove back into molecular biology for the first time since working with Peter Davies. Things had changed. There were a lot more kits and everything had become easier to do. I continued to study anion exchangers, developing a yeast expression system to facilitate expression and developing the tools to use introduced cysteine mutants and cysteine-specific protein chemistry to study anion exchangers. My three years at Stanford taught me how to tackle big research problems and added to my tool kit a new set of cell biological and molecular biological approaches. I also discovered that I love mountains, which contributed to my decision to move to University of Alberta, to join Jim Young's Membrane Transport Group in the Department of Physiology.

Dr. E. Bruce Waygood, Councillor

Since 1996 I have been an Assistant Professor, Department of Physiology and have been an MRC and Alberta Heritage Foundation for Medical Research Scholar and Senior Scholar. With funding from CIHR and Heart and Stroke Foundation, my lab has focussed on the study of structure, function and regulation of plasma membrane anion exchange proteins. Recently we have begun to study a second class of bicarbonate transporters, the sodium/ bicarbonate co-transporters. Since 1997 I have been a member of the CIHR group in Molecular Biology of Membrane Proteins, headed by Marek Michalak. The group has provided an exciting research environment for me and the people in my laboratory. Marek and Jim have been a huge help in guiding my early independent career.

Outside the lab I keep busy with my family and outdoor activities. We now have two children, Sierra (6) and Adam (3). Whenever possible we visit the Rockies for hiking, mountaineering and skiing.

Dr. E. Bruce Waygood, Councillor

Professor Bruce Waygood is the University Coordinator of Health Research, a newly created position with responsibility for health research at the Universities of Saskatchewan and Regina. He is a Professor of Biochemistry at the College of Medicine, where he has maintained an active Medical Research Council (now Canadian Institutes of Health Research)-funded laboratory since his first appointment in 1977. He is currently Chair of the Interim Board of the Saskatchewan Structural Sciences Centre, a major Canadian Foundation of Innovation funded facility.

He has authored over 60 papers primarily concerned with molecular mechanisms of the transport of sugars into bacteria and antibody-protein interactions. He is an active collaborator with researchers who determine protein structures using either synchrotron or Nuclear Magnetic Resonance approaches. Prof. Waygood has had major international collaborations with researchers at the NIH (USA) and the Universities of Cambridge; California, at both San Diego and Los Angeles; Washington (Seattle); Ruhr (Bochum); and Princeton.

He has served on the MRC Biochemistry operating grants committee, Alberta Heritage Foundation for Medical Research Equipment committee, and was recently a member of the executive of the Canadian Society of Biochemistry, Cell and Molecular Biology recently. At the University of Saskatchewan, Prof. Waygood has served on most, and chaired some, of the major committees, and was on the Executive of the Faculty Association in the 1980s. He had a major role in the Biotechnology initiative, and was responsible for reform of the Biochemistry curriculum.

Prof. Waygood holds a B.Sc.(Hons) and M.Sc. in Microbiology from the University of Manitoba, a Ph.D. in Medical Biophysics from the University of Toronto. His postdoctoral work was at Johns Hopkins University, and he has been a visiting professor in Biochemistry at University of Washington (Seattle) and in Microbiology at the University of Ruhr (Bochum).

IUBMB Toronto Congress Update

Joel Weiner
President, 19th International Congress of Biochemistry and Molecular Biology

I am sure you are all aware that the Society will be hosting the next International Congress of Biochemistry and Molecular Biology. The meeting will take place from July 20-24, 2003 at the Metro Toronto Convention Center. This is an outstanding venue for an international congress. Planning for the meeting is moving ahead rapidly. Dr. Peter Lewis is chairing the local planning committee and they are hard at work on all aspects of the logistics of staging a large international meeting.

This is a very exciting time for Biochemistry/ Molecular Biology research. To capitalize on this excitement the meeting will consist of a number of keynote plenary lectures as well as a diverse array of symposia covering the entire field of biochemistry, molecular biology and proteomics. Dr. Mike Walsh is chairing the program committee and he has assembled an outstanding group of Canadian researchers to work with him in planning the program. This committee is advised by an impressive group of international scientists. There will be enough depth in the program to ensure that all participants have an exciting scientific and social experience. This meeting will showcase the best of Canadian science to our international colleagues as well as present the latest international research. A number of satellite meetings are planned to provide additional incentives to attend. We all want this meeting to be an outstanding success. Now is the time to encourage your colleagues from around the world to make plans to come to Toronto in July 2003.

The meeting will have a large commercial exhibit, pre and post Congress tours and an exciting social program. In addition, the CSBMCB will be partnering with the IUBMB and the Pan American Association of Biochemical Societies to host a pre-congress young scientists program. Dr. Fred Palmer is organizing this program and we will be announcing the details in the summer of 2002.

An important component of the success of the meeting will depend on our ability to attract donations from the private sector, government and individual citizens. We are very fortunate that Dr. Gerard Tertzakian has agreed to chair this important committee. Dr. Tertzakian has experience in the biochemical and biotechnology industry and is a well-known venture capital investor. Dr. Tertzakian cannot do all the fund raising on his own. We will need leads, contacts and help. If you are able to help with the fund raising please get in touch with me so that we can discuss it.

Keep an eye on our website for the latest information *www. nrc.ca/confserv/iubmb2003/*

If you have any questions about the congress or suggestions about how to make this the best international congress please contact me directly at *joel.weiner@ualberta.ca*

Minutes of the 44th Canadian Society of Biochemistry, Molecular and Cellular Biology Annual General Meeting

Nottawasaga Inn, Alliston, Ontario, Saturday, June 2, 2001, 4:00 p.m.
Chair: Dr. Frances Sharom, President CSBMCB

Approval of Agenda.

The agenda was approved as circulated on a motion from Dr. B. Waygood, seconded by Dr. H. Schachter

Approval of Minutes of the 43rd AGM Minutes (Published tin the 2000 BULLETIN).

The minutes of the 43 annual general meeting were approved after a motion from Dr B Waygood was received by the Chair.

This motion was seconded by Dr. P. Lewis
CARRIED

Business Arising from the Minutes.

There was no business arising from these minutes.

President's Report.

a) Alliston Meeting

Dr. Sharom made a number of comments dealing with the Alliston Meeting. As this was the first Independent General meeting the Society has undertaken, Dr. Sharom stated a great deal of effort was made In Its planning and organization by the combined efforts of Dr. Peter Davies, who coordinated the meeting and did a superb job in fund raising, Dr. Peter Lewis, who was responsible for organizing the scientific programme, Dr. Reinhart Reithmeier, who looked after the physical aspects of the meeting venue, Mrs. Anna Vanek, who was responsible for administration, and Dr. Sharom who was involved in every aspect of the meeting. Although there was a minor glitch in the first session with the AV system, the meeting appears to be a success from the comments received, however, the final appraisal must await further feedback. The Meeting was oversubscribed, with 230 registrants, 80 Society members, 60 non Society members, 60 Postdoctoral and graduate students, and 30 exhibitors.

The meeting which was opened by the IUBMB Jubilee Lecture presented by Dr. Peer Bork from Heidelberg, has been structured around five symposia with 19 speakers, three workshops, three CSBMCB award lectures and 80 poster presentations.

The experience and success of the Alliston Meeting hopefully will set a standard for future Society AGMs.

b) Membership Recruitment.

Dr. Sharom stated that the Society is still in a challenging situation with regard to membership numbers. Despite signing up 35 new members at the conference registration Web site, our overall numbers are down, primarily because large numbers of long-standing members are not renewing their membership. This probably represents simple forgetfulness, rather than an active decision to leave the Society.

E-mailed dues reminders, with web-based credit card payment for membership renewals, might prevent these losses and this avenue is now being investigated. The Society is only reaching a fraction of potential members.

With new university faculty hiring, and an untapped industrial sector, it should be possible to enlarge our membership ranks significantly. Although the Executive is dealing with a number of solutions, Dr. Sharom encouraged the membership to actively recruit their colleagues to join the Society and to submit suggestions on how to improve the Society's profile in order to stimulate new membership. As for reasons for membership, Dr. Sharom stressed a number of benefits: effective science lobbying which affects Federal and Provincial grant funding, a discounted registration fee for Society-sponsored meetings, graduate and postdoctoral travel grant stipends and journal discounts.

c) Contract with CFBS.

Dr. Sharom stated the contract has now been signed with CFBS which provides for the Society's participation in CFBS's lobbying and science policy. These activities are primarily focussed in Ottawa, but at the Society's urging may be expanded to the provincial levels as well, especially in Ontario.

This contract, which is negotiated annually, calls for a payment of $40 per Regular Society Member and is subject to cancellation by either party, has been extended to 2002.

d) Society's Web Page.

Dr. Sharom reported that with Dr. Uwe Oehler's assistance she will continue to maintain the Society's current Web Page. The Web page will be updated and outdated material removed. She stated that the employment section on the Web was fairly active. Dr. Sharom suggested one way to increase the web page's visibility and make it more of a "two-way street", was to make the Society's Web page available to people seeking employment by making it possible for them to post their resumes as .pdf files. She stated that she would inform our students and PDF members of this feature. It has been decided to charge commercial firms $500 to establish a permanent link on the web page and $250 for an employment advertisement. Work still needs to be done on the French language versions of many of the more permanent parts of the web page and Dr.Sharom offered that assistance from our Francophone Members would be appreciated!

e) Future Society Meetings.

Based on the Society's Membership numbers and limited resources, it has been decided by the Society's Executive that the Society should only hold one meeting annually. Furthermore, this meeting will be held on a three-year rotation cycle, alternating with venues in Banff, Quebec and another site in central Canada preferably in Ontario. This would afford an opportunity for various geographical groups to organize one of these meetings on their own scientific interests.

Next year's AGM will be held in at the Banff Centre, Banff, Alberta, from March 21 - 24. The 2003 Meeting will be held in conjunction with the XIX IUBMB Congress in Toronto, and the 2004 meeting will be in Quebec.

The organization for the Banff Meeting "Membrane Proteins in Health and Disease" is well in hand. The web page (www.csbmcb.ca/2002meeting) will be opened in the next few weeks. The Organizing Committee for this meeting include Drs. J. Casey, C. Cass, L. Fliegel, M. Michalak, B. Lemire, J. Young, X. Chen, C. Cheeseman, R. Reithmeier and F. Sharom. Satellite meetings, topics for which have not been decided, have been scheduled be held prior to the main meeting, Wednesday evening and all day Thursday.

As a result of managerial problems encountered with the last Winternational Meeting, the Executive has decided that the Society will be responsible for the budget of all future meetings. This now means that the Society will negotiate and sign all contracts relating to Society meetings.

f) Negotiations with ASBMB.

Dr. Sharom reported that she has been trying to negotiate a reciprocal membership with the American Society for Biochemistry and Molecular Biology. This matter is now in the hands of ASBMB's Executive awaiting their action. The proposal entails a 20% membership fee discount for Society members who wish to join the other Society.

g) Support of Student Activities.

Dr. Sharom indicated that the Society has allocated $2,000 annually to support undergraduate and graduate student activities in universities across Canada. Four $500 grants are available upon student request to underwrite various activities that fall under the Society's scientific interests. This has not been a very active programme and Dr. Sharom asked the Membership to make this information known in their constituencies.

Past President's Report

a) CFBS Ottawa Lobby

Dr. Davies represented the Society at the May 16 and 17 CFBS science lobbying meetings held in Ottawa. He stated that he participated in seven meetings that were held with various advisors and policy maker in key Government Ministries. In general, the Federal Government seems committed to increasing funding for research. However, it appears that Provincial-Federal cooperation is a big impasse that has to be overcome. For example, whereas the CFI program has been successful at leveraging matching funding, the CRC initiative has not encouraged the Provinces to follow suit. The feeling is that any money the Feds channel to

relieve funding of universities will give the Provinces an excuse to cut their contributions. Thus, although there is general support for indirect costs for research, there is no clear agreement on how this relief should be applied. CIHR is very concerned about their "hand-to-mouth" budget situation. Expansion of their programmes has put them in a precarious situation of making huge future commitments three, four or five years from now, when the reality is that they obtain their funds on an annual basis. This is one reason why this year they are putting so much money ($37m) into one-year programmes. The question of overhead payment to universities and institutions was addressed. Which governmental jurisdiction, Federal or Provincial, should be responsible for this funding? Will this programme be Instituted to the detriment of research funding to the scientific community?

Dr. Davies suggested that the Society strike a subcommittee to explore the ramifications of this matter.

b) Sponsorship of Alliston Meeting.

Dr. Davies reported that he had been very successful in raising funds for the Alliston Meeting. Approximately $80,000 has been raised from governmental and non-governmental granting bodies, pharmaceutical houses, scientific supply companies, universities and scientific societies to support the Meeting's scientific programme, travel awards, social events and the Society's web page. He asked meeting attendees to thank and make welcome the exhibitors, and encouraged them to visit the commercial exhibits.

c) Poster Competition.

The Student Poster Session, presented Friday, June 1, had 42 poster presentations, 38 of which were from students and four from Principal Investigators. Of the 38 student presentations, 29 elected to compete for the two Roche Diagnostics Poster Awards. Dr. Waygood, Saskatoon, headed an adjudication committee (Dr. G. Cote, Queen's; Dr. G. Flynn, Queen's; Dr. F.. Keeley, Toronto; Dr. C. Lazure, IRMC; Dr. D. Litchfield, Western Ontario; Dr. N. Martin Queen's; Dr. L. Mclntosh, UBC; Dr. D. Rose, Toronto; Dr. H. Vogel, Calgary; and Dr. S. Withers, UBC) for these awards. Mr. Tudor Moldoveanu, Queen's University and Mr. Peter Kavsak, University of Toronto were the recipients of the Roche Diagnostics Poster Awards.

The PDF Poster Session was held this afternoon (Saturday, June 2) with 36 poster presentations, 13 by PDFs, 22 from PI's and one from a grad student. Eight PDFs entered the CSBMCB poster competition to contest for the two awarded prizes. These posters were adjudicated by a committee headed by Dr. H. Duckworth, Manitoba; included Dr. H S Chan, Toronto; Dr. E. Pai, Toronto; and Dr. D. Wishart, Alberta. Dr. Denis Daigle, McMaster University and Dr. Laurie Graham, Queen's University were judged to be the presenters of the best posters.

Vice-President's Report

Dr. Browder had no report

Treasurer's Report

a) 2000 and 2001 Budget

Dr. Palmer circulated his financial statement for the year 2000. He reported that the Society's income for 2000 was $82,956.06 and its expenses totalled $70,580.29 resulting in a balance of $12,375.77. The market value of our Special Fund at year's end was $326, 778.82. The total assets of the Society, which included the BULLETIN Editor's computer ($1,190), were $340,344.59. The financial statement for the first five months of 2001 showed an inflow of $211,571.37 and expenses of $23,217,24. The reason for this excess cash was attributed to the yet to be disbursed funds committed in support of the Society's Alliston Meeting. Dr. Palmer then referred to the summary that he prepared on the financial status of the Alliston Meeting. Since there has not been an update on the budget which was presented at the February 3 Executive Board meeting, Dr. Palmer stated that it is difficult to get a definite fix on the bottom line for this meeting. To date, he stated $172,250.73 has been received for payment for hotel and meeting registrations, and funds from Dr. Davies' solicitation efforts and $33,217.24 has been paid out.

Dr. Schachter moved "That the 2000 year budget be approved."

This motion was seconded by Dr. Browder.

CARRIED

b) Special Fund.

Dr. Palmer reported that the Special Fund was performing well considering the downturn in the stock market. As of May 31 the Special Fund stood at $323,646.16.

c) Membership Fees 2002

Dr. Palmer reviewed the membership enrolment trends since 1994. There was an increase

in membership during the first five months of 2001. This he attributed to the differential registration fees instituted for Society Members for the Alliston Meeting. The current membership stands at 395 Regular members, 135 Student Members, 60 Postdoctoral Members and 70 Emeritus Members. Dr. Palmer suggested that membership fees for 2002 be increased to $100 from the current $95, $40 of which are forwarded to CFBS as our contribution to national scientific lobbying.

After discussing this matter, Dr. Palmer moved "That Membership Fees for 2002 be increased to $100".

Dr. Lewis seconded this motion.

CARRIED

d) Using credit cards for membership payment on the Web.

Dr. Palmer brought up the matter of implementing membership fee payment by credit card. Having had good success with Web registration and fee payment for !he Alliston Meeting, it was decided to explore the financial implications of having Management Meeting Services take on this responsibility. Dr. Sharom volunteered to contact MMS and get a cost for their services for both doing the registration for the 2002 Banff Meeting as well as membership fee payment using the Web.

Secretary Report

a) BULLETIN

It has been decided to publish the BULLETIN in the Fall after the AGM instead of the Spring so that it will reflect more recent activities of the Society instead of matters which are a year old.

b) Ballot Report

Dr. Tustanoff reported that Dr. David Andrews, Department of Biochemistry, McMaster University, was elected to the Office of Vice-President. As there were two Councillor resignations, Dr. Turner and Dr. Bouvier, four Councillors had to be elected this year. Dr. Joe Casey, Department of Physiology, University of Alberta, and Dr. Bruce Waygood, Department of Biochemistry, University of Saskatchewan, were elected to a three-year term and Dr. Claude Lazure, Clinical Research Institute of Montreal, and Dr. David Litchfield, Department of Biochemistry, University of Western Ontario, were elected to a two-year term to replace the two Councillors who had resigned. There were 174 ballots cast.

c) Society Awards.

2001 Dr. Tustanoff announced that Dr. Ford Doolittle, Department of Biochemistry and Molecular Biology, Dalhousie University, is the recipient of the 2001 Roche Diagnostics Prize for Biomolecular and Cellular Research and that Dr. Natalie C.J. Strynadka, Department of Biochemistry and Molecular Biology, University of British Columbia, will receive the 2001 CSBMCB's Merck Frosst Award. The competition for both awards was very keen reflecting the high calibre of the nominations.

Merck Frosst-CSBMCB $750 travel stipends were awarded to five students who attended the 2001 Winternational meeting which was held at Mont Ste Anne, Quebec. They were Ms. Kazuko Miyakawa, National Institute of Bioscience & Human Technology, Ibarali, Japan; Dr. Diana Bellovino, Instituto Nazionale de Ricerca per gli Alimenti e la Nutrizione, Rome, Italy; Ms. Edith D. Wong, Section of Molecular and Cellular Biology, University of California, Davis; Ms. LaShaunda King, Department of Medical Chemistry and Pharmacology, University of Illinois at Chicago; and Mr. Julian Guttman, Department of Anatomy, University of British Columbia.

Mr. Roberto Botelho, Hospital for Sick Children, Toronto was the recipient of the Roche Diagnostics Poster Award.

There were 19 travel stipends allocated to students and postdoctoral fellows to attend the Alliston Meeting - eight $750 Merck Frosst CSBMCB Travel Awards, four $375 Merck Frosst CSMBCB Travel Awards, three $750 Perkin Elmer Post-doctoral Travel Awards, and four NANUC Post-doctoral Travel Awards. The $750 awards were given to students who resided outside of Ontario.

d) IUBMB Toronto 2003 Congress Update

Dr. Lewis reported that the XIX IUBMB/ PABMB 2003 Congress Planning Committee met in Toronto on April 27 and a copy of their unapproved minutes were received by the Society Secretary. The first announcement brochure will shortly be distributed. A series of 11 Satellite meetings have been proposed, along with a preliminary list of Plenary lecturers. Dr. Bridger has withdrawn as the Chair of the fundraising committee and a

replacement must be found immediately since there has been little if any activity within this portfolio.

Dr. Palmer, who is in charge of the 2003 Young Scientist Congress Programme, filed his report on the Birmingham Young Scientist meeting along with a preliminary budget of $261,000 for the Toronto Young Scientist Programme. A number of housekeeping matters were discussed.

On behalf of the Society, Dr. Tustanoff reported that he submitted an application to the NRC International Conference Revolving Loan Fund for $25,000, the sum the Society pledged to support the 2003 IUBMB Congress in Toronto. This money will be used instead of Society funds and will have to be repaid only if the Toronto Congress does not generate a sufficient profit to cover this sum. The loan application was discussed at the NRC's CISET meeting and was approved.

e) Dr. Alastair Matheson

It is with regret that the Society has lost a long-standing supporter of our Society. Dr. Alastair Matheson, who was President and member of the Executive Board of our Society from 1979 to 1981, passed away on May 18. Dr. Matheson graduated from the University of Toronto and worked for a number of years in Ottawa at the National Research Council. He was appointed Chair of Biochemistry at the University of Victoria and subsequently Dean of Science. Al established an international reputation for his work on ribosomal proteins and was elected a Fellow of the Royal Society of Canada in 1984. Dr. Matheson was a great friend of Canadian Science and a warm and generous colleague.

f) Report on the 2001 11th Winternational Meeting.

Dr. David B. Williams reported that the 11th Winternational Meeting "Dynamics of Intracellular Organelles and Molecular Machines" was held at Mont Ste. Anne, Quebec, from Feb. 8-11, 2001. The Symposium was very successful in its goal to be a truly international meeting, attracting scientists from Japan, Italy, Germany, Switzerland, France, the United Kingdom, Finland, and the U.S.A. There were 30 speakers (17 Canadian) and 108 attendees for a total of 148 participants. Seven sessions over three days encompassed the following topics: Endoplasmic Reticulum; Protein Folding & Degradation; Protein Machines and Organelle Dynamics; Diseases Associated with Protein Folding & Trafficking; Nucleus; Mitochondria, Peroxisomes & Chloroplasts; and a session to discuss the concept of a Canadian Molecular Biology Organization modelled on EMBO. In addition, 58 posters were presented in two separate poster sessions.

g) 18th IUBMB Birmingham Congress

The Society sent three delegates, Dr. Peter Lewis, Dr. Fred Palmer and Dr. Joel Weiner, to represent the Society at the General Assembly of 18th International Congress of Biochemistry and Molecular Biology which was held on July 16-20, in Birmingham England. Of the three nominations that our Society submitted through NRC for election to the IUBMB Executive Committee, two were successful. Dr. Peter Dolphin, Department of Biochemistry, Dalhousie University, was elected to the post of Treasurer for a six-year period, and Dr. Joel Weiner was elected to chair the IUBMB Nominating Committee. Dr. Michel Chretien's nomination to the position of President-Elect was not successful. The increase in IUBMB unit subscription levy to $500, which is paid by NRC, was approved making Canada's annual contribution $8000.

As our Society is hosting the 19th IUBMB Congress in Toronto in 2003, a number of presentations and activities took place at the Birmingham Congress. Dr. Peter Lewis, Chair of the Toronto LOC, made a presentation to the IUBMB Executive Committee on the status of the Toronto meeting. Dr. Joel Weiner, the President of the Toronto Congress, made two slide presentations, one to the General Assembly and one at the closing ceremony of the Congress. NRC publicized the Toronto meeting, having set up a booth in the Congress Exhibition area where various material was handed out. In addition the Toronto IUBMB Congress Organizing Committee, with the financial help of Tourism Toronto, held a reception in the Birmingham Council House for all national delegates, meeting organizers and Canadians attending the Congress. Dr. Palmer was invited to participate in the Congress's Young Scientist Travel Fellowship Symposium Programme which took place prior to the Birmingham Congress, as he will be organizing this programme in 2003 in Toronto.

h) Society Directory

Dr. Tustanoff suggested that since our Membership Directory is no longer current, the Board should consider issuing an electronic version. The

cost of printing and mailing a hard copy of the Directory, which is out-of-date as soon as it comes off the press, is excessively expensive. It should possible to have Members update their Directory information directly on our Web page and keep it current. The Board was in agreement with this proposal and Dr. Sharom, along with Dr. Palmer, will look into this matter.

Other Reports

There were no reports.

New Business

No matters were brought up.

Change of Chair

Dr. Leon Browder assumed the Chair as President of the Society. Dr. Browder expressed both his and the Society's gratitude to Dr. Frances Sharom for her unflinching devotion, leadership and timeless hours of hard work spent on behalf of the Society during her tenure as President.

Approval of Signing Officers for 2001-2002.

Dr. Davies moved that the President, Treasurer and Secretary be appointed the signing Officers for the Society for the period 2001-2002.

This motion was seconded by Dr. Waygood.

CARRIED

Adjournment

The meeting was adjourned after the Chair received a motion from Dr. Baker which was seconded by Dr.Palmer.

CARRIED

CSBMCB/SCBBMC Financial Statement 2000

BALANCE BROUGHT FORWARD (Jan 1, 2000)		
Secretary's Account	1,463.06	
Treasurer's Account	880.73	
		2,343.79
RECEIPTS		
Bank Interest		88.67
Banquet Tickets		1,460.99
Bulletin Advertising/Subscription		483.50
CFBS Levy (includes GST)		17,513.83
Corporate Sponsorships		19,484.62
GST/HST		1,226.12
Journal Subscriptions		1,373.44
Membership List Sales		1,506.00
Monetary Exchange		94.64
Society Dues		14,880.46
Special Fund		17,500.00
Summer 2001-Sponsor		5,000.00
TOTAL INCOME		**82,956.06**
EXPENDITURES		
Accountant's Fees		5,671.00
Bank Charges		22.00
Bulletin		
Editing (2000)	1,225.00	
Mailing (1999)	822.94	
Printing (2000)	2,424.80	
		4,472.74
CFBS Levy (includes GST)		18,650.17
CFBS Ottawa Meeting		
Awardee Travel	902.61	
Board Meeting	425.29	
Society Banquet	2,626.32	
Student Travel Awards	4,500.00	
		8,454.22
Membership Directory		1,057.05
Industry Canada		30.00
Int. Fed. Cell Biol.		445.21
Journal Subscriptions (NRC)		1,373.44
M. Smith Memorial		250.00
PABMB (98, 99, 00)		2,980.04
Secretary's Expenses		
Mail-Fax-Postage-Courier	179.97	
Office Supplies	599.67	
Printing	250.15	
Telephone (LD)	602.68	
Travel (CSBMCB, IUB)	918.58	
		2,551.05
Service Awards		633.30
Student Symposia		1,000.00
Summer 2001 Meeting (Advance)		10,000.00
Tax (late filing penalty)		204.64
Treasurer's Expenses		
Courier (99)	122.75	
Mail (99)	341.30	
Phone-FAX (99)	27.41	
		491.46
Vice-President's Expenses		206.87
Web Domain Name		230.08
Winter Board of Directors Meeting		5,857.02
Winternational (2001)		5,000.00
Winternational (2002)		1,000.00
TOTAL EXPENDITURES		**70,580.29**
2000 YEAR END BALANCE		12,375.77
SPECIAL FUND (market Value)		326,746.16
Capital asset (Editor's computer)		1,190.00
TOTAL ASSETS		**340,311.93**
OBLIGATIONS CARRIED FORWARD		
Travel Awards (M-F)		3,750.00
Award Lecture Honoraria (2)		2,000.00
GST/HST		1,226.12
TOTAL OBLIGATIONS		**6,976.12**

11th Winternational Symposium Held at Mont Sainte-Anne, Québec

Reinhart Reithmeier

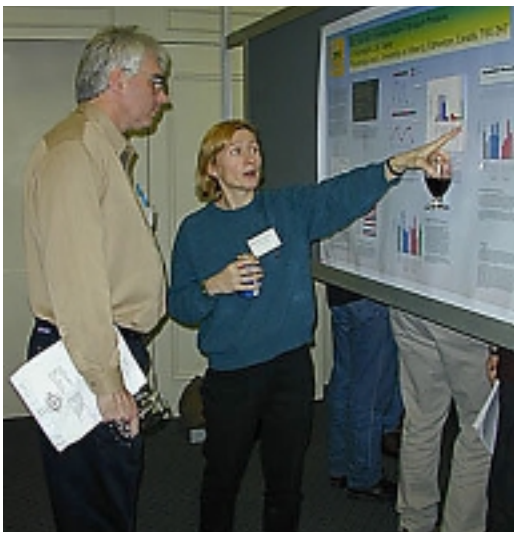
The 11th Winternational Symposium entitled "Dynamics of Intracellular Organelles and Molecular Machines" was held February 8 – 11, 2001 at Château Mont Sainte-Anne, Beaupré, Québec. This exciting meeting, sponsored by CSBMCB, brought together a renowned group of international and Canadian scientists. Topics included protein folding, degradation and trafficking, organellar biogenesis and dynamics, inter-organellar signalling and diseases associated with protein folding. Each session was complemented by oral presentations by a select group of young investigators, students and fellows chosen from the submitted abstracts. The meeting attracted participants from as far away as Japan, Italy, Finland and the UK. The CSBMCB provided Merck-Frosst travel awards to a number of students who submitted abstracts. The winners of the travel awards were Kazuko Miyakawa (Japan), Diana Bellovino (Italy), Edith Wong (UC, Davis), LaSaunda King (U. Illinois) and Julian Guttman (U. British Columbia).

The poster sessions were packed and ran well into the evening. The quality of the presentations was excellent and the poster judges (Michel Bouvier, Richard Wozniak and Reinhart Reithmeier) had a difficult task in selecting the winners of the poster competitions. The winner of the Jake Duerksen Memorial Graduate Student Poster Award, supported by Roche Diagnostics, was Roberto Botelho from the Hospital for Sick Children and the Department of Biochemistry, University of Toronto. The NRC Research Press Poster Award was won by Marie-Eve Paquet from the Department of Immunology at the University of Toronto.

Early Saturday morning a "mini-ice storm" brought down local power lines. The hotel staff rose to the occasion and arranged a candle-light breakfast and provided a gasoline generator to run the slide projector. Our American friends were impressed by the ability of Canadians to adapt so quickly and easily. Saturday evening saw the group in Québec City enjoying the Winter Carnival parade. For many, the bone-chilling weather was tempered with the help of a hot "caribou" drink. The meeting finished with a session organized by John Bergeron who proposed the creation of a Canadian Molecular Biology Organization with the view of creating a Canadian equivalent to laboratories at the EMBL in Heidelberg. Rod McInnis brought us up-to-date on the exciting developments happening with the Institutes at CIHR.

A meeting of this calibre could not be arranged without the untiring efforts of the organizers and the generous contributions of the sponsors. John Bergeron (McGill), John Aitchison (Institute for Systems Biology, Seattle) and David Williams (Toronto) are congratulated for a superb job. We thank the sponsors for their continued support of the Winternational Symposium.

The next Winternational, organized by Joe Casey (Alberta), will be held March 21-24, 2002 in Banff, Alberta. We look forward to another exciting meeting.



(left) Winternational symposium poster presentation. (below) The organizers of the 11th Winternational Symposium

44th Annual Meeting of the CSBMCB, Alliston, Ontario: "From the Genome to Structure and Function"

Report submitted by
Frances J. Sharom,
Past-President CSBMCB

The 44th Annual Meeting of the CSBMCB was held from May 31 to June 3, 2001, at the Nottawasaga Inn in Alliston, Ontario. This exciting conference, which was the first Annual Meeting independently organized by the society, brought together a group of internationally recognized scientists in the fields of proteomics, genomics, and bioinformatics. The meeting accommodated a total of 245 registrants, including 49 graduate students and 18 post-doctoral fellows, and 25 speakers from Canada and around the world.

