
ABSTRACT: Bacteria have mechanisms to export proteins for diverse purposes, including colonization of hosts and pathogenesis. A small number of archetypal bacterial secretion machines have been found in several groups of bacteria and mediate a fundamentally distinct secretion process. Perhaps erroneously, proteins called ‘autotransporters’ have long been thought to be one of these protein secretion systems. Mounting evidence suggests that autotransporters might be substrates to be secreted, not an autonomous transporter system. We have discovered a new translocation and assembly module (TAM) that promotes efficient secretion of autotransporters in proteobacteria. Functional analysis of the TAM in Citrobacter rodentium, Salmonella enterica and Escherichia coli showed that it consists of an Omp85-family protein, TamA, in the outer membrane and TamB in the inner membrane of diverse bacterial species. The discovery of the TAM provides a new target for the development of therapies to inhibit colonization by bacterial pathogens.
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I completed my Honours (BSc) Degree and PhD in the Biochemistry Department at La Trobe University, one of the two best Biochemistry Departments in the country. Before signing on for my PhD, I worked for two years as a research assistant with Dick Wettenhall on ribosomal protein S6 and then took six months off, back-packing around Europe and trying to decide whether science was the right career choice for me. I learned many things as a postgraduate student, one of the most important was to never stay in one place for too long. My PhD supervisors, Nick Hoogenraad and Peter Høj, often talked with us about hothouse flowers, and insisted that the best move for a postdoctoral position was to go to the best place you could find overseas. The second best move for a postdoctoral position was to move to the best place you could find in Australia. There was no “third best” option that didn’t involve moving. So I read papers all around a growing interest in protein targeting, to try and work out “the best place overseas” and in the end settled on three options: Tom Silhavy’s lab at Princeton, Hugh Pelham’s lab at the LMB in Cambridge or Gottfried Schatz’s lab at the Biozentrum in Basel. In large part swayed by a beautiful study by Dietmar Vestweber who was a postdoc in the Schatz lab [1]. I started a fax correspondence with Gottfried (Jeff) Schatz. Jeff was an Austrian who had spent his postdoctoral time at several places in the United States (hence “Jeff”) and was the foundation professor of Biochemistry at the University of Basel’s Biozentrum. With amazing foresight, the University had purpose built the Biozentrum in 1971 to house its Departments of Biochemistry, Biophysics, Pharmacology and Cell Biology and working there was a fabulous interdisciplinary experience, well ahead of “interdisciplinary” becoming a buzz word. When I arrived in 1993, the Schatz lab had just experienced its first “golden age” sketching out the basic principles of protein targeting into mitochondria (as told by Jeff) [2]. His new generation of postdocs (there were ~12 of us in the lab at any given time over the years 1993-1995) had big shoes to fill, and we were charged with determining the mechanisms that drove this protein import pathway in mitochondria. I spent an intense and hugely rewarding time in Jeff’s lab, made some great friends and still sometimes feel home-sick for Basel. I also learned a couple of things that I continue to pass on to students and post-docs who work with me. Firstly, Peter and Nick were right. Staying at La Trobe for my postdoctoral work would not have been a good move. The Department is fabulous, but moving overseas to a different (fabulous) Department gave me experience of things that I continue to draw on today. It forced me to “grow up” as a scientist and it made me feel like a scientist – not a recently graduated student, not an apprentice in training, but part of the international enterprise that is science. This will sound elitist, but science is an elite sport. Living the dream in Europe also allowed me to drop any insecurities that I’d had about whether or not I was as good as the postdocs that student-me had imagined working at places like Princeton, Cambridge etc. We don’t talk about this much, but I suspect that many Australian students have a similar cultural cringe, however unwarranted it is. You can tell yourself you’re as good as the best, other people can tell you this, but if you want to be certain that your PhD really did bring you up to scratch - to an internationally-competitive standard of excellence - there is nothing like working for a few years shoulder to shoulder with the best of your peers from around the world. The experience in Basel also showed me the value of starting in a new area. I moved away from rats and the physiology/enzymology of my PhD to a project bedded in yeast genetics. At that time, I had never seen a plate of yeast colonies and yeast genetics was as voodoo to me. But this was true for all of the postdocs that Jeff recruited. Likewise, very few of the postdocs leaving his lab after their training continued to research the same biological questions as in their postdoctoral project. Ben Glick, Sabine Rospert, Andreas Matouschek, Volker Haucke … all took what they learned in Basel and started work on big, new questions when they moved back home. Important too was a lesson on success (or otherwise) with grant and fellowship applications. It is all too easy, these days included, to be dismayed by unfavourable outcomes. But chasing funding success should not be the driver for the work you will pursue or the approach you will take. I applied for three Fellowships to work in Basel: an NHMRC C.J. Martin Fellowship, a HFSP Long-term Fellowship and an EMBO Postdoctoral award. I was awarded the HFSP and EMBO Fellowships, published some pretty nice “research outcomes” and was ultimately awarded as one of the top ten postdocs in the first ten years of the HFSP. I failed to get the CJ Martin Fellowship, on the grounds that the NHMRC panel saw a lack of feasibility for success. The letter from the NHMRC was the first one I received and it was a hard knock to take. Many of us have had (and still have) many of these letters; don’t let them sway you from your course. Continued page 3
Jeff Schatz is one of the greatest advocates for teaching being part-and-parcel of research. He is famous for his lecturing workload (and the undergraduates in Basel love him) and famous too for having occasionally taken only a stick of chalk to scientific meetings for his plenary presentations. The years under his influence left me inspired and in no doubt that a teaching and research position was the career choice for me. I returned to Melbourne and, after a couple of years as a Level B Lecturer at La Trobe getting my first ARC grants to start up my own laboratory, I took a Level C position at The University of Melbourne. When I arrived, the University was planning the first inception of its Biomedical Sciences degree, and I was given the “opportunity” of designing the curriculum for three new subjects and serving as coordinator (convenor) for all three. That sort of workload should have been terrible for an ECR, but we rolled up our sleeves and ensured that three very special subjects were created. The student feedback was always very positive, and my research ran in a way that I was both happy with and proud of. I enjoyed the ten years that I spent at The University of Melbourne, and I miss teaching.

