Meeting report

A wormer's eye view

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A report on the thirteenth international *Caenorhabditis elegans* meeting, University of California, Los Angeles, USA, 22-26 June 2001.

In the last decade, billions of dollars have been spent on gathering genomic sequence from many genomes, providing invaluable groundwork for informed research. As the first metazoan to have a completely sequenced genome, the nematode *Caenorhabditis elegans* (referred to as 'the worm') has in some sense been the 'animal torchbearer' in the post-genomic world, and examining how the field has advanced in the last three years (since the genome was published) provides some insight into the real impact of genomics on the study of a model organism. The recent International Worm Meeting (IWM), attended by the great majority of 'wormers', gave a very comprehensive overview of the current state of play in the worm field and, rather than concentrate on individual talks, I summarize here some of the genomics-related progress that has been made in this field over the last few years.

Perhaps the most evident (and for the organizers the most difficult) change has been the rapid growth of the worm community, the number of participants having doubled since 1997. Many participants gave as the principal reasons for conversion to the worm the early availability of genomic sequence, the ease with which one can learn how to use worms (many citing the annual course at the Cold Spring Harbor Laboratory as a particular favorite) and, perhaps most of all, the ability to use RNA-mediated interference (RNAi) to generate loss-of-function phenotypes of individual genes.

RNAi

RNAi is now such an integral part of the gene-analysis arsenal in the worm that, although only around five years have elapsed since its discovery, already over 25% of the 1,100 abstracts presented at the IWM involved RNAi-based experiments - a phenomenal rise. Like so much other worm technology, RNAi was pioneered, amongst others, by Andy Fire (Carnegie Institution of Washington, Baltimore, USA), who demonstrated that RNAi can be activated in worms merely by feeding them bacteria expressing double-stranded RNAs (dsRNAs), a miraculously easy and efficient technique.

The Ahringer lab (Wellcome/CRC Institute, Cambridge, UK) has made use of this feeding method to begin the ambitious project of analyzing by RNAi certain loss-of-function phenotypes of every predicted gene. This requires the construction of a library of approximately 19,000 dsRNA-expressing bacterial strains, each targeting a single gene; this library, once complete, can be used for an unlimited number of RNAi-based genome-wide screens for any particular phenotype. Already approximately 45% of genes have been cloned and their RNAi phenotypes screened in wild-type worms by the Ahringer lab, and adding to this the beautiful RNAi screen of chromosome III carried out by the Hyman lab (Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany), the RNAi screen based on expressed sequence tags (ESTs) by Asako Sugimoto (University of Tokyo, Japan) and the smaller-scale analysis by the Kemphues lab (Cornell University, Ithaca, USA), well over half of all *C. elegans* genes have now been analyzed by RNAi.

Large-scale RNAi screens have identified the great majority of embryonic lethal (Emb) genes on chromosomes III and I, and most of the Emb phenotypes have been analyzed by time-lapse videomicroscopy. For example, Fabio Piano from the Kemphues lab showed movies of the phenotypes resulting from RNAi-mediated loss-of-function of mutation of Emb genes present in a set of genes that is known to be expressed in oocytes. These movies have identified the early embryonic defects that give rise to lethality for each gene, thus defining roles for many new genes in embryogenesis as well as providing visually beautiful biology. Moreover, other labs including the Hengartner lab (University of Zürich, Switzerland), the

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Ruvkun lab (Harvard Medical School, Boston, USA) and the Kenyon lab (University of California, San Francisco, USA) are using the RNAi-feeding library developed in the Ahringer lab for other screens, including screens for genes involved in apoptosis, the DNA-damage response, and aging. RNAi-based approaches, whatever the precise RNAi technique, are proving very fertile areas for research, and there seems little doubt that by the time the 2003 IWM rolls around many such screens will have been done.