Dr. Peer Bork, Senior Scientist and Group Leader at the European Molecular Biology Laboratory, opened the meeting by delivering the IUBMB Jubilee Lecture, talking on comparative genome analysis. The high calibre scientific program, organized by Dr. Peter Lewis and Dr. Peter Davies, included symposia focussing on topics such as structural genomics, computational biology and bioinformatics, functional genomics, and proteomics. Such an ambitious program could not have been arranged without the generous support of our sponsors, and the untiring efforts of Peter Davies in rounding up private sector financial support. Many thanks are owed to our numerous private sector sponsors, who covered everything from travel costs for symposium speakers, to coffee breaks and beverages at the poster pubs.

Dr. Peter Lewis, who played a major role in planning the scientific program of the meeting.

Two lively poster competitions were held in the afternoon and evening, assisted by sponsored pubs, with 43 posters presented on Friday, and another 34 on Saturday. The quality of the presentations was extremely high, making it difficult for the poster prize judges to select winners.

Other highlights of the meeting included two afternoon workshops, one on DNA micro-arrays (presented by a team from Toronto, organized by Dr. Jim Woodgett), and the other featuring NMR techniques (presented by NANUC, organized by Dr. Hans Vogel).

Zayna Khayat, a Ph.D. candidate at the University of Toronto, organized an excellent Career Development Workshop for graduate students and post-doctoral fellows, focussing on careers outside academia. Speakers included individuals in careers as diverse as a scientific patent lawyer, and a scientific recruiting firm.

Dr. Rod McInnes, Director of the Institute of Genetics, CIHR, gave a lunchtime address to explain the structure of the new CIHR institutes, and to announce forthcoming training programs.

Presentation of various CSBMCB awards took place during the banquet on Saturday evening.

Roche Diagnostics Award

This prize, donated by Roche Diagnostics, recognizes a Canadian scientist with a record of outstanding achievement in research in biochemistry, molecular or cellular biology. The 2001 Roche Diagnostics Award was presented to Dr. W. Ford Doolittle, of the Department of Biochemistry and Molecular Biology at Dalhousie University. Dr. Doolittle presented his award lecture, entitled "Uprooting the Tree of Life" as part of the first conference symposium.

Merck Frosst Award

The CSBMCB Merck Frosst Prize is an annual award established by the CSBMCB and donated by Merck Frosst Canada to recognize meritorious research in biochemistry, molecular or cellular biology in Canada. The chosen award winner is recognized to have accomplished outstanding research at an early stage of their career (less than ten years of independent research experience). Dr. Natalie Strynadka, of the Department of Biochemistry and Molecular Biology at the University of British Columbia, accepted the 2001 Merck Frosst Award for her work in the area of the structure of bacterial membrane components. After dinner, Dr. Strynadka presented her award lecture "Structure-based antibiotic discovery on the bacterial membrane."

Jeanne Manery Fisher Memorial Lecture

This keynote Lectureship is awarded in honour of the late Jeanne Manery Fisher, Professor of

Biochemistry, University of Toronto. Dr. Fisher, who was not only an outstanding biochemist, but also a remarkable teacher, was instrumental in creating the society's Equal Opportunity Committee and fought diligently for women's position in the field of science. The Lectureship is presented to an eminent Canadian woman scientist who has a distinguished career in biochemistry, molecular or cellular biology resulting from her outstanding contributions to research, teaching or society. The 2000 Lectureship was shared between Dr. Amira Klip of Sick Children's Hospital in Toronto, and Dr. Carol Cass, of the University of Alberta. Dr. Klip presented her award lecture in 2000. After the banquet, Dr. Cass presented her award lecture on "Nucleoside transporter proteins: from membrane biology to therapeutic applications."

Dr. Carol Cass is Professor and Chair of Oncology and Adjunct Professor of Biochemistry in the Faculty of Medicine & Dentistry at the University of Alberta and Associate Director (Research) of the Cross Cancer Institute, the comprehensive cancer centre for Edmonton and northern Alberta. Her research in molecular membrane biology and cancer therapeutics is focused on nucleosides and their mechanisms of cellular uptake. She is a member of the CIHR Molecular Biology of Membrane Proteins Group and the Membrane Transport Group in the Faculty of Medicine & Dentistry and of Experimental Oncology at the Cross Cancer Institute. Dr. Cass received B.Sc. (1963) and M.Sc. (1965) degrees from the University of Oklahoma. Her graduate training was in cell biology (University of California, Berkeley, Ph.D. 1970) with Morgan Harris and her postdoctoral training was in cancer therapeutics and biochemistry (University of Alberta, 1970-73) with Alan Paterson. Her postdoctoral research on nucleoside transporters of erythrocytes laid the foundation for subsequent development of the field and she and her colleagues have made many fundamental contributions, ranging from identification of transporter proteins by molecular cloning to the demonstration that nucleoside transporters are required for pharmacologic activity of many nucleoside drugs used in cancer therapy. She has won a variety of research and service awards, and held NCIC career awards continuously from 1974-1999. Dr. Cass has been active in scientific organizations in Canada and internationally. She was President of the Canadian Society of Cellular & Molecular Biology in 1992-1994, and a founding member of the Executive of the Canadian Society of Biochemistry and Molecular & Cell Biol-

Anthony Duncan, of Roche Diagnostics, presents Dr. Ford Doolittle with a plaque for the 2001 Roche Diagnostics Award.

Dr. Paul Payette, of Merck Frosst, congratulates Dr. Natalie Strynadka on receiving the 2001 Merck Frosst Prize.

Dr. Frances Sharom, then President of the CSBMCB, with Dr. Carol Cass, a co-winner of the 2000 Jeanne Manery Fisher Memorial Lectureship.

ogy. She is currently on the Advisory Board of the CIHR Institute of Cancer Research, the Medical Advisory Board of the Gairdner Foundation, the Selection Committee of the Canadian Medical Hall of Fame, Chair of the CIHR Michael Smith Award of Excellence Selection Committee, Chair of the NCIC Terry Fox New Frontiers Grants Panel, and Chair of the 2001 Gordon Research Conference on Purines, Pyrimidines and Related Substances. Dr. Cass is also an active volunteer in the Canadian Cancer Society and is a member of the Alberta/Northwest Territories Division Board of Directors and the National Board of Directors.

Poster prizes

A judging team led by Dr. Bruce Waygood, of the Department of Biochemistry at the University of Saskatchewan, spent several hours adjudicating 40 very high quality graduate student posters. After a difficult decision, the two graduate student poster prizes, donated by Roche Diagnostics, were awarded to Tudor Moldoveanu, of the Department of Biochemistry at Queen's University, and Peter Kavsak, of the Samuel Lunenfeld Research Institute at Mount Sinai Hospital.

Dr. Harry Duckworth, of the Department of Chemistry at the University of Manitoba, was the leader of the judging team for the CSBMCB postdoctoral fellow poster prizes, for which there were 13 posters in contention. The prize winners were Dr. Denis Daigle, of the Department of Biochemistry at McMaster University, and Dr. Laurie Graham of the Department of Biochemistry at Queen's University.

Travel awards

The CSBMCB, with the sponsorship of Merck Frosst, presented 12 travel awards to graduate students to assist with travel to the conference and the costs of registration and accommodation. The winners were: Laila Singh, Simon Fraser University; Philip Berardi, University of Calgary; Knut Woltjen, University of Calgary; Stephanie Minnema, University of Calgary; Josh Rizak, University of Saskatchewan; Mohammed Hadi, University of Manitoba; Nicolas Bilodeau, Université Laval; Jacquelyn Wood, Dalhousie University; Jason Baardsnes, Queen's University; Tudor Moldoveanu, Queen's University; Greg Vilk, University of Western Ontario; and Rebecca Crane, University of Guelph.

The CSBMCB, with the sponsorship of Perkin-Elmer and NANUC, presented seven travel awards to post-doctoral fellows to assist with travel to the conference and the costs of registration and accommodation. The winners were: Dr. Colin McGowan, Simon Fraser University; Dr. Fiona Mansergh, University of Calgary; Dr. Mike Wride, University of Calgary; Dr. Laurie Graham, Queen's University; Dr. Melanie Tomczak, Queen's University; Dr. Michael Kuiper, Queen's University; and Dr. Peter Ferguson, University of Western Ontario.

Retirement of executive members

Several members of the CSBMCB executive officially "retired" from their positions at the meeting. Dr. Peter Davies, of the Department of Biochemistry at Queen's University, stepped down as Past-President, having worked his way up (or down!) from President-Elect through President over the past three years. Peter played a very large role in the organization of the conference, especially the fundraising from external sponsors. He was presented with a commemorative plaque and an Inuit sculpture. Dr. Marlys Kochinsky, who served as the CSBMCB Councillor in charge of educational activities, also stepped down after three years of service to the society, and was awarded a commemorative plaque.

Next Annual Meeting

The next CSBMCB Annual Meeting (our 45th) will be held in conjunction with the Winternational Meeting at the Banff Centre, in Banff, Alberta, from March 21-24, 2002 (www.csbmcb.ca/2002meeting). Registration for the meeting will start in October 2001. We look forward to seeing you there!

(above) Dr. Frances Sharom, then President of the CSBMCB, thanks Dr. Peter Davies, the outgoing Past-President, for his excellent work on behalf of the society, and presents him with a commemorative plaque.

(right) Dr. Sharom thanks Dr. Marlys Kochinski, who steps down from a three-year position as CSBMCB councillor.

Dr. Reinhart Reithmeier, responsible for the local meeting arrangements, makes his closing remarks to the conference attendees.

Dr. Marie Fraser and Dr. Gilles Lajoie talking about the finer points of protein crystallization.

(above) Dr. Bruce Waygood engaged in a lively discussion at the posters.
(left) More science being actively discussed at the poster session

Winners of the graduate student Roche Diagnostics poster prizes. From left to right: Anthony Duncan, Roche Diagnostics; Tudor Moldoveanu, Peter Kavsak, Dr. Bruce Waygood, coordinator of the poster judging team.

Winners of the post-doctoral fellow CSBMCB poster prizes. From left to right: Dr. Harry Duckworth, coordinator of the poster judging team, Dr. Laurie Graham, and Dr. Denis Daigle.

Graduate student winners of the Merck Frosst travel awards, with Dr. Paul Payette of Merck Frosst (back row, far left).

Post-doctoral winners of the NANUC travel awards for post-doctoral fellows. (l. to r.)Dr. Brian Sykes of NANUC, Dr. Laurie Graham, Dr. Peter Ferguson, Dr. Melanie Tomczak, and Dr. Michael Kuiper.

Post-doctoral winners of the Perkin Elmer travel awards for post- doctoral fellows. (l. to r.) Janice Watkin of Perkin Elmer, Dr. Fiona Mansergh, Dr. Colin McGowan, and Dr. Mike Wride.

Society Awards 2000-2001

Travel Awards

Winternational Meeting, Mont Ste. Anne, Quebec. February 8 -11, 2001

Merck Frosst-CSBMCB $750 travel stipends were awarded to:

Dr. Diana Bellovino, Instituto Nazionale de Ricerca per gli Alimenti e la Nutrizione, Rome, Italy. "Studies on retinol binding protein regulated secretion"

Ms. Kazuko Miyakawa, National Institute of Bioscience & Human Technology, Ibarali, Japan; "An uncleavable signal peptide: Identification of the central hydrophobic region and amino terminal region of FGF-9 as functional domains of secretin"

Ms. Edith D. Wong, Section of Molecular and Cellular Biology, University of California-Davis, Davis, California. "The Dynamin-related GTPase, Mgm1p,Is an Intermembrane Space Protein required for maintenance of Fusion Competent Mitochondria"

Ms. LaShaunda King, Department of Medical Chemistry and Pharmacology, University of Illinois at Chicago. "A DnaK-BiP chimera promotes survival of DnaK-deficient E. Coli cells after heat stress"

Mr. Julian Guttman, Department of Anatomy, University of British Columbia. "Microtubule-Based Motor Transport of a Junction Plaque- Spermatid Translocation by Sertoli Cells"

Travel Award winners at the Winternational Meeting, Mont Ste. Anne, Quebec. February 8 -11, 2001

AGM Meeting , Alliston, Ontario, May 31-June 3, 2001

$750 awards - Merck-Frosst Travel Awards for graduate students

Name	University	Supervisor
Laila Singh	Simon Fraser University	Jenifer Thewalt
Philip Berardi	University of Calgary	Karl Riabowol
Knut Woltjen	University of Calgary	Derrick Rancourt
Stephanie Minnema	University of Calgary	Derrick Rancourt
Josh Rizak	University of Saskatoon	Bruce Waygood
Mohammed Hadi	University of Manitoba	Yeuwen Gong
Nicolas Bilodeau	Universite Laval	Robert Faure
Jacquelyn Wood	Dalhousie University	David Byers

$375 Awards - Merck-Frosst Travel Awards for graduate students

Jason Baardsnes	Queen's University	Peter Davies
Tudor Moldoveanu	Queen's University	Peter Davies
Greg Vilk	University of Western Ontario	David Litchfield
Rebecca Crane	University of Guelph	Janet Wood

$750 awards - Perkin Elmer Travel Awards for post-doctoral fellows

Colin McGowan	Simon Fraser University	William Davidson
Fiona Mansergh	University of Calgary	Derrick Rancourt
Mike Wride	University of Calgary	Derrick Rancourt

$375 awards - NANUC Travel Awards for post-doctoral fellows

Laurie Graham	Queen's University	Peter Davies
Melanie Tomczak	Queen's University	Peter Davies
Michael Kuiper	Queen's University	Peter Davies
Peter Ferguson	University of Western Ontario	Gary Shaw

Poster Competitions

2000 Roche Diagnostics Poster Awards, AGM Ottawa.

Duerksen Award Cell Biology

Sandy Beyko, Department of Biochemistry, Microbiology and Immunology, University of Ottawa. "Cerebral Abnormalities in CX32 Deficient Mice." Supervisor Dr. Steffany Bennett.

Molecular Biology Award

Alex Valencia, Department of Biology Carleton University, "Cloning and Characterization of cDNA Clones Encoding Putative Cell Wall-Associated Proteins of *Neurospora crassa.*" Supervisor Dr. John Vierula

Biochemistry

Karen M. Black, Department of Biochemistry, Dalhousie University, "An Absolutely Conserved Tryptophan; Investigating Its Role In Cytochrome C." Supervisor Dr. Carmichael J.A. Wallace

2001 Roche Diagnostics Poster Award - Winternational Meeting, Quebec

Mr. Roberto Botelho, Hospital for Sick Children, Toronto. "Localized biphasic changes in phosphatidylinositol-4-5-bisphosphaate at sites of phagocytosis".

2001 Roche Diagnostics Poster Awards - AGM, Alliston

The Student Poster Session which was presented Friday, June 1, had 42 poster presentations, 38 from students and four from Principal Investigators. Of the 38 student presentations, 29 elected to compete for the two Roche Diagnostics Poster Awards. Dr. Waygood, Saskatoon, headed an adjudication committee (Dr. G. Cote, Queen's; Dr. G. Flynn, Queen's; Dr. F. Keeley, Toronto; Dr. C. Lazure, IRMC; Dr. D. Litchfield, Western Ontario; Dr. N. Martin, Queen's; Dr. L. Mclntosh, UBC; Dr. D. Rose, Toronto; Dr. H. Vogel, Calgary; and Dr. S. Withers, UBC) for these awards.

Mr. Tudor Moldoveanu, Department of Biochemistry, Queen's University. "Structural Basis for the Activation of Calpain by Ca++."

Mr. Peter Kavsak, Department of Medical Genetics and Microbiology, University of Toronto. "Smad7 binds Smurf2 to form an E2 ubiquitin ligase that targets the TGF receptor for degradation."

CSBMCB 2001 PDF Poster Awards- AGM, Alliston

The PDF Poster Session was held Saturday, June 2 with 36 poster presentations, 13 by PDFs, 22 from PI's and one from a grad student. Eight PDFs entered the CSBMCB poster competition to contest for the two awarded prizes. These posters were adjudicated by a committee headed by Dr. H. Duckworth, Manitoba; included Dr. H S Chan, Toronto; Dr. E. Pai, Toronto; and Dr. D. Wishart, Alberta. Winners are:

Dr. Denis Daigle, Department of Biochemistry, McMaster University. "YjeQ, an essential *Escherchia coli* protein of unknown function, is an unusal GTPase of the Ras/EF-Tu class that contains a circular permutation of the GTPase domain and exhibits marked burst kinetics."

Dr. Laurie Graham, Department of Biochemistry, Queen's University. "Proteomic analysis of the odorant/pheromone-binding protein family in *Drosophila*."

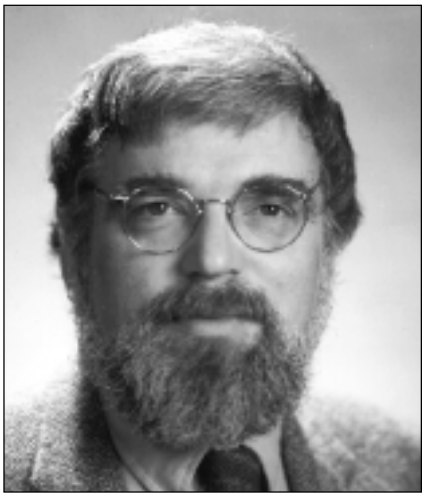
Society's Distinguished 2001 Awards

2001 Roche Diagnostics Prize for Biomolecular and Cellular Research Awardee: Dr. W. Ford Doolittle

Dr. Doolittle, an American by birth, was educated at Harvard University (B.A., 1963) and Stanford University (Ph.D., 1969). First appointed to the Department of Biochemistry at Dalhousie University as an Assistant Professor in 1971, he rose through the ranks to attain a full professorship in 1982. For the past 28 years here in Halifax, he has undertaken an active and productive career in research by demonstrating remarkable scholarship in the area of evolutionary biology. During the last 14 years he has also been the Director of the Evolutionary Biology Program, sponsored by The Canadian Institute for Advanced Research. This has been the most successful of the CIAR-programs and currently includes participating scientists from across Canada and the USA. Dr. Doolittle is working in a highly theoretical field and is widely respected by the international scientific community. His research is directed to the most fundamental level of biology. He is an ardent defender of the pursuit of fundamental knowledge and he is prepared to make the case for this research to university presidents, members of parliament and the lay public. I believe he is one of the most fundamental thinkers in biological sciences in Canada today. His ideas and theories have stood the test of time over two decades in a highly debated and rapidly developing field of theoretical research.

Dr. Doolittle has 150 original publications, 30 review articles and 10 book chapters. His ideas and theories on cellular evolution from the perspective of RNA and DNA sequences and his research accomplishments are widely recognized and cited in the literature. He is frequently an invited speaker at prestigious international meetings on evolutionary biology, such as the Keystone Symposia, the Gordon Research Conferences, and the National Academy of Sciences. He has received numerous honours over the years and is a Fellow of the American Association for the Advancement of Science, the CIAR, the Royal Society of Canada and the American Academy of Microbiology. Most recently he received an honorary doctorate from the University of Ottawa.

Dr. Doolittle' s major research focus for many years has been the use of the techniques of molecular biology for gene mapping and sequencing to determine how Jiving organisms and their genes have evolved. Through his study of archaebacteria he has contributed to the progressive revision of theories of how life evolved via several evolutionary kingdoms. His early studies focused on ribosomal RNA of cyanobacteria and the investigation of the endosymbiont theory for the development of chloroplasts and mitochondria in cells. As data have accumulated to support the concept that archaebacteria are much closer evolutionary to eukaryotes than prokaryotes he expanded his studies to thermophilic and halophilic archaebacteria. The methods developed in his laboratory provide highly flexible and reliable tools for genetic analysis in these organisms, where essentially no previous genetic mapping had been completed. Such studies have helped to define the origin of eukaryote specific genes.



Dr. W. Ford Doolittle

In addition to his ability as an experimental scientist he is an unusually perceptive thinker. He possesses remarkable insight into theoretical issues which results in very creative ideas that stimulate debate on the process of evolution. He has an excellent capacity to critically assess current theories of evolution, construct new theories and develop innovative ways for evaluating those theories. His article in 1980 concerning "selfish DNA," published in *Nature*, was a very important starting point for the idea that the bulk of the DNA which makes up large genomes is the product of genetic processes and selection occurring within the genome and has little to do with the fitness of the organisms. This was the insight that began his rise to international recognition. Important analysis of population genetics provided support for the concept that repeat sequences of DNA in introns play a major role in redesigning development pathways for genes. He was also responsible for developing the concept that introns, which account for 90% of the human genes, are relics of the first assembly of

genes. His greatest contributions have been at the theoretical level and he has become one of the principal contributors internationally to the debate on the genome and genome structure which has led to redefinition of evolutionary relationships.

Dr. Doolittle has been a leader at all levels. He is an excellent mentor for graduate students, some of whom have gone on to post doctoral fellowships in laboratories of Nobel Laureates. Former graduate students and post doctoral fellows that have trained in the Doolittle laboratory are now in demand as faculty members across Canada and in the USA. His research program has become a focus for development locally. Dr. Doolittle and his colleagues in the CIAR program have been the foundation for the Biomolecular Structure-Function, Genomics, Genetics initiative that forms a cornerstone of the Research Strategy for Health Research developed by the Faculty of Medicine and incorporated into the Strategic Research Plan of Dalhousie University. Nationally, he helped initiate efforts to develop a Human Genome Program in Canada and participated development of the CIAR Evolutionary Biology Program of which he became the director. More recently, he has been a key contributor to the development of the Genome Canada Project and to the subsequent establishment of the Atlantic Genome Centre which has evolved from his initial proposal for a study of comparative microbial genomics, broadly based among institutions in all the eastern provinces.

The 2001 Laureate CSBMCB's Merck Frost Awardee: Dr. Natalie C. J. Strynadka

Dr. Strynadka received her university training at the University of Alberta, obtaining her B.Sc. in Biochemistry in 1985 and her Ph.D in 1990 under the supervision of Dr. Mike James.

During the next seven years she stayed on in Dr. James's laboratory, initially as a Post-Doctoral Fellow and then as a Research Associate. In 1997 she was appointed Assistant Professor in the Department of Biochemistry and Molecular Biology at University of British Columbia. Although Dr Strynadka still in the early stages of her career as an independent investigator,she has already established an outstanding international reputation for her work in structural biology. A Burroughs Wellcome New Investigator Award (in 1999) and a Howard Hughes International Scholarship (in 2000) are truly remarkable achievements and amply demonstrate the high regard of the international research community for the pioneering work done by Dr Strynadka and her colleagues.

Dr. Natalie C. J. Strynadka

The quality and significance of her work may readily be judged by the fact that so much of her research has been published in top flight journals such as *Nature, Journal of Biological Chemistry, and Biochemistry*. She has published 42 papers and has participated in numerous symposia as well as receiving many university speaking engagements.

The relevance of Dr. Strynaadka's specific research achievements, which have an immediate and, most likely, longer-term impact on the scientific tableau may be summarised by her following accomplishments.

a) Solving, for the first time in any species, the structure of a membrane-associated signal peptidase (Paetzel et al, *Nature*, 1998). This structure has revealed a novel catalytic mechanism and has provided the basis for understanding substrate specificity. Importantly, the structure provides a template for the design of compounds to inhibit bacterial growth, such that signal peptidase is being explored as an excellent target for antibiotic development.
b) Solution of the structure of the "Tir/Intimin" complex that mediates the adhesion of pathogenic E. coli to mammalian host cells (Luo et al, *Nature*, 2000). This work is the first report of an adhesion complex in any pathogenic species and provides major insights into the binding sites and multivalent character of this cell-cell attachment. It is widely expected that this knowledge will provide a firm basis to design agents to inhibit pathogen adhesion and perhaps to develop anti-pathogen vaccines.
c) Definition, for the first time, of the structure and kinetic properties of the retaining glycosyltransferase from Neisseria meningi-

tidis (Persson et al, *Nature:* Structural Biology, 2001). This enzyme is a key player in the synthesis of lipopolysac-charide in Neisseria species and this structure is the first to reveal interactions of the enzyme with both acceptor and donor sugars. This work is important from the perspective of basic biochemical characterization of this major class of enzymes and also because of the central role of lipopolysaccharides in bacterial pathogenesis.

d) Solution, for the first time, of the structure of a class D beta-lactamase - that of the enzyme from Pseudomonas aeruginosa (Paetzel et al, *Nature*: Structural Biology, 2000). This structure is revealed as a homo-dimer that has a novel catalytic mechanism and site for substrate binding. This structure should provide a template for the design of inhibitors and in this sense is vital because no clinically useful inhibitors of this class of beta-lactamases have yet been developed.

Workshop On:
Myelin Structure And Its Role In Autoimmunity

June 5-8, 2002, Hotel Giubileo, Rifreddo, Potenza, Italy

Sponsored by the International Society for Neurochemistry (ISN), the Italian Association of NeuroImmunology (AINI), and the Multiple Sclerosis Society of Canada.

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OBJECTIVES OF THE WORKSHOP

Myelin, the insulating sheath surrounding nerve axons, has proven to be one of the most difficult membrane systems to study. The reason is that myelin is the product of an intimate contact between two different cell types and because myelin has a compact multilamellar structure that limits the accessibility of its components. Indeed, the organisation of myelin at the molecular level and,

in particular, the high resolution structures of myelin proteins, remain to be clarified. There is also a significant lack of information regarding the putative functions of the various myelin proteins, since they do not seem to possess any detectable biological (viz., enzymatic) activity. However, deciphering myelin is very important in order to understand autoimmune demyelinating diseases, such as multiple sclerosis (MS) in the CNS. Amongst the candidate myelin autoantigens we have: MBP, the basic protein of myelin; PLP, the proteolipid; and more recently MOG, the glycoprotein of the myelin of the oligodendrocytes. Research on the structure of the myelin sheath has long been one of the main topics for the study of physiopathology of myelin. The myelin basic protein (MBP) was isolated and characterized by Eylar in 1969-1970. One important achievement was the finding in 1984 that MBP could be isolated in a form associated with the lipids originally present in the membrane. After a time of very active research on myelin structure, in particular in Canada and in the United States, this declined slowly. So far, the structures of MBP as well as other myelin proteins are still unknown.

In 1992, Ross Smith published a review in *J. Neurochem*. on the structure of MBP, in which he asserted that "further clarification of the structure and function of MBP may have to await development of more powerful techniques for studying proteins bound to large molecular aggregates, such as lipid bilayers". From the references reported in his review, it appears that research on MBP structure declined after the period 1984-1987.

Today, new and powerful techniques are available that were not at the time of Smith's review.

Funding from the European Union of a BIOMED project on "High resolution structure of myelin proteins" (1996-1999) gave a strong impetus to cooperation amongst seven European research groups active in Basel, Strasbourg, Bruxelles, Koeln and Potenza. At present, there is a fruitful cooperation between research centers studying myelin structure in Potenza, Bari, Parma, Rome, and Genoa, in Italy; Strasbourg and Grenoble in France; Uppsala in Sweden; Berlin in Germany; Campinas in Brasil; Boston in the USA; Toronto and Guelph in Canada; and Melbourne in Australia. Interesting results are now appearing regarding the crystallization of myelin proteins, their incorporation into lipid films, and the determination of their structures in solution both in the native and in the unfolded forms. We expect that this workshop will help people to interact better, and aim for presentations that can be understood by researchers with different backgrounds.

In 1994, one of us, Paolo Riccio, organised and funded a workshop on "Proteases involved in demyelination and their origins" at the ESN (European Society for Neurochemistry) meeting in Jerusalem. He felt then that research on the involvement of proteolytic enzymes in demyelination was in a declining phase and had to be revitalised. It is now apparent in the literature that proteases, and in particular matrix metalloproteases, may have important roles in demyelinating diseases. Similarly, we want to provide new impetus to biochemical, biophysical, and molecular biological studies on the structure of myelin and myelin proteins. There is no doubt, indeed, that in order to understand the mechanisms of myelin breakdown, we have to know how the target of myelinotoxic factors is built up, how this target can be made more resistant to degradation, and what the roles are of autoantigens in their native form. Thus, we try to correlate the structure of myelin and myelin proteins/peptides with pathological events. Events involving cell/cell recognition, antibody/protein interaction, and protein/lipid association are revealing more and more the importance of protein structure and a possible role of molecular mimicry. New physical techniques and bioinformatics approaches have to be discussed in the context of myelin function.

The conference on Myelin Structure and Its Role in Autoimmunity could be considered a follow up of a very successful workshop that Paolo Riccio organised 11 years ago in Naples: Antigenic Properties of Myelin and the Role of Myelin in Pathology (Riccio P. and Dal Canto M.C. (1991) Conference Report, Naples, 22-23 October 1990. *J. Neuroimmunol.* **32**, 185-186).

On these grounds, the aims of this meeting are the following.

1) To gather together people who are interested in the architecture of the myelin sheath and, especially, the high resolution structures of myelin proteins.
2) To encourage the synthesis of different kinds of expertise in the fields of biophysics, biochemistry, molecular biology, neurology, neuroimmunology, and bioinformatics. Techniques such as SANS, SAXS, EXAFS, FITR, CD, AFM, Langmuir-Blodgett films, gravimetric analysis, mass spectroscopy, capillary

electrophoresis, zymography, electron and scanning probe microscopies, are specialised and not all well-understood by most researchers. On the other hand, some colleagues working on myelin proteins' structures may be so focussed that they may need to refresh their knowledge on what myelin as a whole really is.

3) To correlate the knowledge of myelin structure with its breakdown in demyelinating diseases, and to elucidate both how myelinotoxic factors can attack the myelin sheath and how myelin proteins can have a role in autoimmunity.
4) Finally, to describe in preliminary sessions selected techniques in biophysics, biochemistry, and bioinformatics, that would be of general interest to all attendees, but particularly young scientists, and provide them with a basis for understanding subsequent sessions and the literature.

Information on the Location of the Workshop:

Hotel Giubileo, Potenza, Basilicata, Italy

http://Giubileohotel.cjb.net
http://www.directa.net/basilicata/potenza/hotels4/giubileo.html
http://www.italyguide.com/giubileo/giubileo.html
http://potenza.pandora.it/giubileo/igiubileo1.htm
http://potenza.pandora.it/giubileo/igiubileo3.htm

The hotel (1200 m altitude), 12 km from Potenza, is situated in the green mountains surrounding Potenza.

Potenza, a town of about 75,000 inhabitants and the capital of Basilicata, is set between Bari and Naples in Southern Italy in the upper Basento Valley near the Appennino Lucano. Of antique pre-Roman origins, it has been conquered by different peoples in the course of the centuries, Lombardian, Swabian and Angevin. Today, Potenza has a vivacious cultural life and a small university with about 6,000 students.

Potenza can be reached by train or by car from Naples (170 km) or Rome (360 km), or by car from Bari (135-170 km), much more easily then Barga (Lucca), which is where Gordon Conferences on Myelin take place in Italy. Pompei (140 km), on the way to Naples, or the rupestrian churches and quarters of Matera (UNESCO monuments) (100 km), on the way to Bari, are also very interesting places to visit. *http://www.emmeti.it/Welcome/Basilicata/Potenza/index.uk.html*

PROPOSED STRUCTURE OF THE WORKSHOP

Type of workshop : Scientific (75%) and educational (25%).