Over that time the focus of research in my lab evolved from following the pathway by which relatively simple “tail-anchored proteins”, like Bcl-2 and its cousins, are inserted into the mitochondrial outer membrane, to looking at the assembly of more complex proteins of beta-barrel architecture. We identified and characterized molecular machines that drive these processes and this, in turn, led to a growing awareness that the molecular machines doing the job in mitochondria were inherited from the bacterial ancestors of these organelles.

My move to our Department at Monash was made possible by an ARC Federation Fellowship awarded to investigate the bacterial and mitochondrial machines in parallel: comparing the mitochondrial system and the bacterial system for insight into evolution, for a better understanding of how they both function, and for any sniff of practical applications that might flow from inhibiting this process in bacterial pathogens. Our research effort is running really well, the team is smiling (pictured) and the facilities at Monash for this sort of work are better than anywhere; I honestly don’t think our current research could be done so well anywhere else. For me though, the best thing about the move to Monash was being part of the new Unit for Host-Pathogen Molecular Biology; for the collegiality between the research groups and the “buzz” of so much exciting new research. The Unit has recruited many new staff and students and has resulted in Monash securing new grants and fellowships from the ARC, NHMRC and international sources. The other five group leaders are ECRs and moved to Monash to start-up new laboratories. They have or are looking towards teaching and research positions. They’ve come from training in the best labs around the world, with new ideas, new research directions and new approaches to big, exciting questions ... stop me if you think that you’ve heard this one before [3].


Prof Lithgow’s Webpage:  http://www.med.monash.edu.au/biochem/staff/lithgow.html
Environmental Sustainability At Monash

Anyone concerned with any environmental issues should either contact: Leigh Yang (li.yang@monash.edu) or visit The Office of Environmental Sustainability (TOES)

STUDENT SOCIETY

2012 Committee
The Student Society is still in the process of establishing a new committee for this year. When this is finalised, their positions and contact details will be published.

POSTGRADUATE MATTERS

Faculty of Medicine Research Supervisor Accreditation Level 1 Workshops - Semester 1, 2012

Online Registration now open.

