University of Otago
Te Whare Wananga o Otago

Centre for Gene Research
NEWSLETTER

Volume 2 Number 1 March 1995

A note from the Director

I am very pleased to report that we now have a new Editor for the Newsletter–Craig Marshall. Craig will be endeavouring to get out three or four more Newsletters this year. Please send any news you have to him in Biochemistry either directly, via his drop box on his computer (login as a guest) or via email to craigm@sanger.otago.ac.nz. The newsletter is our primary form of communication–please make the most of it.

There have been a number of changes on the sequencer front. Firstly in terms of staff, we have been pleased to welcome Vicky Morrison back as a 0.5 time appointment. You will recall the Vicky started last September for a four-month term. We were sorry to farewell Deirdre Dobson last month. Deirdre had set up the sequencer service for us. She has now left us to go to Vietnam to teach English as a second language. Deirdre's place will be taken by Tracee Masson-Lawrence who will start full-time from 20 March. We are very pleased to be able to appoint Tracee to this position. She had earlier worked for us over one summer and we expect to be able to continue with the same high standard of service we have had in the past. Just keep those samples rolling in!

We have recently submitted an application to Lottery Health for funds for a stretch upgrade of the sequencer plus a new thermal cycler. If funded both these items should enhance the level of service we get from the facility.

In February I was required to submit our annual report to the university. It is not my intention to include copies for all our members, but anyone who is interested to read it may uplift it from the Public Out folder on my computer. This folder also contains the list of publications from members of the Centre. This was included with the annual report and ran to over 12 pages. Please feel free to uplift this file also.

Activities for 1995

The Committee is suggesting the following activities for this year.

- GCG computing course (See separate notice)
- Half or Full-day Symposium on topic yet to be settled.-watch out for more information about this.
- Young Scientist Visiting Lecture followed by Centre's Poster Night–probably November. Again watch out for more information in future Newsletters.

If there is anything else you would like to see organized this year, please contact me in the next month or so.

Murray Grigor

****

Editorial

In a moment of weakness I volunteered to edit the Centre for Gene Research Newsletter and Murray took me at my word. We hope to get out at least four issues a year, possibly more, and are always looking for contributions. If you have any articles, reports, advertisements, tips, suggestions, letters to the editor, pet peeves or anything at all, I would be very glad to hear from you. However, to help speed up producing the newsletter and to assist my typing, I would very much appreciate receiving electronic copy as well as the printed version. The printed version helps me to understand how you wanted it laid out; the electronic makes it much easier to bring in the text. There are a number of formats which you can use to supply electronic copy:

- by email to craigm@sanger.otago.ac.nz
- to my dropbox on CraigMarshall in the Biochemistry zone
- by sending me a disk, any format (almost) is acceptable

I look forward to your submissions

Craig Marshall

****

Meeting reports: The Centre has been represented at two of the recent Lorne Conferences

Mike Eccles reports:

The Lorne Cancer Conference was held from February 9-12 1995 at Lorne, Victoria, Australia. This meeting proved to be as exciting and as popular as always with an enticingly dazzling selection of international speakers. The first day kicked off to a fine start with talks from Greg Winter (Cambridge University) on synthetic human antibodies, and then David Goldgar (University of Utah), Mary Claire-King (University of California) and Ellen Solomon

(ICRF) on the cloning and molecular genetic analysis of the breast cancer gene BRCA1. It was interesting to hear about the politics of the upcoming decisions to set up breast cancer screening programmes. The next day we heard a very informative series of talks from Tyler Jacks (MIT), Moshe Oren (The Weizmann Institute of Science), Carol Prives (Columbia University) and Vida Rotter (The Weizmann Institute) on the role of p53 in the cell cycle and apoptosis. This theme was repeated again later in the conference by other speakers such as David Vaux (Walter and Eliza Hall Institute) and Roseanne Hansen, a Ph.D. student working with Anthony Braithwaite. Other talks of note were by Alan Bradley (Baylor College of Medicine) on analyzing tumour suppressor genes in knockout mice, Eric Stanbridge (University of California) on QM, a novel tumour suppressor gene, Joe Sambrook (University of Texas Southwestern Medical School) on genetic instability in ovarian cancer, and Rick Kefford (University of Sydney, Westmead) on the molecular genetics of melanoma. Rick's team has recently found that the familial melanoma gene on chromosome 9 is p16. This finding will be of significance because melanoma is epidemic in both Australia and New Zealand, and is increasing in incidence at a rate faster than any other cancer.