Seven main sessions of about 150-250 min. each, platform presentations of 30 – 45 min. each:

1st session: Physical techniques to determine native membrane and/or protein structures;
2nd session: General aspects regarding structure and physiopathology of the myelin sheath;
3rd session: Reconstituted systems / Crystallography;
4th session: Recent achievements related to the structure of myelin proteins;
5th session: Factors influencing the formation, compaction and swelling of myelin. Myelinotoxic factors. Myelin breakdown.
6th session: The antigenic properties of myelin peptides. Molecular mimicry.
7th session: Bioinformatics and molecular modelling. Data warehousing and databases.

Estimated number of attendees: 80 in total.

40 posters with some posters selected for brief oral presentation. Other posters could be supported by Power Point presentation displayed on laptops.

Conference fees: 450 USD (full) and 300 USD (students).

Registration fee includes participation in all sessions, lodging, full board and transportation from Bari and Naples Airports to the conference venue.

Report on the AUUBC

March 9-11, 2001 at Mount Saint Vincent University, in Halifax, Nova Scotia.

Kathryn Vanya Ewart
NRC Institute for
Marine Biosciences,
Halifax NS

The 32nd Annual Atlantic Universities Undergraduate Biology Conference (AUUBC) was held on March 9-11 at Mount Saint Vincent University, in Halifax, Nova Scotia. This is an annual conference for life sciences students across Atlantic Canada, featuring presentations of Honours project research. It is supported by the Atlantic Provinces Council on the Sciences (APICS) and organised by members of the APICS Biology Committee. Barbara Rao of Mount Saint Vincent University managed this year's conference. The conference featured 20 speakers and 17 poster presentations. In all, 135 people attended, including a number of younger undergraduate students whom we hope will be among the presenters at our future conferences. The AUUBC was held concurrently with the APICS Aquaculture conference (50 attendees) so there was a lively crowd of life sciences enthusiasts when the events were underway.

Oral presentation award winners Karen MacDonald, Dawn Armstrong and Megan Ferguson.

Roger Lee (Memorial University, chair of the APICS Biology Committee), and poster presentation award winners Robert Bishop, Renée Cormier, Michael Taboski and David Chiasson. *Photos: Lois Whitehead (APICS)*

Topics covered at the AUUBC ranged from biological oceanography and field ecology to gene expression and genomic analyses. Oral and poster presentations were judged by three-member panels from the Biology committee and cash prizes were awarded. The diversity made judging a special challenge but it also made the conference an exciting mix for all involved. Prizes this year were awarded as follows:

Oral presentations

First Prize - **Monica Shuegraf**, *St. Frances Xavier University* The effects of temperature, viscosity and growth on Artemia locomotion at intermediate Reynolds numbers

Second Prize - **Melissa Wamboldt**, *Mount Saint Vincent University* Mechanism of oocyte hydration in marine teleosts

Third Prize - **Megan Ferguson**, *Acadia University* Partial sequence analysis of the mtDNA of the deep-sea scallop, *Placopecten magellanicus*

Honourable Mentions -

Karen MacDonald, *Mount Allison University* Cardiac function in diabetic rats treated with a metal complex

Dawn Armstrong, *Dalhousie University* The effect of geographical isolation on the genetic distribution of two American lobster (*Homarus americanus*) sub-populations

Posters

First Prize - **Robert Bishop**, *Memorial University of Newfoundland* Does toxaphene act as an endocrine disruptor in juvenile yellowtail flounder (*Limanda ferruginea* Storer)

Second Prize - **David Chiasson**, *Saint Francis Xavier University* Polyphenols and UV protection in *Aglaothamnion oosumiense*

Third Prize - **Michael Taboski**, *SMU* Cadmium uptake in the marine fungi *Collospora lacera* and*Monodyctus pelagica*

Honourable Mention - **Renée Cormier**, *Acadia University* Distribution and recolonization of pitcher plant-inhabiting insects following defaunation in bogs surrounded by forest, clear-cut and scrub

Atlantic Universities Undergraduate Biology Conference (AUUBC)

The Biology committee of the Atlantic Provinces Council on the Sciences *(http://www.apics.dal.ca/index2.html)* holds an annual conference for undergraduate life sciences students across Atlantic Canada.

The **Atlantic Universities Undergraduate Biology Conference (AUUBC)** features oral and poster presentations by Honours students from universities throughout the area. Each university can send two poster and two oral presenters to the conference.

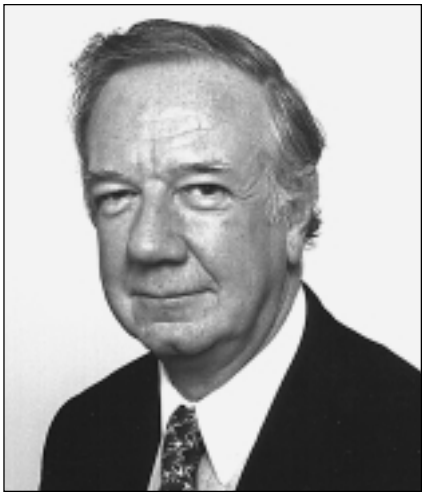
Be sure to encourage your honours students to attend this conference or others like it in other regions – this can be the start of a great career in life sciences.

In Memoriam

Peter Dolphin: A Remembrance

Peter Dolphin, a great friend and supporter of the CSBMCB, died suddenly on Friday, June 22, 2001 at the age of 54. His mother died of a heart attack on the same day 10 years earlier. Peter had been feeling unwell for about a week but refused to consider that it could be anything serious. Barb Bigelow, the departmental secretary, didn't agree and persuaded him to go to the Emergency Clinic on Monday. He was admitted to the hospital right away and was told that he had already had a mild heart attack. By Thursday, he seemed much better and complained that he was bored, asked for visitors and especially wanted to see data from the lab. Then suddenly on Friday morning he suffered cardiac arrest and couldn't be revived. Word spread quickly. His daughter Fiona and his sister Alex flew over from England and the funeral, in beautiful old St. Paul's Church, was packed with colleagues and friends from many walks of life.

We are all finding it difficult to fully imagine life without Peter. He had been at Dalhousie since 1978 and was very active in all aspects of life in biochemistry, in our department, in the university, in Canada and indeed in the world. At the time of his death he was Secretary General of PABMB and treasurer of the IUBMB. He loved to travel and had been to Chile and Brazil several times in connection with PABMB business. One of his goals was to increase interactions between Canadian and South American biochemists and students. Peter was instrumental in securing the huge IUBMB meeting for Toronto in 2003. He went to Tokyo for a meeting of the IUBMB executive and made all of the right arguments. Recently, he was involved in several planning functions for the IUBMB meeting, including the Scientific Program Committee. Peter was active in the former Canadian Biochemical Society in various capacities in the 1980s and early 90s, and in 1996 was president of its expanded successor, the CSBMCB. Later he served as president of the CFBS. He also was Regional Director for the MRC/CIHR in the Maritime Provinces (1998-2001). In this role, he helped many local applicants and grantees with funding issues. For his own research in the area of lipoprotein metabolism, Peter always held grants from MRC/CIHR, as well as from the Heart and Stroke Foundation. He was very involved in peer review and in research policy development for these and other organizations.



Peter Dolphin

Peter had many honours undergraduate and graduate students in his laboratory over the years and was a true mentor to them. One student in particular was a quiet young Malaysian woman who did her honours project in my lab. Eve Teh was terrific at the bench but hesitated about graduate school. Peter could see that she had a real talent for research and persuaded her to join his lab. In the following four years, Peter taught Eve lipoprotein metabolism in all its arcane detail and Eve introduced molecular biology into Peter's lab and cloned a novel mutant gene for cholesteryl ester transfer protein (CETP). Her work won a number of prizes and she is now a CIHR fellow at UBC in Ross McGillvary's lab, doing beautiful structure-function studies on transferrin.

Eve won the Patrick Prize, given by retired faculty member Syd Patrick, for a Ph.D. thesis judged to have had the most impact in the previous two years. She flew here last March for a seminar and presentation of the prize. Peter put on a wonderful party for her. He cooked for days, then donned a French Chef's outfit and served delicious international cuisine to all of us!

Peter liked teaching and often referred to his role model for the ideal biochemistry teacher, Prof. Muhammad Akhtar at the University of Southampton in England. Peter did his undergraduate work in honours physiology and biochemistry at Southampton followed by his Ph.D. in Akhtar's lab, finishing in 1971. I was a postdoctoral fellow in Akhtar's lab at the same time, along with 13 students from all over the world. It was a great place. Akhtar was so dynamic, and full of ideas. He was basically a steroid chemist, but already in 1968 he was intrigued by the notion of using molecular biology to sort out

Peter and Eve Teh at the party celebrating the awarding of the Patrick Prize (March 2001)

steroid hormone action. Akhtar talked to every one of his students every day and loved a good argument. But he was always encouraging and helpful. I could see many of Akhtar's mentoring qualities emerge in Peter over the years. Peter really was interested in students, and not just his own students either. After his funeral, several graduate students from other labs told me stories about how he had helped them in important ways in scientific as well as personal matters. He always took part in student-sponsored activities, and, as an excellent pool-player himself, he organized a popular annual pool tournament for graduate students.

Peter was very pleased to receive a D.Sc. in recognition of his research from the University of Southampton in 1989, and looked splendid at convocations here wearing the red and blue D.Sc. robes. He always enjoyed celebrations, such as a special dinner at Southampton in Akhtar's honour on his retirement in 1998 with about sixty of Akhtar's former students and fellows in attendance. Another special occasion for Peter that I remember particularly well was the CBS annual dinner in 1988 in Quebec City. The Nobel Laureate, Dorothy Hodgkin, had given the first Jeanne Manery Fisher award lecture and was the guest of honour. Peter had always admired Dorothy's many accomplishments in biochemistry and was delighted to have a good long conversation with her about science and politics. I know he especially liked the attached photo of Dorothy and himself.

Dorothy Hodgkin and Peter at the CBS banquet in June, 1988, Quebec City

I have mentioned Peter's generosity with students but I also remember many examples of kindness with his colleagues. He could be generous with his time, ideas, practical technical help and money. A young female colleague has told me that Peter's advice and encouragement were often helpful and that he seemed to have special empathy for younger researchers with family responsibilities. He pushed for more protected research time for young faculty members. And he was kind to older colleagues as well: when I lost grant funding for a while in the mid 90s, Peter found some travel money for me to attend an important conference.

Peter liked to fix things and especially to build small instruments, models for the lab and for home and beautifully finished wooden toys for his children. When Carl Breckenridge moved from department head to the associate deanship, Peter built a very funny model commemorating Carl's early days. He also was an accomplished tailor and made silk-lined ties and vests of every hue and pattern. Waiters at a local restaurant wore his splendid vests and many of us bought his ties for Christmas presents. My son has one with flying cats on it. Peter's own wardrobe often elicited comment, especially the bright pink shirt worn with an equally bright tie and matching handkerchief.

Peter was a very loyal person and was quick to defend colleagues, institutions and programs that he valued. When graduate programs at Dalhousie were threatened, with what many of us in biochemistry considered an ill-considered proposal for reorganization, Peter's response was classic. I can't quote it here, but he said what he thought and it was certainly colourful, as well as effective. He could always be counted on for an honest, forthright opinion. Although he had a lot of opinions, he wasn't doctrinaire and, like Akhtar, loved a good argument. And he loved to laugh.

It was obvious at Peter's funeral that he had friends from many diverse communities in Halifax. In addition to many academics, there also were friends from the world of theatre and costume de-

sign, fellow kite enthusiasts, associates from the Liberal party, pool-players and golfers and many of his son Michael's school friends.

Fiona and Michael buried their father's ashes next to his mother's grave in Sutton Coldfield in England last month. They had a gathering at a local golf club with family and Peter's long term collaborator and good friend from Paris, John Chapman. Some champagne was drunk: Fiona says that Peter would have approved.

The department has established a Peter Dolphin Memorial Fund to provide a graduate student award. Donations may be made payable to Dalhousie University and sent to the Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS, B3H 4H7.

– Catherine Lazier
Department of Biochemistry & Molecular Biology,
Dalhousie University

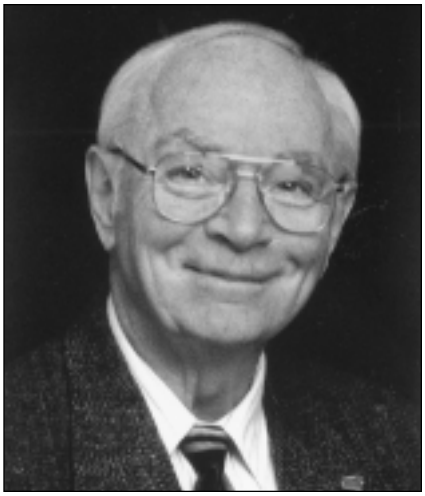
Kenneth Percy Strickland

(August 19, 1927 - August 28, 2000)

Dr. H. B. Stewart and E.R. Tustanoff

It is with sadness we relate to our membership that one of our Society's devoted and long standing members passed away after suffering from Alzheimer's. Dr. Ken Strickland served our Society well. He was a member of the Board as a Councillor from 1972 to 1975, and then took on the onerous task as Society Treasurer from 1978 to 1981.

Kenneth (Ken) enrolled in Honours Chemistry at the University of Western Ontario in 1945, completed his degree in 1949, and proceeded in graduate study in the Chemistry Department to complete his Master's degree in 1950. He then joined Roger Rossiter in the Department of Biochemistry and, with some innovative research involving the incorporation of radioactive phosphorus into neurological tissues and tissue fractions, he finished his Ph.D. in 1953. He then gained two years experience in the laboratories of R.H.S. Thompson in Guy's Hospital Medical School in London, England. He returned to his London (Canada) department as Assistant Professor, supported for two years by a Lederle Medical Faculty award. This support was followed by a National Research Council and later a Medical Research Council Research Associateship as an Associate Professor (1962) and Full Professor (1966). This support continued until 1979 when the Medical Research Council created the Career Investigator post, which he held until 1990. In 1971-72 he spent a year at the John Curtin School of Medical Research, Australia National University and later returned for five months in 1976. The University of Western Ontario granted him tenure in 1989 and he remained on staff until 1993 when he was awarded Emeritus status.



Ken willingly accepted various academic responsibilities outside his research activities. He contributed substantially to the Medical Programme and, when the Honours Biochemistry programme was established, played a major role in the first survey course of the discipline as well as supervision of senior students in various projects. He was eagerly sought after as a supervisor in the Graduate Faculty. Seven Doctoral and six Masters candidates successfully completed their degree programmes under his supervision. Beyond these activities he acted as an external examiner of doctoral students, was a referee on various publications and served as a site visitor on several occasions.

Ken's service to his University was distinguished. As an undergraduate he was interested in athletics and as a faculty member he became a member of the Athletic Directorate for eight years

(President for two years). He served on the Senate and on committees including the Library Council and the Advisory Committee on the Health Aspects in the use of lonizing Radiation. Within the Faculty, he served on the Admissions Committee, the Long Range Planning Committee and in his own Department he served as Acting Chairman for two years as well as on the Promotion and Tenure Committee, the onerous Graduate Studies Committee and others.

From the mid-fifties until he retired, his research was supported by the National Research Council and the Medical Research Council. Following the pioneering work with the incorporation of radioactive phosphorus into phospholipids he expanded his investigations into an examination of the steps in the biosynthesis and degradation of individual phospholipids containing glycerol and inositol. Coupled with these studies was an examination of the metabolic consequences of muscle denervation. This aspect of the work led to studies in normal and myopathic (UM-X7.1) hamsters and L-6 rats. In medical terms these observations provided models for the investigation of demyelinating diseases and the abnormalities observed in muscular dystrophy. In all, with the help of 13 graduate students and five post-doctoral fellows, Ken produced a total of 84 refereed journal articles and seven chapters in books.

In private 1ife, Ken married Isabel and they had four children (three girls and one boy). After the death of Isabel, Ken married Mary with whom he remained until his death. He was a very devoted family man and deeply involved with his children. Every summer an extended camping trip to some remote part of Canada or to the States was intricately planned and of course the family summer cottage was a stated venue.

Ken was a warm and compassionate person – he will be missed by all his student colleagues and friends. He was always ready to pitch in and help and at times was taken advantage of because of his empathy for people. He was a very religious person and very much involved in his parish. He served as a lay preacher and was instrumental in planning and building a new church. He was not evangelistic and if you did not have access to his private life, you would never know he was so deeply involved with his religion. As a quiet and unassuming person Ken Strickland has made his mark on Biochemistry at Western.

Insulin-regulated Glut4 Traffic In Muscle Cells:

A Concerted Action of the Cytoskeleton, Selective Fusion Proteins and Endosomal Sorting Mechanisms

The 2000 Jeanne Manery Fisher Memorial Lectureship Award

Amira Klip, Dailin Li, Zayna A. Khayat, Varinder Randhawa and Leonard J. Foster.

Cell Biology Programme, The Hospital for Sick Children, Toronto, Ont. M5G 1X8.

Preface

It is the greatest honour to receive an award in the name of a foremost Canadian woman scientist and mentor. Dr. Jeanne Manery Fisher, Harvard and Rochester educated, biochemist, taught at the University of Toronto from 1932 to 1948. An expert in the physiology of ion transport and the generation of energy, she has inspired my work on insulin regulation of glucose and Na/K-transport in muscle. Jeanne Manery Fisher's work was supported initially by grants from the Insulin Research Fund, later from the Medical Research Council and the Muscular Dystrophy Association of Canada. Her scientific accomplishments were numerous, as were her efforts on behalf of the recognition of women in the academic world. She worked ardently with the Royal Canadian Institute to promote the cause of science and women's equality in academia. She was the President of the Toronto Biochemical and Biophysical Society and Chairman of the Equal Opportunities Committee of the Canadian Biochemical Society. She trained numerous students who continued to train the current generation of young researchers, and themselves excelled in their scientific endeavours. We humbly follow in her footsteps.

Introduction

Glucose entering the circulation during a meal is rapidly taken up by muscle and fat cells, for both energy generation and storage as glycogen. Glucose enters all mammalian cells via a family of integral membrane proteins, the glucose transporters of the GLUT family. One of these proteins, GLUT4, is specific to muscle and fat cells, and is distinct from all other transporters in that it undergoes both constitutive recycling (Satoh *et al.* 1993) and insulin-regulated exocytosis (James *et al.* 1989). The importance of this phenomenon is highlighted by evidence that insulin-dependent GLUT4 externalization, and hence the stimulation of glucose uptake, is defective in the pathophysiological state of insulin resistance underlying type 2 diabetes (King *et al.* 1992, Zierath *et al.* 1996, Zierath *et al.* 2000). In spite of extensive work, the intracellular GLUT4 donor compartments remain poorly defined, as do the effects of insulin on the route or the velocity of inter-compartmental GLUT4 traffic. This paucity in knowledge is largely due to shortcomings of biochemical approaches to isolate and characterize the diverse intracellular compartments populated by GLUT4, and by the limited intracellular space available in primary fat and muscle tissue and in cultured adipocytes for detailed immunolocalization.

Morphological studies have detected GLUT4 in distinct but inter-related intracellular pools, including sorting endosomes, TGN, recycling endosomes and specialized GLUT4 exocytic vesicles (Aledo *et al.* 1997, Hashiramoto & James 2000, Lee *et al.* 1999, Slot *et al.* 1991). Insulin shifts the subcellular distribution of GLUT4 resulting in a new steady state where a large fraction of GLUT4 resides at the plasma membrane of skeletal muscle (Douen *et al.* 1990, Hirshman *et al.* 1990, Klip *et al.* 1990), primary adipose cells (Birnbaum 1989, James *et al.* 1988), L6 muscle cells in culture (Ramlal *et al.* 1988) and 3T3-L1 adipocytes (Yang & Holman 1993).

An important protein in the insulin signalling pathway regulating GLUT4 traffic is type I phosphatidylinositol 3-kinase (PI3-K) (Kotani *et al.* 1995), an enzyme that possesses lipid kinase activity and has been demonstrated to interact with all the known insulin receptor substrate (IRS) proteins (Shepherd *et al.* 1998, White1998). This enzyme consists of an 85-kDa regulatory subunit, p85, and

a 110-kDa catalytic subunit, p110. The type I_A PI3-K enzyme is required for the insulin-dependent mobilization of vesicles containing GLUT4 glucose transporters to the plasma membrane (Cheatham *et al.* 1994, Kotani *et al. 1*995, Shepherd *et al. 1*998).

In addition to inducing translocation of glucose transporters to the cell surface, one of the early events in the insulin response is the reorganization of actin filaments. Two types of insulin-dependent morphological changes in actin filaments have been described, depending on the cell type studied: an increase in actin stress fiber formation (Goshima *et al.* 1984, Ridley & Hall 1992) and a bundling of actin below the plasma membrane, more commonly referred to as 'membrane ruffling' (Ridley & Hall 1992). The actin cytoskeleton participates in a variety of cellular functions including the compartmentalization of intracellular macromolecules and organelles. It is therefore conceivable that insulin-derived signalling intermediaries might redistribute in response to actin reorganization. However, until recently, the link between the actin microfilament network, the location of insulin signalling molecules, and the location of GLUT4 organelles have remained poorly understood.

Once at the cell surface, GLUT4 is readily removed to enter the endosomal system. GLUT4 removal from the cell surface occurs via clathrin-coated vesicles (Nishimura *et al.* 1993) assisted by the GTPase dynamin (Al-Hasani *et al.* 1998, Kao *et al.* 1998, Volchuk *et al.* 1998). Whether or not insulin inhibits GLUT4 endocytosis in fat cells is still debatable (Czech & Buxton1993, Jhun *et al.* 1992, Satoh *et al. 1*993, Yang & Holman 1993). The contribution of exocytic and endocytic pathways to insulin action in muscle cells has not been explored. Transmembrane proteins removed from the cell surface are directed first to the early endosome. From here, proteins destined for recycling will enter the recycling endosome (Mukherjee *et al.* 1997). This pathway is thought to work in a constitutive fashion but little was known until now about possible regulation of the individual steps, i.e. direction and speed of sorting out of the early endosome, fusion with the recycling endosome and exit from this compartment.

This chapter summarizes results from our laboratory that address the steady state distribution of GLUT4 in basal and insulin-stimulated muscle cells; the rates of externalization and internalization of this transporter; and the mechanisms regulating such traffic. Particular attention is given to the role of the insulin-induced actin remodelling in recruiting signalling molecules and GLUT4 vesicles beneath plasma membrane ruffles, and to the regulation of the transit of GLUT4 through the recycling endosome to reconstitute the specialized vesicle that fuses with the plasma membrane. For these studies we have made use of L6 muscle cells, which differentiate in vitro from myoblasts into multinucleated myotubes by multiple cell fusions (Yaffe 1968). In the myotube stage, these cells contain a large cytoplasmic space rich in actin filaments and are highly amenable to morphological analysis at the light microscopic level. The clonal line used for these studies has been stably transfected with GLUT4 encoding a 14 amino acid myc sequence at its first extracellular loop. This epitope is used to detect the presence of GLUT4 at the cell surface and to follow its journey within the cell.

GLUT4 compartmentalization and insulin-dependent externalization

In the L6 skeletal muscle cell line, GLUT4 expression occurs after differentiation from myoblasts into myotubes (Mitsumoto & Klip 1992). We have previously reported that expression of GLUT4myc in L6 myoblasts leads to the segregation of the protein to a GLUT4-specific pool, conferring insulin sensitivity to glucose uptake (Ueyama *et al.* 1999). This conclusion is based on the finding that in L6-GLUT4myc myoblasts, the intracellular GLUT4myc compartment contains the majority of the insulin-regulatable amino peptidase (IRAP) but less than half of the GLUT1 housekeeping glucose transporter. By expression of GLUT4myc alone the sensitivity of glucose uptake to insulin is markedly improved, compared to parental myoblasts, which do not yet express GLUT4 at this undifferentiated stage.

The exofacial myc epitope of GLUT4myc allowed us to estimate the proportion of this protein exposed at the cell surface and its total cellular content by analyzing intact and permeabilized cells, respectively. The amount of myc epitope exposed at the surface of non-permeabilized cells was determined by a quantitative assay based on the detection of anti-myc antibody bound to a monolayer of L6 cells. The total myc epitope present in L6 myoblasts was determined by permeabilization with 0.1% Triton X-100 before immunolabelling with anti-myc antibody. The primary antibodies bound are then reacted with HRP-conjugated sec-

ondary antibody coupled to a densitometric assay. The intracellular GLUT4myc content was then estimated by subtracting the amount present on the cell surface from the total cellular content. We found that, under basal conditions, 90.0 ± 0.6% of the GLUT4myc resides intracellularly. Stimulation for 30 min with 100 nM insulin elevates the cell surface content of GLUT4myc to 31.0 ± 1.7%, with a commensurate reduction in intracellular GLUT4myc.

We next examined the time course of GLUT4myc appearance at the cell surface in the presence of insulin. To measure GLUT4myc externalization, L6 myoblasts were treated with insulin in culture medium at 37°C for increasing times, and thereafter the cell surface-exposed myc epitope was reacted with anti-myc antibody at 4°C followed by the densitometric detection assay. Insulin triggered a rapid redistribution of GLUT4myc to the cell surface with a $t_{1/2}$ of approximately 4 min. This rapid insulin response peaked by 30 min (Fig. 1).

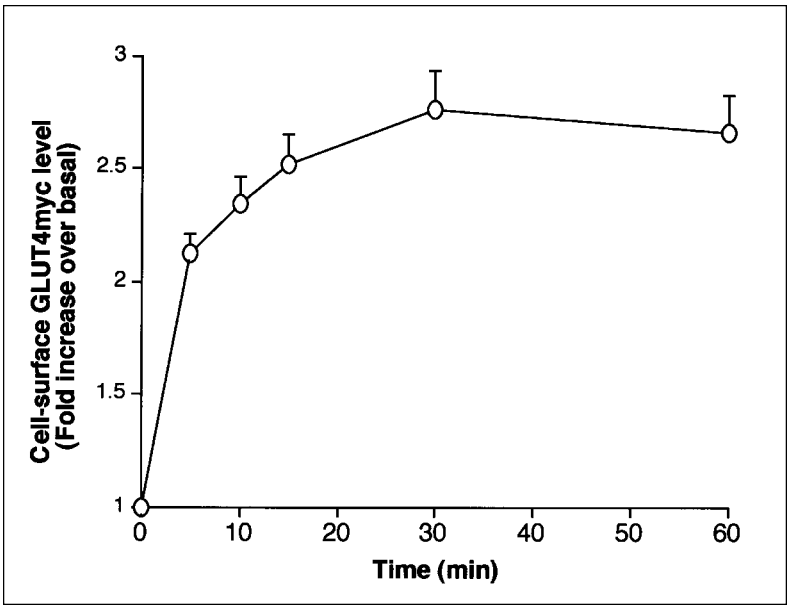


Figure 1. Insulin increases the amount of GLUT4myc at the cell surface with the time. Confluent, quiescent L6-GLUT4myc myoblasts were incubated with or without 100 nM insulin at 37°C for 5 to 60 min. GLUT4myc exposed at the cell surface was reacted with anti-myc antibody and then incubated with enzyme-linked secondary antibody coupled to an orthophenylene diamine (OPD)-based optical densitometric detection assay. $p < 0.05$ by ANOVA.

Insulin-induced actin remodelling and p85 relocalization

Recent evidence points to the potential participation of the actin cytoskeletal network in the insulin-dependent compartmentalization of GLUT4-containing organelles. Indeed, in response to insulin, actin filaments undergo remodelling in L6 myotubes, leading to the formation membrane ruffles (Tsakiridis *et al.* 1994). Pretreatment of L6 myotubes or 3T3-L1 adipocytes with cytochalasin D (CD) or latrunculin B (LB), structurally unrelated drugs which cause actin filaments to depolymerize, prevents insulin-stimulated glucose transport (Tsakiridis *et al. 1*994, Wang *et al.* 1998b). CD and LB also reduce insulin-dependent translocation of GLUT4 vesicles to the plasma membrane of L6 myotubes, 3T3-L1 adipocytes and primary adipocytes (Omata *et al.* 2000, Tsakiridis *et al. 1*994, Wang *et al.* 1998a, Wang *et al. 1*998b). These findings suggest that actin reorganization is involved in the insulin-dependent relocalization of GLUT4 from intracellular storage sites to the cell surface and the subsequent stimulation of glucose uptake. In the experiments that follow we employ fluorescence and scanning electron microscopy to compare the spatial and temporal changes in actin, relocalization of PI3-K (p85) and the changes in the localization of the GLUT4 compartment.

L6 myotubes were treated with insulin and subsequently examined for morphological changes of the cell surface. Scanning electron micrographs of the L6 myotube monolayer were obtained after 0, 3 and 10 minutes of insulin stimulation (Fig. 2). In unstimulated myotubes, very little distortion of the dorsal cell surface was observed. At 3 minutes of insulin stimulation, the plasma membrane showed structures resembling membrane ruffles, particularly above the nuclei. By 10 minutes of insulin treatment, the protrusions from the plasma membrane were more pronounced.

We used indirect immunofluorescence to compare the time-dependent distribution of intracellular actin filaments in response to insulin with the membrane ruffling observed in Fig.2. Under basal conditions (t = 0), rhodamine-phalloidin stained long filamentous structures that were aligned along the longitudinal axis of the cell (Fig. 3, a). Insulin treatment (100 nM) resulted in a rapid reorganization of cortical actin into structures reminiscent of those observed by scanning electron microscopy. The time course and pattern of insulin-dependent cortical actin remodelling (Fig. 3, a-c) was consistent with the dynamics of membrane ruffling observed by scanning electron microscopy.

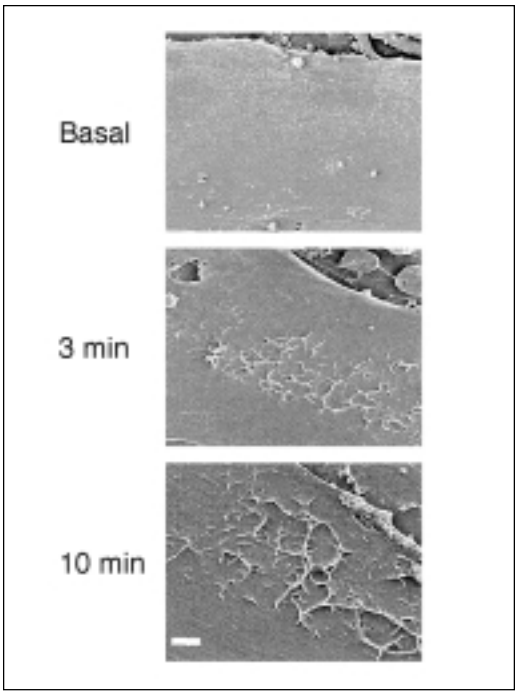


Figure 2. Scanning electron microscopy of insulin-induced membrane ruffles. L6 GLUT4myc myotubes, without (Basal) or with 100 nM insulin stimulation (3 and 10 min) were analyzed by scanning electron microscopy to detect the topography of the dorsal cell surface. Scale bar: 5 μm.