Accreditation is compulsory for:
• staff intending to supervise
• postgraduate research students (with more than 25% supervision load)
• all Honours student supervisors

Intending research supervisors need to complete a series of nine modules.
Approximately 80 per cent of the requirements of these nine modules can be covered by attendance to both of the Faculty’s Research Supervisor Accreditation Training workshops.

You will need to have a mentor allocated to you by your department or centre before you may attend the workshop.
These workshops are run free of charge.

To apply for a place at the workshops, please register online (please note, your Monash login details will be required):
http://www.mrgs.monash.edu.au/seminars/medicine/
*Registrations close Monday 4th June 2012
Please contact Phyllis Di Palma, (Research Degrees Office, Faculty of Medicine, Nursing & Health Sciences) on ext 20047 or email Phyllis.DiPalma@monash.edu with any queries.

Dates and time of workshops
The accreditation program is split into 2 workshops (attendance to both workshops is required) for prospective supervisors (Monash staff members only - your staff ID number will be required from all departments within the Faculty of Medicine, Nursing & Health Sciences).

Workshop 1  -  Friday 8th June 2012
Workshop 2  -  Friday 15th June 2012
Registrations from 9.20am, with each workshop concluding around 12.45pm
Venue:  S2 Lecture Theatre, Building 25
Background

I started my research career upon accepting a postgraduate scholarship in the Yeast Molecular Biology Group at the Department of Biochemistry, Monash University, working under the supervision of Professors Rodney Devenish and Phillip Nagley. This was hard-core molecular biology! My project was focused on the functional relocation of mitochondrial genes into the nucleus to explore the feasibility of correcting hereditary mitochondrial gene mutations causing diseases such as diabetes, muscle pain, deafness and blindness.

After leaving this exciting work, I went along to acquire more skills working in a number of biotechnology projects, one of which was to produce the human glutamic acid decarboxylase GAD65 in the Autoimmunity Group lead by Professors Merrill Rowley and Ian Mackay also in the Department of Biochemistry at Monash University. GAD65 is one of the auto-antigens found in Type 1 Diabetes and Stiff Person Syndrome, and one of the key enzymes that produce gamma amino-butyric acid (GABA), a neurotransmitter inhibitor in the CNS. Our aim was to generate large quantities of GAD65 protein for potential diagnostic development and therapeutic applications. I had a fantastic time building the GAD protein production “factory” whilst juggling to raise our young family. I am still not sure which one was more tricky.

After the GAD project, I decided to do something a little different – working on worms looked good. I was attracted to a parasite called liver fluke because this parasite costs millions of dollars each year through loss of livestock production, stock deaths, human infections and costs of treatment and prevention. More importantly its main effect is in poor rural communities where animals are their main source of income (e.g. Buffalo for meat, milk and to plough their rice fields). For many years, detection of liver fluke infected livestock was not possible, especially in developing countries. I joined the Molecular Parasitology Group led by Professor Terry Spithill, focused on expression and purification of fluke-proteins for the development of vaccines and diagnostic kits for fluke infections. The project was very stimulating and I learnt lots about the areas of vaccine development and parasite immunology. Although we didn’t develop a commercial vaccine and work is still ongoing, I was involved in the development of a diagnostic kit now used in Indonesia. This was very satisfying and I continue to help out with this research in my spare time (which isn’t a lot!).

When my old boss picked up a new post in Canada as the director for the Centre for Host-Parasite Interactions at McGill University in 2003, Professor James Whisstock had just started his new Structural Biology Group in the Department. He kindly invited me to join his laboratory and I moved back to the “hard-core” research projects, firstly working part-time and then gradually full-time, and have been part of his laboratory ever since (some might say I have become part of the Monash woodwork). It has been a fascinating experience from my point of view to see how he started with 2-3 people inside Professor Rob Pike’s laboratory (with one power pack) to a large and fully equipped laboratory of more than 20 people. During this time I have again reinvented myself, switching from a protein chemist to a structural biologist, thanks to James’s patience and understanding. During the past many years, James has been a fantastic leader and mentor and I have learnt a lot and excitingly have been involved in several high impact publications. I have also been very fortunate to work closely many extraordinary colleagues who were always ready to help and share ideas. I owe my special thanks to our very supportive team of research assistants Adam Quek, Dharsh Jeevarajah and Gordon Lloyd, as well as those who have left the laboratory to develop their careers further. Below are some of the exciting projects I am currently involved with in the Whisstock laboratory.