Caenorhabditis briggsae

While the IWM mainly revolves around *C. elegans*, other nematodes are also represented, and one of the most exciting pieces of news at the meeting was the announcement by Richard Durbin (The Sanger Centre, Cambridge, UK) that the *C. briggsae* genome project has at long last been given the green light by funding agencies. *C. briggsae* diverged from *C. elegans* around 20-40 million years ago and, although coding regions are in general well conserved between species, non-essential non-coding regions have drifted greatly. Comparison between the non-coding regions of *C. elegans* and *C. briggsae* can therefore prove incredibly valuable both for identifying regulatory sequences in promoters and enhancers and for finding conserved untranslated RNAs. In particular, the analysis of promoters and enhancers, when coupled to the large amount of microarray data generated by Stuart Kim's lab (Stanford University, USA), should be invaluable in determining the transcriptional circuitry of the worm.

High-throughput approaches

One particularly interesting question arising from multiple array experiments is that of which genes tend to be co-regulated not just under a few conditions but under all conditions. Kim presented a way of depicting these co-regulated clusters as topographical features on a three-dimensional map of gene correlations, revealing 'features' such as 'Mount Sperm', where many sperm-expressed genes co-cluster. Although it may take some time to get used to this new type of representation and understand how such features relate to biology, it is clear that the human eye is far better at understanding images than at poring over tables of numbers, so in the long run this type of representation is likely to be very informative.

An excellent integration of array data, sequence and biology was presented by Jeb Gaudet from Susan Mango's lab (University of Utah, Salt Lake City, USA). PHA-4, a forkhead transcription factor, is expressed in the nematode pharynx, and its expression is both necessary and sufficient for pharangeal cell specification. Gaudet identified by microarray analysis putative targets of PHA-4 and found that these targets have PHA-4-dependent expression that requires predicted PHA-4-binding sites. Furthermore, not only did PHA-4 bind the predicted sites *in vitro*, but also the strength of binding to each site correlated well with the time of expression. This correlation was shown to be significant by altering the PHA-4 binding site from low to high affinity, which converted late expression into early expression.

Aside from RNAi-based techniques and microarrays, there are many other tools, techniques and databases that should increase the speed of data gathering in the worm field. Yuji Kohara's group (National Institute of Genetics, Mishima, Japan) is carrying out *in situ* expression analysis of a large set of genes, a wonderful resource that should prove an excellent counterpart to microarray data; the ability to check tissue expression for many genes at all developmental stages is a huge help in filtering through genomic data. The Plasterk lab (Centre for Biomedical Genetics, Utrecht, The Netherlands) has recently generated a high-resolution single-nucleotide polymorphism (SNP) map of the worm genome, which includes many markers - so-called 'snip-SNPs' - that modify restriction-enzyme-recognition sites. They found a snip-SNP, on average, approximately every 20 kilobases. This should be tremendously useful for the mapping of genetic loci down to a small genomic region. The use of SNP mapping to map a locus down to a small region followed by RNAi of predicted genes in the defined region is already proving a very powerful combination, illustrating perfectly the interlocking and mutually beneficial properties of classical genetics and RNAi.

One of the most useful resources currently being developed is the ORFeome, in an amazing project carried out in the Vidal lab (Dana-Farber Cancer Institute, Boston, USA). The objective is to clone and sequence full-length open reading frames (ORFs) corresponding to each predicted gene in the genome. Each ORF will be cloned into a GATEWAY-compatible vector, thus allowing shuttling of the ORF from this vector into any other merely by carrying out *in vitro* recombination cloning. Thus, any ORF (once available) can be used to generate any desired construct rapidly and easily - no more sequencing, no more restriction maps, and no partial digests and compatible ends are required. In short all gain and no pain. The ORFeome is already over half complete and, once finished, will make work a pleasure.

The enormous amount of data already being generated necessitates an efficient and well-structured database as the bedrock informational resource for the community. WormBase [http://www.wormbase.org] has served as such a repository of mapping, sequencing and phenotypic information about *C. elegans* for the last few years and it is excellent news - announced by Paul Sternberg (California Institute of Technology, Pasadena, USA) - that the scope of WormBase is to be expanded to include expression data, Gene Ontology classification of predicted genes (using a controlled gene-description vocabulary that can be applied to all eukaryotes) and many other key data; in addition, the group of dedicated annotators

is to be expanded, ensuring that WormBase will continue to be the key academic informatics resource for wormers.

