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Meeting Report

The 30th anniversary Crete fly meeting

From individual molecules to 12 genomes, and beyond

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The 16th Biennial Meeting of the EMBO-Sponsored International Workshop on the Molecular and Developmental Biology of Drosophila was held in Kolymbari, Crete from June 23 to June 28, 2008. As in previous years, the weather was hot, the Mediterranean Sea was clear and blue and the presentations were exciting and illuminating.

Thirty years have elapsed since the first International Workshop on the Molecular and Developmental Biology of Drosophila was held at the Orthodox Academy of Crete, which maintains a Conference Center on the Mediterranean Sea, adjacent to the 17th century Gonia Monastery and a few minutes walk from the fishing village of Kolymbari. Since its inception, this premier meeting has gathered together about a hundred Drosophila researchers from around the world in this pastoral and picturesque setting, to discuss recent research findings from their laboratories. This meeting was noteworthy in the broad array of topics covered and in the scope of the various studies, ranging from the examination of basic cell biological processes and signaling mechanisms, through patterning and tissue morphogenesis, to comparative analysis of the genomes of 12 sequenced Drosophila species and examination of modes of evolutionary change. In view of the number of presentations, it is not possible to mention all of the exciting research advances that were reported. Rather, we will report on a sample of talks covering a few specific areas of focus. We apologize in advance to those investigators whose presentations we could not mention.

The two sessions on Cell Biology collected the most presenters and within that topic, many of the talks focused on detailed aspects of RNA biology. More than a decade ago, Ilan Davis (University of Oxford, UK) and David Ish-Horowicz (London Research Institute, UK) identified a class of mRNAs that exhibit localization within the syncytial blastoderm to the apical cytoplasmic compartment lying between the syncytial nuclei and the embryonic membrane.[1] This pattern of localization was shown to be dependent upon the 3' untranslated regions of the mRNAs. The 3' UTR of the *K10* mRNA contains a 44 bp stem/loop structure with 2 unpaired bases that can mediate apical localization when an RNA construct containing it is introduced into embryos.[2] David Ish-Horowicz reported on an NMR solution structure of this localization determinant, generated in collaboration with Simon Bullock (MRC Laboratory of Molecular Biology, UK) and Peter Lukavsky (MRC Laboratory of Molecular Biology, UK). The structure includes several intriguing features, including a novel helical form with similarities to that seen in DNA. Dr. Ish-Horowicz speculated that altered helical conformations contribute to recognition by proteins that facilitate apical localization. Simon Bullock reminded us of the work of Lecuyer et al.,[3] showing that 70% of mRNAs in Drosophila exhibit some form of spatial localization. He went on to describe studies of mRNA apical localization. This process was known to be dependent on the Egalitarian (Egl) and Bicaudal-D (BicD) proteins, although their molecular roles were unclear. Evidence was presented that BicD plays widespread roles in Dynein-based transport. Egl appears to be involved in linking mRNA localization elements to Bicaudal-D, possibly through direct binding to mRNA. This data, together with the previous demonstration that Egl interacts directly with Dynein Light Chain[4] suggests that Egl protein might provide a direct link between RNA cargo and the microtubule motor machinery, with BicD contributing to the formation of the transport complex. In related studies, Beat Suter (University of Bern, Switzerland) reported that immunoprecipitation of BicD protein resulted in RNA-dependent co-precipitation of the PolyA binding protein, PABP, and that PABP binds to the 3' UTR of *oskar* mRNA, which localizes to the posterior of the oocyte during oogenesis. *oskar* mRNA participates in the formation of primordial germ cells and abdominal segmentation. In addition to other pleiotropic phenotypic effects, *oskar* RNA failed to localize to the oocyte during early to mid-oogenesis in *pAbp* mutant germline clone-derived egg chambers. Another protein that was co-precipitated with BicD was Drosophila Imp (IGF-II mRNA-binding protein), a fly orthologue of Xenopus Vg1 RNA-binding protein and chick zipcode-binding protein, both of which have been implicated in mRNA localization.[5,6] Injection of *hairy* mRNA into embryos leads to apical localization of Imp,

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together with BicD. However, while apical localization of *hairy* transcript is unaffected in embryos derived from *imp* mutant germline clones, Hairy protein levels are higher than normal. This suggests that Imp has a role in translational repression of *hairy* mRNA prior to the point at which the mRNA reaches its final apical destination.