Projects

Fibrinolytic system: This work is funded by NHMRC in collaboration with A/Professor Paul Coughlin and Dr Anita Horvath from the Australian Blood Disease Centre; and Drs Tom Caradoc-Davis and Nathan Cowieson from the Australian Synchrotron. Plasminogen is the zymogen form of plasmin and is the most formidable protease in the plasma. It is responsible for the removal of blood clots (fibrinolysis) in the circulating system in order to maintain the blood flow. As well as that, plasminogen can also bind to the surface of cells and become activated to plasmin, which breaks down the basement membrane and extra-cellular matrix hence promotes cell migration and, in the case of cancer, cell invasion. We crystalized and determined the crystal structure of plasminogen, and the results were recently published in Cell Reports (2012). The structure reveals how circulating plasminogen resists activation until it binds to a fibrin clot or target cell surface. It also explains how pathogens (such as Streptococcus) hijack this potent plasma protease during invasion. Our current focus is to understand the molecular interactions during plasminogen activation and plasmin inhibitions, especially in disease conditions such as thrombosis and uncontrolled bleeding. We employ various techniques including mutagenesis, characterization of enzyme activities, protein complex formation, X-ray crystallography and small angle X-ray scattering.

Structure and regulation of function of human glutamic decarboxylase (GAD): Gamma aminobutyric acid (GABA) produced by GAD is the most abundant neurotransmitter inhibitor in the CNS and is critical for the control of movements, and neuropasticity during development, learning and recovery from brain damage. Neurological conditions, such as anxiety, autism and post-traumatic stress disorder, are closely related to the imbalance of GABA homeostasis. This project is a continuation and expansion of my previous work on the GAD project with Professors Merrill Rowley and Ian Mackay. We published the crystal structures of both GAD65 and GAD67 isoforms in Nature Structure and Molecular Biology (2007). We observed that the mechanism through which GABA produced in mammals is regulated by a dynamic catalytic loop. The two isoforms of GAD cooperate to meet different physiological circumstances; GAD67 is a housekeeping enzyme which maintains the basal level of GABA in the CNS and stably binds to the cofactor pyridoxal 5’ phosphate (as a holo-enzyme), whilst GAD65 switches between the apo and holo-form through a process called autoactivation. The current project studies firstly, the structural motifs involved in the autoinactivation and secondly, small molecules which can modulate the rate of GAD activity. These studies involve measuring enzyme activity and using X-ray crystallography; with our outstanding research assistant turned PhD student Chris Langendorf being the key contributor to this project. Our aim is to comprehensively understand the process of auto-inactivation and allosteric regulation of GAD, with the long term aim of developing therapeutic treatments for GAD neurological diseases through the modulation of GAD activities.

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Dr Ruby Law continued

Transmembrane pore formation in perforin: Perforin plays a critical role in the immune homeostasis and surveillance against viral infection and cancer cells and deficiency is associated with impaired cytotoxic T-lymphocyte function. Perforin is a pore-forming MACPF protein (Membrane Attack Complex and Perforin-like) stored as a soluble conformer in the cytoplasmic granules of natural killer cells and cytotoxic T-lymphocytes. Upon conjugation with targets cells, perforin oligomerises and adopts a new conformation during pore formation. The perforin trans-membrane pores mediate the delivery of pro-apoptotic granzymes which induce cytolysis. In collaboration with Professor Joe Trapani and Dr Ilia Voskoboinik from Peter MacCullum Cancer Research Institute, we characterized the X-ray crystal structure of the monomeric perforin. At the same time Professor Helen Saibil’s group from Birkbeck College in London determined the perforin monomer and pore structure by cryo-electron microscopy. Together we were able to model how perforin assembles on the surface of the target cell upon formation of transmembrane pores; these results were published in Nature (2010). This publication is a very fulfilling outcome following our previous paper (in collaboration with Dr Michelle Dunstone) published in Science (2007) describing for the first time that the MACPF proteins including those found in the mammalian immunity defense system adopt the same fold as the bacterial cholesterol dependent cytolysins. Our current objective is to understand at the molecular level how perforin binds to the cell membrane and forms pores on the surface of the target cells.

