From blots to lots of spots: ADNAT symposium on Functional Genomics*

Each year on 23–24 February, the Association for the Promotion of DNA Fingerprinting and other DNA technologies (ADNAT) and the Centre for Cellular and Molecular Biology (CCMB) conduct a two-day symposium. The symposium is followed by a two-week training course. The theme of this year's symposium, the sixth in the series, was Functional Genomics. The 13 talks and 21 posters covered topics in four broad areas: genomics, proteomics, functional analysis and computational methods.

The DNA microarray has become the emblem of genomics. Microarrays are slides on which 10 to 100,000 spots of known DNA oligomer sequences (up to about 60-mer) are arrayed and used to probe complex mixtures of RNA or DNA by molecular hybridization. In his keynote address, Southern (University of Oxford, UK) reviewed some applications of this technique. Microarrays have been used to identify the subset of true genes and true exons from amongst the larger set of genes and exons predicted from the human genome sequence. In fact, one can now define genes as groups of co-expressed exons. Another example was the use of microarrays to profile clinical subtypes of cancers (for example, diffuse, large B-cell lymphoma). Other applications include the analysis of gene deletions, amplifications and point mutations. Southern also reviewed different methods for making microarrays such as pin-spotting, ink-jet techniques, and *in situ* synthesis.

More traditionally, genomics has relied on expressed sequence tags (ESTs). Justen Andrews (Indiana University, USA) described the generation of more than 3000 ESTs from a cDNA library prepared from 1500 *Drosophila* testes. About 33% of the ESTs corresponded to genes that hitherto had only been predicted from the *Drosophila* genome sequence analysis and a significant proportion (13%) was missed in the sequence annotation. Andrews maintained that a shallow analysis of tissue-specific cDNAs still remained the most cost-effective way of genome analysis. His long-term objectives are to look for downstream targets of the germline sex-determination transcription factors encoded by the *ovo* locus. He also gave a brief overview of the effort to use *Daphnia* as a model system for evolutionary genomics. *Daphnia* is interesting because it responds to the presence of fish predators by making more defensive morphological alterations. A map is being prepared using > 2000 microsatellite loci as well as cDNA and arrayed cosmid libraries. Alok Bhattacharya (Jawaharlal Nehru University, New Delhi) reviewed the status of genome studies in *Entamoeba histolytica*. This protozoan's genome has several unusual features: it has both linear and circular chromosomes, the chromosomes do not appear to share the same ploidy and they begin to show considerable size polymorphisms over prolonged periods in culture. The entamoeba retrotransposon-like element (EHRLE) has not yet been seen to move and most genomic copies are degraded. That is, the open reading frame (ORF) is riddled with mutations. But Bhattacharya found evidence for the presence of at least one copy with an intact ORF, which suggests that there is some selection pressure for its maintenance. A. R. Reddy (University of Hyderabad, Hyderabad) described the development of ESTs in the Nagina variety of indica rice. This variety shows drought-induced alterations in gene expression patterns. It is hoped that the ESTs will aid in the identification of useful markers in drought-tolerance breeding programmes.

Proteomics is essentially the analysis of proteins by a combination of high-quality two-dimensional gel electrophoresis together with robotics to help analyse selected protein spots by mass spectrometry. In describing the principles of MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) mass spectrometry, Uwe Rapp (Bruker Daltonik GmbH, Germany) covered topics in sample preparation and in identification of proteins which produce relatively few tryptic fragments. The MALDI-TOF technique is now sensitive enough to pick up signals from femtomolar or sometimes even sub-femtomolar concentrations of proteins and peptides. Tom Slykar (Bio-Rad Labs, USA) discussed the proteomes of two bacterial pathogens of man, *Campylobacter jejuni* and *Helicobacter pylori*, that were obtained with the ProteomeWorks system. Remarkably, the 25 most abundant proteins in each proteome included three of unknown function. He described the efforts to prefractionate membrane proteins of *H. pylori* to enrich the less abundant proteins for use as vaccine candidates. S. Pandey (University of Windsor, Canada) presented the human neuronal teratocarcinoma NT2 as a model for oxidative, stress-induced neuronal cell death that can be prevented by pretreatment with CoQ10. Pandey is now using proteomics to try and identify the proteins that trigger the cell-death process. He presented initial results on the differences found in the mitochondrial proteome of dying cells.

