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James Rennie Bequest Fund Report

I applied for travel support money from the James Rennie Bequest fund to assist me in attending the 12th International Mouse Genome society meeting (39/9/98-3/10/98). A few days before the meeting I submitted my PhD thesis which I shall be defending in a weeks time. This meeting was therefore very timely for me as It gave me the opportunity to present the final data from my project and interact with other geneticists studying related topics. During the meeting I was able to meet up with the collaborators from Oak Ridge National Laboratories, Baylor college of Medicine and the MRC mammalian genome centre that have been involved in, and will continue to work on, aspects of my PhD project. This meeting was therefore an ideal 'handing over' time. I will shortly be taking up a Post doctoral position at Columbia University and the during the meeting I met with other member of the mouse community based in the US which I hope to collaborate with on my Post-doctoral project. Below is a copy of the abstract from the poster I presented at the meeting. I have also attached a copy of a report of the meeting that I have written that will be incorporated into the 12th IMGC meeting report to be published in the societies sponsoring journal, Mammalian Genome. Writing this report was a good exercise for me in examining and evaluating the scientific data communicated. I am very grateful to the Bequest fund for its financial assistance because I feel I gained a great deal from attending this meeting.

Characterisation of genes isolated from the brown deletion complex .

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The specific locus mutagenesis test generated thirty overlapping deletions at the brown (*Tryp*1) locus spanning 7-9cM. Complementation analysis revealed four new functional units in the central to distal part of this region, two of which are early embryonic lethals. Our analysis has shown that one appears to result in neonatal death. The fourth, *baf* (brown associated fitness) has a sub-viable phenotype, demonstrating poor growth, gut abnormalities, nervous behaviour and death at around weaning age. This phenotype appears to be highly variable, with some homozygotes surviving to adulthood. We are currently making a comparative study of the different deletions in there homozygous state to determine if these phenotypes or there severity are genetically separable.

We have generated a fine structure map of a 2.5cM region encompassing these genes containing over 30 markers. Techniques including, sample sequencing, exon trapping, cDNA selection and use of the conserved syntenic region on human chromosome 9 have identified transcripts from this region.. Of particular interest is a transcript containing 13 PDZ domains which maps to the region. PDZ domains are know to interact with a number of proteins at specific junctions and may be responsible for the sub-cellular localisation and clustering of proteins into functional complexes. There is evidence that the protein interacts with the C-terminus of the 5-HT2c receptor. To determine the possible roles of this gene *in vitro* functional assays, behavioural observations and immunohistochemistry techniques will be utilised. Although the gene is not deleted from some deletions that give rise to *baf* animals, the proximity of the gene to the critical region means that it cannot be excluded as a *baf* candidate. Expression studies are currently being undertaken to determine if the quantitative, temporal, or spatial characteristics of expression are altered in these mutant animals.

12 IMGC MEETING REPORT

MODIFICATIONS/ALTERATIONS OF THE MOUSE GENOME

A number of presentations were concerned with the modification of the mouse genome by targeted methods including transgenic and Knock out technologies. The meeting was opened with the Verne Chapman memorial lecture by Prof. Nick Hastie who discussed the use of YAC transgenesis to help dissect the biology of the tumour suppresser gene WT1. The introduction of human wild type and modified genes into the mouse genome has provided a powerful tool for the study of WT1 gene expression and function. Much has been learnt about the developmental function, gene dosage requirements and long distance regulators of the gene. The introduction of fugu clones from the region is expected to unveil more about the function and evolution of this gene.

