University of Otago
Te Whare Wananga o Otago

Centre for Gene Research

NEWSLETTER

Volume 1 Number 4 December 1994

Did you miss the October issue of the Newsletter? Did it get put in someone else's mailbox? Did you throw it away by mistake? The answer to all questions is no. There was no October Newsletter. It had been my intention to get one out round then but I just did not make it. My apologies. Next year we will do better (see below).

It is now a year since I have been Director of the Centre and maybe it is time I gave a sort of annual report to the members of the Centre. I have to do this for the University early next year but that report will take a much different form from this. When I was asked if I would let my name go forward for the directorship last year, I was on study leave in Atlanta in the US. I must have been feeling homesick or something, but in a fit of foolishness, I agree to let my name go forward. The first Director was Tony Robinson who left about then to take up a position in Australia. Tony had set up the Centre and been responsible for raising money for the purchase of the DNA sequencer and getting it going. He had also organised the 1993 Queenstown meeting. I was not sure I could match all Tony did there, but I was keen to try and build up the Centre as a focus for those outside the Departments of Biochemistry and Microbiology and also for the graduate students involved in "gene" research in its widest sense right over the campus. Whatever, I arrived back in Dunedin round the beginning of October 1993. I had received no communication at all from the University about the position, but after some enquiries, I was told that I needed to get a budget prepared. A budget, help, that was not in the original letter sent me by my colleagues. A budget was organised and thanks to the committee for help with that. Towards the end of November and still with no communication from the University authorities about the directorship, I thought maybe I should enquire further about my position. Shortly thereafter, a one paragraph letter came from the Registry saying I was indeed appointed Director but no more information was provided about the position. I then wrote back asking for a job description. In response to this I got a photocopy of the University regulations concerning research centres where the only real requirement was to submit a report once a year. I think you can see why my report to the Registry will be a little different from my report to you! (I suspect I may get a phone call from the someone over there any day now.) By the way, there is no monetary reward for being Director.

Basically, the goal of the Centre is to promote gene research throughout the campus and to, as stated above, act as a focus for all people involved in gene research. The regulations do not require me to have a committee nor is there any requirement for me to consult with the members of the Centre. In fact, there is no real definition of what is required to be a member of the Centre and indeed no indication of how the Director relates to the members - remember all I have to do is write a report to the University. Well, we do in fact have a very effective committee, maybe appointed on a rather ad hoc basis, but nevertheless a committee that I am most grateful for the support of. Without the committees work, the running of the sequencer, our retreat, our poster night and the preparation of this newsletter would be impossible for one person to do. That sentence pretty much sums up our activities for the year. The sequencer is going extremely well. Deirdre Dobson has been joined by Vicky Morrison working part time and we are currently running between three and four gels per week. Alan Crawford keeps close eye on the operation of the sequencer. Recently we have taken out a service contract with ABI for it. Use has almost doubled over the year and we are about capacity there. We try and keep any backlog to a minimum and our apologies to those of you who had to wait for your samples round the middle of the year when we had only one person working in the sequencer facility.

Our retreat in June attracted over 60 people. The scientific sessions were based on technology and it was exciting to find the range of expertise that we have on campus and the willingness of people to share this. Last month we held a poster night. A total of 40 posters were offered. These had been displayed at meetings all over the world and again demonstrate that gene research is alive and well in Dunedin. Two prizes were given: to Boon Low and Bart Challis for the best senior and junior student posters. This is now known as our 1st Annual Poster night.

Our Newsletter has both been a lot of work but also a well supported venture. I appreciate the comments received about it and I appreciate the people like Craig Marshall, Sue Galloway, Murray Broom and Ian Ross who have kept our columns going. I appreciate particularly the people who have submitted material for the Newsletter, and once again my apologies for the delay in getting this particular one out to you. For next year we hope to get maybe 6 issues out starting in February and we are looking for a new Editor. This will be a paid position and if you have some computer skills, don't mind chasing people up for material and would like to be considered for this job, please let me know before Christmas so that we can get the process under way for the new year.