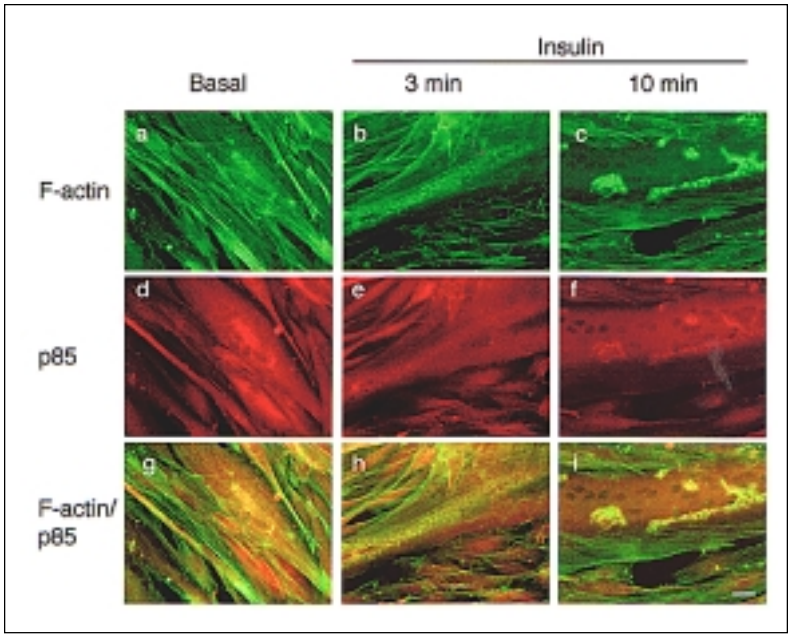


Figure 3. Insulin causes cortical actin remodelling and redistribution of the p85 subunit of PI 3-kinase to actin structures. L6 GLUT4myc myotubes, without or with insulin stimulation (3 and 10 min) were analyzed by indirect immunofluorescence to detect the distribution of filamentous (F) actin (using FITC-labelled phalloidin, green) and p85 subunit of PI 3-kinase (using a polyclonal antibody coupled to a Cy3-labelled secondary antibody, red).

Comparative analysis of the cellular localization of the p85 subunit of PI3-K with actin filaments over the same time period is illustrated in the center panels of Fig. 3, d-f. In the basal state (t = 0), p85 staining was diffusely punctate. Staining for p85 occurred throughout the myoplasm but was more concentrated in the region of the myonuclei, where it appeared to overlap with actin filaments. Within 3 minutes of insulin stimulation however, a fraction of p85 was found to relocate into the newly formed structures of actin filaments. The detection of p85 in these structures continued to parallel temporally actin staining, becoming most prominent at 10 minutes of insulin treatment. Therefore for all time points of insulin examined, a fraction of p85 staining colocalized with remodelled cortical actin. A similar pattern was observed for the p110α subunit of PI3-K, but not for p110 β (results not shown). Neither antibodies against PKC-β or the insulin receptor, nor control antibodies became concentrated with actin at any time after insulin treatment (results not shown).

Given the multiple actions of PI3-K, it is conceivable that changes in its subcellular localization in response to insulin are critical to its ability to elicit the stimulation of GLUT4 translocation and glucose transport. The p85 subunit of PI3-K rapidly associates with the GLUT4-enriched compartment in insulin-stimulated 3T3-L1 adipocytes (Heller-Harrison *et al.* 1996, Wang *et al.* 1998b). It was suggested that this interaction involves cytoskeletal elements because pretreatment of cells with CD or LB prevents the subsequent detection of PI3-K on GLUT4-containing membranes (Wang *et al.* 1998a, Wang *et al.* 1998b). Conversely, PDGF activates PI3-K at the plasma membrane and does not cause GLUT4 translocation (Nave *et al.* 1996, Ricort *et al.* 1996). These results suggest that robust activation of PI3-K alone is not sufficient for glucose transport activation. We propose that the relocalization of PI3-K to actin-rich structures may facilitate the propagation of some PI3-K-dependent signals that are necessary for the translocation of glucose transporters to the cell surface. This hypothesis led us to the following experiments.

Recruitment by insulin of GLUT4 vesicles into the actin-rich structures

Given that the p85 and p110α subunits of PI3-K colocalized with the subcortical actin structures formed in response to insulin, we searched for a possible link between actin remodelling and the

intracellular localization of the GLUT4 protein (Fig. 4). In the basal state, GLUT4myc was concentrated around the myonuclei. GLUT4myc staining remained perinuclear after the first 3 minutes of insulin treatment while actin filaments formed the cortical structures described above. After 10 minutes of stimulation with insulin, a portion of the GLUT4myc immunofluorescence began to colocalize with actin structures. Hence, in contrast to the observations with p85, there was a significant delay in the recruitment of GLUT4 into the cortical actin structures. These findings suggest that as the dorsal actin-rich structures begin to form, GLUT4-containing vesicles are slowly recruited into them, where they come to the vicinity of PI3-K.

Role of Rac in insulin-dependent actin reorganization and GLUT4myc translocation

It was previously shown that insulin-stimulated membrane ruffling in Swiss 3T3 cells is mediated by the Rho GTP-binding protein family member, Rac (Ridley & Hall 1992). To test a requirement for Rac in the actin remodelling events mediated by insulin in L6 muscle cells, we transfected a dominant inhibitory Rac1 mutant, Rac1-N17 (Zhang *et al.* 1995) into L6 myotubes. Cotransfection of Rac1-N17 cDNA with enhanced green fluorescent protein (EGFP) cDNA was performed to facilitate recognition of transfected cells. As expected, expression of Rac1-N17 did not affect the abundance of long actin stress fibres in either the basal or insulin-stimulated states (results not shown). Rac1-N17 expression prevented cortical actin remodelling which was otherwise clearly seen in the adjacent non-transfected cells (Fig. 5). We therefore examined the consequence of Rac1 inhibition on the translocation of GLUT4myc to the cell surface upon insulin stimulation. In the basal state, cells transfected with Rac1-N17 displayed the same density of GLUT4myc staining on the cell surface as adjacent, untransfected cells in the same optical field (not shown). However, the insulin-dependent appearance of GLUT4myc was largely prevented in cells transfected with dominant negative Rac1 (Fig 5). These results suggest that Rac1-dependent cortical actin remodelling may be causally linked to the incorporation of GLUT4-containing vesicles into the plasma membrane in L6 myotubes. However, they do not rule out that the dominant negative Rac1 interferes with GLUT4 traffic by means other than inhibiting the endog-

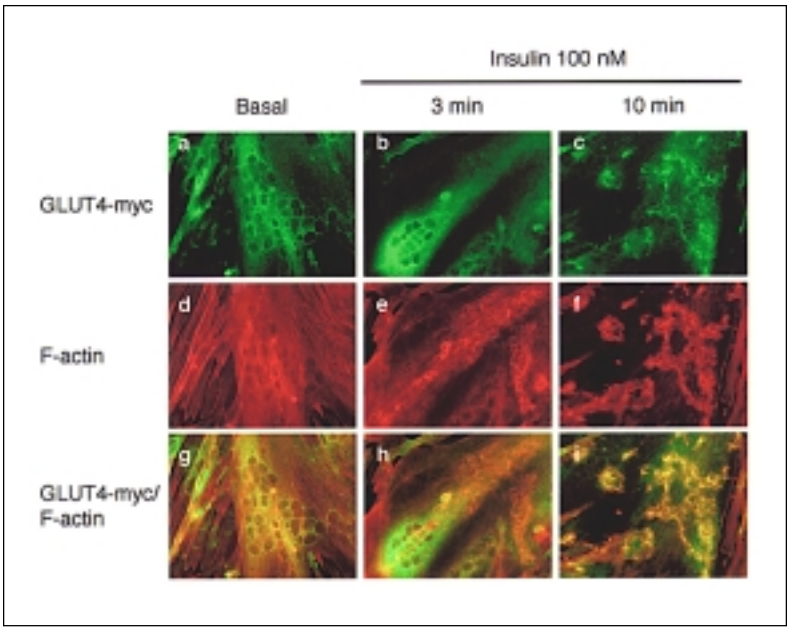


Figure 4. Insulin causes redistribution of GLUT4myc-containing vesicles to cortical actin structures. L6 GLUT4myc myotubes, without or with insulin stimulation (3 and 10 min) were analyzed by indirect immunofluorescence to detect the distribution of GLUT4myc (using a monoclonal anti-myc antibody coupled to a FITC-labelled secondary antibody, green) and filamentous (F) actin (using rhodamine-labelled phalloidin, red).

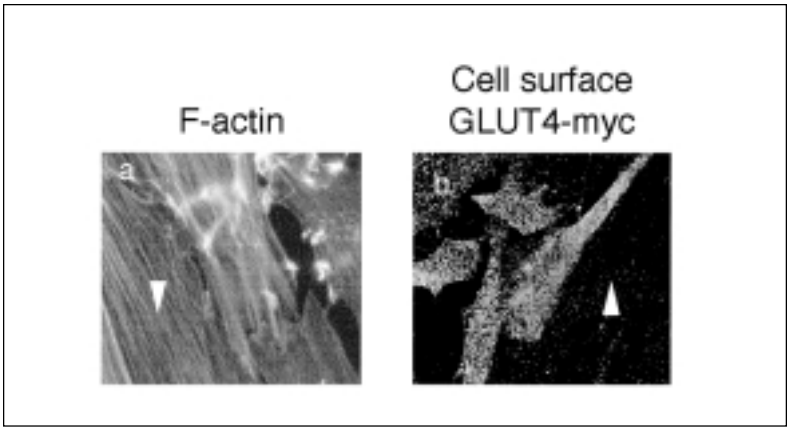


Figure 5. Dominant inhibitory Rac-1 blocks insulin-dependent cortical actin remodelling and GLUT4myc externalization. L6 GLUT4myc myotubes were transiently transfected with 0.3 μg of enhanced green fluorescent protein cDNA together with 1.2 μg of Rac1-N17 cDNA. After 48 hours, cells were exposed to insulin for 10 (a) or 20 (b). (a) F-actin was detected in permeabilized cells, using rhodamine-phalloidin. (b) Surface GLUT4myc was detected in intact cells using anti-myc antibody coupled to Cy3-labelled secondary antibody.

enous Rac1. In particular, it is conceivable that Rac1-N17 may scavenge activators of several small molecular weight G proteins, which in turn may be required for GLUT4 traffic. In this regard, it is possible that G3C, a guanine exchange factor of Rac1 and TC10, among other small molecular G proteins, could be tied up by Rac1-N17 and prevent the GTP loading of TC10. The latter protein has recently been suggested to be required for GLUT4 translocation (Chiang *et al.* 2000).

Fusion of GLUT4 vesicles with the plasma membrane requires VAMP2

Like neuronal synaptic vesicles, intracellular GLUT4-containing vesicles must dock and fuse with the plasma membrane, thereby facilitating insulin-regulated glucose uptake into muscle and fat cells. The backbone of the fusion machinery consists of a v-SNARE protein in the incoming vesicle and two t-SNARE proteins in the target membrane (See Foster & Klip 2000 for review). Specific SNARE isoforms are expressed in muscle and fat cells: the v-SNAREs VAMP2 and VAMP3/cellubrevin (hereafter called VAMP3) (Cain *et al.* 1992, Volchuk *et al.* 1994, Volchuk *et al.* 1995), and the t-SNAREs syntaxin4 (Sumitani *et al.* 1995, Volchuk *et al.* 1996) and SNAP-23 (Rea *et al.* 1998, Wang *et al.* 1997, Wong *et al.* 1997). Biochemically, the intracellular GLUT4 colocalizes in part with the vesicle-SNAREs VAMP2 and VAMP3. The participation of syntaxin4 and SNAP-23 in GLUT4 translocation has been implicated using various molecular and biochemical approaches such as introduction of botulinum toxins and neutralizing antibodies into 3T3-L1 adipocytes (see Foster & Klip 2000). Likewise, there is strong evidence for the participation of a vesicle (v)-SNARE in this process. The *Clostridial* tetanus and botulinum B or D neurotoxins specifically cleave and inactivate VAMP2 and VAMP3 (Jahn *et al.* 1995, Niemann *et al.* 1994, Schiavo *et al.* 1992). Introduction of botulinum neurotoxin D into streptolysin O (SLO)-permeabilized 3T3-L1 adipocytes (Cheatham *et al.* 1996) and microinjection of cytoplasmic VAMP2 soluble peptides and fusion proteins diminished the appearance of GLUT4 at the surface of 3T3-L1 adipocytes (Cheatham *et al. 1*996, Macaulay *et al.* 1997, Martin *et al.* 1998, Olson *et al.* 1997). While these experiments support the notion of a need for VAMPs in GLUT4 translocation, they do not distinguish which one, VAMP2 or VAMP3, is the protein responsible for GLUT4 arrival at the plasma membrane.

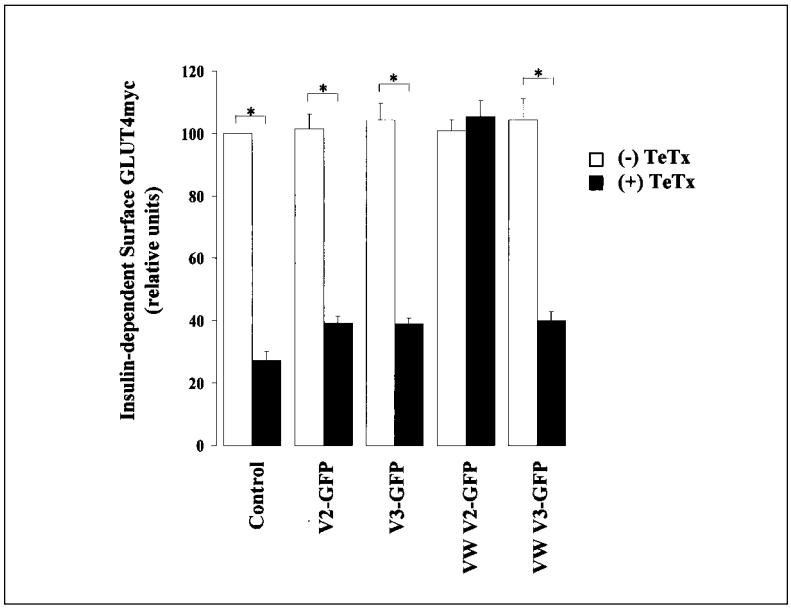


Figure 6. Tetanus toxin inhibits insulin-stimulated GLUT4myc translocation in L6-GLUT4myc myoblasts. L6-GLUT4myc myoblasts were transfected with 0.6 mg of wild type or toxin-resistant (VW) V2-GFP or V3-GFP cDNA in conjunction with 0.9 μg of pcDNA3 or TeTx cDNA. After 48h, cells were exposed to 100 nM insulin for 30 min. Surface GLUT4myc was detected by immunofluorescence in non-permeabilized cells. Results from several experiments were quantitated using NIH Image software. A value of 100% was assigned to the insulin response *above basal* in untransfected cells treated with insulin in each field of view. $p < 0.01$ relative to (*) untransfected cells or VAMP-transfected cells.

In the following experiments, we used GLUT4myc-expressing L6 myoblasts to compare the functional involvement of VAMP2 and VAMP3 in GLUT4 translocation. Myoblasts were chosen because of the ease to transfect these mononucleated cells. Transient transfection of proteolytically active tetanus toxin light chain (TeTx) cleaved both VAMP2 and VAMP3 proteins. Importantly, the transient transfection of TeTx markedly reduced the insulin-stimulated incorporation of GLUT4myc to the cell surface by about 70% (Fig. 6). Upon co-transfection of tetanus toxin with individual vesicle-SNARE constructs, only toxin-resistant VAMP2 rescued the inhibition of insulin-dependent GLUT4 translocation by tetanus toxin. Moreover, GLUT4 and VAMP2, but not VAMP3, were clustered in the insulin-induced remodelled cortical actin mesh (Fig. 7). We therefore propose that VAMP2 is a resident protein of the insulin-sensitive GLUT4 compartment, and that the integrity of this

protein is required for GLUT4-vesicle incorporation into the cell surface in response to insulin.

Insulin does not delay the removal of GLUT4 from the cell surface

Once at the cell surface, GLUT4myc undergoes rapid endocytosis. Half of the surface-labelled GLUT4myc is internalized within 3 min (Fig. 8). The rate of GLUT4myc disappearance from the cell surface was not appreciably slowed down in the continued presence of insulin, showing approximately the same $t_{1/2}$ of 3 min. These results suggest that insulin does not regulate GLUT4 internalization in L6-GLUT4myc myoblasts. This contrasts with observations made in fat cells where a small proportion of insulin-induced gain in surface GLUT4 appears to be due to inhibition of GLUT4 endocytosis (Jhun *et al. 1*992, Yang & Holman 1993).

GLUT4myc travels through the early endosome to the recycling endosome

Morphologically, different endosomes are defined by the presence of marker proteins. The Rab5 effector early endosome antigen 1 (EEA1) is a putative tethering protein that helps to bring vesicles in close proximity with the early endosome and is found solely on early endosomes (Mu *et al.* 1995). Numerous proteins undergo constitutive recycling between the plasma membrane and the recycling endosome. The transferrin receptor (TfR) is responsible for iron entry into the cell via binding to transferrin and is constitutively recycled back to the cell surface (Witt & Woodworth 1978). Myoblasts were again selected for these experiments because they can be manipulated to round up, thereby offering a better opportunity to label and differentiate intracellular organelles by confocal fluorescence microscopy, which otherwise would come too close together in flattened cells.

To follow the transit of GLUT4myc through the early endosome and recycling endosome, surface GLUT4myc labelled with anti-myc antibodies was allowed to internalize at 37˚C for different times up to 20 min. Fig. 9a shows the localization of labelled GLUT4myc and EEA1 at the onset of rewarming. Two min after initiation of internalization, some GLUT4myc could be detected in a compartment positive for EEA1 staining (Fig. 9b) and remained in this compartment for at least 5 min (Fig. 9c). At 10 min, there was no detectable labelled GLUT4myc in the perinuclear, TfR-positive

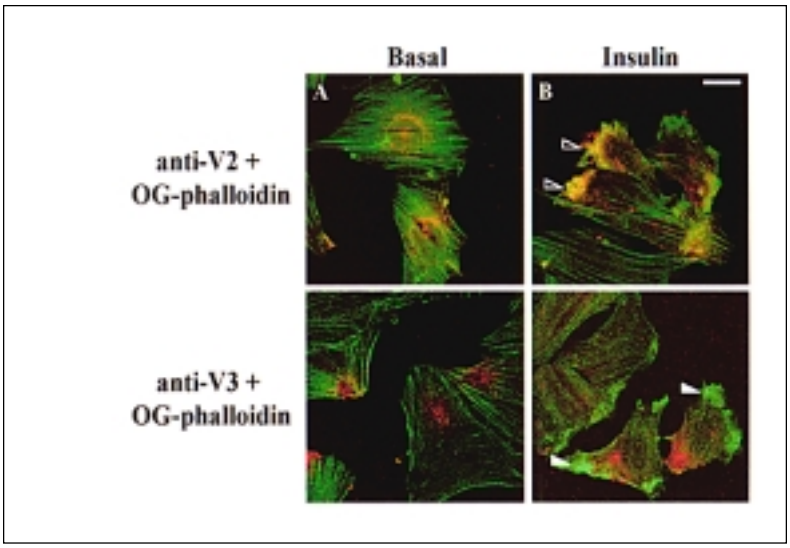


Figure 7. VAMP2, but not VAMP3, colocalizes with the cortical actin structure following insulin stimulation. L6-GLUT4myc myoblasts were left untreated (A) or stimulated with insulin (B), and processed for indirect immunofluorescence for endogenous VAMP2 or VAMP3 with anti-VAMP2 (top) or anti-VAMP3 (bottom) antibodies, along with F-actin staining using Oregon green (OG)-conjugated phalloidin. Open and closed arrows show recruitment of VAMP2 or absence of VAMP3, respectively. Scale bar: 25 μm.

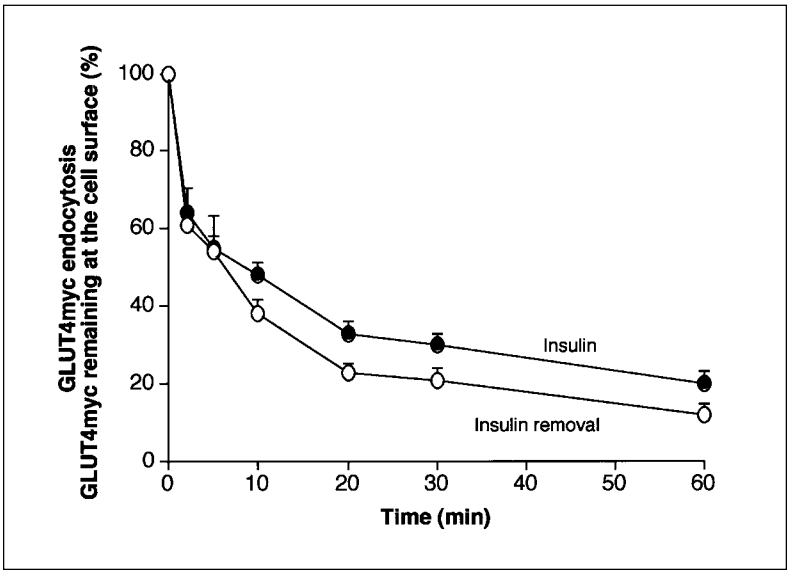


Figure 8. Insulin has little effect on the internalization of GLUT4 myc. L6-GLUT4myc myoblasts were stimulated with 100 nM insulin at 37°C for 30 min, reacted with anti-myc antibody at 4°C for 1 h to label cell surface GLUT4myc and then re-warmed to allow endocytosis in the absence or presence of 100 nM insulin for 2 to 60 min. At the indicated times, the myc antibody-labeled GLUT4myc remaining on the surface was measured by the optical densitometric detection assay.

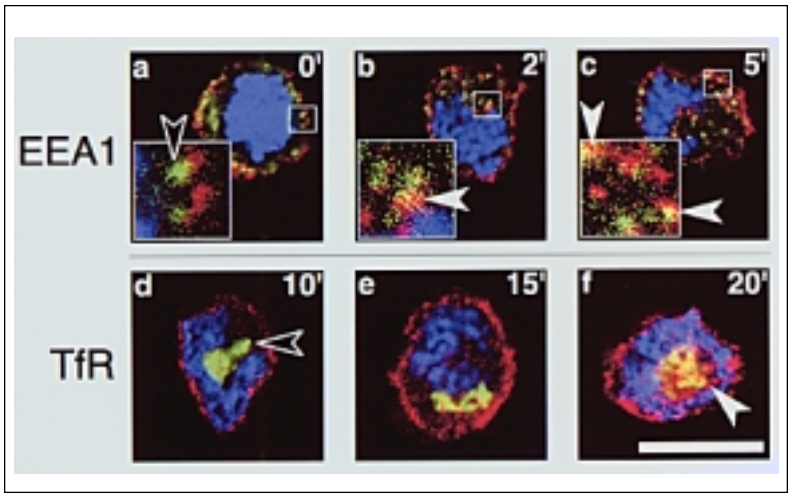


Figure 9. Internalized GLUT4myc travels through the early and recycling endosomes. Rounded-up L6 GLUT4myc myoblasts were stimulated with insulin, GLUT4myc was labelled with anti-myc antibody at 4°C, then cells were re-warmed to allow GLUT4myc endocytosis. Confocal fluorescence images are shown of surface-labeled GLUT4myc after 0, 2, 5, 10, 15 and 20 min of GLUT4myc internalization. At the end of each internalization period, GLUT4myc and other antigens were detected by indirect immunofluorescence. Red staining is GLUT4myc, blue staining is DNA, green staining is EEA1 (a-c) or TfR (d-f). Areas outlined by white boxes (a-c) are expanded 4x in insets. Filled arrowheads indicate areas of colocalization (yellow) while open arrowheads highlight areas of non-colocalization. Scale bar: 10 μm.

compartment (Fig. 9d) but by 15 min (Fig. 9e) GLUT4myc began to collect there and by 20 min (Fig. 9f) it reached a steady-state where a large portion of the labelled GLUT4myc overlapped with TfR. This distribution remained for up to 30 min.

Insulin accelerates the transit of GLUT4 through the endosomal system

GLUT4myc was allowed to internalize in the continued presence of insulin. The hormone did not appear to affect the rate of appearance of surface-labelled GLUT4myc in the EEA1-positive compartment (Fig. 10a-d). As before, in unstimulated cells surface-labelled GLUT4myc was not detected in the recycling endosome by 10 min (Fig. 10a); in the continued presence of insulin, labelled GLUT4myc was detected in the TfR-positive compartment 10 min after initiation of internalization (Fig. 10c). In contrast, labelled GLUT4myc internalized in the absence of insulin required 20 min to be detected in the same compartment (Fig. 10b). Moreover, by 20 min, the labelled GLUT4myc internalized in the continued presence of insulin was no longer detectable in the TfR-containing endosomes (Fig. 10d).

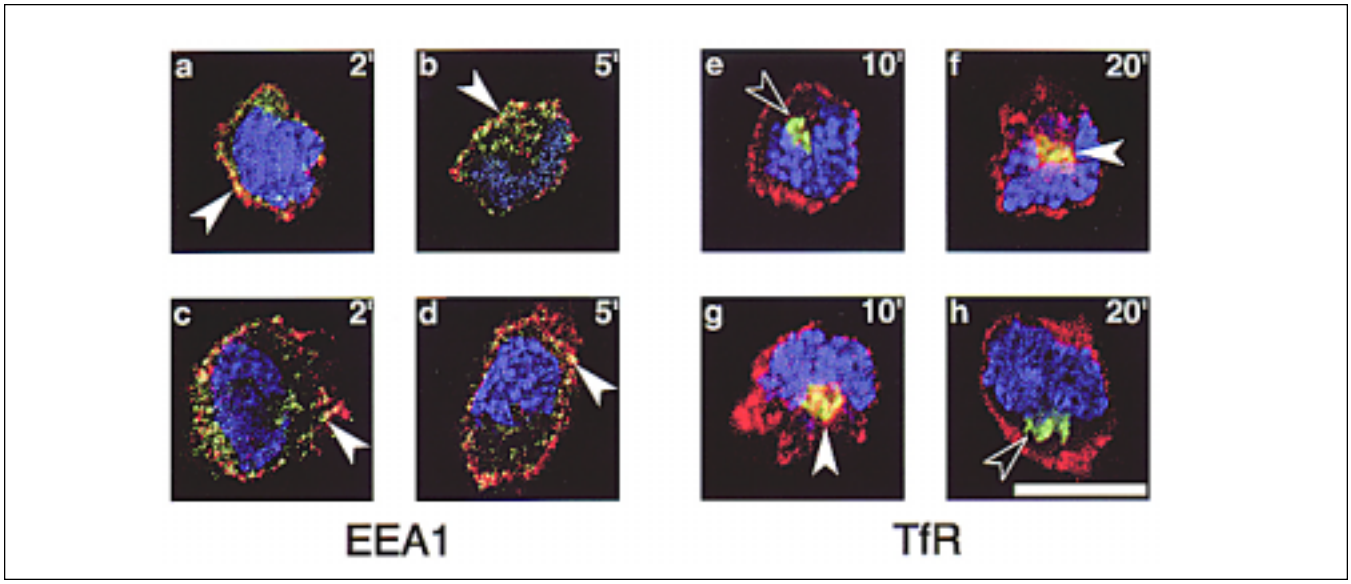


Figure 10. Effect of insulin on inter-endosomal GLUT4 traffic. Confocal micrographs of surface-labeled GLUT4myc after 2 (a, c), 5 (b, d), 10 (e, g) and 20 (f, h) min of endocytosis in the absence (a, b, e, f) or presence (c, d, g, h) of 100 nM insulin (*con*, *ins* respectively). Red staining is GLUT4myc, blue staining is DNA, green staining is EEA1 (a, b, c, d) or TfR (e, f, g, h). Filled arrowheads indicate areas of colocalization (yellow) between GLUT4myc and EEA1 or TfR, open arrowheads highlight TfR staining not colocalized with GLUT4myc. Scale bar: 10 μm.

Acceleration of GLUT4myc traffic due to insulin is dependent on PI3-K and PKB

The exact steps in insulin-dependent GLUT4 translocation directly regulated by PI3-K are not known. To explore whether PI3-K or its downstream effector PKB/Akt are involved in insulin-dependent acceleration of inter-endosomal traffic, dominant-negative DNA constructs of the two enzymes were transiently transfected into L6-GLUT4myc cells. A construct coding for the p85 subunit of PI3-K lacking the intervening SH2 domain that binds the catalytic subunit p110 has been shown to override the insulin-stimulated activation of endogenous PI3-K (Rodriguez-Viciana *et al.* 1997) and to inhibit insulin-induced arrival of GLUT4myc to the cell surface (Kotani *et al. 1*995, Wang *et al.* 1999). We have shown that a construct encoding PKB with three point mutations (K179A, T308A and S473A, called AAA-PKB) overrides the insulin-stimulated activation of co-transfected PKBα (Wang *et al. 1*999) and PKBβ (R. Somwar and A. Klip, unpublished results) and also inhibits insulin-dependent exocytosis of GLUT4 (Wang *et al*. 1999). Transfection of empty pcDNA3 vector had no effect on either the unstimulated arrival of labelled GLUT4myc to the recycling endosome (Fig. 11a) or the insulin-stimulated movement of labelled GLUT4myc through the recycling endosome (Fig. 11b) (images taken 20 min after initiation of internalization). While neither Δp85α nor AAA-PKB affected the arrival of GLUT4myc to the recycling endosome in unstimulated cells (Fig. 11c and d respectively), expression of either mutant prevented the accelerated transit of GLUT4myc into and out of the recycling endosome in response to insulin (Fig. 11e,f). These results suggest that both enzymes participate in the insulin-dependent regulation of this process.

