Centre for Gene Research

September 1999

From your Director

Since the last Newsletter the main focus of activity has been convening and running the Queenstown Molecular Biology Meeting (Aug 13 - 20th). After inviting a panel of speakers from overseas we faced the problem of raising enough money since we did not have our usual sponsors. It was the generous support of the various branches of the Cancer Society that bailed us out. Again I would like to thank the 'A' team consisting of Antony Braithwaite, Paul (Anthony) Hessian, Andy Mercer, Allan Crawford and Anthony Reeve for all the hard work they put into organising the Meeting. Also the other people working behind the scenes were Bronwyn Carlisle (who hassled the Trades people and put together the Abstract book) and Kevin Farnden (who kept us on the straight and narrow with his bottomline budgets).There were about 200 participants at the Meeting which included 44 student registrants, Trades Display people and invited speakers. We had great weather during the week and the overseas visitors were impressed by the "breathtaking" beauty of Queenstown and the quality of the science presented. I believe that having a focused theme: "Control of Cell Life and Death" was successful. There was some convergence from all those apoptotic pathways and after hearing several different versions some of it stuck. Pictorial highlights of the Meeting will be posted on the CGR website shortly, check the website. Next year's Meeting is being organised by the Auckland crowd with Jo Putterill as convenor.

We have had some changes to the membership of the CGR Committee. It seems to have become a tradition (divine-rite-of-rule?) that as members of the Committee resign they suggest someone who could take their place.Allan Crawford and Warren Tate have suggested Teresa Wilson and Chris Brown respectively. Tony Reeve has been invited to join the Committee by his involvement in the new cDNA microarray

facility. Both Allan and Warren were given a vote of thanks for their contributions to the Committee and were presented with a CGR T-shirt!!

In terms of coming events, there will be some focus given to the Model 7700 quantitative PCR, a facility currently under utilised. There will be seminar in early October highlighting some of the recent developments on primers and probes and in February a hands-on bring-your-own sample wet lab workshop. There are several scientific meetings being held in Dunedin in the next few months and its was decided to postpone the Annual CGR Poster Night usually held in November till February and hold it in conjunction with the PE Biosystems workshop on the Model 7700.

Just a reminder, if you did not receive your own personal copy of this Newsletter, join the CGR by the on-line registration option on our website.

James Kalmakoff

Queenstown Molecular Biology Meeting

In mid August another successful Queenstown Molecular Biology meeting was held. The focus this year on cell death seemed to scare a number of molecular biologists away, but numbers were still high with the cell biologists making a good show. A good range of speakers was arranged, and between them and the posters presented, most areas of cell death were covered.

The meeting kicked off on Sunday evening with the keynote talk given by Joseph Sambrook of breast cancer fame. After gaining everybody's attention by describing how his teenage sweetheart's blue knickers lured him into science, he described his intensive efforts to not only understand the biology behind breast cancer, but also personal and social effects concerning breast and such radical surgery as mastectomies.

A number of the presentations centred on the core mechanisms of apoptosis. In particular Don Newmeyer from the USA demonstrated that a lot of useful information can still be gained from cell free systems, and Andreas Strasser gave a very thorough talk on the work they have been doing on their newly discovered pro-apoptotic protein Bim. Mark Hampton also described some interesting work on the redox regulation of cell death, and caspase activation. The pure biochemists were not forgotten with Catherine Day presenting some nice work on the solution

structure and mutagenesis of the CARD domain of Apaf-1, which unfortunately came with an unhappy ending as the complete crystal structure of Apaf-1 was recently published.

The bulk of the talks however centred on cell death in disease. Cancer was discussed by many including Bryan Williams (interferon regulated apoptotic pathways) and Paul Meltzer (amplification of nuclear receptor coactivator genes in breast cancer), who both also described the use, and enormous amount of data that can be gained from micro-array systems. I am sure Tony Reeve was listening keenly! p53 also came up many times and as Wolfgang Deppert pointed out, despite intense study, we still know very little about what determines a cell's fate in response to p53 activation, although he did show that the kinase inhibitor p21warf1 probably plays a role.