So as Director, I am pleased to report that I think the Centre is on track. Whether we match the Universities perception of where we should be going or not we will find when I submit my report to them early next year. Now, for that report I need a full listing of all papers published last year - see item on P10.

Cheers,
Murray Grigor

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NEW ACNGT GUIDELINES, FORMS AND PENDING LEGISLATION

The Advisory Committee on Novel Genetic Techniques (ACNGT) has revised its 1982 guidelines for genetic manipulation research. The "New Zealand Code of Practice for Small Scale Genetic Manipulation Research" issued in November 1994 contains a number of changes, including less emphasis on the "Brenner" system of risk assessment, introduction of a code of practice for containment of experiments involving plants, and introduction of a code of practice for containment of experiments involving animals, including insects and aquatic species. The Code also details requirements for work with live viral vectors. There is little change for experiments requiring C0 containment. Anyone wishing to see the new Code should contact me or Russell Poulter (Department of Biochemistry). I have requested additional copies from the Ministry for the Environment and when I receive them, I will distribute copies to the Safety Officers of Departments involved in genetic manipulation research.

The new Code also suggests new minimum application forms. As well as a standard form for experiments requiring C0 or C1 containment, investigators working with plants need to fill out a supplementary form. These forms (ACNGT applic. form and ACNGT-Plant) are available on the network as MS Word 5 files (Macintosh format) in a folder "ACNGT forms" in the "Public Out (CR Office)" folder on the computer "CR Office" in the Microbiology zone. They can also be obtained on a disk or as a hard copy from me.

Note that the role of the ACNGT is interim until the Hazardous Substances and New Organisms (HSNO) legislation is enacted. The HSNO Bill has been introduced into the House and referred to a Select Committee for consideration. The Bill is available from Bennetts Bookshops or can be perused in all its 205 page glory in my office. The Ministry for the Environment has also provided a detailed Discussion Document on the Bill, copies of which are held by Russell and myself. Basically the plan is to establish a new agency, the Environmental Risk Management Authority (ERMA), that will be responsible for assessing all hazardous substances and new organisms, including the import or laboratory development and field testing of genetically modified organisms. It would seem though that the intention is to delegate approval at C0 and C1 containment levels to local Biosafety Committees. The Select Committee is calling for submissions by 3 February 1995 and, given the likely influence of the Bill on genetic manipulation research, I would encourage interested parties to comment.

With the new ACNGT Code available and the HSNO legislation coming ever closer, **NOW** might be a good time to ensure your genetic manipulation research has received Biohazard Certification from the University of Otago Safety Committee!

- Clive Ronson [X7701]
Department of Microbiology
(University of Otago Honorary Biological Safety Officer)

The discovery of "stuffer" in the Stratagene Uni ZAP XR Vector predigested with both Eco RI and Xho I and dephosphorylated with alkaline phosphatase

An MSc student in my laboratory has prepared a cDNA library from total RNA isolated from cultured cells with a primary titre of 1.2×10^6 pfu. This library has been analysed for insert size, using the *in vivo* excision protocol to obtain Bluescript plasmids originating from individual plaques of the library. Over 20 individual plasmids have been analysed; restriction enzyme digestion by Eco RI or Xho I or Pst I all gave a single 2.9 Kb fragment; there was also no evidence of any inserts with double digests.
Two of these Bluescript plasmids have been sequenced and they contain the **complete cloning cassette**, including the sequence that should have been removed by preparation of the vector arms, ie between the Eco RI site and the Xho I site. In communication from Stratagene, I was surprised to learn, and others may also be interested, that this "stuffer fragment" is **not** removed from the digested vector. (Religation is theoretically prevented by dephosphorylation.)
Religation of stuffer back into the vector would occur if:

(i) inactivated (as this is done by heat treatment at 70°C for 30 min, followed by phenol/chloroform extraction, this possibility seems unlikely).
(ii) the digested vector and stuffer as supplied were not completely dephosphorylated as claimed.
(iii) the lack of any inserts in the library may even suggest that the supplied vector was uncut.