Overall the conference was a great success. Anybody wishing to look at the abstract book is welcome to borrow it from me.

Mike Hubbard reports:

Allowed out of my cage briefly in February, I bolted for sunny Australia. Straight up was the 20th Annual Conference on Protein Structure and Function at Lorne, on the coast near Melbourne. The chasers at Sydney were a Finnigan Mass Spectrometry seminar and a couple of days at Prof. Mark Duncan's Biomedical Mass Spectrometry Unit (Uni NSW) where I received my official training on the Finnigan *Lasermat* instrument, that is the MALDI-TOF-MS we now have here in Dunedin.

Such was the preponderance of mass spectrometry dialogue at Lorne that several people quipped it should be renamed the 'Lorne Biomolecular Mass Spectrometry Conference'. Anyway, it was a great environment for me to learn more about mass spectrometry amongst other things.

Pertinent items of general interest to molecular biologists? I can think of three, all related. First, the databases are now considered to be grossly full of errors - the figure of 70% was mentioned for DNA sequences (yes, 70% of published DNA sequences contain errors) and that for directly determined protein sequences perhaps higher. The mistakes are becoming obvious not only after resequencing by other groups, but also from mass spectrometry, NMR and crystal structures. Second, awareness of the importance, and prevalence, of protein post-translational modifications is growing (remember the flyer from NEB recently which drew attention to this 'new' area of molecular biology - new to gene jocks maybe!!!). In fact, Prof. K Walsh (Seattle) challenged anyone to name a protein known not to be post-translationally modified. Aside from removal of N-terminal Met and postmortem proteolysis, nobody could. Mass spectrometry, with its ability to reveal a broad gamut of modifications, is again a principal player in this field. Third, the (read: my naive) notion that tertiary structure is always a reflection of the primary structure was blown clean out of the nearby emerald-blue ocean. Two groups showed examples of pairs of proteins exhibiting essentially identical tertiary folds but with no (<10%) primary structural identity. Expanding this theme, C. Chothia (MRC LMB, Cambridge) dwelled on the implications for the genome projects which appear to have derivation of primary structures as their goal. His claim that these already huge problems won't be properly solved until the tertiary structures and post-translational modifications are known for every gene product is perhaps excessive. However, the three aforementioned considerations certainly do seem to shift the goal posts back a fair bit.

Craig Marshall reports:

In January the National Science Foundation made it possible for me to spend a month in the Antarctic. The Antarctic Biology Course I attended was designed to allow a group of sixteen (eight from within the US and eight from elsewhere, eight men and eight women, but I am sure these numbers were arrived at coincidentally) to learn something about working in the Antarctic and to become familiar with aspects of the biology in this unique environment. The people on the course were from Brazil, Canada, Germany, India, Korea, New Zealand, Sweden, Switzerland, and ranged from 1st year PhD students to faculty members. In fact the term 'student' caused problems for some of the more senior attendees.

The sixteen of us plus course tutors and support staff (for this was an American expedition) gathered in Christchurch on January 2. I arrived feeling rested and relaxed and flying North but many of the others had been flying for several days and so were looking forward to a little rest. This they were due to get as we stayed in Christchurch for four days until a plane became available. In the meantime we were kitted out in our ECW (extreme cold weather, acronyms abounded) clothing and prepared for the flight. When we finally did leave, it was 30° and wearing our ECW gear was a little trying.

The flight to McMurdo takes about eight hours in a Hercules that resonates like a drum. The most comfortable place is on the cargo provided that you can find a bit without corners. There is a 'honey bucket' for relief and a brown bag containing lunch. There are no cabin crew, but the Navy loadmasters do what they can to make things less uncomfortable.

Landing at Williams' Field is very smooth as you ski to a halt (slowly) on the snow runway. William's Field is named after an unfortunate bulldozer driver who fell though a crack in the ice and died, whilst driving across the sea-ice during the construction of the landing strip. Many other features are similarly named.

