

Centre for Gene Research

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From your Director

A Research Institute for Biomedical Sciences? This is a topic which is exercising the minds of several research groups and the following are some observations from the meeting held November 24th, entitled: Getting the Environment for Biomedical Research Right. The main speakers from outside the University were: Dick Bellamy (SBS, Auckland), John Mattick (IMB, Brisbane), Jim Watson (Genesis R&D Corp). There were three models presented. One of them is to go private like Jim Watson of Genesis said: "put your gonads on the line". Genesis was probably unique in that the Vice-Chancellor of Auckland University allowed the movement of Jim Watson's HRC funding, equipment, and his group to move outside the University to form Genesis. In a sense they were up and running without a lengthy start-up period. Having a functional group carrying out high quality research helped to attract investors and made the offers of stock options to its members a possibility. Jim claims the book value of Genesis is \$200 M with \$63 M in the bank.

John Mattick's development in Brisbane was interesting in that from a modest sum of \$400,000 which was 'top-sliced' from departments he was able to turn that into a large research centre with funding of about \$110 M. This required the dedication by John for about 12 years -- he said that he had to sometimes work till dawn to achieve this. He was able to attract good people to the Centre. To put it into context, there was a growth spurt in Queensland. Growth is able to fund innovation and new developments. He was also able to make good use of the rivalry between the Queensland and Victoria (Walter Eliza Hall group)



state governments for research funding -- each vying to out do the other. The results of this ping pong activity was to raise the research ante to \$110 M.

The third model presented was by Dick Bellamy, School of Biological Sciences at Auckland University.

He took a group of departments working in the biological sciences area, put up a barb wire fence around them and said: you can not get out unless you agree to work together and disassemble the feudalistic departmental structures. In the process Dick was able to release some resources which were previously duplicated and capture the benefits of operations of scale. It is interesting to note that he advocated the most cost effective way of teaching was to put a person in front of 1000 students and deliver the lecture.

In all of this there was a take home message -- recruiting good people was the key to success -- recruit good, successful people, they attract other good people. Give them adequate resources and they will generate research funds. More good people will come and they will establish networks and collaborations. Soon you have a thriving research centre which has the critical mass and can spin off commercial companies.

Otago has some unique problems; the University is an old institution and there are many feudal structures which are not easily breached. Some departments are still able to pull up the drawbridge and man the battlements. It will take someone with a great deal of dedication and energy and mania to overcome these barriers.

The other message I got was that there was venture capital money out there. The problem is that the venture capitalists know that there is no point in parttime funding an of academic. They know that academics are overworked -- academics try to be supermen (sic super persons). Before venture capitalists will put their hands into their pockets, you (as an academic) will need to step outside the academic institution. There is the option as offered by Ian Smith, of taking two years off, without pay, to move to the Centre for Innovation and give your venture a go. It will be interesting to see what the uptake of that option will be and how some of the biotech ventures like BLIS Technologies will fare in the Centre.

To update you on some of behind the scenes activities of the CGR, the following are notes from a recent CGR Committee meeting:

Updating of DNA sequencer -- There was discussion about the replacement of the model 377 DNA sequencer. The current 377 model is no longer being manufactured and although it has about 2-3 years of usable life left, we will have to face replacement. A model which is being promoted to replace the Model 337 is the 16-capillary sequencer, the Model ABI 3100. It can run up to 96 samples in a 24-hour period. A similar discussion was made about the Real-Time Model 7700 PCR machine which is currently heavily utilised. We do not charge depreciation on the use of the equipment. There are service contracts of \$4,000 and \$16,000 for the PCR machine and sequencer respectively. These costs are paid for from the CGR operating costs.

Redesign of the CGR Website -- The University's website is currently being redesigned and there will be an obligation to have the CGR website conform to the new style guidelines and 'branding'. Apart from the changes in the look of the website, I would appreciate any comments on what features the new website should incorporate. The CGR website receives about 1,000 'hits' (files served) a day and the Real-Time PCR html pages receive most of them -- obviously keywords on the search engines. There is usually some follow-on browsing of the rest of the site by the overseas visitors. The current CGR database contains 312 members and this gets some traffic as well.

CGR Activities for 2001 -- post-Theme era? There was some soul-searching discussion about what we can contribute and a reaffirming of our grass-roots origins. The themes are a fact of life and where possible we can work in with them and provide the infrastructure for member-initiated projects. We will continue to run workshops and poster nights --- with a focus on bioinformatics and microarrays in the coming year?

Finally this being the last Newsletter of the year, I would like to wish you all the best for the holiday season and may it be festive and merry one. Make a point of actually taking a break, away from your lab and academic pursuits and from your emails. Soak in some of that leisure time (I was going to say sun, but that as we know is now a no-no) and come back next year, refreshed and ready to do battle with the challenges in the New Year.