Can anyone offer suggestions as to how to check the vector, prior to attempting to ligate cDNA into the vector arms, short of doing a "mock ligation" with the vector (containing stuffer) and packaging; a very expensive option. What would be the easiest way of purifying the vector arms from the stuffer?

Mary Thompson

Can and should cDNA libraries be made from total RNA, or must one first isolate the mRNA?

Most protocols for preparing cDNA libraries start from poly A^+ RNA, but it seems to me, where one wants to maximise the chance that all of the mRNAs, especially the low copy number ones, are represented in the cDNA library, that using total RNA has advantages.
Most of the methods for separating out the mRNA fraction depend on poly(dT) in some way, so it seems unnecessary to do a preliminary purification since the first-strand cDNA is synthesised using poly(dT) as primer with RT; and this in itself should theoretically pick up only mRNAs anyway.
For example, the primer used in the Stratagene ZAP-cDNA synthesis kit is

5' $(GAGA)_5$ACTAGTCTCGAG$(T)_{18}$ 3'

I have received the following comment from the head of the library department at Stratagene: "the first strand primer used in the kit is not just oligo dT, it contains an Xho I site and GAGA sequence so the **priming of total RNA may not be as pure as** you had hoped".
Can anyone shed any light on what other sequences could be primed? There would have to be a good string of A's for polydT to bind to (are long runs of A's found in ribosomal RNA's for example?)
What experiences have others had in using total RNA for library preparation?

Mary Thompson

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PCR Primer design -an update

Like all new technologies, they are subject to refinements in their theory, PCR primer design has also progressed with some refinements as well as some new ideas. Ian Morison gave a seminar to the Biochem. Dept at Otago on some of these new ideas as well as a summary on the computer progams available especially those that incorporate the latest theories. At Otago the available programs include GCG-Prime on

Sanger, and on the Mac's MacVector, Amplify, HyperPCR, and on trial for 4 weeks DNAStar-Primer Select (also at Micro. Dept). Ian has put together a clear summary of his seminar which is available to all on request, Write to me c/-Biochem or E-mail me and I'll send it out. Breifly the recommendations for primers are 1) high overall stability, the GC content should be similar to or greater than that of the template, 2) 3' stability should not be high, dont have CC/CG/GG neighbours in the 3' pentamer, 3) try to keep the Tm's of the primers within 1-2°C of each other, 4) check for all possible hairpins and dimers (Primer Select is the only available program which does this well), 5) primer length is optimal between 17 & 24 (for longer PCR's use about 24-mer), 6) the product Tm and the primer Tm difference should be minimized, 7) engineered mismatches or redundancies are best kept to a minimum and towards the centre of the primer also avoid strings (>2) of mismatched bases, 8) restriction sites can be introduced using mismatching as above. Aviod going too close to the ends and avoid enzymes that don't cut well near ends e.g. Pst I (there is a list of efficiency of cutting near ends in the Stratagene Catalogue p274). Although having cut sites in the primers I often Blunt end clone the PCR product before sub cloning using the specific cut sites, 9) longer ramp times can improve the PCR when using mismatched primers.

Whilst I'm on about PCR and Primers I have been investigating a set of primers for Mycoplasma detection in tissue culture cells. These primers are based on the work of Spaepen, M. et al (1992), FEMS Micro. Lett., 99, 89-94, and are available as a kit ($500) from Stratagene. If anyone has experience of this approach to Mycoplasma detection I would be very pleased to hear from you.

Ian Ross, Ian Morison, Les McNoe

Protein Prattle

Driven by the worthy content of the previous three CGR Newsletters, I feel that it is now time to address the paucity of comment about proteins. After all, it is these gene products that do all the real work.....