A twenty minute ride across the Ross Ice Shelf takes you to McMurdo Base, passing by Scott Base on the way. McMurdo is very bare as it is built on black, gritty volcanic soil which shows the dirt. Living at McMurdo requires that you take a number of courses on how to be a good citizen. Waste is recycled with a vengeance; there are seventeen categories to consider, and there are a number of safety aspects with which to be familiar. For all that, this was part of the arrangements that left a lot to be desired as the information content of the hour long lectures was about ten minutes and those giving the talks were less than gifted communicators.

Our rooms were spartan and curiously enough over-heated to the extent that we all slept with windows open. My room-mate snored (and of course I did not), but that was a minor problem and we all managed to get along very well. The food was very good considering that over a thousand people were catered for although much of what was on offer was not what I would have chosen. By the end of my stay I had figured out most of the items on the menu and how to order eggs or a sandwich to my taste.

The scientific facilities at McMurdo are magnificent. The Crary Laboratory was completed about three years ago and has everything you could imagine and a great deal more besides. Ultracentrifuges (2 L8-80's), five Beckman spectrophotometers, five freeze driers, power supplies and electrophoresis equipment coming out your ears. The main difficulty was finding someone to put the equipment together as much of it had either never been used or was missing some vital, small part. The lab was built mainly to serve NSF grantees who go the Antarctic to work and who need lab space other than that available at a field camp. When we were there, which was at the end of the field season, only three groups were in the labs; penguin ranchers studying emperor penguins, worm herders who were interested in nematodes, and the antifreeze people looking at antifreeze compounds in Antarctic fish.

The potential to do science in the Antarctic is enormous both in terms of the facilities available, and the interesting problems that are there for the taking. Antarctic biology, although not a virgin field, is fairly new and most of the interesting problems have not been studied. The plums are there to be picked out. At present, there is a limitation for New Zealand biologists wishing to work in the Antarctic as the facilities at Scott Base are primitive, but it is hoped that some arrangement can be made with the Americans to allow New Zealanders to use the facilities at the Crary lab.

As well as 'beaking' (what beakers do, beakers being McMurdoese for scientists; the Crary Lab is on "Beeker (sic) Street" the only named street at McMurdo) we were fortunate to visit Scott's Hut at Cape Evans. the Taylor Valley, one of the dry valleys, the ice edge where whales and penguins abounded, and to spend considerable time working on the sea ice fishing and taking temperature and depth soundings.

My overall impression? I thoroughly enjoyed my visit and plan to return to the Antarctic as often as possible, starting next November. I wanted to go particularly to look at the question of how Antarctic fish are able to sustain a near-normal metabolic rate at -2° where most enzymes are essentially inactive. In particular, what are the structural modifications to enzymes such as lactate dehydrogenase that are responsible for this adaptation and what will they tell us about the way proteins are put together. I was able to get to know a number of people working in the area of my interest, to set up two collaborations, and to collect sufficient material to begin work on

isolating and purifying LDH from an Antarctic fish.

The NSF Antarctic Biology Course is likely to run for at least the next three years and I urge that those who are interested in Antarctic biology to consider applying for the course, particularly if you are a senior PhD student.

****

New Faces

Professor Eun-chung (Agnes) Jhee

Visiting Scientist, Cancer Genetics Laboratory, Department of Biochemistry.

A warm greeting is extended to Professor Agnes Jhee, who is visiting the Cancer Genetics Laboratory for 1 year as a visiting scientist from Chonbuk National University, Chonju, Korea. Agnes is Professor of Biochemistry and Director of The Institutes of Dental Sciences at Chonbuk National University, and has a strong research interest on the prevention of aflatoxin-induced hepatocarcinogenesis. Agnes's research now leads her here, under a Korean Government Professor Training Grant. She has traveled extensively, including being a visiting scientist at the Fels Research Institute in Philadelphia. She now wishes to change the direction of her research slightly, and so has come to New Zealand to learn some new techniques. She tells us that her hobbies include, among other things, listening to classical music.

Scott Tebbutt (Postdoctoral Fellow)

Three years ago, two-thirds of his way through his PhD on pollen molecular genetics, Scott thought it might be fun to come and work in New Zealand, specifically in Diana Hill's lab in Dunedin. One of Diana's pet subjects, that of using a sheep as a large animal model to study human genetic diseases, was mooted about, and so on completion of his thesis, Scott returned to Oxford to set up a collaborative project between Otago and Ann Harris' group at the Institute of Molecular Medicine, who specialize in research into the human genetic disease Cystic Fibrosis, caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.