A systematic and comprehensive ENU mutagenesis screen for dominant and recessive mutations is being undertaken in the mouse. This has resulted in the establishment of more than 400 mutant lines (371 dominant and semi-dominant, and 38 recessive) with relevance to human clinical disorders. Johannes Beckers (GSF, Munich, Germany) described these screens and also the phenotyping of some selected ENU-induced mutant lines by high-throughput RNA expression profiling. A high-quality clone set of 20,000 clones containing only the 3′ untranslated sequences without the poly A tails and coding regions has been prepared. Care has been taken to use only those ESTs that give good specific-binding (i.e. showing sigmoidal binding pattern as the hybridization stringency increases); ESTs giving relatively unspecific hybridization profiles have been filtered out. All this contributes to obtaining high-quality expression profile data. M. R. S. Rao (Indian Institute of Science, Bangalore) described his group's

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isolation of an interesting 1.3 kb rat genomic segment (meiotic repair fragment) in experiments designed to isolate rat genomic sequences displaying aphidocolin-insensitive BrdU incorporation. This sequence is rich in sequence motifs associated with replication, recombination and transcription, and it is conserved in mouse and humans. Using radiation hybrid panels the fragments were mapped to mouse chromosome 8C-D and to human chromosome 3p25-26. The sequences have been subjected to intense bioinformatics analysis which resulted in the identification of sequences important for replication, recombination and transcription. They suspect that this fragment might function as a recombination hot spot. A challenge for the future will be to test this prediction in an appropriatively designed transgenic system.

Rakesh Mishra (CCMB, Hyderabad) reviewed the large body of experimental evidence which shows that many non-coding sequences in *Drosophila*, yeast and mammals function instead as 'chromatin domain boundaries'. These boundaries set up higher order regulation and they appear to be composed of specific combinations of small sequences rather than having large consensus sequences. This theme was further developed in two poster presentations from his group [by Vivek Chopra (transgenic fly boundary elements) and Rashmi Pathak (proteins associated with Polycomb response elements found in *Drosophila* boundaries and their interactions with the nuclear scaffold)]. S. Subramanian (CCMB, Hyderabad) described the compilation of a database of human simple sequence repeats (SSRD). Using this database he found that a majority of GATA repeats on the Y chromosome are close to the matrix-associated regions (GATA-MAR) and thus might play a role in coordinate expression of domains in the Y chromosome. GATA repeats were absent from the newly recruited AZFc region of the Y chromosome, which suggested that this repeat may serve as a 'temporal landmark' in Y-chromosome evolution. He also identified more than 2135 genes predicted to contain CAG or CGG triplet repeats. These genes might represent candidates for triplet-repeat expansions that can cause disease phenotypes.

Samir Brahmachari (Centre for Biochemical Technology (CBT), New Delhi) described the PLHost (peptide library based homology search tool) software developed by the CBT. Using this software his group has identified all the peptide modules present in the predicted proteomes of 43 bacterial and ten archael species for which genome sequences are available. All the proteins used a limited number of peptide modules, thus reflecting the limited number of solutions that nature has found for proteins that are compatible with their solubility, foldability and stability as well as function. The aim of this exercise was to identify any protein structural module that was present as an 'Achilles heel' (i.e. present in a pathogen, e.g., *M. tuberculosis* and absent from the host's proteome), that might therefore define a drug target. K. Guruprasad (CCMB, Hyderabad) discussed *in silico* computational techniques to help assigning gene functions.

Not surprisingly, the poster session was thematically more eclectic. The following list is incomplete and intended only to illustrate the range of topics covered: In agriculture and plant biology, screening of more than 15,000 wheat ESTs for SSRs for mapping, SSR-based QTL mapping for grain protein content of wheat.

Identification of wheat varieties containing the short arm of rye chromosome 1 (1RS) that carries many agronomically desirable genes (resistance to fungi, insects and high yield).

Homogenization of inter rDNA gene spacer length at two unlinked wheat loci. Evidence for two genes for fusarium wilt resistance in chickpea – one which maps about 18.3 cM of SSR-4 marker. Chloroplast/mitochondria inheritance in interspecific pearl millet hybrids. Microsatellite markers in coffee. Increased salinity-tolerance of *Arabidopsis thaliana* transformed with the yeast *ENA1* gene.

In poultry and animal husbandry, diagnosis of *E. coli* infection in chicken by PCR (heteroduplex analysis) of 16S rRNA, possible association of infectious bursal disease (IBD)-resistance with a growth hormone (GH) gene polymorphism, estimation of genetic distance between two breeds of cattle by RAPD-PCR.

In human disease, ocular infection by Acanthameba and keratitis, enzymes for beta-ameloid production. In biocomputing, the PARAM 10,000 distributed-shared memory system (a cluster of connected Sun Ultra e450 Workstations) for sequence analysis applications.

In fungal biology, a novel approach to clone fungal meiotic drive elements. It is probably safe to now sound the 'all clear' from the hype and antihype that marked functional genomics in its more juvenile stage.

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Muon anomalous magnetic moment and 'new physics'

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On 8 February 2001, scientists at the Brookhaven National Laboratory in Upton, New York, in collaboration with researchers from 11 institutions from the US, Russia, Japan and Germany announced a precision measurement of the anomalous magnetic moment ($g-2$) of the muon[1]. By itself, this would not have been of momentous consequence if it were not for the fact that the value deviates substantially from that predicted by the so-called Standard Model of Particle Physics. Before we go into the details of the results and the consequences of the measurement, let us summarize briefly some basic ideas about the anomalous magnetic moment of particles.

