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## **A11C6E2–COMPARATIVE FUNCTIONAL GENOMICS**

**Organised by A. El Haj, M. Thorndyke and J. Murray for the Comparative Genomics Group**

### **A11/C6/E2.1–Toxicogenomics of marine brevetoxins**

P.J. Walsh and J. Zaias, Marine Biology and Fisheries, University of Miami; R.J. Bookman, Molecular and Cellular Pharmacology University of Miami; D.G. Baden and A. Bordelais, Marine Sciences, University of North Carolina, Wilmington; M.F. Oleksiak and D.L. Crawford, Biological Sciences, University of Missouri

The marine brevetoxins (PbTx) are multi-cyclic polyether toxins produced by dinoflagellates (e.g. *Karenia brevis*) that are responsible for fish kills associated with harmful algal blooms known as 'red tides'. In humans, one noteworthy route of exposure is via aerosols associated with breaking surf on the beach. PbTx are potent and acute activators of voltage-gated sodium channels, and thus can have a variety of impacts on the nervous system depending upon dose and pharmacokinetics. In the extreme, they can result in respiratory paralysis and death. At lower doses, PbTx can have more indirect and chronic effects, yet these alternative pathways remain to be elucidated. In prior work, we discovered that one isoform, PbTx-6, binds substantially to the aryl hydrocarbon receptor (AhR) with approximately 50% of the avidity of classic dioxin substrates. Thus, there is potential for multiple pathway level responses through phosphorylation/dephosphorylation and by direct activation of gene transcription mediated by AhR. To test this hypothesis and to examine which genes may be effected, two model organisms, mouse and killifish (*Fundulus heteroclitus*) were dosed with sub-lethal levels of purified PbTx-6, and global mRNA expression patterns were assessed by microarray studies, along with classic histological examination of tissues. This presentation will report preliminary analysis of altered patterns of gene expression, including a potentially novel interaction of PbTx with expression of genes encoding proteins that regulate metal metabolism, transport and storage. (Supported by NIEHS.)

### **A11/C6/E2.2–Evolutionary history of the Hox gene complex**

Pedro Martinez, Department of Anatomy and Cell Biology, University of Bergen, Norway

Homeobox-containing genes are a major class of transcription factors regulating many aspects of develop-

ment. Genes with homeoboxes have been found in all metazoans thus far examined. Based on several criteria, they can be grouped into approximately 20 different classes. Particular attention has been given to the class of HOM/Hox genes because these genes seem to provide cells with positional information according to their location along the different axes of the embryo/adult. Our long-term goal is to understand how the Hox gene family controls developmental processes and how their role has changed over evolutionary time.

We have cloned and characterized this complex in sea urchins using all sorts of high-throughput technologies, including arrayed libraries and BAC, PAC large construct mapping. In order to understand how HOX clusters have evolved over time, we plan to characterize comprehensively the genomic regions in which they are embedded, sequencing them at completion, in five different echinoderms. Moreover, we plan to sequence some areas of the mouse HOX cluster for comparison. These should reveal short and long-evolutionary variations in sequence patterns. Sequence comparisons inside the echinoderm groups should give us, specifically, the intraphyletic changes and comparing echinoderms and mammals the interphyletic ones.

A complementary, detailed, study of expression profiles in all these echinoderms should give us insights on the relationship between sequence changes in the genome and altered domains of expression.

Martinez, P., Rast, J.P., Arenas-Mena, C., Davidson, E.H. (1999). Organization of an echinoderm Hox gene cluster. Proc. Natl. Acad. Sci. USA. 96, 1469–1474.

### **A11/C6/E2.3–Abstract is missing**

### **A11/C6/E2.4–The basic helix–loop–helix transcription factors: comparative genomics and phylogenetic analysis**

Valérie Ledent, Belgian EMBnet Node-Service de Bioinformatique, Université Libre de Bruxelles, Département de Biologie Moléculaire. Rue des Professeurs Jeener et Brachet 12; B-6041 Gosselies, Belgium; Michel Vervoort Evolution et Développement des protostomiens, Centre de Génétique moléculaire- UPR 2067

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Basic helix–loop–helix (bHLH) proteins are transcription factors that play important roles during the development of various metazoans, including fly, nematode and vertebrates. They form a particularly large and complex multigenic family. We searched for bHLH sequences in the completely sequenced genomes of the worm *Caenorhabditis elegans*, the fly *Drosophila melanogaster* and the human. We found 39, 58 and 125 different bHLH, respectively, which are likely to represent the complete set of bHLH of these organisms. A phylogenetic analysis of these sequences led us to define 44 orthologous families. Forty-three of these families own both human and fly and/or worm genes, indicating that genes from these families were already present in the last common ancestor of worm, fly, and human. As this ancestor is also that of all Bilaterian animals, our analysis indicate that this ancient ancestor must have owned at least 43 different types of bHLH, highlighting its genomic complexity.