18 months later the ovine CFTR cDNA was cloned and sequenced, northern analysis had shown that the gene was temporally and spatially regulated in an equivalent manner to that seen in humans, and Scott was winging his way over to New Zealand to embark on the project's second phase. This involves screening large populations of sheep DNA samples, in an attempt to identify natural DNA variations within the ovine CFTR gene that may be CF disease-causing mutations. Once mutations are found, classical breeding methods will be employed to create a CF sheep, potentially a valuable resource for study of the CF disease process and therapies.

Peter Farley

Peter Farley returned to the Biochemistry Department at Otago this year after a number of years teaching at a university in Central Java, Indonesia. He writes:

"While in Indonesia I initiated work on the enzymes involved in nitrogen assimilation by the filamentous fungus *Rhizopus oryzae*, which in Indonesia is important because it is used in the food industry.

I'm interested in *Rhizopus* both because it represents a different family (the Zygomycotina) to the fungi traditionally used as model of fungal gene expression and because it could be an acceptable organisms for heterologous expression of eukaryotic genes.

For these reasons, and because the nitrogen metabolite circuit is being characterized in the traditional fungal models, I'm interested in studying the mechanisms which control expression of the enzymes involved in nitrogen assimilation in *Rhizopus*. In particular, I hope this year to go some way to unravelling the regulation of expression of the family of secreted aspartic proteinases, at least one of which is secreted under conditions of nitrogen derepression."

****

Protein Prattle

The **Protein Microchemistry Facility** is now in full swing for 1995. Since recommencing operations in February, the protein microsequencer and amino acid analyser have been revived from their annual shutdown, used to clear a backlog of samples remaining from last year, and set upon several new problems of 1995. A new style of 'service request' form has been produced, enabling multiple procedures (e.g. composition, sequence and mass analyses) to be simultaneously ordered for a single sample. An updated version of the 'General Information' sheet and an information bulletin on the MALDI-TOF mass spectrometer also are now available (call

Diana Carne, 03 479 7542). Hopefully, these alterations will prove to be beneficial for one and all. With the time taken for data processing proving to be a bottleneck, we have been fortunate to gain the assistance of Sarah Jack, a PhD student in this Department, who is helping crunch the amino acid analysis data. Additionally, some project work is now underway on the MALDI-TOF-MS. Our first priority is to analyse samples for those staff members who helped obtain the funds to purchase this instrument, but we anticipate being able to undertake mass analyses for other people later in the year.

In this installment, I would like to briefly overview the **Amino Acid Analysis (AAA) service** that is available, focusing particularly on its potential utility to molecular biologists from the gene and the gene product camps alike. Improvements instituted over the last couple of years now enable us to undertake AAA at similarly high sensitivity as the protein microsequencing - that is, in the low pmol range (<1µg of a 50kDa protein). Hence, **the principal current use of AAA is as a high sensitivity and potentially absolute method of quantitation** (the latter is dependent on purity and a known composition derived from the primary structure). We find that workers submitting samples to the Facility generally have a poor appreciation of the amount of protein present, yet this information is crucial for an efficient and accurate sequence or mass analysis - accordingly, unless well characterized by another reliable approach, we are suggesting that samples routinely be quantitated by AAA. Secondly, **AAA can be used to efficiently establish the identity of a protein.** The AA composition of most proteins is surprisingly unique. For example, overseas labs have recently shown that, given an accurately determined AA composition and additional information such as M_r and/or pI, it is possible to rapidly identify protein spots from 2D gels based on comparison with compositions derived from known structures in the databases. Although this approach only works well for knowns, it also serves to highlight likely unknowns and saves heaps of sequencing which by comparison is slow and expensive. Thirdly, **AAA is used as an adjunct to other analytical procedures such as microsequencing.** For example, an unfortunately common outcome is a much lower-than-expected level of sequence information, or even none at all - this might result from a blocked N-terminus, preventing sequencing, or insufficient sample resulting from inaccurate quantitation or poor retention on the sequencing support matrix. In the case of a low level sequence, it is important to establish whether this is coming from the intended sample or instead a contaminant. By undertaking AAA of 'failed sequence' samples, after removal from the sequencer, these possibilities often can be distinguished. **Summarizing, in one hit, a high sensitivity AAA both identifies and quantitates a protein sample, consuming only a small amount of material.** The Facility can analyse samples in a variety of formats (e.g. from solution, off blots) but there are some preparation constraints imposed by the high level of analytical sensitivity - please contact Diana or myself for further information.