Studies utilising the standard technique of homologous recombination to produce targeted gene disruption included genes in both developmental and neuronal pathways. A targeted disruption of the Creb binding protein, *Cbp* gene resulting in a truncated protein that retained both the trans. activating domain and the Creb binding domain was reported (Yuichi Oike, Kumamoto University). While mice homozygous for this mutation die prenataly, heterozygotes display abnormalities consistent with the clinical features of Rubinstein-Taybi syndrome (RTS). The RTS phenotype in mice is considered to be the result of the truncated protein acting as a dominant negative inhibitor during development. Long term memory of heterozygote mice was deficient as determined by the step through passive avoidance test and the Morris water maze test while the mice performed normally on the Y-maze test, considered to be a measure of short term memory. The learning abilities of another targeted gene knock out, PLCβ1 was also reported (Hee-Sup Shin, Pohang University). An initial characterisation of this null mutation has previously been published, it is now known that PLCβ1 $^{-/-}$ mutants are severely impaired in both context dependant fear conditioning and the Morris water maze task. This implicates PLCβ1 is necessary for normal hippocampal functioning in learning processes.

The targeted deletion of a small region adjacent to a well studied gene, H19, was reported (Michael Reed, Beckman Research institute). Interestingly the deletion results in the disruption of embryonic expression of nearby *igf* only when inherited maternally. This demonstrates the necessity of this region (so called factor X) for the correct monoallelic expression of these two genes (H19 maternally and igf2 paternally). It was therefor proposed that 'factor X' is an oocyte-specific inhibitor of methylation at the H19 locus. This is the first suggestive evidence for a germ line signal involved in the establishment of a methylation pattern and further investigation of this region may help to unravel the complexities of genome imprinting.

Another previously published mouse knock-out was described as a tool identification of genes affected by or interacting with the targeted gene (Catherine Nguyen, INSERM). Expression profiling was achieved by quantitative differential screening using hybridisation of microarrayed cDNA libraries with complex probes prepared from thymus cDNA libraries prepared from MHC knock-out mutants, and wild type mice of several development stages. Clones identified as having differential

expression are isolated for characterisation. This approach utilises a biological model to gain genomic access of the genes also involved in that particular system.

Oliver Smithies suggestion that Essential Hypertension is 'a genetic disease due to many little things' and the discussion of QTL analysis obviated the need for considering the synergistic and epistatic relationships between genes. Already many groups in the mouse genetic community study gene interactions using transgenic mouse models. Four presentations were given describing analysis of the interaction between a transgenic or knockout allele and the mutant allele of one or more other different gene. Smithies example was elaborated on by Nobuyo Maeda (Nihon University) who reported on the interactions observed between mutant alleles of three genes involved in atherosclerosis. Mice homozygous for the targeted replacement of the mouse ApoE gene with one specific Isoform of the human homologue show both a significant increase in blood cholesterol level and a diet dependant susceptibility to atherosclerosis. Homozygous null mutants of the vasodilating free radical scavenger eNOS suffer aortic lesions, which are significantly increased in male ApoE $^{-/-}$ mice. Because the renin/angiotensin system is known to affect Atherosclerosis, mice carrying a targeted deletion of ACE (angiotensin-converting enzyme) were crossed to mice carrying various human alleles but showed no difference in diet induced susceptibility to atherosclerosis. Similarly the detected number of aortic plaques in mice heterozygous for the CBS allele was not affected by homozygous deletion of the ApoE gene. This suggest that the influence of homocystiene metabolism on atherosclerosis does not involve the ApoE locus.