New Biochem Webpage Banner Photo

In April, the new landing page for the Biochem website was released. All linked webpages are in the process of being updated to the new format. http://www.med.monash.edu.au/biochem/

Thanks are extended to Dr Fasseli Coulibaly, who provided the current webpage banner photo

We would like to change the banner photo on a regular basis. If you have photos that maintains high res at 700 x 230 pixels, please forward these to yvonne.dooley@monahs.edu.

The Achilles’ heel of poxviruses

Poxviruses are among the largest viruses infecting humans and have a complex architecture that sets them apart in the virus world. They produce infectious particles that, in contrast to most enveloped viruses, do not acquire their internal lipid membrane by budding through cellular compartments. Instead, poxvirus immature virions are generated from atypical crescent-shaped precursors that appear to form de novo in the cellular cytoplasm. Using a combination of X-ray crystallography and electron microscopy, we provided the first model of the honeycomb scaffold at the surface of crescents and immature virions. This model establishes evolutionary links between poxviruses and viruses infecting all kingdoms of life. Importantly, it also opens new avenues to develop assembly inhibitors as antivirals against poxviruses that may emerge from an animal reservoir or be deliberately released.

Courtesy: Dr Fasseli Coulibaly

Research in the Coulibaly Laboratory

Spheroids: crystalline armours of poxviruses

Another aspect of our research focuses on in vivo crystals that represent the infectious form of potential bioinsecticides including insect poxviruses. Virus particles are protected inside these crystalline armours that are remarkably stable and persist in the environment like bacterial spores. We use innovative X-ray microcrystallography to analyse crystals directly purified from infected cells and elucidate the structure of the most complex of these crystalline armours. Our research will contribute to engineering improved bioinsecticides and novel crystalline microparticles for vaccination.

http://www.med.monash.edu.au/biochem/staff/coulibaly.html
FIRST AIDERS & BREATHING APPARATUS
Reminder for all first aiders to update their training, and check their immunization status.
The Level 2 First Aid course is to be completed every 3 years, also a CPR Refresher/Anaphylaxis every other year.
Anyone interested in becoming a First Aider or a Breathing Apparatus volunteer for the department can send their request to the Safety Officer Irene Hatzinisirio or the Deputy Safety Officer Gavin Higgins.
OHS training sessions are subsidized by Monash OHS but you need to get the relevant approval and fund/cost centre numbers for the Safety Officer before applying online.
Links to apply for the relevant training:
CPR Refresher:
Level 2 First Aider (mixed mode):
Breathing Apparatus:

OHS MATTERS
1. Chemical Segregation
Compliance is required by Victorian State Government Legislation. This has been discussed for some months now and should be completed by Easter 2012. However, there are some lab areas still working on their segregation, and hopefully we will endeavor to help them complete this process by the end of May 2012 and no later!
Our Safety Officer Irene Hatzinisirio and Deputy Safety Officer Gavin Higgins are available for consultation, guidance and assistance.
The SO and deputy SO will do a quick inspection in May 2012, to ensure that all labs have implemented and are maintaining the segregation, as well to help out with any other related issues that may arise. If the SOs find that NO action has been taken. I will be asking the relevant Lab Heads for a please explain and offering the complete removal of the relevant chemicals at the lab’s expense.

2. Leave and After Hours Policy
A. If on “leave” (conference, sick, annual etc) you should not be engaged in laboratory research at Monash as you are not covered for insurance purposes and it is in breach of Workplace legislation.
B. Normal working hours are regarded as 8am-6pm Mon-Fri. If a protocol / procedure is deemed as high risk (such as liquid Nitrogen handling, use of radioisotopes or primary sources of radiation, dangerous chemicals), it should only be carried out if approved by the Supervisor and the Safety Officer with the requirement that a second suitably trained person is available to assist in the case of emergency. Documentation must be kept to support the granting of such approvals.