As 'minder' of the **Protein Microchemistry Facility** in the Department of Biochemistry, I feel that many CGR members will benefit by being better informed of the protein analytical procedures available to them through our service operation and/or collaboration. Hence, I have taken the unusual step (for me at least) of volunteering a regular contribution to these pages, initially focussing on the available services one by one.

In this first instalment an overview seems appropriate - and consistent with its designation as prattle, things will be kept simple (although we can't yet offer it in a kit!!). The Facility contains a range of equipment dedicated to the microanalysis of protein structure. Due in large part to the demands of my own project work, the focus is very much on high sensitivity analyses. The upside of this is that we are often able to obtain structural information from vanishingly small amounts of sample - to my knowledge, only a couple of labs in Australasia work at such high sensitivity. The downside of working at these sensitivity levels is the increased demands on stringency of sample preparation placed on all users, not just those seeking the highest sensitivity. However, gene jocks need not panic since in most cases this just involves (moderately accurate) quantitation with which we can provide assistance. The routinely available services are protein microsequencing and amino acid analysis - all you need is a microgram or so of sample, in solution or on a blot. By arrangement we can offer microbore-HPLC with diode array spectral analysis, and high performance electrophoresis chromatography. MALDI-TOF mass spectrometry will be available soon. In subsequent instalments I will cover what these respective techniques have to offer.

First stop for most service users-to-be should be with **Diana Carne**, Technician in Charge of the Facility (**Room 127, ext 7542, e-mail: protchem@otago.ac.nz**).

For workers less experienced in protein analyses but capable of doing their own thing, Diana will endeavour to provide useful guidance such as stock protocols and pertinent references while for experienced 'protein guns' she will act simply as an instrument operator - the latter users are therefore able to stipulate their own experimental approaches (selection of controls etc) subject to our sample preparation requirements. Diana can also help out those researchers with no experience in protein work (and who have no desire to get any) by arranging liaison with Biochemistry staff members who may be able to provide collaborative assistance: Alan Carne, Pat Sullivan, John Cutfield and Warren Tate are amongst those well experienced in getting 'regular range' samples successfully analysed, while yours truly probably is most experienced with the 'bottom of the barrel' stuff. Further information about services available through the Facility, including charges, appears on a bulletin available from Diana or myself.

Mike Hubbard, Department of Biochemistry, Room 237, ext 7831, e-mail: mother@otago.ac.nz

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News from Department of Physiology Grants received 1994

Jean Fleming has been busy applying for funding to establish some research. Application to the Lottery Board (Health) resulted in $30,000 for equipment to be used for RNA analysis in the new Developmental Biology Unit/Centre/Laboratory. $15,000 was received from the Otago Medical Research Foundation for a study on the growth hormone responsiveness of pituitary adenoma cells, in collaboration with Dr Patrick Manning, a consultant endocrinologist in the Department of Medicine. An Otago Research Grant of $34,500 was provisionally awarded for a -80°C freezer and radioimmunoassay equipment to be used in research on activin and clusterin expression in sheep, along with a PhD Fellowship for 1995. Jean's office is now crowded with boxes of tips and tubes and Schott bottles, waiting for the move to the new lab.

Meanwhile we welcome Dr Rey Garcia, who has a one year Physiology Department Postdoctoral Fellowship to work on nerve growth factors and their receptors in velvet antler. Rey is currently working in Jean's "old lab" at Invermay, but will also be based eventually in the Developmental Biology Laboratory in the Wellcome Institute building.

Other grants: Drs **Ian McLennan**[1], Oorschot[1], Hammond-Tooke[2] and Hendry[3] have been awarded a one year grant of $49,000 by the Neurological Foundation. Drs McLennan and Koishi have recently discovered that transforming growth factor-beta 2 (TGF-β2) is highly localised at the neuromuscular junction and this grant is a collaborative effort of four laboratories to begin to investigate whether TGF-β2 is a survival factor for adult motoneurones. 1: Department of Anatomy and Structural Biology; 2: Department of Medicine; 3: Australian National University.

