University of Otago
Te Whare Wananga o Otago

Centre for Gene Research
NEWSLETTER

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GUEST EDITORIAL Cinderella went to the ball but will she live happily ever after? Molecular biology is the Cinderella discipline that now underpins many of the biological sciences. It brings the ability to ask questions that could not otherwise be posed and a pragmatic urgency about getting things done. I suspect that this last attribute worries many people who have not practiced the discipline. Like most fast-moving science, molecular biology requires a strong awareness of the activities of others in your research area and an appreciation of the broader spectrum of scientific activities - in other words, a degree of inclusion within a cohort of scientists. The tools of molecular biology have a breadth of application that makes it possible for an individual to contribute to many areas of biological research during their career. This is a particularly attractive feature, given the uncertainty and changing nature of scientific employment in New Zealand. Tenure is rapidly disappearing and the short term research contract is making a nonsense of the notions of strategic research and of career structures. Government scientists are biting the well gnawed soft money bullet - and they are lumbered with the Ugly Sisters of inferior peer review and inappropriate competition between CRIs.

The geographic isolation of Dunedin and it's necessarily limited representation of disciplines means that scientific survival here mandates the efficient use of local expertise and an active interaction with the national and international scientific communities. The common language of molecular biology and the pragmatism of its practitioners has ameliorated some of the problems of isolation. Other aspects have been partially addressed through the growing appreciation by funding bodies (like the HRC) of the need to support collaborative ventures, greater flexibility in the provision of sabbatical leave, and by the availability of resources to fund student and staff attendance at conferences. The advent and increasing utility of electronic mail has also created a greater sense of community by giving more immediate access to colleagues and hot experimental information. Locally, the Centre for Gene Research fosters communication between molecular biologists at Otago and with their wider community. In addition, its cross-campus nature may prevent some of the fragmentation and myopia caused by the devolution of power to divisions and departments within the University. Unfortunately this devolution is part and parcel of the EFT trap that colours the education system and which pits department against department, division against division. From my perspective, this outcome has not been balanced by a coherent plan that seeks to produce and nurture molecular biologists at Otago so they can confidently expect to contribute the scientific and technological expertise required for an internationally competitive New Zealand. For too many of our graduates, the only convincing reason for working in New Zealand is the lifestyle rather than the opportunity to carry out good science. Neither academia nor government science yet offers the right environment!

So what can be done? First, the molecular biologists, from DNA jockeys to protein crystallographers, must recognise the pivotal position that their discipline occupies in the future of the biological sciences. Second, the dissemination of knowledge about molecular biology must be supported at all levels; from schools through to high quality post-graduate training, through the media and into the minds of the general public, corporate executives, university officials and government. Third, the progress of molecular biology is sustained by an amalgam of basic research and technological application that is powered by highly skilled and motivated people. Some real vision is required if the University of Otago is to remain part of, and reap the benefits from, one of the fastest growing scientific disciplines. We need a mechanism that will attract high quality molecular biologists and bring back our best graduates - to share their skills and knowledge, help refurbish some of the more moribund university departments and attract to the university a greater slice of

outside research funding. I believe that the best way to achieve this end would be to establish a research institute that focuses on basic molecular biology and its application in agriculture and biomedical research. There are many successful precedents for this way of creating a suitably nurturing environment, much of the infrastructure and many of the necessary skilled personnel are already here, and the time seems about right for positive action.....or should we just wait until the Handsome Prince comes around checking out slipper sizes?

Brian C. Monk
Senior Research Fellow
Experimental Oral Biology Laboratory

Please feel free to comment about Brian's editorial. Letters to the Editor are welcomed and will be published in our next Newsletter. Please address these to Murray Grigor in Biochemistry. Use the Gene Centre Drop Box on my Macintosh or email (grigor@sanger.otago.ac.nz) or steam mail.

Meeting Reports

Two reports from Clive Trotman and John Cutfield recently returned from the other side (Tony Reeve has also recently returned from a meeting in Sweden that he will tell au about in our next newsletter):

The Gordon Conference on Oxygen Binding Proteins was held at Plymouth State College, about two hours north of Boston, in a wilting late July. From almost the first moment a lively debate broke out over the issue of the origin (or multiple origins) of introns, haemoglobins being one of the favourite experimental systems for the exploration of this issue. Did globin introns support the exon theory of genes, i.e., "introns early" (yes, of course) or did they support the insertional or "introns-late" theory (yes, of course)? At the start of the conference I still hadn't decided where to place the emphasis of my talk, on either protein structure or gene structure, but as a first-evening speaker it was an easy decision to pull on the heavy boots and wade straight into introns argument, armed with hot results from Anna Jellie. At least it made the question session after the talk an easy and somewhat entertaining affair: like several speakers before me, it was a matter of folding one's arms and attempting to mediate as the early and late factions hurled their verbal missiles across the lecture theatre.