Thank you for your assistance in meeting our obligations for these important matters.

QUICK OVERVIEW OF WHAT TO DO WHEN AN EMERGENCY ARISES:
1. Remain CALM…
2. Yell out for a First Aider (don’t go looking for one yourself, get someone else to go looking)
3. First Aiders: Read MSDS before treating any chemical injury
4. First Aiders: Call Med Centre if necessary ext. 53175
5. First Aiders: Call the Safety Officer and/or Safety Representative as soon as possible
SPOTLIGHT ON: Andrey Shubin

Background

After completing both a Bachelors and Masters Degree in Chemical Technology and Biotechnology at Moscow State University of Fine Chemical Technology, I was enrolled in a PhD course in Biotechnology at the Institute of Molecular Genetics of Russian Academy of Sciences (IMG RAS). From the beginning of my research activity I have been working at the Laboratory of Protein Engineering (IMG RAS) under the supervision of Dr. Ilya Demidyuk. The main objectives of investigation in our laboratory are proteolytic enzymes; with a core focus on their maturation and activation pathways, mechanisms of substrate recognition and functioning, as well as possible applications of proteases in medicine and industry.

My current PhD project considers proteases as potential therapeutic agents with the capability to induce death of cancer cells. In the course of the project we have found that one of the tested enzymes - 3C protease of human hepatitis A virus - has a prominent cytotoxic effect, accompanied by massive cytoplasmic vacuolation. Since the vacuolation might result from the impairment of autophagy pathway and could reflect a natural role of the protease during infection, I was interested in uncovering a deeper characterisation of this effect. Luckily, due to the Australia Awards Fellowship Program, I have an excellent opportunity to carry this out at the Yeast Laboratory here at Monash University under the supervision of Professor Rod Devenish.

Projects

All the research projects I participate in are related to different aspects of proteolytic enzyme functioning. One of the investigations considers the information value of expression profiling of proprotein convertases (PC) and matrix metalloprotease (MMP) genes in malignant tissues of human lungs. The key function of PC is processing and/or activation of numerous proteins and peptides, many of which are associated with malignant diseases. MMPs are substrates of PCs and key factors in tumor invasion and metastasis. Systems of PCs and MMPs respond to malignant transformation, which suggests their expression status can be utilised as a possible marker for cancer typing and prognosis. The most interesting result of the project obtained so far is evidence that expression of PCs and MMPs genes in human lung tumors changes in a limited number of scenarios. These scenarios are characterised by a sharp increase in the expression level of a single PC without significant changes in expression of other proteases. We believe this result reflects the different pathways of tumor development and hope to confirm this finding in other types of cancer.

Our laboratory is also focused on the investigation of the roles of propeptides in protease functioning. Most proteases are synthesised as propeptide-containing precursors. These structural elements can determine folding of the cognate protein, function as an inhibitor/activator peptides, mediate enzyme sorting, and the interaction of a protease with other molecules and supramolecular structures. Even minor changes in propeptide structure can crucially alter protein function in the living organism. Modulatory activity coupled with high variation allows one to consider propeptides as specific evolutionary modules that can transform biological properties of proteases without significant changes in the highly conserved catalytic domains. As the considered properties of propeptides are not unique to proteases, propeptide-mediated evolution seems to be a universal biological mechanism.

For our investigations, we use the thermolysin-like M4 protease family as an experimental model. We have revealed that according to structure of their propeptides, M4 proteases are divided into two distinct groups, even though the mature enzymes have largely similar sequences. Proteases of the first group have relatively long propeptides, are known to act as intramolecular chaperones and inhibitors of cognate mature proteins. Proteins of the second group have short propeptides and resemble protealysin from Serratia proteamaculans. We have obtained a crystal structure of protealysin (which was the first example of thermolysin-like peptidase precursor crystal structure) and revealed an inhibitor function of its short propeptide. However, the existence of two different groups of propeptides makes one think that short propeptides have other specific functions which are still to be clarified.

In spite of our focus in the fields mentioned above, we are always interested in collaborations and new projects related to different aspects of protease functioning and keen to broaden our research horizons.
**RECENT PUBLICATIONS**