James Kalmakoff

Functional Genomics, Gene Expression & Proteomics

The first meeting of the Functional Genomics, Gene Expression & Proteomics research theme was held in the Biochemistry Department this week. The meeting opened with an excellent overview of the present state of play in Proteomics from Stuart Cordwell from the Australian Proteomics Analysis Foundation (APAF) in Sydney. This facility has some of the world's best facilities for doing proteomics work. Stuart described what is and isn't possible, outlining each of the limitations of 2D analysis and how they try to get around them. Stuart's own work has been mainly on bacteria and he explained that it has been possible to identify 20% of the total proteome of a bacteria on one gel.

"The essential thing is that the question you ask has to be good enough for you to be able to make sense of the answer", he said. At APAF their initial approach is to make theoretical arrays from the genome information before they start and then to overlap the found data with these. Also, a cell fractionation approach is seen as beneficial in helping to identify protein function.

After that they employ a three level analysis, increasingly narrowing the pH gradients, until on the third pass they span about one pH unit. In reality 32% of proteins are outside the scope of 2D analysis, either because they are too basic (15%), too hydrophobic (14%) or too low in abundance (3%). How do they overcome this? The narrow Ph gradient 2D analysis and pre-fractionation allows a greater number of low abundance proteins to be seen as more can be loaded. Basicity is a big problem. Work shows that even on a 11-12 range gel everything above a PI of 9.77 is washed out of the gel. They are working on new buffering reagents and new equipment to try and overcome this.

Other speakers at the meeting discussed the Yeast two hybrid system and x-ray crystallography and their usefulness as high throughput protein analysis tools.

Michele Coleman

If DNA is Life, What About the Details?

The last year has seen the official announcement of the completion (although a little premature) of the human genome, as well as the release of further prokaryote genomes. The number of completed

genomes is now quite impressive and new genome projects are announced every day. At the same time, a second sequel to the film Jurassic Park (titled, with some originality, Jurassic Park 3) is being made in Hollywood starring Sam Neil among others. I never saw anything except fragments of Jurassic Park (and I've never seen the Simpson's either) but I gather the story involves the recreation of a number of dinosaur species from DNA recovered from the intestinal tract of Jurassic mosquitoes preserved in amber. Quite apart from the question of whether Jurassic mosquitoes existed and whether they bit dinosaurs, it seems unlikely that fragmentary DNA could be used to recreate a whole living creature. But what about whole genomes? Could we recreate a creature by synthesizing its genome and putting it into a suitable cellular container? Can we create virus or a bacterium by simply knowing its genome? What about a yeast, a lily or a hippopotamus?

Although this is rather a trivial question at first sight, it hides a much more interesting question. Is the genome of an organism sufficient to completely and explicitly describe that organism? If we were synthesize a piece of DNA corresponding to a simple virus, would this virus be viable? What other information would be required to make this simple organism animate? If it is possible to make a live virus, what extra things would be necessary to make a bacterium viable: a cell membrane, some ribosomes, a complete set of cytosolic enzymes?

It seems pretty clear that more than just the genome would be necessary to make something even as simple as a bacterium. Somehow it would be necessary to provide a minimal set of cellular machinery to interact with the DNA so as to interpret the instructions and to build the appropriate structures they encode. If this is true, then it seems pretty clear that an organism is not just a construct of its DNA but that a mechanism for translating that genetic information is essential as well. All of this is neither terribly surprising nor original but it does focus attention on the old questions of where and how did life originate.

Before Darwin it was widely considered that there must be underlying physical laws and principles which accounted for both the similarity and diversity of living things. The discovery of the laws of heredity and of genes diverted attention away from these kinds of studies for it seemed clear that essential information about an organism was transmitted from generation to generation and that no underlying physical laws were necessary to explain the way things were. Although this view is true as far as it goes, it overlooks the fact that underlying physical laws are essential to the expression of the information in the genome. In fact,

many of the really interesting questions are all about how the instructions in the genome are translated into structures within organisms. Careful consideration of the underlying physical laws is likely to greatly assist in understanding how genetic information is translated into a living thing.

Craig Marshall

Real-time PCR News

Due to the success of the first workshop on real time technology in February this year a second workshop was held recently in the Microbiology department. Already a waiting list has started for third. The open seminars updated us all on the recent and future developments in real time technology, there are many exciting advances due out in the new year. The participants in the "hands-on" workshop carried successful amplifications of their own targets. Thus the numbers of people using the machine has greatly increased and booking is essential, its now time to plan well ahead to ensure a space on the machine. The diversity of uses of the Taqman technology again was evident in the workshop.