The interaction between genes involved in a similarly common human disease, obesity was discussed by Karen Moore (Millenium) during the late breaking session held. The action of Mahogany (Mg) and mahoganoid (md) as negative modifiers of the Agouti coat colour gene and the suppression of both coat colour and obesity of the agouti lethal allele A^{Y} by these mutations has previously been published (Miller et al, 1997). However this ability of mahogany to suppress obesity does not occur in mice mutated for the *tub*, *fat* or *ob* genes. The mahogany allele does however appear to suppress diet induced obesity convincingly in males while the trend is less strong in females. *(A positional candidate approach has lead to a strong candidate gene for the mahogany allele Sally?)* Mahoganoid appears to show the same gene interactions yet is linked to a different chromosome suggesting that mahoganoid may be an agonist or binding partner for the predicted mahogany receptor. A third presentation concerned with multiple gene interactions involved analysis of the Micropthalmia family of transcription factors. Three closely related bHLHZip transcription factors, *Tfeb*, *Tfec* and *Tfe3* have previously been shown to interact in vitro with the mouse micropthalmia gene *Mitf*. Null alleles of all of three genes have been generated by targeted homologous recombination and only one of the homozygous knockouts *Tfeb* displays a detectable phenotype which is embryonic lethality. The possible in vivo interactions of these genes has been studied by making double mutants which display interesting phenotypes (Eirikur Steingrimsson, Univeristy of Iceland). *Tfec*$^{-/-}$ *Tfe3*$^{-/-}$ and *Tfec*$^{-/-}$ *Mitf*^{mi}/ *Mitf*^{mi} mice show no phenotype suggesting Tfec is not involved in Mitf function. However, *Tfe3*$^{-/-}$ *Mitf*^{w}/ *Mitf*^{w} mice display the osterpetrosis typical of some *Mitf* mutant alleles which is more severe in *Tfe3*$^{-/-}$ *Mitf*$^{eyes\ white}$ *Mitf*$^{eyes\ white}$ animals. This is evidence for the dominant negative action of proteins from those mutant *Mitf* alleles on TFE3 in osteoclasts.

Surprisingly, only two presentations were given data generated using restricted transgenic technology, the use of temporally or spatially regulated transgenesis is becoming a common technique to overcome homozygous lethality permitting study of the affect of particular gene products in certain tissues and at certain stages. The number of presentations including this type of analysis is certain to increase in future meetings, especially after the announcement during the business meeting that Du Pont now permit the use of their Cre-loxP technology for academic purposes. Both reports used Cre-loxP-mediated recombination based strategies. To circumvent neonatal death due to respiratory failure in Glucocorticoid receptor (GR) knock-out mice a point mutation in the receptor dimerisation domain was obtained which permitted dissection of the activating and repressing functions of the receptor. (Cristophe Kellendonk, Deutches Kresforschunginstitut) Cell specific Knockouts were also generated, the most interesting of which was achieved by Nestin driven Cre expression which resulted in disruption of the GR gene in almost exclusively neuronal precursor cells. These mutant mice displayed a phenotype strongly resembling the defects displayed by human Cushing's syndrome patients. Behavioural analysis of these mutant also revealed poor performance in four independent tests believed to be indicators of anxiety (The restrained stress, Porsolt swim, light-dark and elevated zero tests). This evidence strongly implicates the involvement of GR in emotional behaviour and could not have been identified without the restricted targeting approach taken. Temporal restriction of the disruption of the gp130 gene was used to overcome the prenatal lethality exhibited by gp130 Knock-out homozygotes (Werner Müller, University of Cologne). Inducible inactivation of gp130 Via Cre-loxP-mediated recombination was achieved by injection of new-borns with the inducing agent, Ifn. Treated mice exhibited a wide range of defects demonstrating the importance of gp130 dependant cytokines in neurological, cardiac, hematopoitic, immunological, hepatic and pulmonary systems.

To facilitate targeted mutagenesis programmes The NIH priority setting document recommends that $0.1M should be invested in the next five years for the validation of ES lines from different mouse strains for specialised uses. Over the next three years $0.5M should be invested in the study of mutations on genetic backgrounds other than mouse strain 129. These are both important issues that should expand the quality of genetic and biological information that can be obtained from the targeted mutagenesis technology that is currently widely used in the mouse community.