#### **A11/C6/E2.5–Unraveling the Australian lungfish genome**

J. Rock, Biology, University of Wales, Bangor; J. Joss and T. Longhurst, Biology, Macquarie University, Sydney, Australia

The Australian lungfish (*Neoceratodus forsteri*) has one of the largest genomes known among extant fauna, with a diploid chromosome number of  $2n=54$  and nuclear DNA content of 99–112 pg/nucleus. From paleontological evidence of change in cell size, it has been estimated that Devonian lungfish had smaller genomes than either group of extant lungfish. Within this lineage, *N. forsteri* is the oldest species and it has the smallest genome; this suggests that a trend towards increasing genome size may be characteristic of, and perhaps influential in, lungfish evolution. In most vertebrates, functional exons make up a very small percentage of the genome. Accordingly, we hypothesised that extremely large portions of *N. forsteri* genome would be comprised of unexpressed DNA components, such as repetitive elements or gene duplications. To date, preliminary data indicate a surprising lack of such components; for example, relatively few blocks of constitutive heterochromatin have been revealed. A variety of techniques are also currently being employed to define repetitive elements within the genome (e.g. using reassociation kinetics, and characterisation of RFLPs) and to describe aspects of the genome signature (e.g. estimates of GC content). Results from these analyses will clarify the structure and functional significance of genome size in the Australian lungfish and provide several parameters that can then be compared with other vertebrate genomes. Such comparisons have the potential to provide significant contribu-

tion to our knowledge of the functional importance of genome size in vertebrate evolution.

#### **A11/C6/E2.16–From protein to function: the chloroplast proteome network**

W. Gruissem, S. Baginsky, F. Kessler, T. Kleffmann, D. Russenberger, A. Siddique and A. von Zychlinsky, Institute of Plant Sciences, Swiss Federal Institute of Technology, ETH Centre, 8092 Zurich, Switzerland (wilhelm.gruissem@ipw.biol.ethz.ch; <http://www.pb.ethz.ch>)

Chloroplasts are unique plant cell organelles. Their differentiation from proplastids is regulated by tissue-dependent and developmental signals. In the absence of light, proplastids differentiate into photosynthetically inactive etioplasts. After illumination, etioplasts are converted into chloroplasts, which can also differentiate directly from proplastids. We are investigating chloroplast differentiation in rice and *Arabidopsis*. Using a combined proteomics and functional genomics approach, we explore the proteomes that determine plastid function in a cellular context and the role of proteins involved in chloroplast differentiation.

We have analyzed the proteome of chloroplasts and proplastids, and identified several hundred proteins using multi-dimensional HPLC fractionation and subsequent LC-ESI-MS/MS identification. The proteins are part of a functional network that we construct with computational methods. To identify plastid proteins that are regulated during chloroplast differentiation, we displayed etioplast and chloroplast proteins by two-dimensional PAGE. Proteins that differed in abundance were subsequently sequenced by mass spectrometry. Together, we have identified several candidate proteins of potential importance for plastid function and differentiation. The function of these proteins is being assessed in a high-throughput phenotype screen of *Arabidopsis* T-DNA lines, in which the genes of interest are disrupted. We focus primarily on hypothetical proteins or proteins of unknown functions. Mutants that revealed a chloroplast-related phenotype, such as the lack of chlorophyll accumulation or loss of autotrophic growth, are selected for closer analysis to gain insights into the molecular mechanism that is affected during chloroplast development.

#### **A11/C6/E2.17–NEMBASE: dealing with fragmented genomes**

J. Parkinson and M. Blaxter, University of Edinburgh

The advent of high-throughput sequencing has led to the development of a growing number of genome sequencing initiatives. Although this is leading to the generation of entire genomes for a few model organisms, the majority of other sequencing projects are limited to obtaining only incomplete (fragmented) genome data (involving just hundreds or thousands of sequences). Due to the

lack of a genomic scaffold, sequence data associated with fragmented genomes are often poorly organised, making it difficult to perform comparative analyses.

The Edinburgh parasitic nematode expressed sequence tag (EST) project has recently been initiated to generate ~350 000 sequences from 14 different species of parasitic nematodes. As part of this project, we have developed a unique fragmented genome database system—NEMBASE—which is being used to organise this data. In brief:

1. Sequence data is collated and clustered into homologous groups which are presumed to derive from the same gene.
2. The clusters are assembled to form consensus (putative 'gene') sequences.
3. Putative gene sequences are translated into peptide sequences and annotated with, e.g. functional/location predictions.
4. Data arising from these analyses is stored in a central database resource based on PostgreSQL.