Eventually Gerald Bergtrom suggested a round-table conference to "resolve" the issue and next day in poster time 17 souls including Mitiko Go, Claire and Austin Riggs, Tom Vandergon, Bill Pohajdak and Cyril Appleby, armed with a makeshift whiteboard, succeeded in resolving rather little, least of all which question we were trying to resolve. Talk of counting heads was discouraged by yours-truly on the grounds that it would have no bearing on the origin of introns and in any case we were all on the same side - the facts.

And the answer? Being totally objective about it, and in a spirit of fair and balanced assessment of all the evidence, and above all avoiding any temptation to take sides, the introns-late folk must be hallucinating.

Clive Trotman

"Crystallography of Molecular Biology" Meeting, Erice, Italy.
May 27 - June 5 1994

I count myself fortunate to have been invited to this particular Meeting which is held every six years at the Ettore Majorana Centre for Scientific Culture, Erice. Isolation, a wealth of archeological sites, and the echoes of 3000 years of history and prestige are the main attractions of Erice, a small, walled town sited on a hilltop at the north-western tip of Sicily. Restricted to 200 participants the conference (or "School" as the Italians call it) covered, via 64 talks and 110 posters, a wide variety of themes as well as many new structures. Topics included structure-based drug design, immune recognition, viruses and viral proteins, protein folding and mobility, allosteric regulation, and proteins involved in DNA cutting, replication, transcription and translation. There were also sessions devoted to methodology, theory and new technology. Structural studies of engineered proteins, designed to answer fundamental questions on catalysis and ligand binding and in many cases leading to new proteins with novel functions, were in strong evidence. Indeed the rate at which new protein structures are being solved is now

extraordinary - five new journals devoted to structures of proteins and nucleic acids, including "Nature Structural Biology" have appeared in the past few years.

Some of the highlights of the meeting were the structures of a eukaryotic DNA polymerase clamp (Kuriyan, Rockefeller), picornaviruses (Rossman, Purdue), the chaperonin groEL (Sigler, Yale), the *lac* repressor (Steitz,Yale), MHC protein HLA-DR1 with bound peptide antigens (Jardetzky, Harvard), cholera toxin (Hol, Seattle), influenza neuraminidase binding drug (Colman, Melbourne), HIV reverse transcriptase (Stuart, Oxford) and the cryo-crystallography work on ribosomes (Yonath, Weizmann). One of the surprises was the number of structures revealed that had a pronounced hole in the middle, 'doughnuts with a purpose'; *for example* the *Trp* RNA-binding attenuation protein (11-fold symmetry), groEL (7-fold symmetry), human serum amyloidP component (5-fold symmetry), the phospholipid-binding annexin XII (6-fold symmetry) and the ribonuclease A inhibitor (a horse-shoe).

My enduring memories are of an extraordinarily successful and stimulating meeting, of cloudless skies, marsala wine, juicy black olives and last but not least the mystique and history of Erice. Support from the University of Otago Medical School and the Health Research Council of New Zealand is gratefully acknowledged.

John Cutfield

Editors Note: I suspect there are others out there who go to meetings - please send us in a report so we know what is happening in the big wide world.

Grants received.

Mike Hubbard and Tony Reeve have been busy chasing grants for purchase of two major items in the Department of Biochemistry. Mike has fronted up on applications for a laser mass spectometer and Tony for a phosphoimager. Thanks to the HRC and Lottery Board grants-in-aid totaling $145,000 for the mass spec and $122,500 for the phosphoimager have been made. The mass spec is now in Dunedin and the phosphoimager will be ordered shortly. Once the machines are up and running both Mike and Tony will provide a description of them for our next newsletter.

Mike also got a grant from the Lottery Board for the purchase of a high resolution 2D gel system.
Any other grants? Please let mw know so that they can be acknowledged in our next newsletter.