However, many presentation demonstrated that straight forward targeted gene disruption, as currently used, provides less information than analysis of either multigenic interactions or expression restricted disruption. Targeted single gene knockouts also provide more limited information than the multiple allele series that can be obtained from genotype directed mutagenesis which several presentations described. The generation of new ENU induced alleles of two different genes required for embryonic development, (*Quaking* and *Axis*) has provided insights into the possible functions of these genes. New recessive alleles of *Axis* were obtained by screening ENU induced mutations over chromosomes carrying radiation induced deletions generated by the Specific Locus Test (SLT) that encompass the *Axis* locus (Rinchik et al, 1990). This strategy is now being used to saturated two other SLT loci, Screening of 7 cM surrounding the Tyrp1 Locus on Mouse chromosome 4 and screening of a 4-5cM interval surrounding the Pink-eyed dilute locus on chromosome

7. In the later screen, over 1218 gametes have been tested and 19 new mutations which represent 8 complementation groups have already been Identified (Gene Rinchik, Oak Ridge). The goal of this project is to take a 'Parallel processing' approach by using three generation screens with inversions to recover mutations whilst at the same time developing deletions in ES cells to cover the majority of chromosome7 which will be used as powerful mapping and gene identification tools. These deletions will be generated using the technology presented by the Schementi lab which involves the γ-irradiation of ES cells. A series of deletions on chromosomes 5 and 17 have already been generated using this approach and in order to generate deletions over the whole genome an ES cell deletion bank (DelBank) is being generated. This consists of ES F1 hybrid cell lines that have a reporter cassette integrated in different positions in the genome. Deletions can then be generated anywhere in the genome using the cassette as the specific targeting loci, which will be mapped to approximately one cM resolution. This will permit genotype directed mutagenesis screening of recessive alleles anywhere in the mouse genome, in contrast to the majority of large scale mutagenesis projects currently underway which are phenotype driven for dominant alleles. Screening for recessive phenotypes using a deletion complex generated by this technology is already underway for a proximal portion of mouse chromosome 5 (Maya Bucan, University of Pennsylvania.)

An update of all the large scale, genome wide, phenotype driven mutagenesis screens currently underway was provided. The GSF ENU mutagenesis project taking place in Neuherberg includes both a dysmorphology and clinical screen for heritable dominant mutations (Hrabé de Angelis and Balling, 1998). The dysmorphology screen, which analyses 39 parameters which evaluate the CNS, sense organs, limbs, axial skeleton and pigmentation of the mice, has so far identified 93 confirmed mutant lines from the 16000 F1 offspring screened. Only a tenth of this number of mice have so far been screened for clinically relevant phenotypes, which includes basic haematology, plasma enzyme activity, metabolites and electrolytes. Heritability testing is currently underway and confirmed mutant lines are now emerging. The ENU project at Harwell which aims to screen 40,000 F1 progeny has already generated over 7,000. Of the 217 mutant phenotypes initially identified using the SHIRPA screening protocol (Rogers et al 1997). Approximately half of those that have been tested for have proved to be heritable mutations which means that approximately 1 in every 100 progeny tested are carrying heritable dominant mutations of known or novel genes.

A project which spans the Neurherberg and Harwell programmes is the search for new mouse models of genetic deafness, co-ordinated by Karen Steel, MRC institute of hearing research. A total of 18,000 mice from both sites have been tested for hearing impairment and 7 mutants have been identified A further 20 mutants with vestibular defects have also been found and all of these mutants are currently being tested for heritability.

A mutagenesis discussion was held and the majority of questions related to practicalities of the various programmes, particularly accessibility of mutant phenotype information and the actual mice themselves. Most answers are already available on the Harwell and GSF web sites which are available at http://www.mgc.har.mrc.ac.uk/mutabase/ and http://www.gsf.de/isg/groups/enu-mouse.html respectively, although the GSF site is still undergoing development. After

the meeting a two day workshop devoted solely to ENU mouse mutagenesis was held in nearby Schloss Hohenkammer. The programme included discussion on mutagenesis protocols, assays for mutant identification, mutant database management, and the archiving and distribution of mutants. The importance of mutagenesis programmes for the future of functional analysis of mouse biology is reflected in the NIH priority setting document. This recommends that of the total direct cost of $17.1M spent on functional analysis, in the first year alone $0.5 M should be invested in standardisation of mutagenesis protocols and $9.0 M on establishing centers for ENU mutagenesis and phenotyping. Over a duration of 5 years $6.0M is recommended for technology development and a fifth of this again on technology transfer.