The organisation of sequence data within a single resource, allows the formulation of complex queries (both locally and remotely via the use of web based forms), to perform comparative genomic analyses.

### **A11/C6/E2.18—Comparative approaches to understanding plant cell biology and cell proliferation**

J. Doonan, John Innes Centre, Norwich, NR4 7UH

The basic mechanism of cell proliferation is conserved throughout eukaryotes: cells remain quiescent until they receive a signal to initiate DNA synthesis; subject to certain checks, DNA is replicated; replicated DNA is checked and resolved and the cell enters mitosis during which time DNA is separated into two nuclei. In most cases, mitosis is immediately followed by cell division. Although the basic process is conserved, the details vary considerably. We have found that entry into the cell cycle in plants involves the D-cyclin/Rb pathway, analogous to that of animal cells, but responsive to plant specific growth factors. Plants contain a diverse family of D-cyclins and I will discuss their regulation and possible functions during leaf development in *Arabidopsis* and *Antirrhinum*.

A c-Myb/B-cyclin pathway seems to control mitotic entry in plants but, in animals, this pathway is implicated in S-phase progression. A model of gene expression controls during plant cell division and development will be presented.

Finally, I will outline systematic approaches aimed at understanding the control of gene expression during the plant cell cycle and development.

### **A11/C6/E2.19—Identification of low prevalence differentially expressed sequences using the Genetix Gene Subtractor kit**

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For organisms where there is extensive sequence information available, differentially expressed sequences that are important in cellular growth and differentiation can be identified using microarrayed gene libraries on glass. Microarray technology is, however, expensive and labour intensive, and cannot be easily applied to organisms where there is little sequence information available. We have developed a kit to allow the identification of differentially expressed sequences from macroarrayed cDNA libraries of organisms where there is minimal sequence information.

The kit is based upon the methodology described in a paper by Rast et al. (2000). [Develop. Biol. 228, 270–286]. Although the methodology they describe was used to identify low prevalence differentially expressed sequences in the sea urchin, the techniques can be applied to any organism from which cDNA libraries and probes can be obtained. The cDNA library is arrayed in the form of bacterial colonies onto large (22×22 cm) nylon membranes (macroarrays) using a Genetix QPix robot. In contrast to microarraying, macroarraying requires little labour and is relatively inexpensive. However, the size of the macroarrays means that they are relatively insensitive because of the larger volumes required for hybridisation. Through a combination of total message amplification and subtractive hybridisation their sensitivity can be improved sufficiently to detect low prevalence differentially expressed sequences. A software package known as BioArray is also included in the kit for the analysis of hybridisation images. An important feature of this software is its ability to automatically detect spots and overlay a grid onto a hybridisation image with minimal intervention by the user.

### **A11/C6/E2.20—Bridging genetics and physiology: candidate gene approach to the study of the habitat-related variation in enzyme activities in intertidal snails (*Littorina* spp.)**

I.M. Sokolova, E.G. Boulding, Zoology, University of Guelph, Canada

Marine gastropods of the genus *Littorina* are key species in many intertidal benthic communities and a model object for numerous ecological, physiological and genetic studies. In some *Littorina* species, the parallel expres-

sion of distinct phenotypes associated with particular habitats in different geographic areas was reported and was suggested to be a result of parallel evolution due to local adaptation in response to similar selection pressures. In Pacific *L. subrotundata* and *L. sitkana*, two distinct ecotypes were found associated with salt marshes and the wave-exposed rocky shores.[EGB1] Our studies have shown that the periwinkles from the salt marsh and open shore habitats significantly and consistently differ in salinity tolerance and in their resistance to dehydration, suggesting local adaptation to the salinity regimes of the respective habitats. In order to investigate the genetic and physiological basis of the observed dif-

ferences in salinity tolerance, we are measuring the activities of key metabolic enzymes involved in isoosmotic cell volume regulation (aspartate and alanine aminotransferases and glutamate dehydrogenase) in the snails from salt marsh and open shore populations. We also develop degenerate primers that will be used to amplify the variable parts of the respective enzyme-encoding genes from the snail genomes. The gene sequences will be analysed for the single nucleotide polymorphisms (SNPs) that will be further used as anonymous *cis*-markers and correlated with the variation in enzyme activities in snails from different populations and habitat types. The preliminary results of this study will be presented.