Computer Corner - Gopher, The World-Wide Web and Mosaic

There are a number of tools designed to make the finding and getting of information on the Internet much simpler. Among these are the World-Wide Web (WWW), Mosaic and gopher. To take the last first, gopher is a tool which can be used to move around the internet finding things and downloading them to a local computer. Many places have a gopher hole where such things as phone lists, address books, lists of software, tools for searching databases, and stores of software which can be downloaded to your machine. A gopher "client" is available on sanger by typing `gopher` and following the instructions. The University of Otago Computing Services Centre maintains a gopher hole with links to other useful gopher holes around the world and is well worth a look. In preference to using the gopher on sanger, try using the gopher client TurboGopher for the Macintosh[1] . This is a point and click application with which you can just fossick around–surfing is the name the afficionados use apparently. Places that you like can be saved as bookmarks which makes it easier to go back there again. TurboGopher is pretty easy to use and allows you to download programs and image files relatively painlessly.

The WWW is an effort which originated at CERN in Geneva to merge a number of systems to allow access to documents using hypertext links. It is the first example of multimedia that I know of to really work and reach a general audience. A number of programs are available to navigate around the WWW; perhaps the best known ones are those from NCSA (the same people who brought you Telnet) called Mosaic and available for Macintosh, X-Windows and Windows. Mosaic is even easier to use than gopher and it makes less network traffic. The HTML documents that it uses can include pictures and maps which also carry information that can be
clicked on. Mosaic is an even better interface than gopher and I urge you to try it.

Practical Details

[1] Instructions for getting this are included at the end of this piece

Copies of TurboGopher and Mosaic are available from my machine. To get them, if they are not already installed on your machine you will need to log on to my machine and download the files, and then install and configure the software. Explicit instructions for this are below, but if you find the prospect daunting, try and find someone else in your lab to do it for you.

Requirements

The prime requirement for using gopher or Mosaic from a Mac is an ethernet connexion. If you don't have this, find another machine that does.

Getting the Software

- Go to Chooser and select AppleShare
- Choose the Biochemistry Zone and select the computer CraigMarshall
- Login as a the registered user Rhino password ceros
- Select Public Out
- Close the Chooser
- Open Public Out and drag the TurboGopher or Mosaic folders to a convenient place

Installing Software.

Gopher

For gopher it is as simple as click and go. The gopher will automatically go to the University of Otago gopher hole and from there you can go anywhere. One place you might want to start is the biology gopher at Indiana. A bookmark to this is in the gopher folder, so try that and gopher around there.

Mosaic

After getting Mosaic, read the readme file before proceeding, especially if you have on older version installed. Mosaic uses a "home" page. By default this is set to the NCSA Mosaic Home Page in the US. However, they receive several million connexions a week and are keen that people nominate an alternative home page. Such a page is provided in the Mosaic folder and can be selected by choosing Open Local from the File menu. Select the file LocalHome.html and fix this as your home page by selecting Use this URL for Home from the Options menu.

Also included in the Mosaic folder is a hot list of interesting sites. Select Hotlist from the Navigate menu and double click on the place of interest

When to Surf?

I have found the best time to Surf the Internet is either early in the morning or late at night. The limiting factor seems to be the network traffic out of New Zealand. surfing in the morning has the advantage that most of the American and European sites are in their quiet times so the pressure on the resource is not as great.

Craig Marshall

Retreat

On the weekend of June 17 and 18 members of the Centre went on retreat to Orokonui to discuss technology, funding and just get to know each other. Over 65 people were at the Saturday morning session, almost as many on the Sunday morning session and rather fewer stayed over each night. Those that did stay over had the pleasure of knowing that they did not have top brave the icy roads the next morning. Our guest for the weekend was Professor Jim Watson from Auckland (now of Genesis Research and Development). Jim gave a talk in the Department of Biochemistry on the Friday. We were also pleased to have Mike Berridge and Craig Hilton from Wellington joining us. Feedback about the weekend was positive and our thanks to Allan Crawford who looked after the catering for us. For those that did not come - tough. Probably we will try and repeat the event every second year at some venue outside Dunedin - maybe Orokonui again but other suggestions were Tuvalu or Fiji.

Peter Stockwell's overheads: Many people at the retreat expressed interest in getting copies of the overheads that Peter used for his talk on computing. These are now available in the Public Out folder on my (Murray Grigor's)Macintosh. Help yourself to them. Just follow Craig's instructions in his Computer Corner to access that folder. If you dont have a Mac, let me know and I will send you a copy.

