Meeting report

Physiology engages with functional genomics - at last

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A report on the XXXV International Congress of Physiological Sciences, held together with Experimental Biology 2005, San Diego, USA, 31 March - 6 April 2005.

A massive meeting with an estimated 15,000 delegates and accompanying persons brought together a world physiology congress, seven national American learned societies, and a further 30 guest societies in San Diego in March. The byline for the meeting was 'From genomes to functions', so a high degree of relevance to genome biology could be hoped for. The standard of talks in the 1,024 sessions was high, and many described the use of post-genomic technology in their work. This was refreshing, given that the disconnect between genomic technologists and functional biologists, such as physiologists, is probably the largest obstacle to true functional genomics at present. It was good to see the widespread deployment not only of arrays and proteomics, but also of comparative genomic approaches to the evolution of development (evo-devo), together with the use of simpler model organisms where appropriate.

High-throughput genetics

The most impressive contributions to high-throughput functional genomics came from combined genotype/phenotype approaches in model organisms. Perhaps unusually for such meetings, the presidential address by Allen Cowley (Medical College of Wisconsin, Milwaukee, USA) was the most spectacular research contribution. The premise was simple: to perform quantitative trait locus (QTL) analysis for heart and renal phenotypes in wild-type and established mutant rat strains. The scale of execution is remarkable: essentially a whole physiology department [http://pga.mcw.edu] has converted to phenotyping hundreds of thousands of rats in unprecedented detail (more than 200 traits measured for each rat, and over a third of a million measurements to date). The project has already generated interesting results, for example that most of the hypertension-related traits in rats are sexually dimorphic. It has also generated valuable resources, including a collection of rat chromosome substitution strains (CSS, also called 'consomic' strains). They are based on the Dahl salt-sensitive strain; in each line, one chromosome of the Dahl strain has been individually replaced (by repeated back-crossing) with the corresponding chromosome of another strain, BN. Some of these strains are now available commercially [http://www.criver.com] and provide a valuable resource for rapid identification of chromosomes carrying a particular genetic trait.

Another example of the heroic use of genetics to provide a functional analog of high-throughput research is 'Neuromice' [http://www.neuromice.org], part of the mouse initiative currently being carried out with National Institutes of Health funding [http://www.nih.gov/science/models/mouse]. The collaboration, described by Joseph Takahashi (Northwestern University, Evanston, USA), between three mutagenesis programs - at Northwestern University, the Jackson Lab (Bar Harbor, USA), and the University of Tennessee (Knoxville, USA) - is relying on ethylnitrosourea (ENU) mutagenesis and a three-generation screen to provide a range of more subtle phenotypes than simple knockouts. Mice are being screened for a wide range of neural and behavioral phenotypes at the rate of 10,000 per year (40,000 planned over 4 years) and 155 strains are searchable online so far. The phenotypes found to date include circadian defects, circling, and satiety defects.

Automated screening and tissue harvesting

Moving to simpler model organisms, Todd Lamitina from Kevin Strange's group (Vanderbilt Medical Center, Nashville, USA) reported the use of the COPAS sorting system [http://www.unionbio.com] to powerful effect to

study an osmoregulation phenotype in the nematode *Caenorhabditis elegans*. COPAS is effectively a multiparameter cell sorter, but can also be used to detect different phenotypes in whole transgenic organisms, including the fruitfly *Drosophila melanogaster*, the zebrafish *Danio rerio*, and the cress *Arabidopsis thaliana*. It is possible to sort not just on size or fluorescent intensity, but even on the profile of fluorescence along the length of an organism. Small organisms can be sorted - live - into multiwell dishes for further analysis. For traditional 'forward' genetics, the advantage is that a saturating mutagenesis screen can be performed almost unsupervised. For example, 300,000 ENU-mutant worms were screened for altered expression of enhanced glycerol dehydrogenase tagged with green fluorescent protein (eGFP), indicative of failure to osmoregulate, and 80 lines were isolated. In a further screen but this time working 'in reverse', from gene to phenotype, worms carrying a GFP reporter for an osmotically relevant gene were exposed to the worm RNA interference (RNAi) feeding library and then screened for suppression of enhancement of fluorescence. (The feeding library is a collection of *Escherichia coli* containing plasmids expressing RNAi constructs against virtually the whole predicted worm transcriptome [http://www.openbiosystems.com/clones_c_elegans_orf.php].) More than 130,000 worms were screened in a little over a day, leading again to around 80 genes being implicated as relevant. It is thus clear that, with a little ingenuity in experimental design, this equipment allows effectively saturating genetic screens (forward or reverse) to be performed with great speed.