Summary and Conclusions

The findings described above lead us to propose a model for the exocytic and endocytic transit of GLUT4 and its regulation by insulin, as illustrated in Fig. 12. On the right hand side of the figure, it is shown that insulin-activated PI3-K, acting through Rac1 (or a Rac1-like protein), induces remodelling of actin filaments into a mesh-like structure below the cell surface. Subsequently, a subpopulation of vesicles containing GLUT4 and VAMP2 are recruited to the newly formed actin structures. The close proximity of PI3-K and GLUT4 vesicles allows for PI3-K to phosphorylate lipid substrates in the vesicles. The products of this

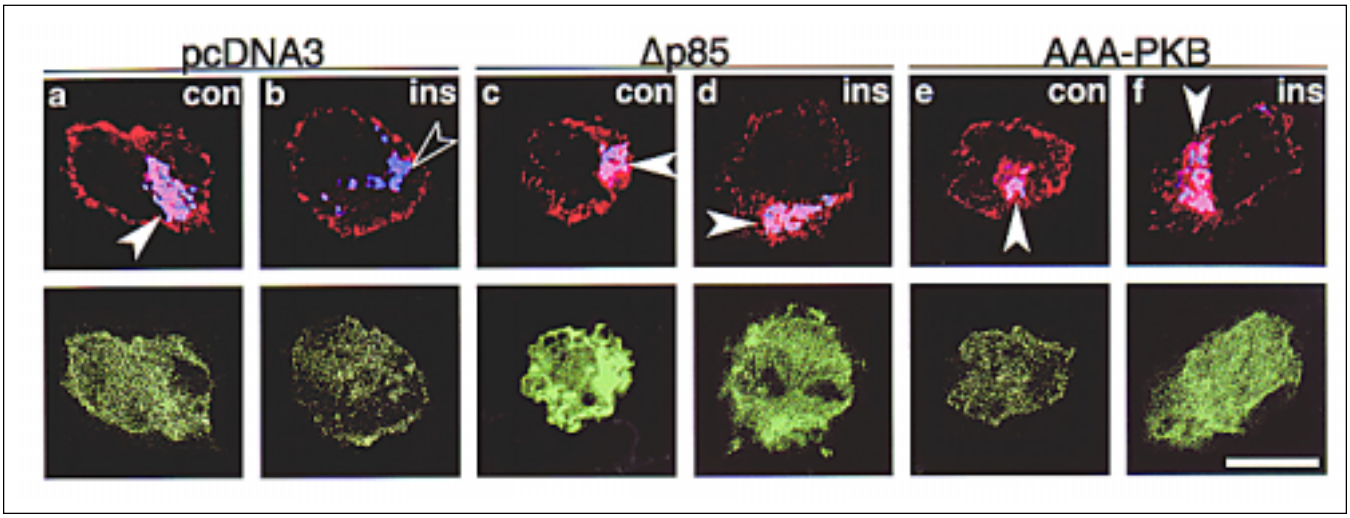


Figure 11. **Acceleration of inter-endosomal traffic by insulin is dependent on PI 3-K and PKB**. Staining patterns of surface-labeled GLUT4myc (red) in relation to whole-cell TfR (blue) after 20 min endocytosis in the absence (a, c, e, g, i) or presence (b, d, f, h, j) of 100 nM insulin as indicated (*con, ins* respectively). Untransfected cells were treated with DMSO (a, b) or 25 μM LY294002 (c, d) as indicated. Remaining panels represent cells transiently transfected with empty vector (e, f), Δp85 (g, h) or AAA-PKB (i, j). Transfected cells were identified by the presence of cotransfected eGFP shown in panels (green, e-j). Filled arrowheads indicate areas of colocalization (purple) between GLUT4myc and TfR while open arrowheads point out TfR not colocalized with GLUT4myc. Scale bar: 10 μm.

reaction, PI 3,4,5-P_3 and/or PI 3,4-P_2 may then bind and activate downstream effectors such as Akt/protein kinase B or PKC-λ/ζ to trigger the insertion of GLUT4 vesicles into the plasma membrane. This last step is mediated by the v-SNARE VAMP2 and t-SNAREs on the membrane ruffles.

The left hand side of Fig. 12 illustrates that internalized GLUT4 travels through the early endosome, defined by the presence of EEA1 and progresses to the recycling endosome defined by TfR. Insulin accelerates GLUT4 arrival at the recycling endosome. From the recycling endosome, surface-labelled GLUT4myc would generate specialized exocytic vesicles destined for the cell surface. Our model implies that sorting of GLUT4 likely occurs in the recycling endosome, but does not rule out that a portion of the specialized vesicle pool may form directly from the early endosome.

The results presented raise the hypothesis that insulin input is required at distinct loci in the cycle of GLUT4 traffic. PI3-K (and PKB) appear to be

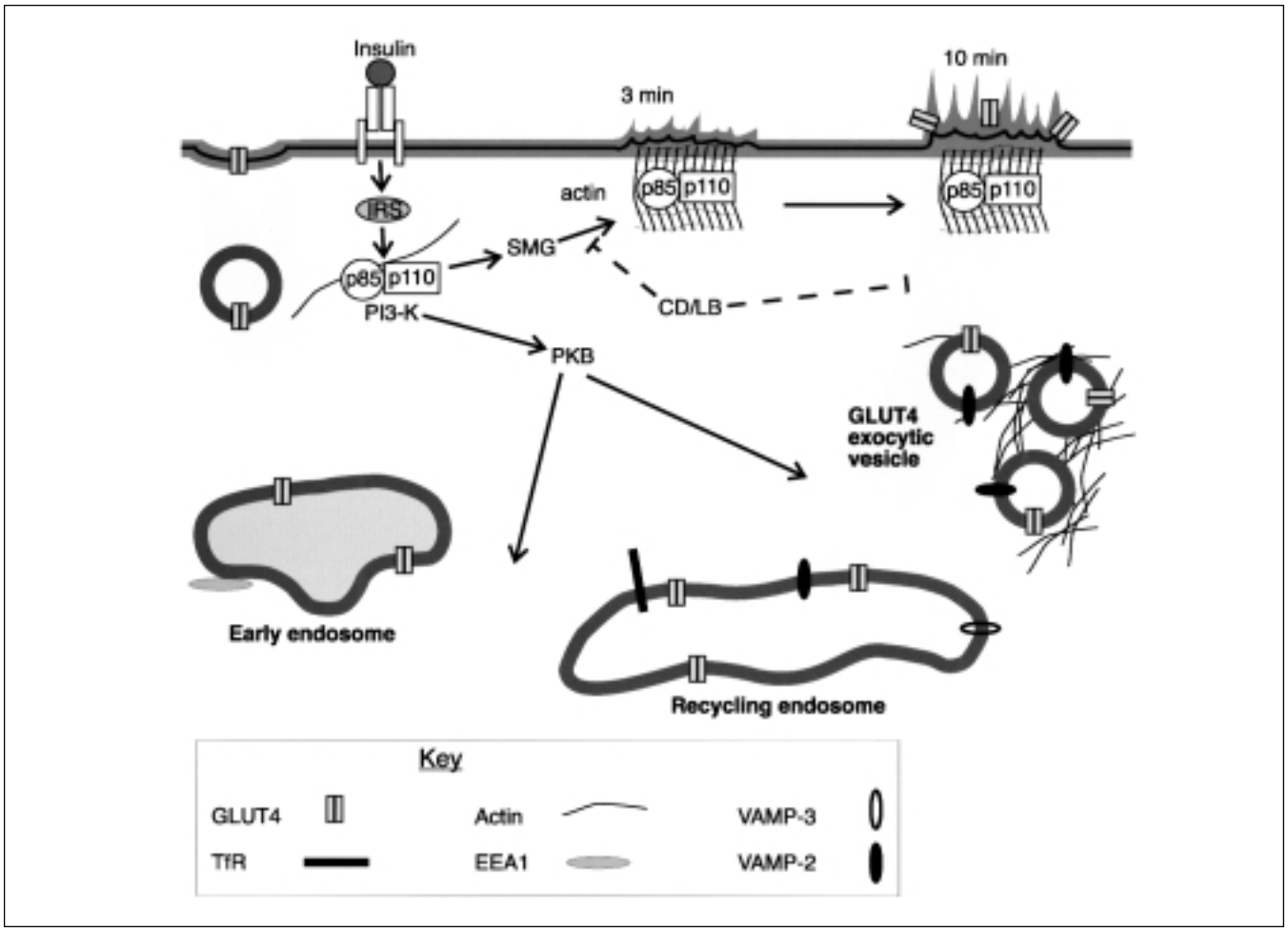


Figure 12. Model of GLUT4 traffic and sites of insulin input. GLUT4 is internalized in an unregulated fashion from the plasma membrane via clathrin-coated pits. After removal from the plasma membrane, GLUT4 moves to the early endosome characterized by early endosome antigen (EEA1). From the early endosome GLUT4 can travel to the juxtanuclear, recycling endosome marked by transferrin receptor (TfR) or to the specialized vesicles. Transit to the recycling endosome is regulated by a PI 3-K- and PKB-dependent signal from insulin. Once in the recycling endosome GLUT4 is presumably packaged into specialized vesicles and this step may also be accelerated by insulin in a PI3K- and PKB-dependent manner. Insulin can cause cortical actin remodelling through the action of PI3K and a small molecular weight G-protein (SMG, possibly Rac or TC10). Actin remodelling is blocked by cytochalasin D (CD) and latrunculin D (LB). The remodelled actin brings the exocytic GLUT4 vesicles to the vicinity of the plasma membrane containing t-SNAREs, for vesicle incorporation into the membrane.

required for the acceleration of GLUT4 transit through the recycling endosome, as well as to facilitate arrival of GLUT4 vesicles to areas beneath the cell surface, aided by the remodelled actin mesh. VAMP2 on these recruited vesicles would then participate in forming a SNARE complex with the plasma membrane t-SNAREs for the ultimate fusion of the vesicles with the membrane. The overall effect of insulin on inter-endosomal GLUT4 traffic would be to expedite movement of GLUT4 through the endosomal system, culminating in the genesis of the GLUT4 vesicles. These diverse inputs of insulin would provide the cell with a means to maintain levels of plasma membrane GLUT4 in the presence of a continued insulin challenge by regulating the production of plasma membrane-destined GLUT4 vesicles.

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Nucleoside transporter proteins

From Membrane Biology to Therapeutic Applications

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Introduction

Because this award lecture, given in memory of Jeanne Manery Fisher, celebrates my lifetime research achievements, I will give a historical overview of the field of nucleoside transport, which coincides almost exactly with my academic career at the University of Alberta. I will trace the history of the nucleoside transport field, from our early functional studies in human erythrocytes to our current studies of nucleoside transporter proteins and their importance in nucleoside biology and therapeutics.

Nucleosides are central metabolites in all life forms and, as precursors of nucleotides, play an essential role in intermediary metabolism, biosynthesis of macromolecules and cell signaling through interaction with purinergic receptors. Nucleoside drugs are used to treat hematologic malignancies, certain solid tumors and many viral diseases. A natural nucleoside, 2'-deoxycytidine, and three of its analogs that have important applications in either antiviral therapy (lamivudine) or anticancer therapies (gemcitabine, cytarabine) are shown in Fig. 1.

Since most nucleosides are hydrophilic molecules and don't cross cell membranes readily by diffusion, cellular utilization of extracellular nucleosides is dependent on the activity of specialized membrane proteins that translocate organic solutes across lipid bilayers.

In humans, the physiologic nucleosides and most nucleoside drugs enter cells via one or more of the five known nucleoside transporter proteins (NT) that have been identified during the past decade by molecular cloning and functional expression of cDNAs encoding the NT proteins. Their characteristics will be described in detail later. All of the known nucleoside transporters play an important role in nucleotide metabolism by their catalysis of the first step in nucleoside "salvage" pathways and the bidirectional transporters also probably play an important role in cellular release of nucleosides (Fig. 2).

The beginning: studies of nucleoside transport in human erythrocytes

The story begins in the late 1960s, when Dr. A.R.P. Paterson (McEachern Cancer Research Laboratory, University of Alberta) discovered that uptake of uridine and thymidine by human erythrocytes was mediated by a process that exhibited the hallmark characteristics of facilitated diffusion (1, 2). I joined the Paterson research group as a postdoctoral fellow in 1970, just as the nucleoside transport project was gathering momentum. At the time, nucleoside analogs were being aggressively examined as potential anticancer drugs in the U.S. National Cancer Institute's drug discovery program and there was considerable interest in the possibil-

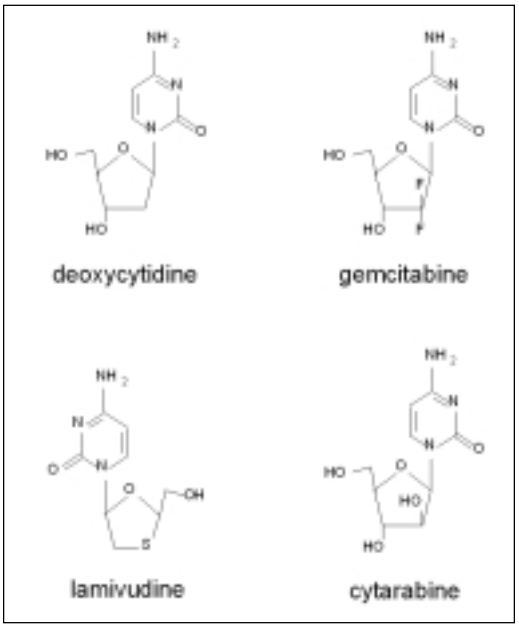

Figure 1. Deoxycytidine and analog drugs

ity that cellular uptake might be an important determinant of their pharmacologic activity.

During the 1970s, the major focus of research was on the functional characterization of nucleoside transport processes. Human erythrocytes, which lack intracellular organelles, have only a single membrane type, thereby enabling analysis of processes of the plasma membrane without the necessity of membrane fractionation. Human erythrocytes also lack many enzymes of nucleotide metabolism, including the kinases required for salvage of uridine and thymidine, thereby enabling kinetic studies of nucleoside transport in the absence of metabolism. Because of these characteristics, erythrocytes of humans, and other species, were extensively used for functional characterization of nucleoside transport processes. Although we didn't know it at the time, human erythrocytes were also ideal for functional studies of nucleoside transport because they are one of the few human cell types that possess a single, rather than multiple, nucleoside transport processes. During the 1970s, the nucleoside transport process of human erythrocytes was shown to be equilibrative in nature, was characterized kinetically and was shown to exhibit broad permeant selectivity.

A key discovery, which subsequently led to the identification of equilibrative nucleoside transporter proteins, was the demonstration that a group of S-substituted thiopurine ribonucleosides, which had been synthesized as potential anticancer drugs, exhibit potent nucleoside-transport inhibitory activity in erythrocytes (2). We established that nitrobenzylthioinosine (NBMPR) binds to a single set of high-affinity sites (K_d, 1 nM; 10^4 sites/cell) on erythrocyte plasma membranes, the occupancy of which is directly correlated to inhibition of uridine transport (3-5). The erythrocytic nucleoside transporter has subsequently been extensively studied and is the prototypic equilibrative NBMPR-sensitive (*es*) transporter, now known through molecular cloning to be the hENT1 protein (Fig. 3).

We defined the broad substrate selectivity of the *es* transporter in kinetic studies in erythrocytes (6, 7) and subsequent work by ourselves and others extended these observations to erythrocytes from other species (4, 8), and as rapid-assay technologies were developed (9, 10), to more complex cell types, including cultured cancer cell lines (11-15). Our studies of the relative abilities of structural analogs of NBMPR to inhibit nucleoside transport in cells (14-19) and high-affinity binding of NBMPR in membrane preparations (5, 18, 20-23) provided the basis for the wide-spread use of NBMPR as a molecular probe for quantification and identification of the *es* transporter protein in cell membranes. We also demonstrated that two potent, structurally unrelated inhibitors of nucleoside transport, dipyridamole and dilazep, inhibited binding of NBMPR to the *es* transporter (22, 24), thereby stimulating interest in the nucleoside-transport inhibitory capabilities of these, and related, compounds.

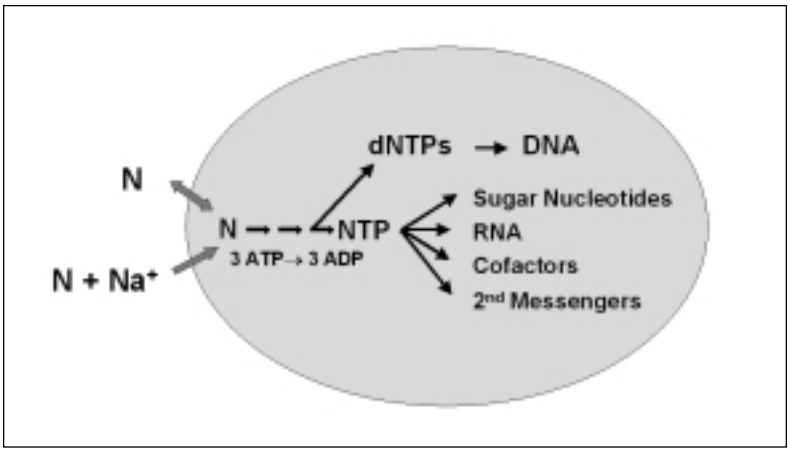

Figure 2. Role of plasma membrane transporters in nucleoside (N) metabolism in mammalian cells

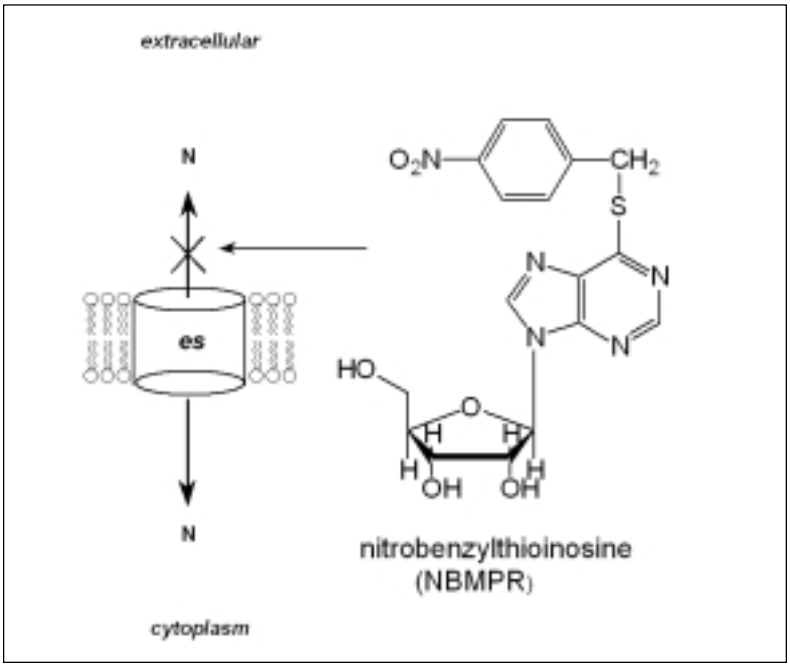

Figure 3. NBMPR, a tight-binding inhibitor of the equilibrative sensitive (*es*) transporter

Multiple nucleoside transport processes

During the 1980s, it became increasingly evident that mammalian cells possess multiple nucleoside transporter types, based on permeant selectivities, inhibition by diagnostic agents, and mechanisms of transport (for reviews, see 25, 26). We now know that these processes comprise two functionally distinct groups that differ in their fundamental mechanisms of transport. The equilibrative processes, which exhibit the classic features of a bidirectional, non-concentrative process, have been subdivided on the basis of their sensitivities to nanomolar concentrations of NBMPR into the *es* (equilibrative-sensitive) and *ei* (equilibrative-insensitive) processes. The *es* and *ei* processes transport a structurally diverse group of nucleosides and the *ei* process also transports nucleobases.

The concentrative processes are Na^+-dependent, moving nucleosides into cells against their concentration gradients and have been subdivided into several functional groups. The *cit*, *cif* and *cib* processes are insensitive to NBMPR and accept, respectively, pyrimidine nucleosides, uridine plus purine nucleosides or pyrimidine and purine nucleosides as permeants. Two minor processes (*csg*, *cs*), which are inhibited by NBMPR, have also been described.

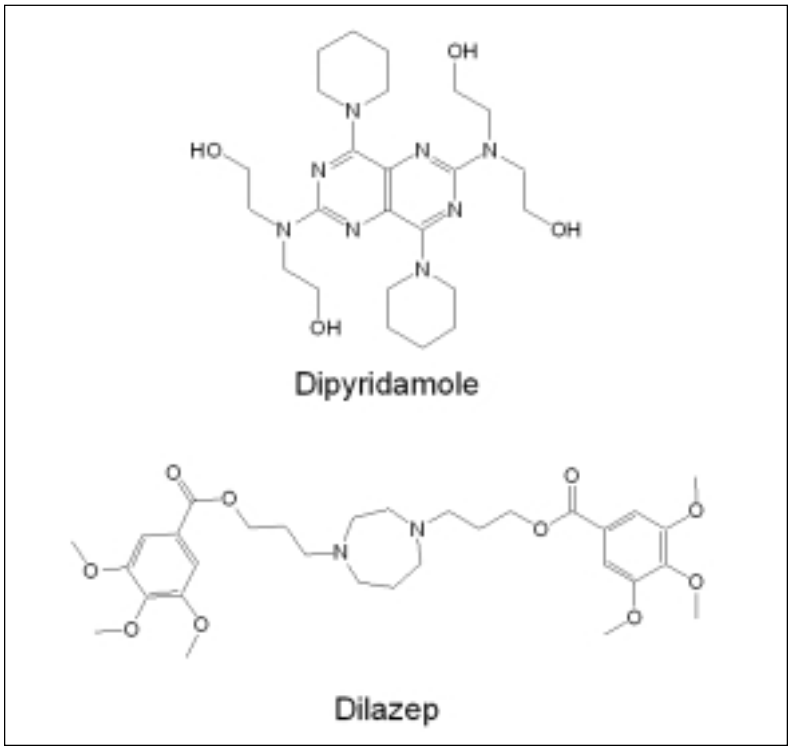

Figure 4 Inhibitors of equilibrative nucleoside transport and NBMPR binding

Transporter discovery by molecular cloning: two new membrane protein families

The nucleoside transporters of mammalian cells are hydrophobic membrane proteins of low-abundance that have been notoriously difficult to study biochemically. With the exception of the transporter of human erythrocytes, which was purified in very small quantities in the late 1980s (27), efforts to isolate the proteins responsible for various nucleoside-transport activities failed. Molecular cloning strategies became increasingly attractive as an approach to identify the elusive nucleoside transporter proteins. In 1990, we established a long-term collaboration with Dr. J.D. (Jim) Young (Physiology, University of Alberta) and Dr. S.A. (Steve) Baldwin (Biochemistry & Molecular Biology, University of Leeds) to identify nucleoside transporter proteins by molecular cloning. Because we had physical information about only one of an uncertain number of different nucleoside transporter subtypes, we invested heavily in strategies for functional expression selection of candidate cDNAs, recognizing that expression methods would be essential for the subsequent characterization of newly discovered nucleoside transporter proteins. The Young group focused on functional expression of nucleoside transporters in oocytes *of Xenopus laevis*, and their system has become the 'gold standard' for nucleoside transporter identification. We focused on the use of the yeast *Saccharomyces cerevisiae* and cultured mammalian cells for functional expression of nucleoside transporter proteins.

Identification of the first nucleoside transporter protein occurred in 1994 by functional-expression cloning in Dr. Young's laboratory, and its sequence revealed a new membrane protein family with representatives among bacteria (28). The next major breakthrough came in 1997 when our three-laboratory consortium successfully cloned a cDNA encoding the first recognized member of a second family of membrane proteins (29). Since then, our collaboration has been extraordinarily successful with the discovery of five nucleoside transporters from human cells, an equal number from rodents and several from lower organisms, including yeast, protozoan parasites, nematode worms and bacteria.

The nucleoside transporter proteins are members of two evolutionarily old protein families: the Equilibrative Nucleoside Transporters (ENTs) and Concentrative Nucleoside Transporters (CNTs). cDNAs encoding representatives of the two ENT subfamilies (ENT1 and ENT2: exhibit *es* and *ei* characteristics, respectively) were cloned from human and rat tissues (29-32), and there is evidence from the human EST data base that a third ENT family member may exist (33). cDNAs encoding representatives of three CNT subfamilies (CNT1, CNT2 and CNT3: exhibit *cit*, *cif* and *cib* characteristics, respectively) were cloned from human, rat, pig and/or mouse tissues (28, 32, 34-39).

The ENT and CNT protein families do not share sequence identities and are architecturally quite different (Fig. 5). The ENT proteins are predicted to have 11 transmembrane domains (29, 30, 32, 33) whereas the CNT proteins of mammalian cells are predicted to have 13 transmembrane domains (40, 41). Studies with N-glycosylation mutants and antibody preparations raised against the large hydrophilic domains of hENT1 have established that the large hydrophilic loop between transmembrane domains 1 and 2 is extracellular and the large hydrophilic loop between transmembrane domains 6 and 7 is intracellular.

There has been a rapid increase in the identification of nucleoside transporter cDNAs since the isolation of the first cDNAs encoding representative members of the CNT and ENT families. Cloning strategies based on sequence similarities and functional analysis of recombinant proteins have recognized in excess of 20 nucleoside transporter cDNAs from eukaryotes and prokaryotes. Additionally, homology searches of sequence databases using predicted protein and/or cDNA sequences have identified a number of structurally related proteins that are candidate nucleoside transporters. The variability in the properties of the ENTs and CNTs among different cells and species has expanded beyond the scope of the existing nomenclature. Currently, nucleoside transporter proteins are categorized on the basis of their structural similarities as either CNTs or ENTs; their molecular and functional properties must be determined experimentally. Phylogenetic trees of known and putative nucleoside transporter proteins have been developed (33, 40, 42) and are constantly changing.

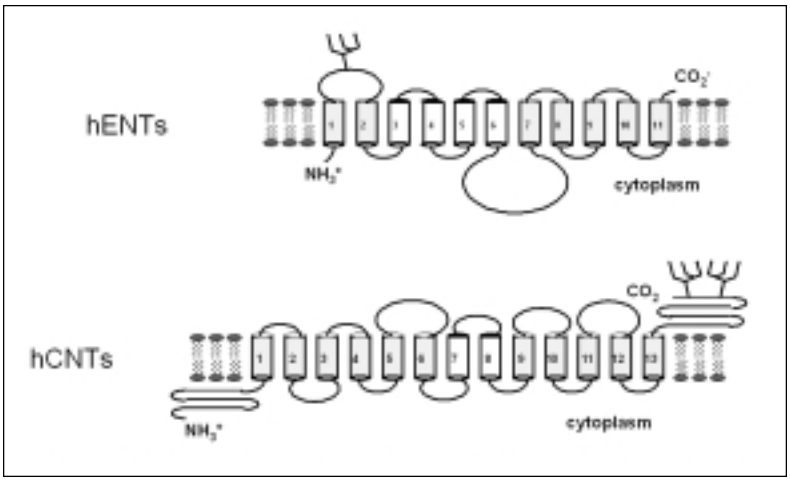

Figure 5. The architecture of the ENT and CNT protein families: different predicted membrane topologies

Therapeutic relevance: nucleoside transport activity is required for cytotoxicity of nucleoside drugs.

Our studies of nucleoside transport in transplantable murine tumors and cultured murine and human cell lines led to the concept that cells require membrane 'carriers' for salvage of extracellular nucleoside precursors of nucleic acids, and for manifestation of cytotoxicity by anticancer nucleoside drugs. We demonstrated that inhibition of nucleoside transport by NBMPR afforded protection of cells against cytarabine, an anticancer nucleoside, by reducing drug uptake (43). This work, which was conducted with a mouse leukemia cell line (L1210), suggested that nucleoside transport activity is a critical determinant of cytotoxicity of nucleoside drugs. The requirement for nucleoside transport for cytotoxicity was extended to human cells in a subsequent study in which we demonstrated that treatment with non-toxic levels of NBMPR protected cultured human cells (RPMI 6410) from a large series of structurally diverse cytotoxic nucleosides by inhibiting cellular uptake of drug (13). We also demonstrated that resistance to nucleoside drugs in a murine lymphoma cell line that had been mutagenized and selected for resistance to cytotoxic nucleosides was due to the loss of a functional nucleoside transporter in cell membranes (21).

Our results established that either pharmacologic or genetic nucleoside-transport deficiency is accompanied by high-level resistance to a variety of nucleoside drugs and thus provided

experimental evidence for the concept that the presence of a functional nucleoside transporter in cell membranes is essential for manifestation of cytotoxicity of many nucleoside drugs. In recent studies with gemcitabine, we have shown that a panel of mutant murine leukemia L1210 cell lines that differ in their nucleoside transporter phenotypes exhibit different levels of gemcitabine sensitivity (Table 1).

Table 1. Gemcitabine Cytotoxicity in Murine L1210 Leukemia Cells with Deifned NT Activites

Cell line	Origin	es	ei	cit	cif	IC_{50} (μM)	Relative resistance
L1210	wild-type	+	+		+	0.0071	**1.0**
B23.1	mutant	+				0.092	**13**
DU-5	transfectant			+		0.012	**1.7**
MA27	mutant				+	5.2	**730**
DNC-3	null mutant					13	**1800**

We have also demonstrated that normal human hematopoietic progenitor cells can be protected from *ex vivo* nucleoside cytotoxicity by treatment with nucleoside transport inhibitors, thereby providing experimental evidence for a therapeutic rationale based on the use of nucleoside transport inhibitors to selectively protect dose-limiting normal tissues during nucleoside chemotherapy (44). Our most recent studies (26) have shown a strong correlation between nucleoside transport activity and cytotoxicity against human cancer cell lines by gemcitabine, a widely used nucleoside drug with therapeutic activity against a variety of solid tumors (e.g., breast, pancreas, head and neck, lung). Fig. 6 compares the cytotoxicity of gemcitabine against cultured human leukemia cells that possess the capacity for nucleoside transport (CEM cells) with that against a mutant cell line (CEM-AraC-8C) that lacks the capacity for nucleoside transport.

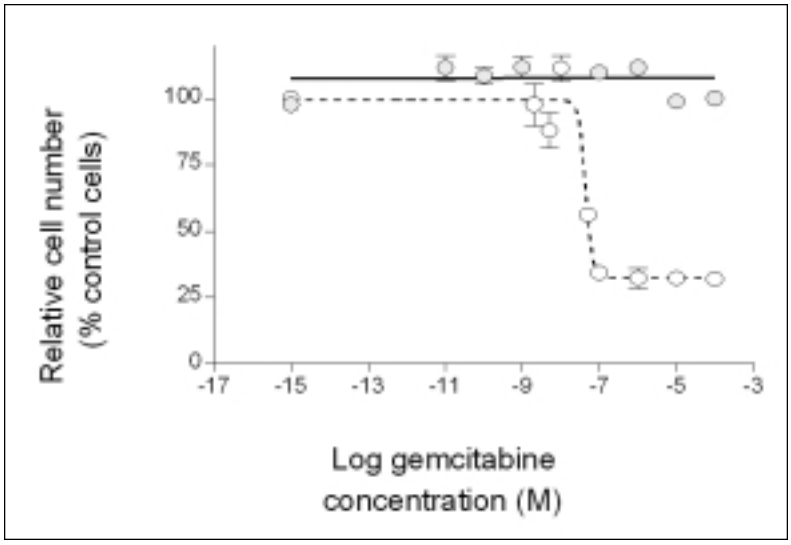

Figure 6. Gemcitabine toxicity against NT-deficient (CEM-AraC-8C) and NT-competent (CEM) cells

Future studies

We have proposed that cellular resistance to anticancer nucleoside drugs is related, in part, to low nucleoside transport capacity (45). That several anti-cancer cytidine analogs are transported differently by recombinant human nucleoside transporters (46, 47) is consistent with the importance of drug transportability as a determinant of therapeutic activity. We are collaborating with Dr. J.R. Mackey (Oncology, University of Alberta) on the role of nucleoside transporters in clinical resistance to gemcitabine therapy in solid tumors, with a special focus on cancers of the breast, head and neck and pancreas, and resistance to nucleoside therapy in hematologic malignancies. On-going and future projects include development of immunohistochemical assays for detection of nucleoside transporters in clinical samples (48), assessment of relationships between expression of nucleoside-transporter mRNA by cells and responsiveness to gemcitabine, and definition of the nucleoside-transporter phenotype of hematopoietic progenitor cells.