Information to hand suggests that a number of members of the Centre have been successful in the latest HRC and Lottery Health granting rounds. Rather than publish an incomplete list of these, we will wait until it is all official and publish the list in the next Newsletter. In the meantime, congratulations to those people who were successful.

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Meeting Reports

In early September **Jean Fleming** attended the Second Clusterin Workshop in Couer d'Alene, Idaho, USA. Clusterin is a highly sulphated glycoprotein which has been purified and characterised under a variety of names and which appears to function in tissue remodelling and response to injury, programmed cell death, cell aggregation and recognition, cholesterol transport and complement inhibition. Between 80 and 100 people from more than a dozen countries attended this workshop, which was held in a large resort hotel in a splendid pine wooded, lakeside setting. Since the 1st workshop in 1992, little progress has really been made on the major function of this protein, but some nice experimental models have been developed to characterise clusterin function.

Neural tissue:
Alzheimer's Disease, Scrapie and Bovine Spongiform Encephalopathy plaques contain clusterin, as well as ß amyloid, TGFß, various interleukins and apolipoprotein E. Evidence was presented that that clusterin bound ß-amyloid 1:1 and reduced ß-amyloid aggregation and fibril formation *in vitro*. There is also up-regulation of clusterin expression in glia of BSE infected spinal cord. Clusterin also features in retinosa pigmentosa, in that expression is increased in *rds* mutant mouse retina, associated with increased glial cell numbers, the onset of "apoptosis" (neurodegeneration) and infiltration of complement components. Ablation of the olfactory bulb in rats leads to massive neuronal loss in olfactory epithelium and intense clusterin gene induction, concomitant with neurodegeneration, not in the dying neuroepithelial cells, but in underlying glial and neural structures.

Gonadal tissues:
Clusterin is thought to be important in the maturation of sperm. The Sertoli cells secrete large quantities of the protein, which binds to sperm in the seminiferous tubule. However the epithelium of the efferent ducts appears to sequester clusterin, removing it from the seminal fluid until, in the epididymis, a different form of clusterin is secreted. In the human, the presence of clusterin on the sperm surface is an effective marker for damaged or abnormal sperm in the ejaculate, where it appears to cause aggregation of the abnormal sperm.

In many hormone-dependent secretory tissues clusterin expression increases on removal of the hormone, as the tissue enters apoptosis. There is still two schools of thought on its function in these tissues: is clusterin acting as a biological detergent to "mop up" the membrane components and degraded nucleic acids from the dying cells, or does clusterin protect cells from apoptosis? In mammary duct regression after pup removal clusterin is expressed in the dying luminal epithelial cells, whereas in LNCaP cells (an androgen sensitive human prostate cell line in which TNF induces programmed cell death), clusterin expression preceded DNA laddering. Furthermore over-expression of clusterin in transfected cells inhibited the TNF effect, whereas transfection with a clusterin antisense oligo increased cell death and decreased clusterin expression.

Mouse development:
Clusterin expression is first seen at day d9.5 in heart outflow tract (valve precursor cells) and by d12 it is expressed strongly in liver and small intestine. A transgenic mouse with 1.7 kb of 5' flanking DNA, including the promoter, 1st exon and intron and the splicing signals, plus a CAT reporter gene, shows high expression in most, but not all tissues where previously reported (liver > testis > brain), but notably also in white blood cells and bone marrow. Unfortunately the clusterin knockout mouse didn't quite make it in time for the workshop: chimeric mice have been created and are being bred to create the homozygous knock-outs. Participants in the workshop were asked to write down their predictions for the knockout phenotype; the best guess wins a prize at the next workshop, to be held in early 1997, in Switzerland. Most predictions suggested that the knockout would be fatal, but the group who are actually doing the work are hoping that the phenotype will be relatively normal until "stressed" in some way.

After an intense three days of full immersion in clusterin research, participants decided that the real function of clusterin was:

> To stimulate the aggregation of scientists from diverse fields in memorable locations.
> To promote fellowship and exchange of ideas amongst people who would not have otherwise met.