Technological frontiers

Despite the tremendous progress made over the last few years, there are still key techniques missing from the worm field. The most glaring holes in 'wormology' are, first, the continuing nuisance of being unable to make targeted knockouts and, second, the absence of any transposon system that is suitable for carrying out exon and/or enhancer traps. Targeted-knockout technology was discussed by many people at the meeting, who thought it should be possible; the next few years will tell whether it is. But whenever someone cracks this problem, it will be a tremendous advance for the community; the rather disappointing rate at which deletion worm strains are being created at present means that the creation of genetic null mutations is a real bottleneck. The transposon technology is still advancing, in particular thanks to work by the Jorgensen lab (University of Utah). But, although *mariner*-based transposons now appear to jump in the worm with an efficiency that would permit exon and/or enhancer-trap screens, there remains a major problem: the current transposons still cannot carry sufficient DNA to allow tagging with green fluorescent protein (GFP) or lacZ reporter genes; there is thus still some way to go. Again, though, progress in this area appears to be incremental and consistent, so the long-term prospects for the resolution of this problem are encouraging.

Biological frontiers

Ultimately, whatever the tools and reagents available in the worm, these are simply the means to the end of carrying out careful and detailed biology. There is not sufficient space to present more than a fraction of excellent talks from the meeting, but what follows may give some idea of the tremendous breadth of the worm field, ranging from axon guidance to DNA damage, from lipid metabolism to early embryogenesis, and from meiosis to chemotaxis.

Particularly remarkable at the meeting was the large number of presentations concerning chromosome biology, including meiosis, mitosis, regulation of centrosomes, and DNA-damage sensing and repair. For example, Kenneth Hillers from the Villeneuve lab (Stanford University) presented intriguing data showing that meiotic crossing over in the worm is regulated, such that a single crossover occurs per homolog pair in meiosis, and Amy MacQueen from the same lab set out to do a detailed analysis of genes required for the establishment of homolog pairing and the formation of the synaptonemal complex. Simon Boulton from the Vidal lab presented an awesome, comprehensive two-hybrid interaction map of known homologs of all genes known to be involved in DNA-damage response in other organisms; novel interactors that were identified were further analyzed using RNAi.

Many groups presented diverse work on signal transduction in the worm looking at a range of pathways, including the Wnt, Ras and TGF-β pathways. Perhaps most progress occurred by analysis of G-protein signaling pathways: G-proteins play a role in locomotion and egg-laying and, earlier in development, both in spindle positioning and in the establishment of left-right polarity in the early embryo.

Finally, any account of a worm meeting would be incomplete without mentioning the beautiful work on axon guidance and neuronal function, as this is one of the areas in which the worm is at the forefront of research. Again, the range of approaches and analyses is great, and I would like to mention just a single outstanding presentation, that of Maria Gallegos from the Bargmann lab (University of California, San Francisco, USA), presenting her cloning of *sax-1* and *sax-2* genes, which suppress neurite outgrowth. Worms that carry mutant *sax-1* display normal initial axon outgrowth and guidance, but later, ectopic neurites form. The *sax-1* gene turns out to encode a serine/threonine kinase protein that has a homolog in flies (Tricornered) that, when mutated, results not in additional ectopic neurites, but in ectopic winghairs. Thus, there appears to be a conserved biological module that regulates some aspect of the initiation of focal growth. Furthermore, *sax-2* encodes a homolog of *Drosophila* Furry, which, when mutated, causes a similar phenotype to *tricornered*, confirming the biological similarity.

In summary, one will soon be able to target approximately 90% of worm genes by RNAi simply by feeding dsRNA-expressing bacteria to worms, to order any full-length ORF in a GATEWAY vector; map any genetic locus down to a few candidate genes within a couple of weeks by SNP mapping; examine the *in situ* expression patterns of many genes just by dialing up a database; and look at the expression profiles of any gene in any one of hundreds of different chip experiments. The technologies are all there, the reagents are coming fast, and when these are combined with detailed knowledge of the biology, the worm field will no doubt continue both to grow and to accelerate.