Technical Tips Technical Tipsicals Tipnical Techcicals

Can you help us with your nifty innovations, with your solving of frustrating technical problems. If you have cracked it, pass it on so others don't crack up.

For instance, do your Southern blots suffer from acne, non specific hot spots. Did you know that talc powder from gloves can cause this?

Time savers: recently we had the laborious task of producing 150 agar plug pools of our YAC library. Each plug had to be rinsed 20 times in various reagents, thus requiring 3000 caps on/caps off. Repetitive strain material. Solution: puncture the lids of the Falcon tubes with a red hot wire to create a seive enabling the tubes to be drained and the new solution to be added without cap removal.

Every lab has one/ someone who knowingly uses the last of some vital reagent and fails to reorder etc or someone who leaves a horrendous mess in a communal work area. In our lab this is a Food offence. The lab rule is that the culprit provides morning tea for the rest of the crew. It works!

John Harris has commented that thorough vortexing of X-gal reagent prevents crystal formation during B-gal detection. Thank you John for this technical tip.

That it for this month
Murray Broom/Sue Galloway

Kit Comment

A request from Lynn Slobbe
I am interested to hear if anyone has used the new Amersham 'Rediprime DNA labelling system'? The idea of a system that can be stored at room temperature appeals to me as I have recently experienced my Klenow loosing activity! I currently use the Boehringer 'Random Priming Kit' with my DNA fragments still in agarose. It is working well, however, if this new kit is all it is supposed to be I would be interested in changing. If anyone has used or heard of its use I would appreciate hearing from you. I can be contacted via AppleTalk on the Microbiology 'DRL office LC2', phone 479-7710 or Fax 477-2160.

Ian Ross adds:
Regarding Klenow losing activity, we have always treated Klenow as a highly unstable enzyme and as such its time outside the -20°C freezer is minimal i.e. about 30 seconds to remove a 1 µL sample.

On the subject of preparing probes, how have you all been getting on with your isotopes, especially the new Amersham 'Redivue'?

Sequencer

Currently Deirdre reports that usage of the service has grown way beyond that we had budgeted for and we are working to capacity. Major users are the Departments of Biochemistry and Microbiology, AgResearch Molecular Biology Unit and the Virus Research Unit. We also are doing close to 20% of our work for people outside the campus and this proportion is growing. We plan to hire a part-time person (see Advertisement on p6) and this should allow us to expand from three gels per week to four gels per week to avoid any additional delays in the service. Your Committee has also agreed that we should take up a service contract with ABI to minimise any down time that might occur for sequencer breakdowns. Shortly Deirdre will be circulating a questionnaire to all users to try and anticipate usage into next year. She will also be seeking comments about the service. Please be frank with these so that we can ensure the best service we can to our clients.

New Faces.

Congratulations to **Robin Olds** who has recently returned to New Zealand from Oxford and last month was awarded a Chair of Pathology.

Robin writes:

I am a medical graduate of Otago, and completed a PhD here while undertaking my post-graduate training as a Pathologist specialising in Haematology. In May ot this year I returned to the Pathology Department after spending the last five

years at the Institute of Molecular Medicine in Oxford. There I concentrated on the molecular basis for thrombotic disease, principally by identifying the genomic basis for inheritied deficiencies of naturally occurring anticoagulant proteins, such as antithrombin, protein C and protein S. By correlating the genotypic abnormalities with phenotypic behaviour of variant proteins, in particular antithrombin variants, functional domains of the normal inhibitors were identified. I hope to set up a group in Dunedin to further examine the aetiology and pathogenesis of thrombosis, and to delineate in greater detail how the anticoagulant proteins function. The analysis of the function of variant proteins expressed in mammalian cell cultures will be used for this latter aspect of the project.