The proportionality constant between the magnetic moment $\vec{\mu}$ and spin $\vec{s}$ of a particle is given by the gyromagnetic ratio g

$$\vec{\mu} = g\left(\frac{e}{2m}\right)\vec{s}, \tag{1}$$

which for the muon can be written as

$$\mu_\mu = (1+a_\mu)\frac{e\hbar}{2m_\mu}, \text{ where } a_\mu = \frac{(g-2)}{2}. \tag{2}$$

The Dirac equation, which is the relativistic generalization of the Schrödinger equation for spin-half particles, predicts $g = 2$ which is a consequence of the minimal coupling of the photon to the electron or muon, ($\bar{\psi}\gamma_\mu\psi A^\mu$). However quantum electrodynamics (QED) predicts that

$$G = 2 + O(\alpha) + \ldots,$$

where α is the electromagnetic fine structure constant. This correction comes from a term generated at higher order in QED ($\sigma_{\mu\nu}F^{\mu\nu}$) that is not present in the Lagrangian and gives essentially a spin-orbit coupling of the spin to the magnetic field $\vec{\sigma}\cdot\vec{B}$. This is the anomalous magnetic moment, the deviation from the relativistic quantum mechanical value of 2 and comes from diagrams of the kind shown in Figure 1.

The electron magnetic moment has been measured to a few parts per billion and is described by QED to $O((\alpha/\pi)^4)$, and is presently limited only by our knowledge of the fine structure constant. In fact, the electron magnetic moment measurement is one of those used to determine the value of α – the others being the measurements of the quantum Hall effect and AC Josephson junction.

Today QED is the most stringently tested and most dramatically successful of all physical theories. Thus *any new particle that couples to e or* μ *will produce a correction to* $g-2$. *Since the QED prediction is so precise, it allows us to severely constrain the coupling strength of these new particles.* Although a_μ is not competitive in precision with a_e, it is much more sensitive to electro-weak loop effects as well as 'new physics' which give contributions which are more sensitive by a factor $(m_\mu/m_e)^2$, i.e. an enhancement of 4×10^4 in sensitivity. This is the reason muons rather than electrons are used in this experiment.

We briefly describe next the experimental set-up. Longitudinally polarized μ^+ at 3.09 GeV/c (we will see later the reason for choosing this energy) from a secondary beamline is injected into a storage ring 14.2 m in diameter with a homogeneous perpendicular magnetic field of 1.45 T. An electric quadrupole field is used for vertical focusing. The cyclotron frequency is given by the well-known result

$$\omega_c = \frac{eB}{mc},$$

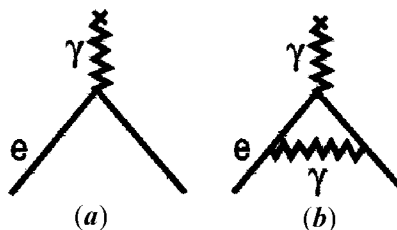

Figure 1. QED corrections to ($g-2$).

which is also the frequency of rotation of the momentum vector. The Larmor spin precession frequency is

$$\omega_s = g\left(\frac{eB}{2mc}\right) = (1+a_\mu)\frac{eB}{mc}.$$

Note that for $g = 2$, these two frequencies are equal! This means that the muon maintains its initial polarization vector with respect to the momentum vector. For any $g > 2$, the spin turns faster than the momentum vector. For relativistic muons, these formulae change appropriately and one can write down the 'difference angular frequency' ω_a between the spin precession frequency and cyclotron frequency

$$\vec{\omega}_a \equiv \vec{\omega}_c - \vec{\omega}_s = -\frac{e}{m}\left[a_\mu\vec{B} - \left(a_\mu - \frac{1}{\gamma^2-1}\right)(\vec{\beta}\times\vec{E})\right]. \tag{3}$$

Note that the above result is true for relativistic muons. The dependence of ω_a on the electric field can now be eliminated by storing muons with 'magic' $\gamma(\mu) = 29.3$, which eliminates the second term above and corresponds to a muon energy of 3.09 GeV/c. A measurement of ω_a and B now determines a_μ.

We now turn to the theoretical aspect of the contribution $g-2$. The Standard Model value of a_μ comes from all corrections from within the standard model of particle physics and can be written as

$$a_\mu = a_\mu(\text{QED}) + a_\mu(\text{hadronic}) + a_\mu(\text{weak}), \tag{4}$$

and as we have already stated, any discrepancy between the measured value and above value will indicate new physics. Since the electron $g-2$ agrees with the QED value to within a few parts per billion, it is used to determine α, which in turn gives us the value of the first term[2].