Is that why I remain fascinated with this multi-faceted protein?

Warren Tate reports from a meeting for Howard Hughes Fellows:

One of the compulsory requirements of being an International Fellow of the Howard Hughes Medical Institute is to attend one of the designated 4 or 5 meetings for their investigators, for which the Institute covers the travel costs. While this seems like a 'pleasant' compulsion, there is a slight sting-namely that at least every second year you have to present

your achievements to this select group of US high flyers.

The meetings are held at the Institute Headquarters and Conference Centre in Chevy Chase, Maryland. This represents a group of elegant buildings with great hallways, high ceilings and significant art work. The accommodation is befitting this and, although I was told by a fellow particpant that one can charge phone calls to the Institute while staying there, I couldn't go quite that far this year. Maybe next year. The 75 TV channels available in your room were useful for me personally since I was unable to sleep until about 4am, and I found the 'Court Channel' fascinating veiwing. It focussed mainly on a case very similar to 'The Philadelphia Story' while I was there, ironically involving a young lawyer from Philadelphia, incredibly articulate, who was shabbily treated by his 'prominent and upstanding' law firm.

What about the science! Halfway through the first power-packed session of outstanding talks, full of exciting new information on 'the analysis of gene expression', I leaned over to the person next to me and asked how frequently people talked. "Every year " was the reply-gulp!. I was relieved later when one speaker apologised for not showing the same slides he had shown for the last seven years-he had some data this year. This was a report of the discovery of the obesity gene expressed in white adipose tissue, responsible for the obese mouse phenotype (recently reported in Time magazine). Unlike anything in the data bases it is a secreted protein and the human equivalent is highly similar. Interestingly evidence that this gene product was the ligand for a receptor responsible for the db obese mouse was presented, and it is believed that the system may operate via the hypothalamus to dampen down hunger. Another report relevant to human disease was the demonstration that Hirsprung disease, where affected infants have part of their large intestine 'aganglionic' so that the intestine blows up, was due to the absence of an endothelin receptor. Again studies of mouse mutants was the key to understanding the lesion, and two known natural mutations in mice 'Piebald lethal' and 'Lethal spotting' turn out to be the endothelin 3 receptor and its ligand , having the same phenotype as the human disease. Gene knockouts confirmed the conclusions.

There was strong Drosophila contingent at the meeting demonstratingthe power of this organism as a model for developmental biology in eukaryotes. With topics ranging 'signal transduction during eye development', 'neuronal connectivity in the visual system' and 'genetic dissection of neurotransmitter release' enormous progress is being made in cloning 'pathways'. "This year my postdoc has cloned 8 genes in this signal transduction pathway"! was a typical comment. Structure/function studies of important protein systemns were not forgotten and a discussion of how the Chaperonin groEL is involved in folding proteins, and spitting them out to try again if unsuccessful was a highlight for me. There was a lot of serendipity in this (and in other breakthroughs reported at the meeting-there is a message here somewhere!) in that the group were trying to crystalline one subunit of the chaperonin, and after many unsuccessful attempts they tried the whole complex and it worked!

What set this meeting aside from the usual international meeting one attends? Well I found myself getting hyper-excited as each presentation was like **the highlight** of a typical meeting. I kept thinking-not another one. Perhaps it was just the lack of sleep, or the herbal 'homeopathic' 'no more jet lag' I took every 2 hours on the plane on the way to the meeting which clouded my judgement, but the meeting was a resounding endorsment for the Hughes philosophy to support 'the person' rather than worry too much about what she or he is doing. As a senior administrator said to me 'everyone talks about doing this but Hughes is the only organisation that has actually done it. It breeds eliteism but it also breeds excellence. And the question that was running through my mind throughout the whole meeting-how did I become part of this? Well a couple of people came up to me and said that they had nominated me because I was the only New Zealand scientist they knew-brings you down to earth a bit!