Another not-so-new-face is **Michael Sullivan**, Air New Zealand Repatriation Fellow in Cancer Research now working in the Cancer Genetics Laboratory in the Department of Biochemistry. Michael writes:

In January this year I returned to New Zealand on a Cancer Society of NZ Repatriation Fellowship, and rejoined the Cancer Genetics Group in the Dept of Biochemistry. This continues a long association with this group and the laboratory in room 224. I was first in this lab in 1980 as a final year Hons student with Prof Petersen and Dianna Hill working on bacteriophage DNA, and later while I as medical student I returned over 2 summer vacations (1982 and 1983) to work with Tony Reeve on "oncogenes". After graduation from medicine in 1985 I specialised in Paediatrics and for the last 4 years I have subspecialised in Paediatric Oncology. In 1992 I joined the Cancer Molecular Biology Research Laboratory of Prof Peter Smith, which is part of the Joint Experimental Oncology Programme of the University of Queensland, where I started a PhD, and I also worked part-time as a Fellow in Paediatric Oncology at the Royal Childrens Hospital. This group has had close collaboration with the Cancer Genetics Laboratory as they shared a common interest in the molecular biology of Wilms Tumour. My main research interest is role of novel molecular mechanisms such as genomic imprinting in the pathogenesis of child cancers especially Wilms Tumour and Neuroblastoma, and I am currently working on the association of DNA methylation with genomic imprinting at the IGF2/H19 locus.

Also a not-so-new face **Jerome Demmer** has recently arrived back to a position of postdoctoral fellow in Biochemistry. Jerome's story:

I started out at Lincoln College graduating with an honours degree in Agricultural Science majoring in the animal sciences. Timing this perfectly to coincide with the agricultural depression of the mid l980s meant a change in direction. I shifted to Otago, and completed a Diploma of Science and a PhD in biochemistry cloning sheep insulin-like growth factor cDNAs with George Petersen and Diana Hill. Following this I spent a productive year with Warren Tate and Cliff Abraham studying the "mechanisms of persistence of phenomena which are thought to be representitive of memory". During this year I was awarded a Wellcome Trust Overseas Fellowship which I spent at the AFRC Roslin Institute (Edinburgh). I worked on the regulation of milk protein gene transcription by prolactin, gaining skills in cell culture and transcription factors. The HRC have recently repatriated me from Edinburgh and I am currently considering my future options while cloning a few genes from possums (SRY and UBE1Y), located on the Y chromosome, which are thought to affect their fertility.

Advertisement

A part-time position is available in the Sequencer facility working 4 or 5 mornings a week for four months. This person should have science training and preferably have a completed qualification (NZCS or degree). However, students completing a degree should also apply. For further information please call Murray Grigor (479 7840). Applications close 16 August.

A date for your diary - Poster Night

Please make a note of Wednesday, 19 October. This will be our Poster Night and everyone who has prepared a poster in the last 12 months is invited to come along and display it. This is the way to find out what really is happening within the Centre. THe venue is yet to be fixed - it will be determined by the number of posters offered. Refreshments will be available and prizes will be awarded in two categories; (a) for students up to the end of their 1st year PhD and (b) for students in their 2nd and further years of their PhD. More details will be circulated later.

GROUP PROFILES

DR KEVIN FARNDEN

DR ERWIN LAMPING

JENNIFER SMITH

BRETT REDDINGTON

DR MICHAEL LEE

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

Dr Kevin Farnden

My research group is studying the expression of plant genes in a variety of plants. Our immediate aim is to clone and characterise several promoters that control the expression of plant genes in a tissue specific and/or temporal manner.

We have used the legume-*Rhizobium* symbiosis as a model system and more recently we have begun characterising genes and promoter regions for genes expressed in seeds and leaves of legumes as well as nodules. In the longer term it is planned to use these promoters to control and target the expression of agriculturally and horticulturally useful plant genes. With this in mind three projects in the lab are now based on broccoli or asparagus and a further will use *Arabidopsis* as a model system to identify other genes of interest.

The promoter analysis work involves firstly the construction of vectors containing various promoter fragments and reporter genes (luciferase or ß-glucuronidase). Plants are then transformed using *Agrobacterium tumefaciens* or *rhizogenes* with these genes to study promoter function. Similarly, antisense contructs have been made to turn off plant genes in a controlled mannner in studies of plant gene function.

Dr Erwin Lamping

Erwin holds an Austrian post-doctoral fellowship and has been in the group for nearly two years. During this time he has cloned and sequenced the gene for L-asparaginase in *Lupinus albus* and characterised the expression and regulation of the potassium dependent form of L-asparaginase in different tissues at the mRNA and activity level. Currently Erwin is involved in a project to purify this enzyme and is using sense and anti-sense technology to establish transgenic plants which constitutively express a second copy of the gene or down-regulate the expression respectively.

Jenny Smith

For her Molecular and Physiological Plant Biology honours project Jenny is working with Erwin on the potassium dependent asparaginase from *L. albus*. The aim of this work is to purify the enzyme to obtain some protein sequence. We hope this will confirm that the gene Erwin has sequenced is indeed the potassium dependent form of asparaginase.