Mike Hubbard,

Department of Biochemistry, Room 237, phone 479 7831, e-mail: mother@otago.ac.nz

Tips

****

Computer Corner

New Software. A new World Wide Web (WWW) browser is now available. It is called Netscape 1.0 and it is a significant improvement over NCSA Mosaic although it is related. The people who made Mosaic have split into two camps and are looking to commercial release of their products. Netscape is the first of these and it is to be made available free of charge to browsers. Apparently those wishing to use the software to create and manage WWW sites will pay. Netscape is available within the University from the CWIS server in the Central Services zone of the AppleTalk network. Login as guest and fetch the software. Arrangements to supply Netscape can be made for those who do not have access to the CWIS server.

Mailing List. A mailing list has been created to assist in passing on interesting tips and asking for help. You can subscribe to this list by sending a message to `majordomo@stonebow.otago.ac.nz` with the message `subscribe cgr-list your_name` where `your_name` should of course be your name. Help in using the server can be obtained by sending the message `help` to the address `majordomo@stonebow.otago.ac.nz`. To use the list itself, you should mail your contribution to `cgr-list@stonebow.otago.ac.nz` where it will be distributed to the rest of the list. At present, this will be a moderated list meaning that I will be responsible for checking everything distributed on the list. In the future, we may set up

a place on a WWW site to r
but that will depend on the use the list gets.

I urge you to use this list as a place to discuss technical questions, names of suppliers, arranging meetings and the like.

Database Errors. Users of such databases as Genbank and EMBL should note Mike Hubbard's comments on the accuracy of entries in these databases. If you are using data from these sources, double and triple check the information especially if it is critical for some application. Suggested checks include translating the sequence and comparing the protein sequence so derived with that expected (I know of at least two sequences where the complement of the correct sequence was deposited in Genbank). Consider also doing an electronic restriction map and comparing that with the data reported for the sequence. You should also get the paper recorded in the entry and cross-check that with the sequence of the entry itself.

Protein databases are also prone to errors but probably at a lower rate than their nucleic acid counterparts, and it is more difficult to check them, but look at the sequence carefully and do what you can.

Craig Marshall

****

CGC Workshop

GCG course: A hands-on workshop on the use of the GCG (Genetics Computer Group of the University of Wisconsin) set of programs will be held for four mornings during the first week of the mid-year break (12 June to 16 June). The venue will be the North Cal Computer Lab (Science Library building) and Macintosh computers will be used as terminals to the Department of Biochemistry's Unix system. Course coordinators are Mark Dalphin and Peter Stockwell and a comprehensive course book will be prepared. The GCG system version 8 is [illegible]
ning on the Department of Bioch
puter and is accessible to anyone on the network at Otago. Although based on the Unix operating system the course will be valuable to people who have access to GCG on Vax systems. The course charge will be $100 for staff members and $50 for bona fide students. A maximum of 20 places is anticipated and people who wish to sign up for this should contact Mark directly in the Department of Biochemistry or email him at mdalphin@sanger.otago.ac.nz. Please register your interest quickly as space will be limited

****

Advertisement

Research Fellow in Biochemistry

Applications are invited for a research fellowship in the Department of Biochemistry. The successful applicant will investigate the role of mitochondrial oxidative stress in human pathologies, a project funded by the Health Research Council of New Zealand. Applicants with a PhD in a relevant discipline and expertise in Molecular Biology are particularly encouraged.

This position will be available from September/October 1995 for an initial period of one year with the possibility of extension for one further year. Starting salary will be in the range $(NZ)37,440–$40,872 per annum depending on experience.

Further particulars are available from the Registrar, Dr DW Girvan, University of Otago, PO Box 56, Dunedin, New Zealand, fax 64-3-474 1607. Informal enquiries may also be made to Dr Michael P Murphy, Department of Biochemistry, University of Otago, fax 64-3-479 7866, email murphy@sanger.otago.ac.nz.

Applications quoting reference number A97/26 close with the registrar on June 1 1995.

Equal Opportunity in employment is University policy