Warren Tate

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Group Profile

CANCER GENETICS LABORATORY

The Cancer Genetics Laboratory has been studying Wilms tumour for approximately 10 years now, and their research has taken them into a number of unexpected areas ranging from genetic alterations in cancer cells through to developmental biology, embryology, and clinical genetics. Wilms tumour is, in a sense, embryogenesis gone wrong, so there is a vast amount of background reading and knowledge that is required to keep up in this fast moving field. Two major research areas have emerged over the past 3 years. The first area is coordinated by Tony Reeve and involves the role of genomic imprinting in tumour onset. The second area is coordinated by Mike Eccles and involves studying the role of the PAX genes in normal and neoplastic kidney development.

Tony Reeve (Professorial Research Fellow)
Hasn't handled a pipettman for a year or two now. Was once known to say "the only important thing is what happens at the bench". Now wanders around suggesting sane and not so sane experiments. Often talks to himself saying "what's the title of your paper?"

Garry Grubb (PhD student)
Is in the throes of finishing his PhD, having worked on the regulation of the Wilms tumour suppressor gene WT1. He has found an antisense transcript to WT1 which could be involved in the onset of some Wilms tumours. The lab oenologist. Loves RNase Protection.

Ian Morison (PhD student)
Loves designing PCR primers. He is addicted to RNA-PCR and is using this to look at the role of IGF2 imprinting in hematopoiesis. Lurks around at odd hours whistling oboe concertos whilst waiting for unsuspecting bone marrow donors.

Ann Schofield (Post doctoral Fellow)
Is looking at the complex pattern of IGF2 promoter imprinting. She also has a big interest in the way that the H19 and IGF2 genes interact to regulate their imprinting. Suffers from withdrawal symptoms when not either sequencing DNA or climbing mountains.

Tak Taniguchi (Postdoctoral Fellow)
Alias MacTak, he devours Macintosh manuals. Used to enjoy chopping up kidneys in vivo but has now opted for the life of research. Can PCR anything out of anything. These feats of wizardry are often performed during "father's hours" when most of the laboratory is sleeping.

Mike Sullivan (PhD student)
Rumoured to be the first to do the southernmost southern blot, many years ago. Perfectionist by nature, he still strives for the perfect southern blot which is being used to examine the role of DNA methylation patterns in the imprinting of the IGF2 gene. Often seen racing out of seminars accompanied by beeping sounds. Helps children with cancer as a side-line occupation.

Mike Eccles (Research Fellow)
Mike is directing an investigation of a gene called PAX2 , which is a new thread in the Cancer Genetics Laboratory The PAX genes are an unusual group of genes in that mutations have been shown to result in either congenital abnormalities or in cancer. The PAX2 gene is relatively new on the scene, and it is not known if it is involved in any disease. Indeed, it could be said that we are doing this the wrong way round; i.e. cloning a gene and then looking for the disease. However, this might not be as silly as it seems... With an interest in kidney development and kidney cancer, one would have to ask does Mike like kidneys. His answer to this; "I can't stand them, but they're interesting to look at". When Mike is not in the lab he is back at the ranch stabling horses, or playing with his two year old daughter.

Phaikasame Sanyanusin (alias Sam; Ph.D. student)
A sometime mystic, rumoured to be a Thai monk in a previous life. Sam routinely produces marvelous feats of biochemical wizardry between the hours of 5pm and 9am. He is looking for a PAX2 mutation.

Melanie McConnell (Ph.D. student)
Melanie is hoping to CAST away soon, on a fishing expedition to find a gene that PAX2 regulates. She has been spending recent weeks perfecting the finer details of CASTing and will

attempt her first CAST soon. (CAST=cyclic amplification of selected targets). Aside from her Ph.D. Melanie has been known to do the odd spot of ballet.

Teresa Ward (Research Assistant)
Teresa is a FLAG expert. Flower arranging and designer "FLAG" proteins are an unusual combination, but if you need advice Teresa is the one to ask. She's looking for DNA targets of the PAX2 transcription factor protein.