Two very interesting talks were given by immunologists on a facet of cell death I personally had never really considered before. David Tarlinton and Phil Hodgkin both discussed the very tight regulation of cell death required during lymphocyte differentiation, and also the removal of excess lymphocytes after an immune response, while still keeping a small population in case a secondary response is required.

Cell death in much less obvious places was also discussed, including bacteria, and plants. One of the more interesting was by ex-Otago graduate Murray Grant, who showed that some disease resistant genes in plants show homology to mammalian apoptosis genes, suggesting a similar pathway may exist in plants.

A very enjoyable and pleasant conference dinner was held on the Thursday night, however most people still managed to make it in for the last sessions on Friday morning, amongst many jokes on ethanol induced cell death. Friday morning kicked off with two very good talks on cell death in neurobiology by Mike Dragunow, and adult motoneurones by Ian McLennan. Michael Meyer from the USA Tripler Army Medical Centre also talked about cell death in response to neuromuscular blocking agents. His abstract came complete with US Government, Army, and Department of Defense disclaimer.

All in all I believe it was a very useful and successful conference, and once again the organisers should be congratulated on a fine effort. The programme of the Meeting is available as a Adobe Acrobat pdf file.

Jared Scarlett

Real-time PCR News

All is running smoothly. The SYBR green technology has now successfully been used giving good results. Probe prices have dropped, it is now only $532 for 40nm scale (500 reactions). There have been several groups expressing an interest in using the machine over the last few months. Many of the groups have had slightly different requirements and have received help from the technicians in Melbourne; their 0800 number is well utilised. The machine is currently under- utilised so groups from outside Otago are welcome to take advantage of the technology. The SYBR green technology was first used by a group from the North Island.

It is hoped that Brant Bassam (the PE Biosystems product manager) will visit Dunedin early in October. He will be able to update us on the latest technology used by the ABI 7700 and would be able to answer those difficult questions that I have not been able to. Notification of his visit will be published when it is finalised.

Please contact me for any information on the ABI 7700. Look forward to hearing from you.

Lynn Slobbe

Real-time PCR: The Revolution is well Underway!

Principles of real-time PCR, Chemistry options, Assay design principles, Qualitative (+/-) and Quantitative detection, Genotyping (SNP's), Miniaturisation and biochips, DNA dissociation (melting curve) analysis and Automated DNA preparation and reaction setup. To be presented by Dr Brant Bassam, Product Manager - PE Biosystems, Australia.

"SNP typing for Parasite Resistance in sheep using the 7700 Sequence Detector" to be presented by Korena Paterson - AgResearch.

"Quantitative RT-PCR for Cervine Cytokine expression using the 7700 Sequence Detector" to be presented by Lynn Slobbe - Department of Microbiology, Otago University.

Where: 4th floor Seminar Room, Department of Microbiology, Otago University When: Wednesday 6th October, 1999 Time: 3pm.

Refreshments will be provided.

Marsden Fund

The following members of the CGR were successful in the latest round of Marsden Fund grants:

CONGRATULATIONS to our researchers who have been successful in the 1999 Funding Round for Marsden Fund grants. All grants but one are over 3 years.

Professor Warren Tate and Dr Liz Poole; Biochemistry; $795,000 The mammalian translational termination signal: a sequence element involved in gene regulation? The solution of the genetic code for protein synthesis provided an explanation of how information in DNA is interpreted to provide specific proteins. A critical feature was the definition of very specific 'start' and 'stop' signals for the synthesis process. Subsequently, there have been indications that the 'stop' signal is much more complex. We will define the signal for humans and other mammals, and investigate hints that a larger 'stop' signal is important for regulating amounts of specific mammalian proteins.

Professor Anthony Braithwaite and Dr Merilyn Hibma; Pathology and Microbilogy; $570,000 A co-factor requirement for p53 mediated apoptosis.