A major goal of our translational research program is to define nucleoside structural determinants for permeants and inhibitors of the human transporters, to guide the development and use of therapeutic nucleosides. We have taken advantage of our ability to functionally produce the human nucleoside transporters in yeast (49, 50) to develop assays to assess the potential transportability of nucleoside drugs by assessing the ability of test nucleosides to inhibit uptake of ^{3}H-uridine (a universally accepted permeant of the known human transporters). The determination of relationships between nucleoside transportability and cytotoxicity will be undertaken in human cell lines, since there are substantial differences in sensitivity to cytotoxic drugs between yeast and human cells. We

have created a series of stable transfectants each of which possesses a different human transporter subtype in otherwise genetically identical human cell lines. The transportability and inhibitor-sensitivity assays in yeast and transportability-cytotoxicity assays in human cell lines are being used to develop 'transportability guidelines' for development and application of therapeutic nucleosides for use in human diseases.

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Signal peptide cleavage in the *E. coli* membrane

The 2001 Merck Frosst Prize Award Address
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Abstract

Our laboratory uses x-ray crystallography and other structural biology techniques in the analysis of bacterial membrane proteins and membrane protein complexes. We are particularly interested in bacterial protein secretion machinery, including the Sec-dependent translocation apparatus which is essential to the viability of all bacteria, as well as the type III secretion apparatus which is specific to bacterial pathogens. The latter is a fascinating system which apparently acts as a "molecular syringe" to inject virulence proteins directly from the pathogenic bacteria into the host cell. In this paper we focus on one step of the Sec-dependent translocation of proteins, the essential cleavage of the signal peptide from membrane embedded pre-proteins during the translocation cycle.

Introduction

Many of the proteins that are essential for the survival of the bacterial cell function on the outside (or trans side) of the bacterial inner membrane. For example, proteases and beta-lactamases involved in defense mechanisms and transpeptidases involved in the synthesis of the cell wall must first be exported across the lipid bilayer of the inner membrane.

The majority of the proteins that are destined for translocation across the bacterial inner membrane are synthesized as pre-proteins with an amino-terminal peptide extension called the signal

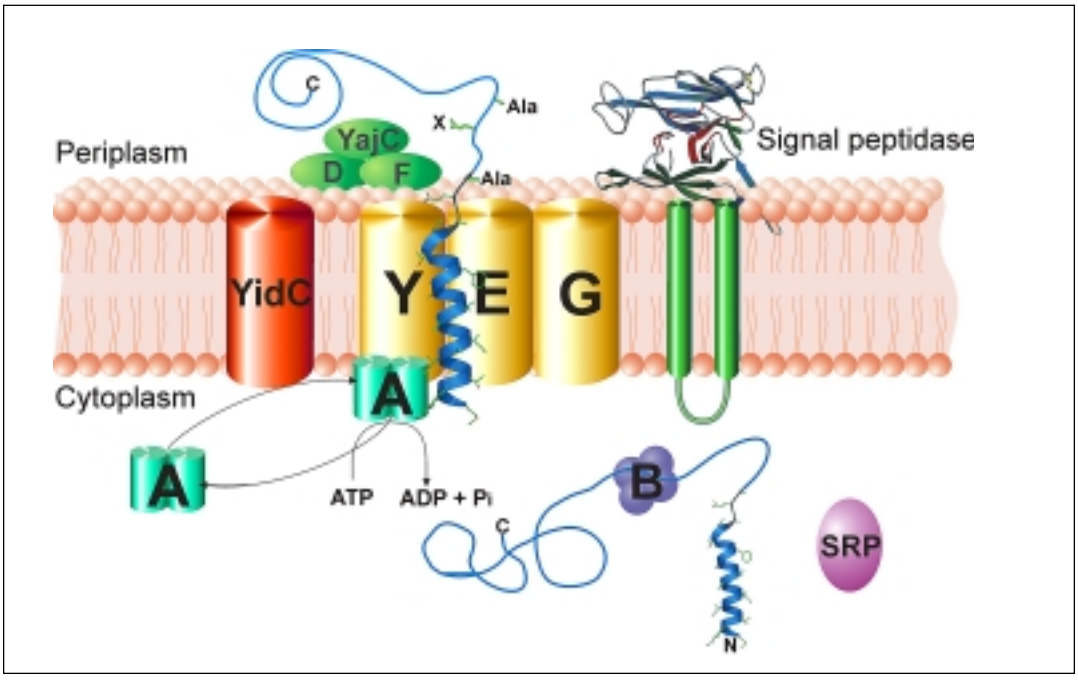

Figure 1. A schematic diagram of the Sec dependent protein translocation system. The pre-protein with N-terminal signal peptide interacts with the homo-tetramer SecB which chaperones the protein to the homo-dimer SecA which works as a molecular motor to push the pre-protein through a pore formed by SecYEG. The proteins SecDF and YajC function to refold the protein on the trans side of the membrane. Once translocated across the membrane the signal peptide is cleaved off by the type I signal peptidase.

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or leader peptide. The signal peptide is essential for both targeting the proteins to the membrane and their subsequent translocation across the membrane. A molecular machine called the translocase recognizes the secretory proteins and assists in their travel across the membrane (Fig.1).

Once translocated across the inner membrane, the secretory pre-protein is tethered to the membrane by the signal peptide. A membrane bound endo-peptidase, called signal or leader peptidase, functions to cleave off the signal peptide, thereby releasing the mature secretory protein from the membrane and allowing it to proceed to its final destination. Although the crystal structure of the catalytic domain of the signal peptidase has been solved in our laboratory (Paetzel et al., 1998) and numerous spectroscopic studies (NMR, EPMR, and CD) have been performed on synthetic signal peptides in model membrane systems, the actual environment in which signal peptide cleavage occurs (i.e. within the membrane or not) still remains unclear. We present here a brief analysis of known features of the signal peptide, signal peptidase and bacterial membrane structure and the resultant implications for the likely location of the scissile bond cleavage.

Signal peptide primary structure

In general, signal peptides share little sequence identity, but they do contain some common features in size and distribution of electrostatic and hydrophobic residues (Fig. 2). All have a short, positively charged amino-terminal region (n-region, 1-5 residues), a longer hydrophobic middle region (h-region, 7-15 residues), and a carboxy-terminal protease recognition region (c-region, 3-7 residues). The protease recognition region contains small aliphatic or polar residues at the -1 and -3 positions relative to the cleavage site (or P1 and P3 in the Schechter and Berger (1967) nomenclature). Alanine is most often observed at the -1 and -3 positions (von Heijne, 1983, 1985; Perlman & Halvorson, 1983, Fig. 2). The –6 position of the signal peptide is most often occupied by a Pro, Gly or Ser residue and it has been suggested that this position defines the transition from the hydrophobic h-region to the c-region (von Heijne, 1990). There are a number of computational methods for identifying signal peptides and their cleavage sites from the sequence of proteins. One of the most popular and accessible is the SignalP World Wide Web server (http://www.cbs.dtu.dk/services/SignalP/), which is based on a combination of several artificial neural networks (Nielsen et al., 1997a,b). The signal peptides from gram-positive bacteria are significantly longer than those from other organisms, and they have a much longer h-region (von Heijne & Abrahmsen, 1989). The average eukaryotic signal peptide is 22.6 amino acids in length, the average gram-negative signal peptide is 25.1 amino acids in length, and the average gram-positive signal peptide contains 32.0 amino acids (Nielsen et al., 1997a,b). The gram-positive signal peptides in general have an amino-terminus containing more lysine and arginine residues (Edman et al., 1999). The positively charged n-region is thought to play a role in the proper orientation of the signal peptide in the lipid bilayer.

Signal peptide secondary structure

A number of studies have focused on the structure, orientation, and interactions of signal peptides in lipid bilayers or membrane mimetic environments (Cornell et al., 1989; Bruch et al., 1989; Jones et al., 1990; McKnight et al., 1991a,b; Wang et al., 1993; Rizo et al., 1993; Jones & Gierasch, 1994; Bechinger et al., 1996; Keller et al., 1996; Voglino et al., 1998; Voglino et al., 1999). Most studies to date are consistent with the central h-region adopting an a-helical conformation when in a lipid or hydrophobic environment (McKnight et al., 1989; Chupin et al., 1995; Voglino et al., 1998; Batenburg et al., 1988; Rizo et al., 1993; and Wang et al., 1993). The boundary between the h-region and the c-region (-6 to -4), which often contains proline or glycine residues, has been suggested to

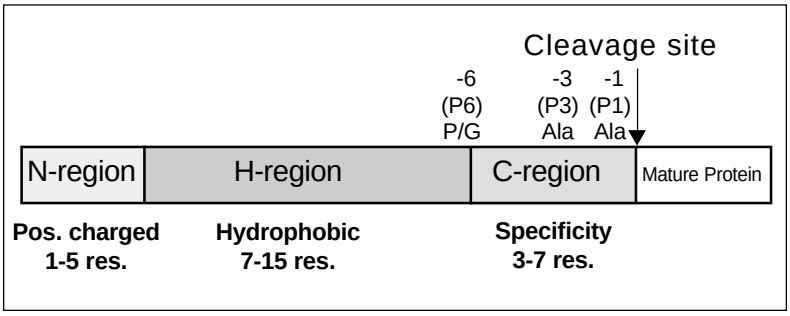

Figure 2. The features of a typical bacterial signal peptide. Signal peptides have a positively charged amino-terminus (n-region), a hydrophobic central region (h-region), and a neutral but polar carboxy-terminus (c-region). The boundary between the h-region and the c-region is usually marked by a helix-breaking residue (Pro or Gly) at the –6 (P6) position relative to the cleavage site. The cleavage recognition sequence consists of small residues at the –1 (P1) and –3 (P3) positions relative to the cleavage site. By far the most common residues at these positions is alanine.

have a β-turn structure (Rosenblatt et al., 1980; Perlman & Halvorson, 1983). The recent conformational, statistical and mutational analysis by Karamyshev and coworkers (1998) is consistent with the signal peptide having an extended β-conformation in the -5 to -1 region while bound to the signal peptidase binding pocket.

Signal peptidase structure

Escherichia coli type I signal peptidase is by far the most thoroughly studied signal peptidase. Typical of many gram-negative species, *E. coli* signal peptidase (323 amino acids, 35,988 Da) has two predicted amino-terminal transmembrane segments (residues 4-28 and 58-76), a small cytoplasmic domain (residues 29-57), and a large carboxy-terminal catalytic domain (residues 77-323) (Fig. 1, 3).

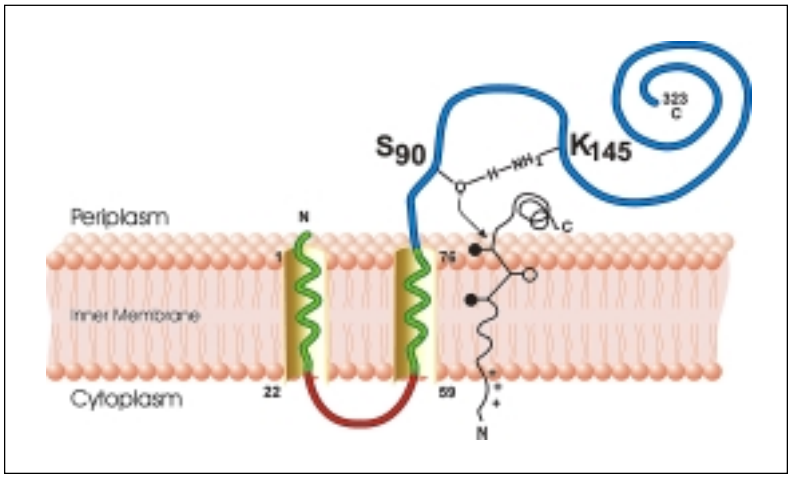

Figure 3. The membrane topology of *Escherichia coli* signal peptidase.

The crystal structure of the catalytic domain of *E. coli* signal peptidase has been solved in our laboratory (Paetzel et al., 1998). Although the N-terminal membrane anchor was deleted in the construct used for the structure determination, appropriate concentrations of detergent were still required for optimal activity (Tschantz et al., 1995) and for crystallization (Paetzel et al., 1995). The structure revealed for the first time the catalytic residues (a Ser/Lys dyad) and substrate binding site of a signal peptidase. The extended, shallow hydrophobic binding cleft is consistent with the observed substrate specificity and prediction of an extended beta-conformation for the c-region of signal peptides (Fig. 4). Also observed is an unusually extensive exposed hydrophobic surface that runs along the full length of the central β-sheet and includes the hydrophobic substrate binding site. Exposure of significant areas of hydrophobic surface is rarely observed in soluble proteins and we proposed this unusual surface would be involved in membrane association (Paetzel et al., 1998). Located on this predicted membrane association surface are a number of aromatic residues, including Trp 300, which was shown to be essential for optimal activity in *E. coli* signal peptidase (Kim et al., 1995a,b), even though our structure maps it to a position more than 20 Å from the enzyme catalytic center (Paetzel et al., 1998, Fig. 4). Aromatic amino acids are thought to play an important role in protein/membrane interfaces (Landolt-Marticorena et al., 1993) and presumably Trp 300 facilitates the insertion or association of *E. coli* signal peptidase into the membrane. A tryptophan residue was also found to be essential for the interfacial catalysis of phospholipase A_2 at the membrane surface (Gelb et al., 1999). Sequence alignments indicate that several conserved aromatic or hydrophobic residues exist in the proposed membrane-association domain in both gram-positive and gram-negative bacterial type I signal peptidases.

Lipid membrane structure

X-ray and neutron diffraction data on fluid phospholipid bilayers (dioleoylphosphatidylcholine) show that the hydrocarbon core (the hydrophobic fatty acid chains) of the bilayer is approximately 30 Å thick and that the interfacial region (the phospholipid head groups and glycerol-fatty acid ester backbone) is approximately 15 Å on each side (Wiener & White, 1992, Fig. 5).

Even though the overall bacterial membrane is in a bilayer (lamellar) structure, it is essential for the membrane to be near to the bilayer-nonbilayer transition point. This is achieved by organisms maintaining a certain proportion of phospholipid in their membrane that have a strong preference for nonbilayer states. These phospholipids have a small head group compared to their acyl-chains that give them an overall conical shape. One of these nonbilayer phospholipids is phosphatidylethanolamine. The *Escherichia coli* inner membrane is made up of approximately 75 % phosphatidylethanolamine.

Evidence for the insertion of the catalytic domain of signal peptidase into the membrane

Using the catalytic domain of *E. coli* signal peptidase (as described in our crystallographic study) van Klompenburg and colleagues showed, using monolayer and vesicle binding experiments, that despite the absence of the transmembrane segments, the signal peptidase catalytic domain penetrated deeply into lipid monolayers with a marked preference for the nonbilayer lipid dioleoylphosphatidylethanolamine (van Klompenburg et al., 1998). Recently this preference for nonbilayer lipid has been shown to be independent of the exact chemical structure of the lipid head group (van den Brink-van der Laan et al., 2001). It is interesting that NMR (Killian et al., 1990) and ESR (Sankaram et al., 1994) experiments implicate the signal peptide in promoting non-bilayer lipid structure (deVrije et al., 1990) and non-bilayer lipid structure as important for proper function of the translocase (Rietveld et al., 1995; van der Does et al., 2000).

Based on the evidence that the catalytic domain of *E. coli* signal peptidase penetrates into lipid membranes as well the extensive hydrophobic surface on the signal peptidase catalytic domain we have modeled the *E. coli* signal peptidase bound to a signal peptide. We have modeled the c-region of the signal peptide substrate in an extended conformation that can form beta-sheet hydrogen bonds with the beta-strands that line the binding site (typical of a many known proteases). The peptide substrate then transitions into the alpha-helical h-region, and finally to the n-region. The model is presented along side a pair of phospholipid molecules taken from a molecular dynamics simulation of a bilayer system (Heller et al., 1993). The location of the signal peptidase hydrophobic binding-site, the extended hydrophobic surface, as well as the location of the amino-terminus, gives us an approximate orientation of signal peptidase relative to the plane of the membrane. Given that the average gram-negative signal peptide is 25.1 amino acids in length, the helical, hydrophobic segment (h-region) would be at most 15 residues in length. Calculating the length of this helix using the typical values of 3.6 residues/turn and 5.4 Å in pitch estimates an overall length of 22.5 Å for the h-region. Clearly, in this model, the h-region of the signal peptide would be too short to span the aliphatic portion of the fatty acid chains of a typical lipid bilayer. Presuming the positively charged n-region of the signal peptide keeps the amino-terminus on the cis-side (or cytoplasmic side) of the inner membrane, we predict the cleavage-site of the signal peptide would reside in the hydrophobic acyl-chains of the fatty acids or at the more polar region of the glycerol backbone of the phospholipid (Fig.5).

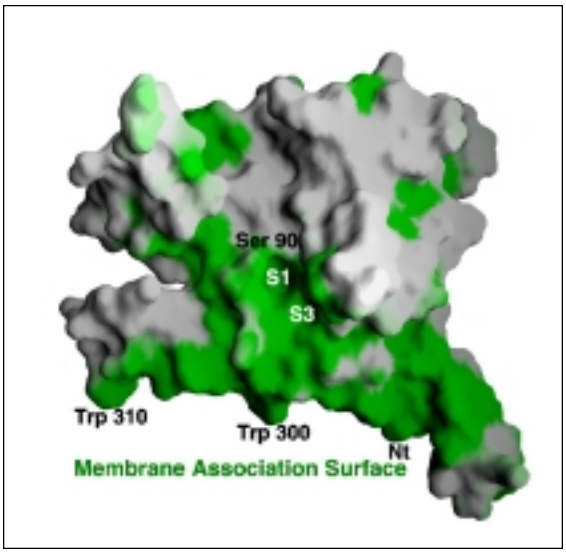

Figure 4. The extended hydrophobic surface on the catalytic domain of *Escherichia coli* signal peptidase.

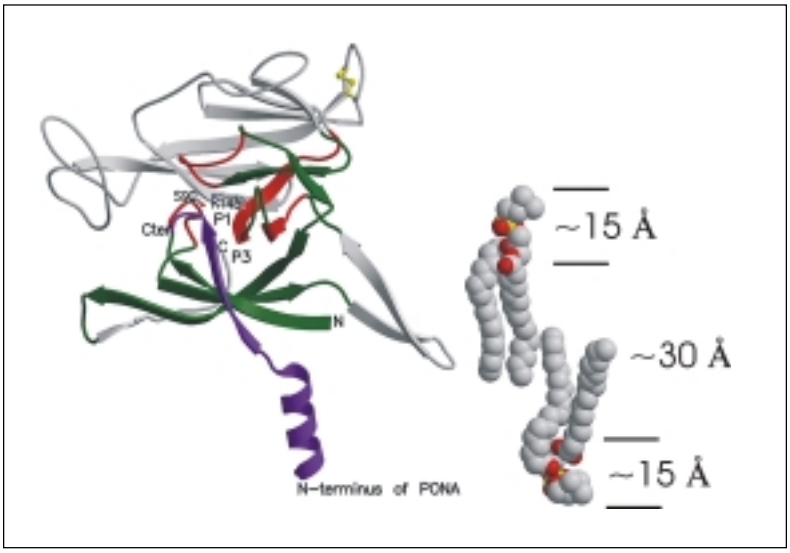

Figure 5. A model of a signal peptide bound to the catalytic domain of *Escherichia coli* signal peptidase. The dimensions of a typical lipid bilayer are shown adjacent to the model.

Future Experiments

To date there is no direct experimental evidence to confirm whether the bacterial signal peptide is associated with lipid and/or the proteins of the secretion machinery (translocase) during the cleavage event *in vivo*. It is also presently unclear whether bacterial signal peptidases cleave the signal peptide during or after translocation and in what phase the membrane lipids exist at the time and place of protein translocation. These are obviously questions that need to be addressed experimentally in order to further refine our view of the essential cleavage of signal peptides in bacteria and higher order species. Work is now underway to solve the crystal structure of the full-length *E. coli* signal peptidase in complex with a signal peptide. Solid state NMR experiments will be performed to investigate the structure of the signal peptidase transmembrane segments and the signal peptide in a lipid environment and EPR and fluorescence experiments will be utilized to measure the depth of the insertion by the catalytic domain of *E. coli* signal peptidase.

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The Implantation Serine Proteinases: Potential Therapeutic Footholds in Female Fertility

Review Article

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Abstract

Hatching of the blastocyst from the zona pellucida *represents an important first step in implantation and the establishment of a successful pregnancy. Investigation in the mouse model system has revealed two serine proteinase systems associated with hatching: strypsin, a localized blastocytic enzyme responsible for the focal hatching of the embryo* in vitro*; and lysin, a uterine lumenal serine proteinase that lyses the* zona pellucida *at the time of hatching. Due to limitations in applying biochemistry to the study of hatching, much confusion has arisen over the respective roles of these two proteolytic systems. Recently, we have used RT-PCR to reveal two novel genes (denoted implantation serine proteinases, ISP 1&2) encoding tryptases that are expressed during the initiation of implantation in mouse. Based on functional studies, we suggest that ISP1 and ISP2 encode strypsin and lysin respectively. We have noted that ISP1/ strypsin plays two critical roles in implantation. Although it is essential for hatching* in vitro*, more importantly, ISP1 is required for the initiation of blastocyst invasion into ECM. We suggest that the localized expression of strypsin/ ISP1 is necessary for the orientation specific invasion of the blastocyst. In the absence of lysin/ ISP2 (i.e. during assisted reproduction), strypsin plays a critical role in hatching. Hatching disorders are common in women of older reproductive age. In demonstrating a potential link between hatching and implantation, our work helps to explain why artificial hatching of these embryos often fails to yield successful pregnancies. Having identified the mammalian hatching enzymes, potential therapeutics and indicators of pregnancy success may be developed. Moreover, these proteinases represent ideal targets for the development of non-steroid based contraceptives.*

Implantation in the Human and Murine Experimental Model

Implantation is a critical stage of reproduction where the embryo attaches to the uterine wall in order to garner nutrition from the mother's blood supply. Failure results in spontaneous abortion of the fetus. Based on similarities with humans, implantation has been modeled in the murine experimental system. Modern molecular genetics techniques, such as targeted gene disruption, have begun to provide insights into the potential genetic causes for implantation failure (for recent reviews see Carson, 2000; Rikenberger *et al*, 1997).

Implantation is a complex process composed of several developmental phases: blastocyst hatching, attachment and invasion (Fig. 1). Prior to implantation, the mammalian blastocyst is maintained within a proteinaceous coat, the *zona pellucida*, which prevents polyspermy and ectopic implantation. Hatching from the *zona pellucida* is considered to be the first step in implantation. It is generally accepted that hatching is mediated by two proteolytic systems (Gonzales and Bavinster, 1995; Perona and Wassarman, 1986). First a serine proteinase(s), lysin, is secreted into the uterine lumen in response to progesterone, which lyses the *zona pellucida* externally on day 4 of pregnancy (Orsini and McLaren, 1967; Joshi and Murray, 1974; Denker, 1977). A second serine proteinase, strypsin, is secreted by the embryo one day after lysin and acts more as a factor in implantation. However, in situations where embryos are removed from the uterus (i.e. during assisted reproduction), embryonic strypsin plays a primary hatching role in the absence of lysin. *In vitro*, hatching occurs focally due to the localized activity of strypsin at the abembryonic pole. As it is this pole that first attaches to uterine tissue, it has been suggested that strypsin's primary role may be in initiating implantation (Gozales and Bavinster, 1995).

The hormones estrogen and progesterone are necessary to synchronize the embryo and uterine receptivity in implantation. Prior to hatching on day

four, estrogen and progesterone are important in preparing uterine proliferation and differentiation. After hatching, an estrogen spike creates a period of receptivity between the embryo and the uterus, allowing the embryonic trophectoderm and uterine epithelium to interact (Paria *et al*, 1993). This embryo-uterine dialogue is orchestrated by the local traffic of cytokines between the embryo, lumenal, epithelium and endometrial glands. In mice, EGF and LIF have been found to play principle roles in blastocyst attachment and invasion, lying downstream of this estrogen spike (Das *et al*, 1994). Interestingly, the invading (abembryonic) pole of the blastocyst is rich in the heparin sulfate proteoglycan (perlecan), which is necessary for blastocyst invasion *in vitro* (Carson, 1993). Attachment of the invading pole of the blastocyst is thought to be mediated by the expression of heparin binding EGF, which is tethered to the surface of the uterine epithelium (Raab *et al*, 1996). EGF, in turn, has been found to promote blastocyst invasion, which is thought to occur by signaling through the EGF receptor, ErbB4, which appears on the apical surface of the invading trophoblasts (Paria *et al*, 1999, Wang *et al*, 2000). LIF is a regulatory cytokine that is secreted from endometrial glands at the onset of implantation and is necessary to sustain adhesion and invasion (Stewart *et al*, 1992). In the absence of LIF, luminal EGF family members are not expressed and blastocysts fail to attach (Song *et al*, 2000). As LIF expression is estrogen-dependent, it has been suggested that the window of implantation may be LIF-mediated (Song *et al*, 2000).

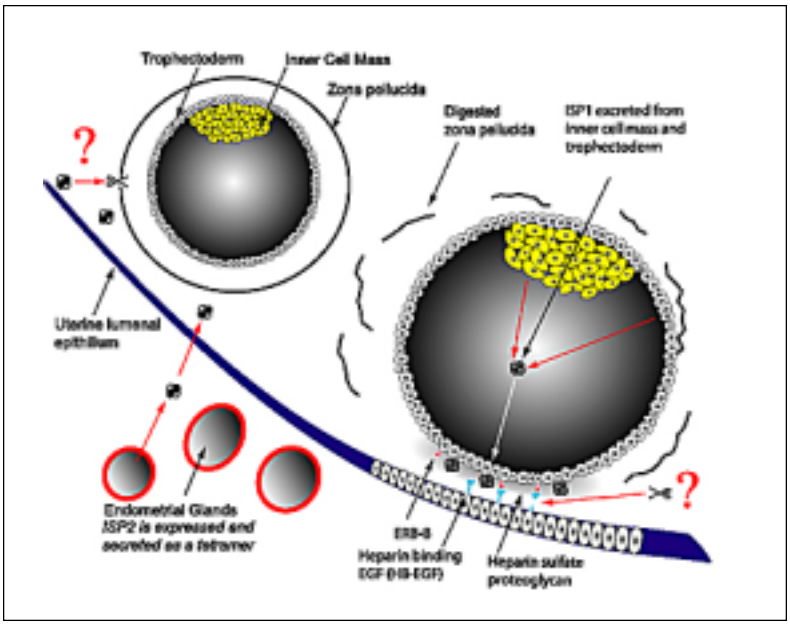

Figure 1. Prior to implantation, the blastocyst is maintained within a proteinacious coat, the *zona pellucida*, which prevents polyspermy and ectopic pregnancy. Implantation is initiated by hatching, whereupon the blastocyst is free to interact with the uterine lumenal epithelium. Hatching is mediated by a progesterone regulated serine proteinase that is secreted into the uterine fluid. Based on the expression pattern of ISP2, we hypothesize that ISP2 tetramer may represent the hatching enzyme. Following hatching, blastocyst invasion is initiated by the interaction of the heparin sulfate proteoglycan-rich, abembryonic pole with the uterine lumenal epithelium. Heparin binding-EGF, which is tethered to this epithelial surface, is important in invasion. Here, ISP1 tetramer may be recruited to the heparin sulfate bed in order to participate directly in invasion or via the proteolytic liberation of lumenal HB-EGF.

Proteinases and Embryonic Invasion During Implantation

Under cytokine instruction, proteinases and their corresponding inhibitors are thought to mediate the fine balance between trophoblast invasion and decidual anti-invasion. Classically, proteinases secreted by trophoblasts, uPA and MMP9, have been thought to be the main orchestrators of invasion (Rinkenberger *et al*, 1997). Their expression is upregulated by cytokines such as LIF and EGF and their enzymatic activity is regulated by the inhibitors PAI 1& 2 and TIMP3, which are expressed on the border of trophoblast invasion (Harvey *et al*, 1995; Teesalu *et al*, 1996, Leco *et al*, 1996). Inhibitor studies have suggested that both uPA and MMP9 play principle roles in embryo outgrowth during implantation (Behrendtsen *et al*, 1992; Werb *et al*, 1992). In sharp contrast, however, targeted mutagenesis studies have indicated that either proteinase is dispensable in implantation (Carmeliet *et al*, 1994; Vu *et al*, 1998). These latter observations have questioned the importance of uPA and MMP9 within the presumed implantation proteinase cascade and have suggested that other proteinases may be important in implantation. Indeed, other proteinase and antiproteinases have begun to be localized to the embryo-decidual border (Afonso *et al*, 1997; Lefebvre *et al*, 1992; Hurskainen *et al*, 1998).

The ISPs: Novel Tryptases Expressed During Implantation

Using serine proteinase active site RT-PCR, we have identified two murine genes encoding novel tryptases that are expressed at the time of blastocyst hatching and implantation (O'Sullivan *et*

al, 2000a; O'Sullivan *et al*, 2000b). We have coined these implantation serine proteinase genes as ISP1 and 2. Sequencing of a full-length ISP cDNA clones demonstrated that the ISP genes encode a novel serine proteinase related to tryptases. In BLAST identity searches, we found that the ISPs shared a moderate amount of sequence similarity with haematopoietic tryptases and shared conserved His and Ser active site moieties, in addition to the common N-terminal sequence (IVVG) of mature tryptases. Maximum parsimony analysis suggests that the ISPs represent distinct branches of the S1 proteinase superfamily that diverged from the elastase/chymotrypsin and mast cell proteinase clusters at approximately the same time (Fig. 2). The evolution of these tryptases is consistent with our suggestion that the ISPs play overlapping roles in hatching and implantation.

ISP 1: A Novel Tryptase Involved in Embryo Hatching and Invasion

We have demonstrated that the ISP1 gene is expressed in pre-implantation embryos and is necessary for successful blastocyst hatching *in vitro* (O'Sullivan *et al*, 2001). Accordingly, we have hypothesized that ISP1 encodes the elusive mammalian hatching enzyme, strypsin. Prior to implantation, strypsin is localized to the abembryonic pole of the blastocyst (Perona and Wassarman, 1986). Based on inhibitor studies, strypsin activity is necessary for hatching *in vitro*. In antisense studies, we have demonstrated that specific abrogation of ISP1 gene expression can prevent hatching *in vitro*. This affect on hatching is specific, dose-dependent and is completely reversible. In the absence of ISP1 expression, strypsin activity no longer localizes to the abembryonic pole. Interestingly, it is this pole that first becomes adhesive *in utero* and orients the embryo for invasion (Kirby *et al*, 1967). As tryptases are tetramerized on beds of heparin sulfate proteoglycan (Lindstedt *et al*, 1998), we hypothesize that ISP1 monomers that are expressed throughout the embryo must tetramerize at the abembryonic pole to form strypsin (Fig 3).