One of the problems in both proteomics and transcriptomics is the difficulty of obtaining distinct tissues in sufficient quantity and purity for useful analysis. R. Lance Miller (University of Utah, Salt Lake City, USA) also reported the use of COPAS, in this case to select kidney collecting-duct regions enriched for principal or intercalated cells, based on GFP reporters driven by aquaporin or V-ATPase promoters, respectively. The yield obtained by this method was sufficient for proteomic analysis. The average length of collecting duct regions was 260 μm, and was obtained with good morphological preservation. This technique addresses the problem of trying to study a compact but immensely complex tissue like the kidney; such screens could thus be valuable for a range of physiological, pharmacological or molecular endpoints.

Bright prospects in imaging

Roger Tsien (University of California at San Diego, USA) and Atsushi Miyawaki (RIKEN Brain Science Institute, Wako-city, Japan) described novel reporters for imaging in relation to large-scale screening. Tsien described the re-engineering of the cameleon series of calcium reporters to make them brighter, less sensitive to attenuation by endogenous calmodulin and suitable for use as stable reporters for imaging the endoplasmic reticulum. Lisa Leon Gallegos (University of California at San Diego) described a new fluorescence resonance energy transfer (FRET) reporter (called CKAR) for protein kinase C (PKC) that enables PKC activity to be imaged within a cell. Miyawaki revealed a set of marine photoproteins that switch between two colors upon exposure to light of a particular wavelength. Subsequent to the description in 2002 of the original 'Kaede' fluorescent protein from coral, which switches from green to red fluorescence under UV or violet light, different proteins have now been found that can switch reversibly or irreversibly, suggesting applications anywhere from transgenic cell marking to organic memory for computers. Indeed, the writing lasers used to switch Kaede proteins from one state to another in single cells were those from 'blu-ray' DVD drives. Kaede transgenic mice have already been made.

In the 'breakthrough technologies' session, Robi Mitra (Washington University, St Louis, USA) described progress in single-molecule DNA sequencing. Typically, single PCR amplimer molecules are suspended in a gel on a microscope slide to provide individual PCR colonies ('polonies'). Incorporation of single fluorescently tagged nucleotides is alternated with reading each colony by fluorescence microscopy. Although the maximum length achievable at present is of the order of tens of bases, the massive parallelism of the approach offers potential advantages both for genotyping and *de novo* sequencing.

Elsewhere in the genomics track that ran through the meeting, Hans Westerhoff (Free University, Amsterdam, The Netherlands) nicely illustrated the utility of the systems biology approach in providing models for predicting cellular functions, currently being developed in the 'silicon cell'. He was able to illustrate that by integrating separate models into systems-level analysis, predictions could be made that are sufficiently counterintuitive that they would not have been arrived at by human insight alone. As well as describing work modeling yeast cell behavior, he showed that critical steps for pharmacological intervention could be predicted by this approach, for example in the parasite *Trypanosoma brucei*. Several other speakers clearly had an eye on the transition to systems biology. The need to produce models in an integratable, generic form was highlighted by Poul Nielsen (University of Auckland, New Zealand), who discussed the development of the language CellML, based on the XML markup language, for the description of biological models [http://www.cellml.org]. Mike Cherry (Stanford University, USA) illustrated the progress made in improving the already excellent annotation of the yeast genome [http://www.yeastgenome.org], by comparing yeast sequence with shotgun sequences of a range of related species. A large number of ATG sites had been reassigned, and 800 genes from the original annotation had been reassigned as dubious. Improved links to gene ontology, and to pathway tools such as Peter Karp's collection of pathway/genome databases [http://www.biocyc.org], were also continuing to add utility to the yeast sequence.

Back in humans, Kelly Frazer (Perlegen Sciences, Inc., Mountain View, USA), described the use of biochips for detecting single-nucleotide polymorphisms (SNPs) to look at differential expression of the two haplotypes for each gene. Such differential expression had been thought to be the property of relatively few imprinted genes, but a systematic analysis of several thousand genes suggests that as many as 20% of human genes might be differentially expressed in any individual, and that this percentage varies widely between individuals. The implications for pharmacogenomics - the tailoring of drugs to the individual patient - are obvious. Eric Green (National Human Genome Research Institute, Bethesda, USA) described the ENCODE project [http://www.nisc.nih.gov/open_page.html?/projects/encode/index.cgi], which seeks to identify all the functional elements in the human genome by massive parallel sequencing of related species. The project has provided evidence that it is possible to delineate not just the 1.5% of the human genome that is protein coding, but a further 3.5% that is non-coding but still under selection.

Overall, the meeting provided clear evidence that functional biology is not just exploiting, but adding value to, post-genomic endeavor.