Anne Ephrussi (European Molecular Biology Laboratory, Heidelberg, Germany) presented a beautiful electron microscopic analysis that combined in situ hybridization to *oskar* mRNA-containing ribonucleoprotein complexes (RNPs) with immunohistochemical localization of various components of the localization machinery. These studies indicated that *oskar* is transported in the form of RNP particles that are not surrounded by membranes or associated with any other organelles and that coalesce with one another during the process of movement from the anterior to the posterior of the oocyte. These studies also enabled the demonstration of associations between the *oskar* mRNA-containing RNPs, microtubules and microtubule-dependent motor proteins. Finally, her analysis also identified factors likely to be involved in assembly of *oskar* into transport-competent RNPs or in the recruitment of motor proteins to the RNPs.

The experimental investigations outlined above, together with a large body of previous research, implicate a polarized microtubule network within the egg chamber as a crucial component of mRNA localization within the developing egg.[7] Many of us are familiar with the textbook image of a stage 10 egg chamber containing a parallel array of microtubules with their plus ends at the posterior of the oocyte and their minus ends at the nurse cell/oocyte border.[8] The actual picture is much more complex. In collaboration with Hiro Ohkura (The Wellcome Trust Centre for Cell Biology, University of Edinburgh, UK), Ilan Davis' group (University of Oxford, UK) has been examining this issue in living egg chambers, using as a marker EB1-GFP, which decorates the plus ends of growing microtubules.[9] A movie of the dynamic behaviour of EB1-GFP presented by Dr. Davis did not show conspicuous microtubule polarity along the anterior-posterior axis of the oocyte. However, quantitation of EB1 dynamics showed 56–57% of EB1-GFP movement to be in the posterior direction with 43–44% of movement anteriorwards, consistent with a mechanism for *oskar* mRNA localization that involves a random walk that is slightly biased in the direction of the posterior end of the oocyte.

Michael Welte (University of Rochester, USA) also presented studies of microtubule-based trafficking, in this case of the movement of lipid droplets within the developing embryo. The Drosophila embryo contains a microtubule network with minus ends at the periphery of the embryo and plus ends directed towards the interior. Movement is bidirectional, involving both plus- and minus-end motors. Prior to nuclear cycle 14, movement in the two directions is balanced. Over a span of 10 minutes during nuclear cycle 14, plus-end motion is upregulated and lipid droplets move to the interior of the embryo.[10] The Halo protein is expressed at this time and is required for this movement to the interior. In the absence of Halo, the movement of lipid droplets on microtubules is minus end-directed[11] rather than plus end-directed. Halo coprecipitates with the droplet motors, suggesting that it might participate directly in determining whether lipid droplets are subject to plus- versus minus-end directed movement. Dr. Welte also described optical tweezer-mediated measurement of force generated during the movement of lipid droplets, experiments that make it possible to estimate how many motors are simultaneously active per droplet.

Figure 1. The Orthodox Academy of Crete. In the foreground is the main building of the Orthodox Academy and the balcony overlooking the Mediterranean Sea where meals are served. The more recent building that houses the conference hall is not visible in this image, but the large courtyard that extends from it is seen. Note the skylight that illuminates the library, which is underground. In the background at left is the Gonia Monastery, in the background on the right is a small chapel. Image courtesy of Ilan Davis.

Several workers described studies of the role of small RNAs in development. Martine Simonelig (Institut de Génétique Humaine, Montpellier, France) described studies of the translational control of Nanos, the posterior determinant in Drosophila. A relatively small proportion of *nanos* mRNA becomes localized to the posterior pole of the oocyte, late in oogenesis.[12] It is essential that translation of *nanos* mRNA that is not localized to the posterior be repressed, in order for anterior development to occur. Smaug is an RNA-binding protein that negatively regulates *nanos* mRNA at the levels of polyadenylation[13,14] and translation initiation.[15] Dr. Simonelig's group tested the effects of mutations in genes involved in the generation and function of the three classes of small RNAs found in Drosophila (siRNA, miRNA and piRNA) for their effects on *nanos* translational repression, finding that genes encoding proteins that participate in the piRNA pathway contribute to this process. Howard Lipshitz (University of Toronto, Canada) described studies of the involvement of Smaug in the destabilization of mRNAs during the maternal-to-zygotic transition (MZT), the time during embryogenesis at which zygotic gene products take over developmental control from maternally-deposited transcripts. About 30% of maternal transcripts are eliminated at this time. Destabilization of about 2/3 of these transcripts is dependent on Smaug[16] and for a subset the effect is direct, with Smaug-mediated recruitment of CCR4/Twin deadenylase being essential for transcript destabilization. However, Smaug also seems to have indirect effects on some maternal transcripts. The zygotically-transcribed *miR-309* microRNA is absent from the progeny of *smaug* mutants. Moreover, of over 400 maternal mRNAs that exhibit stablization in a *miR-309* mutant background, 85% also exhibit stabilization in a *smaug* mutant background. This suggests the existence of a large class of transcripts that are indirectly regulated by Smaug via the destabilizing effects of the *miR-309* microRNA, the expression of which is dependent on Smaug.