This proposal aims to test the hypothesis that the p53 tumour suppressor needs to bind a co-factor in order to be competent to cause cell death. This hypothesis derives from a number of observations with adenoviruses which suggest that p53 dependent death induced by the virus requires binding of a viral protein (Elb55k) to p53. The experiments described herein aim to explore this (new) paradigm of p53 mediated cell death with adenoviruses and to extend it to other p53 binding proteins (E6 from human papilloma viruses and the cellular mdm 2 protein) that function similarly to Elb55k.

Professor Christine Winterbourn; Pathology, CSM; $540,000
Oxidant targets in cell signalling.
External signals regulate many functions of cells, including growth and

death, by initiating a cascade of molecular responses. Reactive oxygen species such as hydrogen peroxide can initiate some of these responses. They may also transmit the effects of other molecules that bind to cell surface receptors. it is not known how these oxidants act. We aim to determine whether hydrogen peroxide selectively oxidises specific thiol proteins to change their properties in a way that promulgates the signalling cascade. Our objective is to identify such proteins and probe the molecular mechanism of this fundamental cell process.

Dr Brian Monk; Oral Sciences & Orthodontics; $300,000 (over 2 years)
Breaking the mould through structural resolution of a prototypic P-type ATPase.

Extensive work on the genetic manipulation, function, isolation and crystallisation of the yeast plasma membrane proton pump positions us to tackle the 3-dimensional structure of this prototypic P-type ATPase. High-resolution X-ray crystallographic models of this enzyme will illuminate how the membrane ATPase functions. The analysis of fungal ATPase-inhibitor complexes will pioneer the application of structure-directed design for surface-active inhibitors of individual P- type ATPases. This, in turn, will provide new mechanistic classes of medicines and agrichemicals, including fungicides, and circumvent the intracellular detoxification and multidrug resistance mechanisms that are curtailing the "age of antimicrobials".

Dr Richard McKnight; Biochemistry; $360,000

Isolation of novel flowering time genes using transposon launching pads.
Little is known about how the time at which a plant flowers is controlled. In recent years, substantial progress has been made in our understanding of flowering through the characterisation of plant genes that when mutated cause a change in the time the plant flowers. In this project we employ a new method (using plant transposable elements) to isolate and characterise two novel flowering- time genes. We will determine the function of these genes using genetic and molecular biological techniques. Understanding how the genes work to control when a plant flowers will provide a significant advance in our understanding of flowering and plant development.

Dr Geoff Tompkins and Associate Professor J Tagg; Oral Sciences & Orthodontics and Microbiology; $384,000

Bacteriocin-facilitated gene acquisition.

Dr Michael Roy and Professor P Mladenov; Zoology; $440,000
Using unique New Zealand fauna to examine the evolution of animal development.

Starfish are excellent models for studying body plan evolution amongst animals. We will document the role of body patterning genes amongst a diversity of starfish families and construct a robust starfish phylogeny by combining a battery of datasets using novel computational methods. This project aims to elucidate: 1) if changes in the expression of body patterning genes has provided the mechanism for the evolution of new body plans and life history strategies in starfish and 2) the order and tempo of evolutionary events relating to changes in body plan and whether the development of basic body plans remained labile late in starfish evolution.

Associate Professor Clive Ronson; Microbiology; $414,000
Evolution of a Microbial Genome.
With our discovery of the symbiosis island of Mesorhizobium loti, we demonstrated a novel mechanism through which bacteria grow their genomes and adapt to environmental niches. The symbiosis island is neither phage nor plasmid but represents a novel class of acquired genetic element that integrates into the chromosome at a tRNA gene. In this proposal, we examine the hypothesis that such "fitness islands" are widespread and play a more significant role in bacterial evolution than currently recognised. We will determine the extent of acquired DNA adjacent to a tRNA locus in members of a population of soil bacteria, and characterise the acquired genetic elements. The outcome will be fundamental new insight into mechanisms of bacterial adaptation and evolution, and the nature of the genetic elements involved.

Dr Hamish Spencer; Zoology; $415,000

Genetic models of the evolution of genomic imprinting.

Otago has won $8,224,000 in this round (Auckland $5,175,000) and this represents 28.8% of the total pool of $28,574,000. Well done and congratulations again to all!

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