Brett Reddington

Brett is currently writing his PhD and planning a career move into commercial horticulture on a property near Wanaka. His PhD project involved complementing yeast mutants with plant cDNAs, which were thought to encode asparaginase, in order to confirm their identity. In this work we formed a valuable collaboration with Russell Poulter and later Chris Winefield (now at Levin) was also able to use this system to express a plant aspartate aminotransferase in a yeast mutant.

Dr Michael Lee

Originally a graduate of this Department, Michael's PhD research

JANE CAMPBELL

RICHARD MOYLE

CRAIG GRANT

BERNARD LAEVENS

DR JAMIE DAY

involved a study of a linear plasmid in the seaweed *Porphyra spp.* Michael returned to Dunedin last year after post-doctoral positions in Uppsala, Sweden (fish immunology) and then in Durham, U.K. (carnitine acetyltransferase in plants). Michael is working on a FRST funded project "Nitrogen metabolism in plants" and specifically he is cloning and studying the expression of the glutamate synthase multigene family in legumes.

Jane Campbell

Jane is a research assistant working with Michael Lee on the FRST project. Jane has worked previously in Geochemistry, Food Technology, Water and waste water management and Pharmaceutical quality control. Biochemistry is a new field for Jane but she assures us she is enjoying working in a research lab. and learning new techniques.

Richard Moyle

Crop and Food Research is funding Richard's PhD research aimed at isolating genes displaying harvest inducible and harvest repressible expression in asparagus. So far Richard has isolated the 5' region of an asparagine synthetase gene that is up-regulated in harvested asparagus spear tips. He is also attempting to isolate a proline rich cell wall protein gene that is down-regulated post harvest. Future work will involve promoter analysis of these genes to identify elements regulating post harvest expression.

Craig Grant

Craig is also working on asparagine synthetase, but on a PhD project funded by Hort Research. Craig is isolating asparagine synthetase genes from the model legume *Lotus japonicus*. Craig says "at present I am going through the subcloning and sequencing routine and eventually hope to make trasgenic plants to check out the promoters and kill off a few plants with some antisense constructs".

Bernard Laevens

Bernard took his first degree in France and then completed a MSc in Quebec. He is being funded by Hort Research to continue with a project on Nodulin45 for his PhD. This protein is highly expressed in legume nodules but its function is unknown. Bernard hopes to regulate the expression of antisense constructs to this gene in transgenic legumes using a copper inducible promoter. This hopefully may give some clues as to the function of Nodulin45. In addition he plans to express the protein for antibody production and to use the antibodies for cellular localisation studies.

Dr Jamie Day

Jamie arrived in the Department early this year from PhD research at Adelaide and a two-year post-doc at the University of Auckland. Jamie works in the area of plant development and his previous research has been on environmental and plant growth regulator control of flowering in Australian native plants. He has also researched changes in leaves and branching patterns during ontogeny of New Zealand native trees. He is presently an assistant lecturer in the department but in October he will take up a FRST post-doctoral fellowship investigating the genetic control of branching in plants.

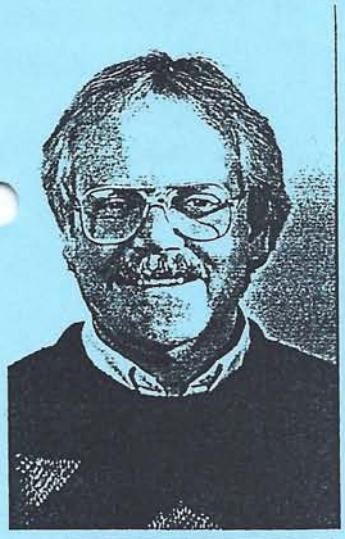
ROBYN ROXBURGH

JOHN MACKAY

Robyn Roxburgh
Robyn is employed on a short term CRI project to isolate and sequence promoters that could be of use in driving high level constitutive expression of genes in asparagus.

John Mackay
John is also working on a short term CRI project, in his case aimed at isolating cDNA clones encoding proteins involved in senesence of broccoli.