Judy Norrish (Junior Research Fellow)
YACS and FISH are probably not two words that Judy thought she would come to use routinely. She is a cytogeneticist by training who came into biochemistry two years ago. However, Judy is still making chromosomes, but now she is also mapping genes.

Les McNoe (Junior Research Fellow)
Over the past year Les has been analyzing various families for syndromes involving PAX2. He thinks he might have something, so right now Les's fingers are crossed. After hours Les is a family and home handy man

Stop Press: Congratulations, Tony on your election as a Fellow for the Royal Society of New Zealand. Ed.

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Wanted: An Editor for the Centre for Gene Research Newsletter.
For 1995, we wish to appoint an Editor for the Newsletter. This person will be responsible for gathering news and getting it typed up and printed for five or six issues starting in February. Word precessing skills, preferably using a Mac, are necessary. This is a paid position at standard universtiy hourly rates but to an agreed maximum time input. We anticipate that approximately 8h should be spent on each issue. Final editorial policy remains the perogative of the Committee of the Centre. If you think this sounds like you, please write to or email me - or use the Centre's drop box on my computer - a letter of application. The closing date is 27 January 1995. We are planning the first issue for 1995 coming out by the end of February.

Murray Grigor

(email: grigor@sanger.otago.ac.nz)

DNA sequencer news

The seqeuncer service will run up till Christmas, close down over the holidays and then start up again on 9 January. Deirdre took part of her holidays in November and is now back on deck. Vicky Morrison has been working part - time in the sequence facility since September and will continue from the beginning of February. In the meantime, Deirdre and Vicky will be pleased to receive your smaples - so keep them flowing in. Over the next couple of weeks they will be treated on a first come-first done basis and any that are not done before Chirstmas will be held over until January.

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TGF-β1 null mutant mice

A colony of mice with a disrupted transforming growth factor-β1 (TGF-β1) gene is being established at the University of Otago. The mice or their tissues can be obtained by contacting either Ian McLennan (x7346) or Kyoko Koishi (x7440).

TGF-β1 is the archetypical member of the TGF-β superfamily, of which there are at least 25 members. Most cells respond to TGF-β1 in vitro by altering either their rate of proliferation, state of differentiation and/or synthesis of extracellular matrix proteins. The relevance of these response in vivo is unknown. TGF-β1 has been variously postulated to control the development of all of the major body organs, but TGF-β1 null mutants mice are grossly normal at birth. The TGF-β1 null mutant mice over express both class I and II major histocompatibility antigens and generally die within 3 to 4 weeks of birth as a result of progressive inflammation of their tissues (Shull et al, 1992. Nature 359:693-699; Geiser et al, PNAS 90:9944-9948). They may prove to be a valuable model of autoimmune diseases.

TGF-β1 null mutant embryos lack TGF-β1 mRNA but contain some TGF-β1 protein! It

appears that TGF-β1 embryos and neonates receive TGF-β1 directly from mum (Letterio et al, 1994 Science 264, 1936-38). A superb result: doctrine says that proteins like TGF-β1 can not pass through the placenta and that TGF-β1 is an autocrine or paracrine factor. Now TGF-β1 is also an endocrine factor that passes through the placenta and GI tract of neonates (milk contains TGF-β1). This presumably explains why the tissue locations of TGF-β1 mRNA and protein are not identical.

Embryos without TGF-β1 protein can be made by mating null-mutant females with heterozygote males. The secret is to keep the mums alive by suppressing the inflammation with dexamethasone. Does anyone know a better way of doing this? The hearts of null mutant embryos from null mutant mothers are abnormal but otherwise they appear to be grossly normal. Our hunch is that the tissues of the null mutants are microscopically, biochemically and functionally abnormal.

A Final Request: Please send me copies or references for any papers you have published this calendar year. These are required for the Centre's Annual Report due in March. May I have them by 10 February please.

Murray Grigor.

Happy Christmas Everybody!!!