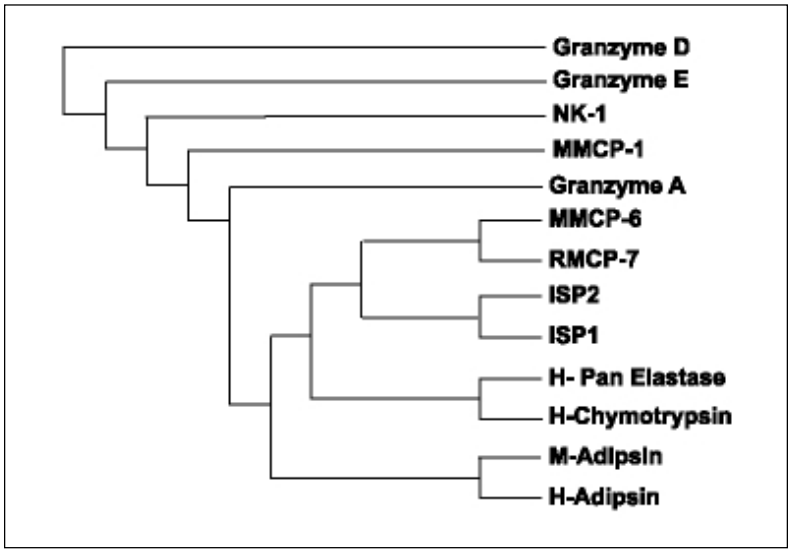

Figure 2. Dendrogram showing the relationship of amino acid sequences between representative serine proteinases. Serine proteinase sequences identified from a BLAST identity search were aligned using Clustal W and an unrooted tree was constructed using maximum parsimony analysis. The ISPs represent a distinct branch of the S1 proteinase superfamily that first diverged from the elastase/chymotrypsin and mast cell proteinase clusters at approximately the same time.

Through our discovery of ISP1, we have confirmed the prediction that the enzyme responsible for focal hatching *in vitro* might really be the enzyme responsible for facilitating blastocyst attachment and invasion (Gozales and Bavinster, 1995). In the absence of ISP1 expression, we observe that blastocysts also fail to outgrow *in vitro*. We hypothesize that ISP1 participates in a continuum that connects blastocyst hatching to ECM attachment and outgrowth. Historically, hatching and outgrowth have been viewed as unrelated molecular phenomena. While serine proteinase inhibitors have been demonstrated to affect both hatching and ECM invasion, these studies have focused on the respective roles of strypsin in hatching and uPA in invasion (Perona and Wassarman, 1986; Kubo *et al*, 1981). We have noted that most, if not all of these inhibitors, are effective against tryptases and have suggested that their action in affecting outgrowth may be directed against the ECM-degrading potential of strypsin.

Interestingly, heparinase and heparin sulfate has been found to abrogate embryo attachment and outgrowth *in vitro* (Carson *et al*, 1993) and may act, in part, by interfering with strypsin activity. In hindsight, it seems reasonable that a localized proteinase involved in degrading the *zona pellucida* might also be involved in initiating the degradation of ECM that occurs in blastocyst outgrowth. ISP1/strypsin may also participate indirectly in ECM degradation through the activation of other proteinases, such as uPA and MMP9. As EGF has previously been found to activate blastocyst out-

growth *in vitro* (Das *et al*, 1994), localized strypsin could also participate by freeing bound HB- EGF for signaling to the ErbB4 receptor. Indeed, other serine proteinases have been found to facilitate HB-EGF signaling (Kalmes *et al*, 2000), and support this idea.

ISP2: A Novel Progesterone Regulated Tryptase in Uterine Glands

Recently the action of strypsin during hatching has been characterized an artifact, since focal hatching does not occur *in utero* (Gozales and Bavinster, 1995). Instead, it is generally accepted that release of the embryo from the *zona pellucida* occurs via the action of lysin proteinase (Gonzales *et al*, 2001). Although not well characterized, lysin is secreted into the uterine lumen on day 4. Pregnancy manipulation studies in mouse and hampster have demonstrated that lysin activity in uterine fluid is embryo independent, yet is dependent upon progesterone signaling (Gonzales *et al*, 2001; Orsini and McLaren, 1967; Joshi and Murray, 1974; Rosenfeld and Joshi, 1981). Based on functional studies, we hypothesize that ISP2 encodes the elusive lysin proteinase, and that like ISP1, ISP2 may multimerize to form a lysin (O'Sullivan *et al*, 2001b). ISP2 expression is limited to the uterine endometrial gland, which is the major source of uterine secretions in early pregnancy. Based on *in situ* hybridization staining, ISP2 expression is first detected in day 4 pregnancy. In artificial pregnancy, we have demonstrated that ISP2 is expressed in oil-induced deciduomas, and in control uteri treated with estrogen and progesterone alone. These observations suggest that, like lysin, ISP2 expression is embryo-independent and hormonally regulated. Indeed, when the day 4 estrogen pulse is prevented by ovariectomy, ISP2 expression is only observed when progesterone is administered. Similarly, administration of antiprogestin prevents ISP2 expression, suggesting that ISP2, like lysin, is progesterone regulated.

Based on all of the above functional data, including the putative relationship of ISP2 to ISP1 and strypsin, we hypothesize that the ISP2 gene encodes lysin. Our observation that two related tryptases are derived separately from the embryo and uterus to effect hatching reiterates the hypothesis that genetic redundancy has evolved to ensure successful implantation. Although hatching and implantation have been viewed as unrelated molecular phenomena, our data suggests that these two events may be intimately connected. While ISP1/strypsin may facilitate implantation, the possibility exists that lysin could also participate in the early stages of implantation. Indeed, Mintz and colleagues first suggested that the uterine enzyme responsible for zona lysis is also an implantation initiation factor (Mintz *et al*, 1972; Pinsker *et al*, 1974). Here, we envision that after lysis, the abembryonic bed of heparin sulfate could also recruit lysin to the site of apposition and invasion.

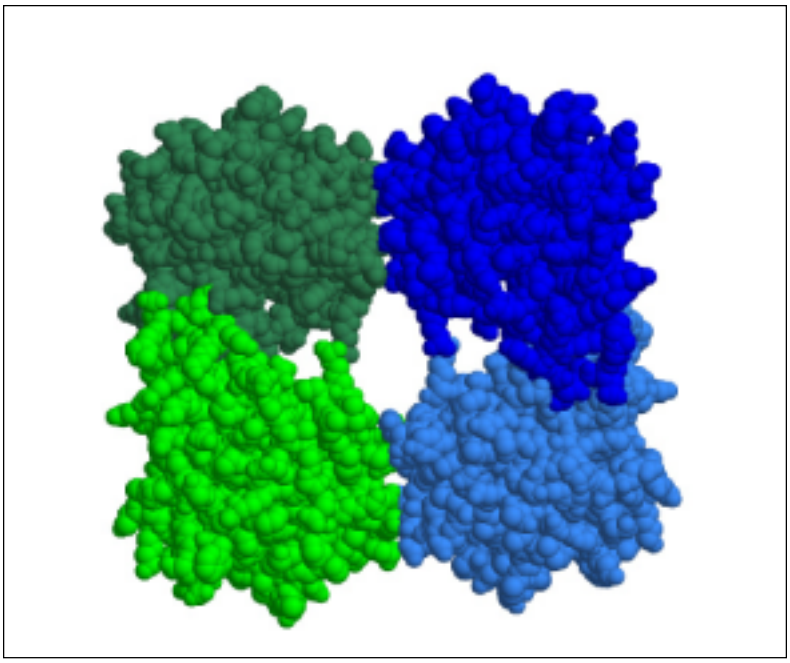

Figure 3. An ISP1 tetramer was modeled using SwissPDB Viewer and RasMol. The mouse ISP1 protein sequence was blasted against the ExPdb database and high sequence similarity to human beta tryptase was discovered (BLAST score: 2 X 10-60). Human beta- tryptase was then used as a modeling template. The mouse ISP1 sequence was superimposed over the different chains of the human beta-tryptase tetramer using SwissPDB structural alignment algorithms.

Footholds in Female Fertility

One of the most pressing issues of the new millennium is overpopulation. Although the birth control pill has been available for many years, it is only used by 18% of women of childbearing age in the U.S., and an even smaller percentage in the developing world, due primarily to concerns over side effects. Having identified two proteinases responsible for hatching of the mammalian embryo and having demonstrated that that hatching is essential for embryonic development, we have discovered important targets for the generation of nonsteroidal

contraceptives. Since a number of specific tryptase inhibitors have recently been developed (Sanderson, 1999), it should be possible to identify pharmaceuticals, which inhibit hatching.

While contraception has given women the freedom to postpone childbearing until later in life, this has created it own set of problems. In women of advanced childbearing years, more than half of normal pregnancies fail. Chronic pregnancy failure, especially in older women, has led to the field of assisted reproduction, whereby embryos are fertilized, cultured *in vitro* and subsequently transferred back into the mother. However, less than 15% of embryo transfers result in successful pregnancy. Here it has been recognized that embryos derived from women of older childbearing years frequently fail to hatch (Bider *et al*, 1994). While physical methods for hatching embryos have been developed, these have not been found to significantly improve pregnancy outcome (Mandelbaum, 1996). Our observation that strypsin is important both for *in vitro* hatching and the initiation of implantation, may help to explain why, in assisted reproduction clinics, human embryos that fail to hatch *in vitro*, also fail to implant, despite the successful implementation of artificial hatching. Accordingly, we anticipate that the ISPs may be important prognostic indicators for successful implantation. In addition, administration of the hatching proteinase may improve pregnancy outcome, both by emulating the normal hatching process *in vitro* and potentially enhancing implantation.

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Reminiscences From Montreal (1954-1956)

Harry Jellinck
Department of
Biochemistry
Queen's University
February 2001

Much has been written already about the "early days" of Biochemistry in Canada and therefore I will limit my recollections to two short but memorable years at McGill University between 1954 and 1956. This was before the Canadian Biochemical Society had emerged from its parent Physiological body. Those were halcyon years when the relatively small number of biochemists working in that vibrant city knew each other well and would meet regularly at various Montreal restaurants to present their latest findings. Many of those from this club went on to become leaders in their field.

I arrived into this stimulating environment from England in October 1954 after a stormy, week-long Atlantic crossing on the SS Atlantic. I was still fresh with Ph.D., my wife (Mary) and fortified by the youthful enthusiasm that goes with such accomplishments! I had spent the previous years working on the metabolism of synthetic estrogens related to diethylstilbestrol under the nominal direction of Professor Sir Charles Dodds who was Director of the Courtauld Institute of Biochemistry at the Middlesex Hospital Medical School. He was a supportive but extremely busy man so that my de facto Ph.D. supervisor became Wilfred Lawson, an exceptionally talented organic chemist who had been Sir Robert Robinson's (President of the Royal Society) personal research assistant. He had already synthesized more than a hundred stilbene derivatives to test for estrogenic potency in an extensive structure-activity study. This was just prior to the establishment of the estrogen receptor concept. Lawson was a strict disciplinarian and used to give me a hard time whenever I wanted to take Saturday morning off to play in an "away" rugby football match! Another person who had an impact on me was Sylvia Simpson who with her subsequent husband Jim Tait, were involved in the isolation of the mineralocorticoid, aldosterone, before its novel structure had been elucidated. I remember that we had a "pool" to predict its structure but none of us were anywhere near to envisaging the unusual aldehyde side-chain in this steroid.

The question of where to go for my "postdoctoral" was pre-ordained by the emerging use of radiolabelled compounds to study metabolism. The chemical synthesis of ^{14}C-containing steroids had been accomplished recently and presented me with the opportunity of building on my expertise with estrogens. Here was an exciting new field of research in which the biogenesis of the naturally-occurring estrogens could be studied in vivo under near-physiological conditions! R.D.H. (Don) Heard at McGill University and his group, which included Sam Solomon, Vince O'Donnell, Jim Webb, Graham Bligh, Malcolm Cann and Judith Saffran, to mention a few, had already laid some of the groundwork in this area. A postdoctoral position had become available and my previous experience with estrogens – albeit synthetic ones – helped me in being selected. In those days funding for research was no problem, particularly in well-established Universities such as McGill.

The problem assigned to me was the biosynthesis of estrone, which is excreted in horse urine in very large amounts. Enough could be isolated in a day from a pregnant mare that it could be crystallized. It was already known from non-isotopic evidence that androgens were the probable estrogen precursors, but this could now be firmly established by administering a very small dose of [4-^{14}C] testosterone and measuring the specific ^{14}C-radioactivity of estrone in the urine. I had become used to working with rats and rabbits to identify metabolites, but I was not quite prepared for the logistics of isolating steroids from almost 13 litres of urine. The radiolabelled androgen, dissolved in a small volume of ethanol, was injected into the jugular vein of a mare in the 6th month of gestation. She was one of many kept on a farm in Alexandria, Ontario, which provided a source of "Premarin" – a mixture of equine estrogens – for Ayerst laboratories based in Montreal at the time. Five decades later, it is still the most commonly prescribed estrogen preparation for replacement therapy in estrogen-deficient women. Gallons of urine were collected by the company, initially employing speedy young men with buckets, which they used

whenever a tail went up, and later by a more elegant method – truck tires, split in half, harnessed under the horses.

Our mare's reaction to the alcohol passing directly to its brain was predictable, but after an initial head droop, it recovered and went on as if nothing had happened. The next procedures, which involved acid hydrolysis to release steroids from their conjugates and extraction with liters of toluene was not only a challenge, but a health hazard and a source of great unpopularity in the Department of Biochemistry. The whole building was permeated by the pungent aroma of the refluxing urine, which also impregnated hair and clothing. The air flow in the fume hoods was checked repeatedly after that, but it took many months to be forgiven! However, the experiment was a success and I managed to isolate over 150 mg of pure estrone, containing significant amounts of ^{14}C-radioactivity. It took me another month of repeated crystallizations, conversion to the benzoate followed by regeneration of the estrone with no decrease in specific radioactivity before I managed to convince Don Heard that the conversion of testosterone to estrone had really occurred. I remember going into the Laboratory on Dec. 25 – we lived nearby, at the corner of University and Pine – to count the last batch of estrone crystals, which by now had dwindled to 5 mg, and the joy of finding that they had still retained their radioactivity. It was probably the best Christmas gift that I ever received! This experiment was part of the studies by Don Heard's group on elucidating the pathway of biosynthesis of the ring B unsaturated estrogens, equilin and equilenin, which bypass cholesterol involved in the formation of all the other classical steroid hormones. The problem of this alternate pathway of steroidogenesis was taken up more recently, by Bhagu Bahvnani while still at McMaster University.

Naturally, not all experiments are a success and one of my more memorable failures in Montreal was missing the boat to show that human placental microsomes provided an exceptionally active aromatizing system. Not only was my anatomical knowledge inadequate to dissect out the appropriate areas from the large mass of blood-engorged tissue but I was disheartened by the procedure needed to obtain the placentas. I would be informed by phone from the delivery room at the Royal Victoria Hospital that a birth was imminent and would then rush up the hill from McGill during the humid mid-summer days with a large, ice-filled bucket. On one occasion, while waiting with the nervous father, I heard one of the nurses shout: "It is O.K., you can forget about the baby, it is only the placenta that he wants"! I need hardly add that this was said as a joke.

I have not mentioned many other memorable events or some of the well-known scientists such as J.H. Quastel and Hans Selye who were active in Montreal at the time. Others, such as Tim Parsons who recently won the prestigious Japan prize and Andrew Schally (who took over our Pine St. apartment) were at the beginning of their careers. The latter shared the 1977 Nobel Prize in Physiology and Medicine with Roger Guillemin and Rosalyn Yallow. Those indeed were the "good old days"!

Biography

Harry Jellinck was born in 1928 in Paris, France and is married with 3 daughters. He obtained his undergraduate Degree in Natural Sciences at Trinity College, Cambridge in 1948 and an M.Sc. and Ph.D. from the University of London (1952 and 1954). After 2 years as a Postdoctoral Fellow (National Research Council) at McGill University and 3 years as a Lecturer at two Medical Schools in London, he returned to Canada to join Dr. R.L. Noble at the newly established Cancer Research Centre, University of British Columbia. He was promoted to Full Professor before moving in 1967 to Queen's University as Head of the Department of Biochemistry. He stepped down from this position in 1978 and became Emeritus in 1993. He has also been a Visiting Professor at Rockefeller University, since his first sabbatical in New York in 1978. He has published 3 textbooks, over 100 scientific papers and is still active in research, albeit at a reduced pace.

News from Biochemistry Centres

University of British Columbia

Department of Biochemistry and Molecular Biology

Correspondent: Dr. E. Peter M. Candido

The Department continued its strong record of teaching and research in 2000/2001. The present graduate student population stands at approximately 65, and 2000 saw the graduation of 4 M.Sc and 5 Ph.D students. The Department's research support totalled approximately $5.3 M in 1999-2000. In addition, the Department had a major role in funding the innovative Laboratory of Molecular Biophysics (LMB; further details below), which received combined funding of $8.75 M from the Canadian Foundation for Innovation, the Blusson Endowment to UBC, and the BC Knowledge Development Fund.

Faculty News

Ian Clark-Lewis, Professor and member of the Biomedical Research Centre, received a CIHR Scientist Award in 2000. Dr. Clark-Lewis is interested in the structure and function of protein growth factors and their receptor interactions, with special focus on the structure-activity relationships of cytokines and their receptors, and protein engineering using chemical synthesis.

Pieter Cullis, Professor, was the recipient of the Alec D. Bangham Award at the Liposome Research Days Conference at Napa Valley, CA., April 13, 2000. This is a lifetime achievement award and is bestowed upon scientists who have a highly productive and illustrious history of research, publication and teaching in the liposome field.

Shoukat Dedhar, Professor, was appointed a Terry Fox Cancer Research Scientist of the National Cancer Institute of Canada. He is a Senior Scientist at the British Columbia Cancer Agency/ British Columbia Cancer Research Centre. His research interests focus on integrins, their interactions with the extracellular matrix, and on receptor-mediated signal transduction.

Patrick Dennis, Professor, is currently on a two-year leave of absence in Washington, DC, where he is serving as a Program Officer for the National Science Foundation. His research focusses on ribosomal RNA processing, ribosome biogenesis and molecular evolution of archaea.

Dana Devine, Professor, Department of Pathology and Laboratory Medicine and Associate Member of our Department, has been appointed Director of Research and Development for Canadian Blood Services. Recently, Dr. Devine was instrumental in founding UBC's Centre for Blood Researchz, a new initiative comprising an interdisciplinary group of UBC faculty members (see further details below). She also received this year's award for Science, Research and Medicine from the YWCA at their annual "women of distinction" awards ceremonies. Her laboratory is interested in the activation and regulation of the complement system.

Brett Finlay became a CIHR Research Scientist and, more recently, received a CIHR Distinguished Investigator Award. Brett was also the recipient of a renewed Howard Hughes International Scholar Award, and was recently elected a Fellow of the Royal Society of Canada. Brett is a member of the Biotechnology Laboratory at UBC and holds appointments as Professor of Biochemistry and Molecular Biology as well as Microbiology and Immunology. His research focusses on the processes by which pathogens adhere to, enter, survive in and exit host cells.

Philip Hieter, Professor of Medical Genetics and an Associate Member of our Department, has assumed the Directorship of the UBC Biotechnology Laboratory. The objective of the Laboratory, which was established in 1987 with our late colleague **Michael Smith** as Director, is to foster research and teaching in interdisciplinary areas, with particular emphasis on fermentation and process engineering, on protein engineering, on forest, plant and fungal molecular genetics, and on animal and human molecular genetics.

Grant Mauk, Professor, was awarded a Canada Research Chair in Blood and Transfusion Medicine. His research interests centre on metalloprotein structure and function, including mechanisms of hemoprotein electron transfer and catalysis, protein-protein interactions, and multicopper oxidases. Grant also provided major lead-

ership in establishing the UBC Laboratory of Molecular Biophysics (LMB). The LMB provides investigators at UBC with sophisticated research instrumentation that will also be accessible to neighbouring universities and companies. These facilities will include macromolecular NMR spectroscopy, macromolecular X-ray crystallography, a laser laboratory, a light scattering laboratory, a microcalorimetry facility, a surface science and clean room facility, and a kinetics laboratory. Shared instrument facilities will include FT-Raman spectroscopy, fluorescence spectroscopy, and circular dichroism spectroscopy. New service laboratories will provide access to electrospray and MALDI mass spectrometers, analytical ultracentrifuge, and surface plasmon resonance (BiaCore). Other members of the Department on the LMB Steering Committee are **George Mackie** (Professor and Department Head), **Lawrence McIntosh** (Associate Professor, Chemistry and Biochemistry and Molecular Biology), and **Steve Withers** (Khorana Professor, Chemistry and Biochemistry and Molecular Biology).

Ross MacGillivray, Professor, has been appointed Chair of the Senior Scholar and Scientist Committee for the newly created Michael Smith Foundation for Health Research (MSFHR), an important new source of support for biomedical research in BC. The MSFMR succeeds the former BC Health Research Foundation. Dr. MacGillivray was also recently appointed Director of the new UBC Centre for Blood Research (CBR). His research interests lie in the structure, organization and expression of genes coding for clotting factors and metalloproteins. The mission of the CBR is to apply cutting-edge methods of biotechnology to the study of blood and blood processing in an integrated, interdisciplinary manner. The CBR approach will combine laboratory and clinical research with engineering innovations to create new knowledge in transfusion science. In partnership with Canadian Blood Services and Bayer Canada, the knowledge emerging from the CBR research innovations will be used to enhance the blood system in Canada. Other members of the Department are also involved with the CBR, including **Dana Devine, Grant Mauk, Chris Overall** and **Natalie Strynadka**.

Lawrence McIntosh, Associate Professor, received a CIHR Scientist Award in 2000, and spent 1999-2000 on sabbatical at the European Molecular Biology Laboratory in Heidelberg, working in the group of Michael Nilges. Dr. McIntosh was supported as an Alexander von Humbolt Research Fellow during this period. His laboratory uses NMR spectroscopy to study a variety of biological problems and materials, including macromolecular structure and dynamics, eukaryotic gene regulation, signal transduction, polysaccharidases and carbohydrate binding proteins.

Robert Molday, Professor, was awarded a Canada Research Chair in Vision and Macular Degeneration, and was elected a Fellow of the Royal Society of Canada. His research interests include the identification and characterization of proteins involved in signal transduction pathways, membrane-cytoskeletal interactions and specific inherited degenerative diseases in vertebrate rod and cone photoreceptor cells.

Chris Overall, Professor of Oral Biological and Medical Sciences, and Associate Member of the Department, was the recipient of a Canada Research Chair in Metalloproteinase Biology. His research explores the biological activity of matrix metalloproteinases and tissue inhibitors of metalloproteinases in cancer, inflammation and development.

Natalie Strynadka, Assistant Professor and MRC/CIHR Scholar, received a Howard Hughes International Scholar Award. Natalie was also the winner of the year 2001 Merck Frosst Prize of the Canadian Society of Biochemistry and Molecular and Cellular Biology. Her research is aimed at the

P.D Bragg Dinner (Front row, l. to r.): C.T. Beer, I. Clark-Lewis, P.D. Bragg, B.E. Tiberiis, N.C.J. Strynadka. (Back row, l. to r.): M. Roberge, L. McIntosh, R.W. Brownsey, R.T.A. MacGillivray, G.A. Mackie, C.R. Astell, E.M. Trip, E.P.M. Candido, G.M. Tener, A.G. Mauk, P.R. Cullis, R.E. Barton

structure-based design of novel, therapeutically useful antibiotics and inhibitors of antibiotic-resistance mechanisms.

On April 30, 2001, members of the Department gathered at the Royal Vancouver Yacht Club for a dinner honouring **Philip Bragg**, Professor Emeritus. Dr. Bragg, who retired officially in 1998, continued to contribute to teaching and maintained an active research group until the fall of 2000. Indeed his research, which centred on the structure and function of NADH/NADP transhydrogenase, ATPase/ATP synthase and the bacterial respiratory chain, was continuously supported by the MRC/CIHR for approximately 38 years. Dr. Bragg joined the Department in 1964, and served as Head from 1987 until 1994. The dinner was ably organized by **Gordon Tener**, Professor Emeritus of the Department, a long-time friend of Phil's and member of the yacht club.

Graduate Studies

Natalie Rundle, a Ph.D student in the laboratory of Michel Roberge, was the recipient of the year 2000 S.H. Zbarsky Scholarship, a prize given for the best graduate seminar by a Ph.D student in their second year, as chosen by the graduate student body of the Department. The year 2001 Zbarsky Scholarship was awarded to **Michael Page**, a Ph.D student in the laboratory of Ross MacGillivray. This award was established by donations from family, friends and colleagues at the time of Dr. Sidney Zbarsky's retirement from this Department in 1985. Dr. Zbarsky is one of the founding members of our Department.

University Of Calgary

Dr. Leon Browder

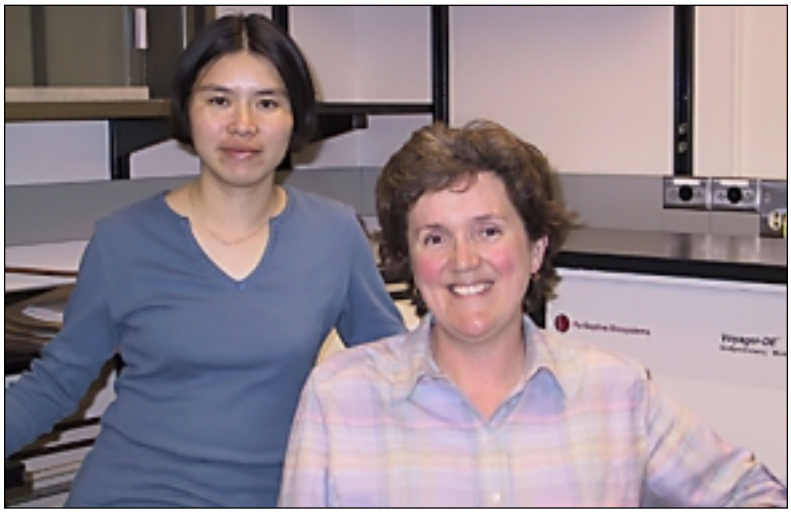
Department Of Biochemistry & Molecular Biology

Leon W. Browder, Professor and Head

The Department of Biochemistry & Molecular Biology is committed to excellence in research, teaching and service. Over the years the department has received considerable recognition for its achievements in basic research as well as in training of graduate students and postdoctoral fellows. We are a diverse department, with members belonging to eight different interdepartmental Research Groups. At the present time, 45 faculty members hold primary or secondary appointments in the department. There are three Emeritus Professors and seven adjunct appointees.

Our research activities are supported by a number of excellent core facilities, including UCDNA Services, the Peptide Synthesis Facility, the Mass Spectrometry Laboratory, the Southern Alberta Microarray Facility, the Embryonic Stem Cell/Targeted Mutagenesis Facility, the Hybridoma Facility, the Microscopy and Imaging Centre and the Bio-NMR Facility.

These are exciting times as the department embraces enthusiastically the post-genomics era. Annotation of genomes and determining the identities and functions of the proteins encoded in the genome presents unprecedented opportunities to understand the living world and will provide novel solutions to preventing and combating disease. Two programs are particularly noteworthy. These are the Proteomics & Functional Genomics Program established by the Alberta Cancer Board and the Alberta Network for Proteomics Innovation established through funding from the Alberta and Federal governments. These province-wide programs have enabled us to establish state-of-the-art facilities and to recruit outstanding scientists in proteomics, functional genomics and bioinformatics.



Dawn Chen and Susan Lees-Miller. Dr. Chen operates the MALDI Mass Spectrometer, and Dr. Lees-Miller coordinates proteomics facilities at the University of Calgary.

Faculty Transitions

Dr. Gil Schultz served during 2000 as Acting Associate Dean (Research). He now serves the Faculty of Medicine as Assistant Dean (Research).

Dr. Joe Goren returned from a very productive sabbatical in the Joslin Diabetes Center at Harvard University. He used a combination of genetics, physiology and molecular biology to study glucose homeostasis in mice. He learned a variety of new experimental techniques, which he is now using to enhance his own research program.

Dr. Kostas Iatrou completed his sabbatical in the Department of Biology at Athens University in Greece, studying the molecular biology of insect oogenesis. Kostas is currently on a leave of absence, serving as Director of the Institute of Biology in The National Centre for Scientific Research (NCSR) "Demokritos".

Dr. Marvin Fritzler is currently on sabbatical in Edward Chen's laboratory at The Scripps Research Institute in La Jolla, Ca. He is acquiring new technology and skills in gene and protein array analyses, laser dissection microscopy and bioinformatics.

Dr. Randy Johnston has been named Associate Vice-President (Research) with responsibilities in the biomedical and health research areas.

Dr. Karl Riabowol completed his term as Chair of the Cancer Biology Research Group. During his tenure as Chair, this group grew significantly in number of investigators and stature. The group also played a major role in the development of our program in proteomics, functional genomics and bioinformatics.

Dr. Chris Brown has replaced Karl as Chair of the Cancer Biology Research Group.

Dr. Kamela Patel has been named a Tier 2 Canada Research Chair.

Dr. Steve Robbins has been named a Tier 2 Canada Research Chair.

Dr. Mike Walsh has been named a Tier 1 Canada Research Chair. In addition, Mike spearheaded a successful application to establish a Canadian Institutes for Health Research Group in Vascular Contractility.

Dr. Norman Wong has assumed the role of Director of the Libin Gene Therapy Unit and Chair of the Gene Therapy Advisory Committee.

Dr. Gil Schultz

Dr. Joe Goren

Dr. Kostas Iatrou

Dr. Marvin Fritzler

Dr. Randy Johnston

Dr. Karl Riabowol

Dr. Chris Brown

Dr. Kamela Patel

Dr. Steve Robbins

Dr. Mike Walsh

Dr. Norman Wong

Richard Pon, Director of UC DNA and Protein Services


Dr. Susan Lees-Miller


Dr. Christoph Sensen


Dr. Dave Schriemer


Dr. Sarah Childs


Dr. Xi-Long Zheng


Dr. Tara Beattie


Dr. David Lau

New Members of our Department

Dr. Susan Lees-Miller has become a full member of this department; until now, she had an adjunct position in the department. Susan is coordinating proteomics facilities at the University of Calgary in both the Faculty of Medicine and in the Department of Biological Sciences.

Dr. Christoph Sensen has joined the department to spearhead our bioinformatics initiative. Before joining the University of Calgary, Christoph was Project Manager of the Canadian Bioinformatics Resource, located at the National Research Council's Institute for Marine Biosciences.

Dr. David Schriemer has become a member of the department as a key player in our proteomics program. Dave came to us from MDS-Proteomics, where he was Director of Screening Technologies.

Dr. Carol Schuurmans joins the department after a highly successful postdoctoral fellowship in the laboratory of François Guillemot in Strasbourg, France. She brings to the University of Calgary additional strength in the study of neural development and enhances our strong group of investigators who generate and study genetically modified mice.

Dr. Sarah Childs becomes the first investigator at the University of Calgary to study development of the zebrafish. Sarah completed her Ph.D. with Dr. Victor Ling. After an initial Postdoctoral Fellowship with Dr. Ling, Sarah moved to Dr. Mark Fishman's laboratory at Harvard University, where she began working on the zebrafish system. She has been studying vascular development and angiogenesis using this highly tractable system.

Dr. Xi-Long Zheng joins us to undertake innovative research on smooth muscle proliferation. Xi-Long has had two very productive postdoctoral fellowships. The first was in in Dr. Craig Malbon's laboratory in Stony Brook, where he discovered that streptozotocin-induced diabetes is ameliorated by expression of a subunit of the inhibitory G-protein of adenylyl cyclase. The second was in Norman Wong's laboratory at the University of Calgary.

Dr. Tara Beattie joins us from the Amgen Institute in Toronto. Tara studies the function of telomerase using an innovative *in vitro* system.