Currently it is used for Allelic determination, quantitative PCR/RT-PCR and now quantitation of bacteria from biological samples. I have used the ABI Real Time PCR machine for quantitative detection of Bifidobacteria in pure cultures and in faecal samples. The method has been rapid, sensitive and highly correlated with standard plate counts of the same samples. These comments are from Teresa Requena who attended the workshop.

The software for SYBR green analysis (melting temperatures) has at long last been made available. The Cancer group in Biochemistry has tested this system and if you have any questions regarding this technology please contact Tumi Toro ext. 7868. As many of you will know the Primer design software is now located on the CGR server, at the PCR 7700 site. This has allowed easy access for any one wishing to design primers/probes. Unfortunately with the weak NZ dollar the prices have had to increase for reagents. I have received the latest price list. We still have our discount & a special offer to people setting up for the first time.

Please feel free to contact me for any information on the ABI 7700.

Lynn Slobbe

Conference Report

The Vascular Research Group was represented by **Dr. Eugenie L. Harris** at two recent conferences: GEHH2000, the Genetics of Experimental and Human Hypertension symposium held in Toledo, Ohio August 17-18. ISH2000, the International Society of Hypertension conference in Chicago August 21-24.

In a talk at the GEHH symposium, Jean Harris reported the results of a collaboration with Ross Barnard of the University of Queensland to determine whether the New Zealand genetically hypertensive (GH) rat had a putative A1079T transversion in the $\alpha 1$ isoform of the Na⁺, K⁺-ATPase gene (*Atp1a1*). This gene lies within hypertension QTLs in a number of crosses between hypertensive and normotensive rat strains. A paper published in *Science* in 1990 claimed that a point mutation in the *Atp1a1* gene found in the inbred salt-sensitive strain (SS/Jr) would result in a Q276L substitution in the

ATPase protein. Such a substitution was posited to alter the membrane conformation of the Na⁺, K⁺-ATPase, resulting in a change in the Na⁺:K⁺-pumping ratio and a concordant increase in Na⁺ reabsorption in the kidney. The existence of the transversion was challenged, but the challenge was apparently rebutted.

Atp1a1 lies with a hypertension QTL found in a cross between the GH and Brown Norway (BN) strains. Therefore we sought to determine whether the GH rat carried the controversial A1079T transversion. Since the controversy over the transversion centered on the possibility of PCR bias, we chose a method, first nucleotide change analysis, that can detect point mutations in a mixed population of PCR products, even in the presence of PCR bias. We confirmed our analysis using direct sequencing and restriction enzyme digestion of PCR products. To assure the validity of our analyses, we used site-directed mutagenesis to create positive controls containing the mutation. Surprisingly, we found that, not only does the GH rat not have the A1079T transversion, the SS/Jr strain doesn't either. Indeed, the transversion was not found in any strain tested. As an incidental observation, we sequenced the intron preceding the exon containing the putative A1079T transversion. Within

this intron, a single base, C/T polymorphism was observed at base 132. Our results definitively eliminated the putative A1079T transversion in Atp1a1 as a causative factor underlying hypertension in the GH, SHR and SS/Jr strains and indicated alternative candidate genes within the region defined by the chromosome 2 hypertension QTLs should be examined.

The talk was of great interest and was very well received since the Atp1a1 transversion is so well known in hypertension circles and had many important implications. One delegate was overheard remarking, "Imagine. They got 10 years of NIH funding for that!" We have recently submitted a paper on our findings to Hypertension.

A poster on the mapping of a gene causing breaks in the internal elastic lamina (IEL) of the abdominal aorta of the BN strain was presented at ISH2000. The IEL lesion gene is independent of hypertension, though at least one gene from the GH rat greatly increases lesion severity in F1 and F2 rats from the BN X GH cross made to map the lesion gene. Since it is more within the realm of vascular research, this work was not of such great interest to hypertension researchers as the ATPase results. However, the conference allowed Dr. Harris to talk with all the relevant people, resolve some issues concerning a paper on the lesion gene and set the stage for some future collaborations. Submission of a paper on the lesion gene is awaiting the signatures of our collaborators at the Medical College of Wisconsin on the copyright transfer agreement. Meantime, anybody wishing further information on the mapping of the lesion gene is welcome to consult [Dr. Harris](#).

The conference venues were excellent. Though the huge Chicago conference was a bit disorganized and didn't allow electronic slide presentations, the Navy Pier setting was outstanding. The Toledo Art Museum made a wonderful venue for the GEHH banquet. Likewise, the Field museum with Sue, the famous Tyrannosaurus rex exhibit, was an imaginative setting for the ISH2000 banquet.

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