Meanwhile from Lab 607 Microbiology, CLIVE RONSON reports:



Our research activities are focussed on molecular genetic and ecological studies of plant-microbe interactions and are conducted in collaboration with scientists from AgResearch at Invermay working on legume inoculation. Much of the research focuses on rhizobia nodulating "new" legumes being introduced into dryland environments where the use of white clover is marginal. The legumes *Lotus corniculatus* and *Trifolium ambiguum* have significant potential for these environments but both have highly specific rhizobial requirements and their agronomic use is currently limited by nodulation problems. Projects include the genetic basis of host-specificity of the rhizobia and aspects of their ecology and population genetics. Other projects relate to the regulation of expression of *Rhizobium* genes required for symbiotic nitrogen fixation and to the role of specific genes in competitive root colonisation and nodulation.

Rhizobia nodulating *T. ambiguum*
Helen McIntyre, Rachel Elliot and Bart Challis
Trifolium ambiguum (Caucasian clover) is closely related to white clover but has highly specific *Rhizobium* requirements. There are only three strains (including strain 105) of *R. l.* bv *trifolii* available that effectively nodulate *T. ambiguum.* These strains also nodulate white clover rapidly but the nodules formed are ineffective; in contrast, strains of white clover rhizobia form only a few ineffective nodules on *T. ambiguum* after several weeks. Helen's research aims to identify and characterise the nodulation gene(s) responsible for host specificity in *T. ambiguum* rhizobia. Helen has also characterised strains nodulating *T. ambiguum* in the field and found strain 105 is the only strain present, even in stands established for 15 years. Rachel is characterising the competitive interactions between strain 105 and white clover rhizobia in the nodulation of white clover in soil and determining the genetic basis of the ineffective nodulation of white clover by strain 105. The basis of host specificity at the level of nodule function is not understood in any system. *T. ambiguum* oversown in adverse environments often fails to nodulate, even when the seed is heavily inoculated. Bart's research aims to follow rhizobia from initial inoculation of the seed until root hair penetration, to identify the critical steps in the nodulation of oversown seed and provide a selection for improved inoculant strains.

Rhizobia nodulating *Lotus corniculatus*

John Sullivan, Vernon Trainor and Clive Ronson

Inoculation of *L. corniculatus* with *R. loti* is essential for establishment of the plant in NZ tussock grasslands as there are no compatible rhizobia present in the soil. Recently *Rhizobium* isolates with diverse chromosomal types were isolated from a stand of *L. corniculatus* originally inoculated with a single strain of *R. loti*. We found that the symbiotic genes in these diverse strains were identical to those in the original inoculant strain. This indicates that the strains arose by symbiotic gene transfer from the inoculant strain to non-nodulating rhizobia already present in the environment. These novel results shed new light on microbial ecology in soil. We are now determining the nature and extent of the genetic material transferred. Plasmid transfer is not involved and we currently favour the idea of a symbiotic transposon. We are also determining the phenotypic and genotypic diversity and symbiotic properties of some of the field isolates to further our understanding of the population genetics of *R. loti* and identify strains suitable for use as inoculants for hill country environments.

Influence of the *dct* regulon on the competitiveness of *Rhizobium.*

Rachael Studholme

Rhizobium strains added as inoculants usually fail to form nodules due to competition from indigenous rhizobia. The genetic factors influencing competition in rhizobia are poorly characterised. Rachael is looking at the role of the *dct* regulon in competitive ability. The *dct* regulon is responsible for the transport of C4 organic acids, which are major components of plant root exudates and an important carbon source in the rhizosphere. Results show a *dct* mutant had to be present in numbers 100-fold greater than wild-type in co-inoculation experiments to obtain 50% nodule occupancy. Dct^+ revertants regained competitive ability. Rachael is also isolating strains that overexpress the *dct* regulon to determine whether they show increased competitiveness.

Molecular genetics of the thioredoxin reductase operon of *R. leguminosarum.*

Chris Hamilton and Rachel Elliot

We have discovered the thioredoxin reductase (*trxB*) operon of *R. leguminosarum* and Rachel has found that it is expressed during symbiotic nitrogen fixation and that it is regulated by a *lrp*-like gene. Chris sequenced a gene downstream of *trxB* in the operon that shows homology to the LysR family, a family of transcriptional regulators. Insertion mutants in the *lys*R-type gene have been made and are now being tested to see if they fix nitrogen in symbiosis. The *trxB* gene itself seems to be essential. The presence of the *lrp*-like gene upstream of the *trxB* gene and a regulatory gene immediately downstream suggest that the operon may have a global regulatory role.