Dr. David Lau has a joint appointment in this department, with a primary appointment in the Department of Medicine. David also serves as the Chair of the Diabetes and Endocrine Research Group and as the Director of the Julia McFarlane Diabetes Research Group.

I am pleased to welcome all of these outstanding scientists to the Department of Biochemistry & Molecular Biology.

Training Opportunities

The Department of Biochemistry & Molecular Biology offers graduate training leading to Ph.D. and M.Sc. degrees in Biochemistry and Molecular Biology. We invite potential graduate students and postdocs to give Calgary careful consideration. Members of this department conduct exciting, leading edge research, are well funded by international, national and provincial agencies and publish extensively in the very best journals. More details about the department can be found at www.ucalgary.ca/bmb. Not only do we offer excellent training opportunities for young scientists, but the natural beauty surrounding Calgary is breathtaking, providing year-round recreational opportunities.

Extraordinary science in an extraordinary location!

Dalhousie University

Department of Biochemistry and Molecular Biology

Correspondent: Catherine Lazier

A reprise of the events here in 2000-2001 is overshadowed by the recent unexpected death of Peter Dolphin, who figured so greatly in this department. A separate remembrance of Peter is published in this issue. A special memorial lecture in his honour was given by Dr. Robert Ryan in the Tupper Building at Dalhousie on October 25th. A prize for graduate students in Peter's name is planned (for information contact F. Palmer).

Last September, we were very pleased to welcome **Doug Hogue** to the department as an assistant professor. He did his Ph.D. with **Carol Cass** in Edmonton and post-doctoral work with **Vic Ling** in Vancouver. Doug received grants from both the CIHR and CFI on his first applications.

Good news on the evolutionary biology front is that **Mike Gray** and **Ford Doolittle** have both been awarded Canada Research Chairs and are principal investigators on the two major programs funded by Genome Canada as part of the Genome Atlantic endeavor. This should mean significant expansion of these well-recognized research programs, including new faculty and post-doctoral positions.

Roger McLeod is busy organizing the Canadian Lipoprotein Conference to be held at a beautiful resort in Digby NS this fall. Roger's lab is in full swing with funding from the CIHR and the Dairy Farmers of Canada Foundation.

Our undergraduate science classes have expanded tremendously in the past few years. Numbers of honours, combined honours (usually with microbiology) and advanced major students have doubled at least. Our third year classes fill up within days of registration opening. Part of this may be due to increased interest in biotechnology but we don't think it is the only factor involved.

Rick Singer and **Gerry Johnston** (Head of Microbiology and Immunology) were jointly awarded the Max Forman Senior Research Award recently. This is the main Faculty of Medicine research award at Dalhousie. It was very good to see Rick and Gerry's long and very fruitful collaboration on yeast cell cycle and gene expression recognized in this way.

Harold Cook has been appointed Vice-Dean (Research) in the Faculty of Medicine and **Carl Breckenridge** is now the Vice-President (Research) for Dalhousie. Unfortunately, both of them are so busy we hardly ever see them anymore.

Your correspondent (now the department dowager) is happy to still be active in research and teaching. This doesn't leave much time to contemplate retirement though, which is only a couple of years away.

University of Guelph

Guelph-Waterloo Centre for Graduate Work in Chemistry and Biochemistry

Correspondent: Dr. Frances J. Sharom

University of Guelph, Department of Chemistry & Biochemistry

The past year has been good one for the department, with recruitment of new faculty to fill retirements proceeding apace, and substantial involvement in CFI and ORDCF initiatives. We welcomed **Dr. Marc Coppolino** as a new Assistant Professor on May 2001. Marc was born and raised in Waterloo and received his B.Sc. (Co-op, 1990) from the Department of Biology at the University of Waterloo. After graduating, he continued his laboratory training and became interested in biochemical research while working as a junior technician in the Department of Pharmacology at Merck Frosst Canada in Montreal. Marc then completed his Ph.D. (1998) under the supervision of Dr. Shoukat Dedhar in the Department of Medical Biophysics at the University of Toronto. Prior to joining the Department of Chemistry and Biochemistry at the University of Guelph, he finished a three-year MRC Fellowship in the laboratory of Dr. Sergio Grinstein at the Hospital for Sick Children, Toronto. During this period, Marc was awarded the 2000 John Charles Polanyi Award for Physiology and Medicine.

Dr. Dev Mangroo was recently awarded a Premier's Research Excellence Award (PREA). The additional funding will be used to support research directed at understanding the mechanism of nuclear tRNA export.

Graduate news:

Dr. Souzan Armstrong graduated with her Ph.D. from Rod Merrill's lab in September 2001, and has recently joined PTI (Photon Technology International) Inc. in London, Ontario, as a research scientist. Souzan will be running the Biofluorescence Laboratory under the direction of Dr. Alex Siemiarczuk.

Dr. Marty Lehto graduated with his Ph.D. from Frances Sharom's lab in August 2001, and has recently taken up a position as a post-doctoral fellow in the research group of Dr. Neil Cashman, who holds the Diener Chair of Neurodegenerative Diseases at the University of Toronto's Centre for Research in Neurodegenerative Disease.

University of Waterloo, Department of Chemistry

Dr. Lewis Brubacher, whose research involved protease enzymology and kinetics as well innovations in biochemistry teaching, has officially retired from the Chemistry department at Waterloo. He will continue with serving as editor of the highly successful publication *Chem13 News***. Dr. Michael Palmer** joined the department in September 2001. His research is focused on the study (by fluorescence) of the reversible conformational changes that occur in recombinant monomeric streptolysin O upon binding to erythrocyte membranes. Dr. Palmer was recently a research scientist in the Institute of Medical Biochemistry at Texas A&M. **Dr. Gary Dmitrienko's** research group is involved in the design, synthesis and enzymology of inhibitors of bacterial zinc-dependent beta-lactamases as well as the development of new structural classes of HIV-1 reverse transcriptase inhibitors. **Dr. Guy Guillemette's** research group investigates the structure and function of metalloenzymes including nitric oxide synthases and aldolases. **Dr. John Honek's** group is involved in the area of mechanistic enzymology of metalloenzymes as well as the structure-function of enzymes involved in methionine biochemistry. He was appointed an associate editor of *Biochemistry and Cell Biology (NRC)* this year. **Dr. Elizabeth Meiering's** group is conducting research on the folding, structure, function and dynamics of medically and biologically important proteins. **Dr. Susan Mikkelsen** is interested in biosensors and bioassays. Her group invented the world's first voltammetric sensor for DNA sequence detection, and is now actively developing a new electrochemical antibiotic susceptibility assay for microorganisms; technology available includes screen-printing for disposable sensor design and atomic force microscopy for surface characterization. **Dr. Scott Taylor's group** is continuing research in the areas of enzyme inhibitors and catalytic antibodies.

The department has completed setting up the new 600 MHZ NMR spectrometer and is also completing the installation of the Q-Tof electrospray mass spectrometer. A new ultrasensitive differential scanning calorimeter and a cell disrupter has been added to the group's equipment.

Graduate Scholarships:

NSERC graduate scholarships were awarded to **Jennifer Lapierre** and **Mark Vaughan**; OGS and OGSST scholarships were awarded to **Heather Montgomery, Jennifer Steere, Nicole Sukdeo**, and **Jason Wu**. **Susan Clugston** was awarded a NSERC postdoctoral fellowship and she is now in the laboratory of Professor Christopher Walsh, Harvard University.

University of Lethbridge

Department of Chemistry and Biochemistry

Correspondent: Marc R. Roussel, Associate Professor and Biochemistry Coordinator

The Department of Biological Sciences has recently hired **Igor** and **Olga Kovalchuk** in tenure-track positions. Olga's research focuses on DNA damage and repair processes, especially in relation to radioactive and chemical environmental contamination. She also studies the biology and toxicology of herbicides, work which dovetails nicely with local agricultural concerns. Olga holds a Ph.D. from the Ukrainian Scientific Genetics Center in Kiev. She comes to us from the Toxicology/Cell Biology Department of the Human Safety Division of Novartis in Basel, Switzerland.

Igor's focus is on the effects of both biotic and abiotic stresses on the genomes of higher eukaryotes, with a particular interest in transgenic plants. His research ranges from studying the effects of ionizing radiation on mutation rates to virus-induced genome instability. Igor is also a graduate of the Ukrainian Scientific Genetics Center. He was most recently a postdoc in the Friedrich Miescher Institute in Basel.

The Department of Chemistry and Biochemistry is in the process of building a biomolecular structure group. Our first step toward this goal was the hiring last fall of **Steven Mosimann**, a macromolecular X-ray crystallographer. Steven learned his trade as a graduate student at the University of Alberta and was a postdoc at the University of British Columbia. Shortly after arriving in Lethbridge, Steven was made an Alberta Heritage Foundation for Medical Research (AHFMR) Scholar. This award liberates Steven from some of his teaching duties for a period of five years. Steven also received a major equipment award from AHFMR with matching funds from the Canada Foundation for Innovation Young Investigator pool totaling $500,000. He has used this money to purchase a macromolecular X-ray diffractometer which will be installed in an annex of the new Life Sciences building. Steven will be using this facility to study RNA processing enzymes.

The Life Sciences annex will also house a new wide-bore 500 MHz NMR spectrometer, which has been funded by the Government of Alberta. This spectrometer, which is expected to be operational later this year, will have both solids and liquids capabilities. Funding for a microimaging accessory is currently being sought by the University's neuroscience group.

University of Manitoba

Department of Biochemistry and Medical Genetics

Correspondent: Jane A. Evans

The last year or so has been an exciting time for the Department as a merger took place that brought the previous Departments of Biochemistry and Molecular Biology, and Human Genetics together to form the Department of Biochemistry and Medical Genetics. The new Head of the merged department is Dr. **Jane Evans**, while the previous head, Dr. **Pat Choy** has moved up the administrative ladder to become Associate Dean Research for the Faculty of Medicine.

The Department has been fortunate enough to recruit several excellent new researchers: Dr. **Nasrin Maeseli** who works on molecular mechanisms of vascular development and remodeling by calreticulin; Dr. **Etienne Leygue**, who is interested in the molecular biology of precancerous breast lesions; Dr. **Spencer Gibson**, investigating signal transduction pathways leading to apoptosis and cell survival; Dr. **Geoff Hicks**, who is working on functional analysis of the genetic determinants of cancer and human disease; Dr. **Jeff Wigle**, who has interests in lymphangiogenesis using mouse models, and Dr. **David Merz**, who is using *C. elegans* to determine the regulation of cell migrations and axon guidance. This means that the Department now has active programs throughout the University and several of its related Institutes, including The St. Boniface Research Institute and the Manitoba Institute of Cell Biology, where Dr. **Jim Davie** is now Director.

We have also been very successful with respect to career awards, with Dr. Maeseli receiving

a Heart and Stroke Scholarship; Dr. Gibson, a CIHR New Investigator Award, and Dr. Leygue, a US Army Breast Cancer Scholarship. Our pleasure and pride in seeing Canada Research Chairs awarded to Dr. **Arnold Greenberg** and Dr. **Geoff Hicks**, were tempered with sorrow when Dr. Greenberg succumbed to cancer earlier this year.

A Canadian Foundation for Innovation major award to Dr. **Leigh Murphy** and the Breast Cancer Group, as well as a New Investigator Award to Drs. Leygue and Gibson have allowed our scientists access to more sophisticated equipment, including a microchip reader, laser-capture microdissection equipment, a light cycler system and an auto-immunostainer.

On a final note, our activities, like those of all Departments, have been much enhanced by our graduate students and other trainees. We are phasing in a merged graduate program in Biochemistry and Medical Genetics, while continuing to oversee students completing their degrees in the Biochemistry and Human Genetics programs. Currently, we have 3 premasters students, 24 working on their MSc degrees, and 7 PhD students, as well as postgraduate medical trainees in Medical Genetics, BSc (Medicine) students, a Canadian College of Medical Genetics fellow in molecular diagnostics and several BSc Honours projects students.

Highlights of our current and previous activities, as well as more information on faculty members and their interests, can be found on our web site at *http://www.umanitoba.ca/faculties/medicine/units/biochem/faculty.html*

McMaster University

Department of Biochemistry

Correspondent: Dr. V.S. Ananthanarayanan

With **John Capone** moving up to the position of Associate Dean of Research, Faculty of Health Sciences, in September, 2000, the department had **David Andrews** as acting chair until June, 2001. From July, 2001, **Gerry Wright** took over as the Chair. At 38, Gerry is perhaps the youngest chairperson of a biochemistry department in Canada. Gerry also is the Director of the McMaster University Antimicrobial Research Centre housed in our department. With his excellent research record and his keen interest in undergraduate and graduate education, we expect Gerry to act as an enthusiastic leader and take the department to newer heights.

Bernardo (Dino) Trigatti joined us as a new faculty member in September, 2000. Dino is an alumnus of our department and did his Ph.D. with **Gerhard Gerber** who is now on sabbatical leave after serving as the Vice-President of Research and International Affairs at McMaster for the past 5 years. Dino did his postdoctoral studies in Monty Krieger's lab at MIT, where he and others knocked out the high density lipoprotein receptor, SR-BI, in mice and demonstrated its critical role in high density lipoprotein metabolism and atherosclerosis. He has been an MRC postdoctoral fellow and is currently the William T. McEachern Fellow at McMaster University. His current research uses a combination of cell biological and targeted genetic approaches in mice to decipher the molecular and physiological roles of scavenger receptors in lipoprotein metabolism and atherosclerosis. His expanding laboratory (currently five members) is supported by the CIHR, CFI and OIT.

Ray Truant, who joined us in July 1999, is continuing his research on the pathways for protein nuclear import in mammalian cells, focussing on proeins involved in Huntington and related diseases. He has recently won a CIHR New Scientist award and a CFI award of $425,000 for buying a computerized microscope and other cell biology equipment. He also holds an operating grant from the Hereditary Disease Foundation, U.S.A.

Richard Epand, who won the prestigious Avanti Award last year from the Biophysical Society, is currently the Chairman of the Membrane Structure and Assembly Subgroup of that Society. He is also the current Vice-President of the Canadian Biophysical Society. He was an invited speaker and chair at symposia in Norway and Hungary that dealt with membrane structure and interactions. He delivered seminars at the University of Stockholm and the Institute für Biophysik in Graz.

Paul Berti who joined us in 1999 as a joint appointee in our Department and the Chemistry Department, has recently been awarded $500,000 from CFI for drug design. His research interest lies in understanding enzyme mechanism using kinetic isotope effects and other techniques with a focus on potential antibiotic targets. Paul also holds grants from CIHR and NSERC.

A most notable accomplishment for the Department this year has been the award of Canada Research Chairs to four of our members: **David Andrews**, **Gerry Wright**, **Eric Brown** and **Yingfu Li.** As a national level acknowledgement of the excellence in research of these scientists, these awards rank among the most grabbed by any single Canadian University this year.

We have recruited **Michelle MacDonald** as Assistant Professor as of September 2001. Michelle did her undergraduate program in our department and had done a project in Richard Epand's laboratory. She did her Ph.D in Medical Sciences under the supervision of Dr. **George Heigenhauser** at McMaster. She was an NSERC postdoctoral fellow in kinesiology at the University of Waterloo, looking at the fatty acid transport proteins in muscle and adipose tissues with special reference to their role in type 2 diabetes. While Michelle's major task now will be teaching several undergraduate courses, she will also continue her collaborative research with Dr.Heigenhauser's group at McMaster.

Finally, the department bid farewell to one of our young and very successful crystallographers, **Albert Berghuis**, who sought greener pastures and left us to join the biochemistry department at McGill. One person's loss is another person's gain! On the plus side, we will soon be having **Murray Junop**, who obtained his Ph.D. working with Dr. David Haniford at UWO in 1997 and is currently doing his postdoctoral research in x-ray crystallography in the laboratory of Dr. Wei Yang at the NIH in Bethesda. Murray's expertise lies in the structural and mechanistic aspects of proteins involved in DNA repair processes.

In sum, 2001 has been a very good year for us with new faculty and prestigious awards and a new Chair.

McGill University

Biochemistry at McGill

The Department has three new faculty members. **Dr. David Thomas**, from the National Research Council of Canada, took over as the new Chair commencing in January, 2001**. Dr. Albert Berghuis,** from McMaster University, is an X-ray crystallographer and will also direct The McGill Centre for Structural Biology. **Dr. Imed Gallouzi,** from Yale U, studies mRNA stability and transport.

The former Chair, **Dr. Philip Branton**, remains with the department and has been appointed as the first director of the Cancer Institute of the CIHR. **Dr. Michel Tremblay** has been appointed to head the McGill Cancer Research Centre. During the pasr year, several other members of the department were recognized for their contributions. **Dr. David Thomas** was awarded a Canada Research Chair. **Dr. Nahum Sonenberg** and **Dr. Philippe Gros** were appointed as Distinguished Investigators of the CIHR while **Dr. Jerry Pelletier** and **Dr. Morag Park** were appointed as Senior Investigators.

Construction has started on the building that will house The McGiIl Genomics and Proteomics Centre, which has strong ties with the department. A 600 MHz NMR awarded in the last CFI competition and scheduled for installation in late 2001, will be directed by **Dr. Kalle Gehring.**

University of Alberta

Department of Biochemistry

The Department of Biochemistry lost two faculty members this past year, but also grew in size with the addition of six new faculty members.

Departures from the Department included Dr. Robert Ryan and Dr. Robert Hodges. Dr. Ryan returned to the United States, and Dr. Hodges retired from the University of Alberta.

New members to the department are:

Dr. Andrew MacMillan – The research in Andrew's laboratory is focused on the chemistry and biochemistry of nucleic acids with an emphasis on biologically important reactions involving RNA.

Dr. Leo Spyracopoulos – Leo's research interests include the determination of the solution structures of proteins and protein complexes, elucidation of the internal dynamics and thermodynamics of proteins, and determination of the kinetics of interaction of proteins with their target molecules.

Dr. Luis Schang – Luis' lab is interested in the roles that cellular proteins play in viral replication and pathogenesis. As a model for his studies, he uses herpes simplex viruses (HSV). Three major areas of research: 1) the mechanisms whereby cellular cdks regulate transcription of viral genes; 2) expression of cell-cycle proteins in non-cycling neurons; and 3) the possibility that cdk inhibitors may be useful as antiviral drugs.

Dr. Kevin Wilson – Kevin's research is directed at understanding fundamental mechanisms of translation conserved in all organisms. The central enzyme of translation is the ribosome, a large and complex assembly of RNA and protein components.

Dr. Carlos Fernandez-Patron – Carlos is launching an exciting and interdisciplinary research program to study the molecular basis of hypertension. He is studying the molecular basis of hypertension and applying functional proteomics to identify mechanisms of vasoregulation that are unique to this condition.

Dr. Howard Young – Dr. Young will join the Department early in the new year. Howard has concentrated on the role of Ca^{2+} in biological systems, including hearing and muscle contraction. As a PDF he added cryo-electron microscopy and image reconstruction to his list of skills as a biophysicist, and membrane biochemist.

Recent initiatives in Biochemistry

- The National High Field Nuclear Magnetic Resonance Centre (NANUC). This is a National research centre with a mandate to further NMR science development; and
- The Institute of Biomolecular Design (IBD). This new research institute has a strong commitment to providing services in certain areas that are directly related to Biochemistry and other departments. IBD is a campus wide institute and is industrially linked with the Alberta Peptide Institute (API).

University of Toronto

Department of Biochemistry

Correspondent: David Williams

Faculty News

Peter Lewis will be completing his second five-year term as Chair of the Department at the end of December, 2001. During this period, Peter has been remarkably successful in recruiting new faculty members despite very trying fiscal constraints. Eight new faculty joined the campus-based Department within the Medical Sciences Building, plus nine other members were cross-appointed from various Research Institutes. Coupled with twelve retirements this has resulted in quite a dramatic transformation of the Department! Peter also spearheaded the creation of the multi-departmental Program in Proteomics and Bioinformatics, which involves the Banting and Best Department of Medical Research, Biochemistry, Laboratory Medicine and Pathobiology, Molecular & Medical Genetics, Medical Biophysics, Medicine and three affilated Research Institutes. This is a program to train undergraduates and graduates in these rapidly growing disciplines. Six new faculty have been recruited to various Departments through this initiative and four additional tenure-stream positions have yet to be filled. We look forward to the prospect of further faculty recruitment. On top of his

duties as Chair, Peter also served as Vice President and President of the CSBMCB. We are all very appreciative of Peter's exceptional contributions to the Department, and we are sorry to see his term as Chair drawing to a close. Peter will be embarking on a well-deserved sabbatical early in 2002 with Ruedi Aebersold at the Institute for Systems Biology in Seattle to work on mass spectrometry of protein complexes. We wish him every success in his research efforts.

A search for the next Chair of the Department is ongoing. **David Isenman** will assume the position of Acting Chair in the interim.

The Department is pleased to announce the arrival this summer of our newest faculty member, **Boris Steipe**, who was recruited through the Program in Proteomics and Bioinformatics. Boris comes from the Ludwig-Maximilians-Universitat, München, and joins us as an Associate Professor. His research interests include antibody engineering, green fluorescent protein engineering, phage display, nanotechnology and bioinformatics.

Many of our Faculty were honoured with awards in the 2000-2001 academic year. **Amira Klip**, **Sergio Grinstein**, and **Charles Deber** were all elected as Fellows of the Royal Society of Canada. Congratulations to all three on this exceptional achievement! **Grant Brown** and **Walid Houry** received CIHR New Investigator awards, and **Liliana Attisano** and **Lynne Howell** were the recipients of CIHR Investigator awards. **John Glover**, **Hue-Sun Chan, Walid Houry**, **Christopher Yip** and **Craig Smibert** all won Premier's Research Excellence Awards. Several of our members were successful in obtaining Canada Research Chairs with **Lewis Kay** and **Brian Robinson** being awarded Tier 1 Chairs and **Hue-Sun Chan**, **Règis Pomès**, and **Christopher Yip** receiving Tier 2 Chairs. **David Williams** was the recipient of the U. of T. Dales Award for sustained research excellence at the University of Toronto. **Harry Schachter** was honoured with the Plenary Lecturer Award of the Pan-American Biochemistry and Molecular Biology Society.

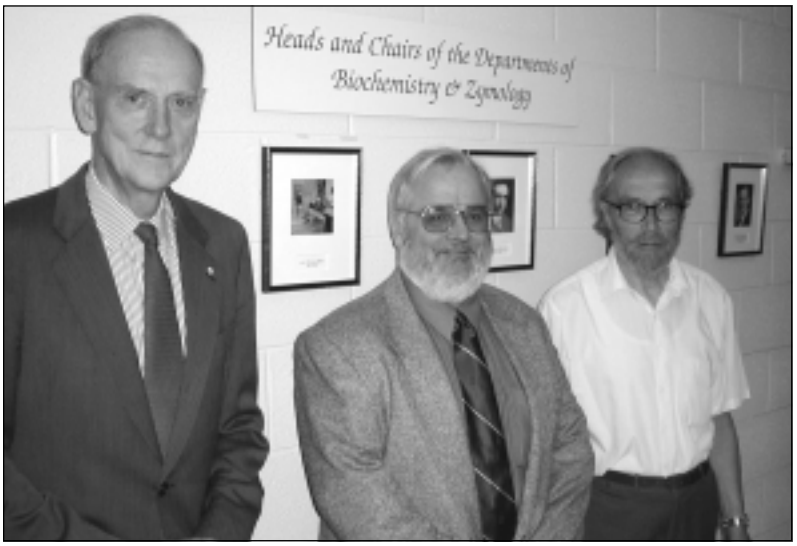
In addition to awards, several of our Faculty were very popular on the international lecture circuit, with **Hue-Sun Chan** giving talks in Sweden, Denmark, the U.K., Taiwan, Italy, Japan, and the U.S. **Harry Schachter**, rumoured to be retired but carrying on at his usual pace, was an invited speaker at meetings in Sweden, France, the Netherlands, and Singapore and also organized the Second International Symposium on Glycosyltransferases in Toronto. **David Williams** co-organized the CSBMCB Winternational meeting on "Dynamics of Intracellular Organelles and Molecular Machines" held at Mont Ste. Anne, Quebec, and **Peter Lewis** was Chair of the Scientific Program Committe for the 44th CSBMCB summer meeting, "From the Genome to Structure and Function", held at Alliston, Ontario. Both meetings were very popular and attracted diverse international audiences.

Events

Professor Emeritus, **Theo Hofmann**, conceived of and undertook the daunting task of created a pictorial history of Departmental Chairs, dating back to founding Chair A.B. Macallum in 1908. The photo gallery was unveiled at our Annual Poster Day on May 8, 2001. Several past Chairs attended the event. The gallery provides a wonderful perspective on the long history of the Department.

Appointments

We are pleased to welcome **Liliana Attisano** as a cross-appointed member of the Department. Liliana is a member of the Department of Anatomy and Cell Biology and is interested in the role of Smads in TGF beta Signalling. Liliana was also promoted to Associate Professor this year.



George Connell, Peter Lewis, and Theo Hofmann at the unveiling of the gallery of Departmental Chairs

We are also happy to announce that **Christine Bear**, a Senior Scientist in the Cell Biology Division in the Research Institute at the Hospital for Sick Children and Associate Professor in the Department of Physiology, has accepted a cross-appointment to our Department. Christine is interested in membrane associated ion transporters in general and CFTR in particular.

Congratulations also to **William Trimble** who was promoted to the rank of Full Professor.

Graduate Studies

The Department held its annual graduate student poster day on May 8, 2001. This annual event is very popular among faculty and students and provides an excellent opportunity for students to showcase their research. The judges faced a difficult task given the high quality of the graduate student posters, but after prolonged debate the following winners (who receive cash awards) emerged:

M.Sc. 1st Prize - **Victoria Stronge** (supervisor David Williams) *Relationship between the chaperone functions of calnexin and BiP using glycosylated and non-glycosylated substrates* in vitro.

M.Sc. tied for 2nd Prize - **Jianfei Qi** (supervisor Chi-Hung Siu) *Role of Cell Adhesion Molecules in the Transendothelial Migration of Melanoma Cells*

and **Jeffrey Lee** (supervisor Lynne Howell) *MTA/AdoHcy nucleosidase structure reveals homology with purine nucleoside phosphorylases*

Ph.D. 1st Prize – **Christopher Lemke** (supervisor Lynne Howell) *Insight into the catalytic mechanism of argininosuccinate synthetase.*

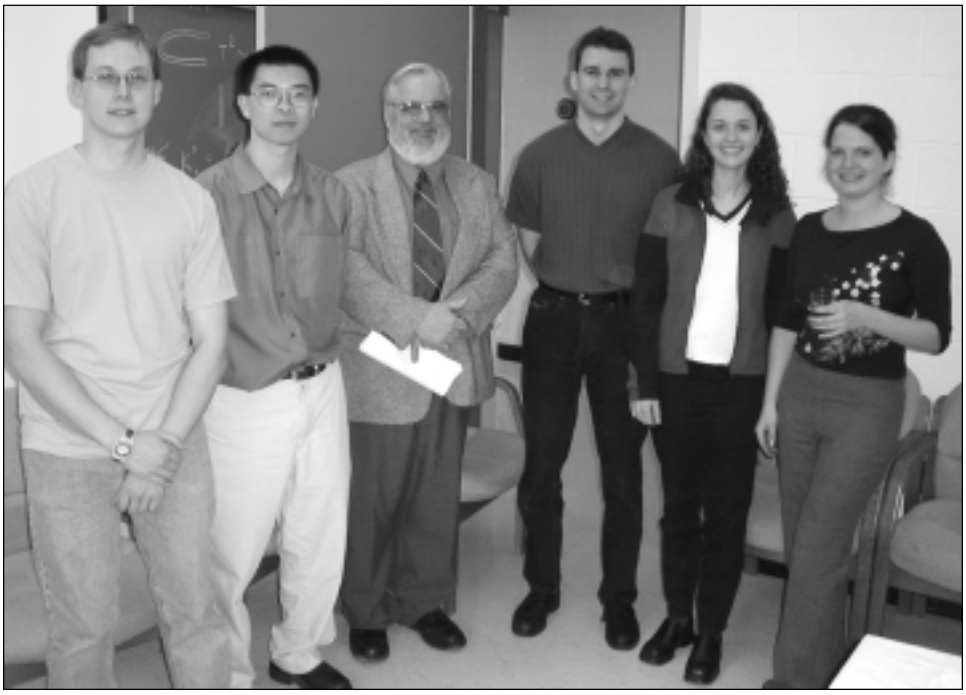
Ph.D. 2nd Prize – **Voula Kanelis** (supervisors Daniela Rotin/Julie Forman-Kay) *Solution structure of a NEDD4 WW domain - ENaC PY motif complex.*

Ph.D. 3rd Prize – **Janne Quilty** (supervisor Reinhart Reithmeier) *Trafficking of distal renal tubular acidosis mutants of the anion exchanger AE1.*

Additional graduate award:

The annual David Scott prize for outstanding all-round graduate student was awarded this year to **Zayna Khayat** (supervisor Amira Klip). Criteria for selection include performance at seminars, readiness and willingness to assist fellow graduate students and staff, and abilities as a teaching assistant.

Congratulations to all winners on their achievements.



Poster award winners (from left): Janne Quilty, Jeffrey Lee, (Peter Lewis), Voula Kanelis, and Victoria Stronge

University of Saskatchewan

Biochemistry Department

The Head of the Department, **Dr. Louis Delbaere**, was awarded a Tier 1 Canada Research Chair in Structural Biochemistry for seven years starting July 1, 2001. His research involves the study of protein structure by the method of x-ray crystallography and the correlation of structure with the function of the particular protein. The Canadian Light Source synchrotron is scheduled to be in operation at the University of Saskatchewan in January 2004 and Dr. Delbaere is expected to make extensive use of this national facility for his research.

In addition, the Department of Biochemistry welcomes **Dr. Gordon R. Gray**, who was appointed as Assistant Professor on July 1, 2000. Dr. Gray received his Honours B.Sc. (co-op) in Biology from the University of Waterloo and a M.Sc. and Ph.D. in Plant Science from The University of Western Ontario. His graduate studies involved a detailed examination of photosynthetic acclimation to low temperature and energy 'sensing' in response to environmental stress. Prior to his arrival at the University of Saskatchewan, Dr. Gray completed a post-graduate training as a NSERC postdoctoral fellow in the U.S Department of Energy Plant Research Lab at Michigan State University. During his postdoctoral tenure Dr. Gray undertook a molecular-genetic analyses of mitochondrial TCA cycle metabolism and alternative pathway respiration. Since his appointment, Dr. Gray has been successful in acquiring a 4-year NSERC research grant as well as a NSERC equipment grant. He was also awarded a CFI New Opportunity Award to fully equip a Plant Metabolism Laboratory. In addition, he is a co-principle investigator on a Genome Canada Proposal for the study of 'Abiotic Stress in Crop Plants', playing a role in the proteomic analyses component of the proposal. His laboratory is presently investigating metabolic adjustment of redox homeostasis in response to fluctuating environmental conditions in the model plant *Arabidopsis thaliana*. Dr. Gray currently has one graduate student who is screening various T-DNA insertion lines of *Arabidopsis* to identify mutants with altered mitochondrial or chloroplastic metabolic processes that affect redox balance. In addition, he is responsible for teaching BIOCHEM 220.3 'Introductory Plant Biochemistry'.