On the topic of mRNA localization, Maria Leptin (University of Cologne, Germany) unveiled a powerful new strategy for the identification of mRNAs that undergo spatially-regulated patterns of localization, using tracheal morphogenesis as a test case. This approach makes use of a modified EP element[17] that, when inserted upstream of a gene, leads to Gal4-inducible expression of a transcript to which an RNA-stem loop corresponding to the MS2 bacteriophage coat protein binding site has been attached. The distribution of the transcript can be visualized by co-expression of a fusion protein containing MS2 coat protein and GFP. Dr. Leptin's group carried out a pilot screen in which a collection of tagged genes were expressed in tracheal cells with the goal of identifying genes that encode mRNAs that are specifically transcribed at the tracheal branch sites, possibly in response to local demand for oxygen. Among 250 tagged genes examined, 12 exhibited a spatially-localized distribution of mRNA. In addition to identifying genes with interesting roles in tracheal morphogenesis, this general approach provides a powerful opportunity for the identification of mRNAs that undergo subcellular localization in a wide variety of other biological processes operating in the fly.

Following invagination of the presumptive mesoderm into the ventral furrow, the mesodermal cells associate with, and spread out in a dorsal direction over the underlying ectoderm. Although specific genes and signaling pathways, notably the FGF pathway, have been implicated in this process,[18,19] the mechanism by which the cells move in a coordinated way remains largely a mystery. Both Angela Stathopoulos (Caltech, USA) and Arno Müller (University of Dundee, UK) showed beautiful live-image movies of this process, obtained through 2-photon microscopy of GFP-expressing cells in gastrulating embryos. Dr. Stathopoulos' observation of the behavior of both mesodermal and ectodermal cells during the process allowed her to define the overall spatial organization of the migrating mesoderm collective, and to dissect the migratory behavior and cell division patterns of individual cells and groups of cells during the process. She was also able to distinguish the differential responses of distinct groups of mesodermal cells to perturbations in FGF receptor expression using *heartless* (*htl*) mutants. This analysis would not have been possible through the examination of fixed sections, the standard method for analysis of this process in the past. Dr. Müller's data suggest that coordinated movement towards the ectoderm requires the expression of both of the FGF ligands Pyramus and Thisbe.[20,21] In contrast, differentiation of dorsal mesoderm is dependent only upon Pyramus, while Thisbe is dispensible for this process. In Dr. Müller's movies, cells were observed to extend actin-rich protrusions, with cells at the dorsal edge of the migrating population repolarizing and extending lamellipodia and filopodia. Adding to the potential for major advances in our understanding of mesodermal morphogenesis, Eileen Furlong (European Molecular Biology Laboratory, Heidelberg, Germany) described an analysis of the gene regulatory network that operates during mesodermal development. In these studies, six transcription factors implicated at various stages of mesodermal development were employed in a CHIP-on-chip analysis[22] of transcription factor binding to genomic target sites over a time course of development. A large set of cis-regulated modules (CRMs) were identified. These CRMs will form the basis for a detailed study of patterns of transcriptional regulation during mesodermal development. Moreover, the genes associated with these CRMs should provide valuable insights into the various cellular events occurring over the course of mesoderm specification, morphogenesis and differentiation.

Dr. Jennifer Zallen (Sloan-Kettering Memorial Cancer Center, New York, USA) described her group's studies of germband extension, another major morphogenetic movement that occurs during early embryogenesis, which results in the lengthening of the body along the anterior-posterior axis. In vertebrates such as Xenopus, body axis elongation during gastrulation results from polarized cell migration, in which cells moving in a direction perpendicular to the long axis intercalate between one another, which leads to extension of the long axis of the body. In contrast, Dr. Zallen has previously demonstrated that elongation of the Drosophila body axis during germband extension results from a mechanism in which groups of cells form multicellular rosette structures that assemble and resolve in a directional manner.[23] The rosettes are initially elongated in an orientation that is perpendicular to the long axis of the body. As germband extension progresses, the rosettes change their shapes to become elongated in parallel to the long axis of the body. Dr. Zallen presented data showing that Actin and Myosin are enriched at cellular interfaces at the center of the rosettes. By using a laser microbeam to sever the actomyosin cable and observing the resultant recoil of the cells, she was able to monitor the changes in mechanical tension within the cable that are responsible for changes in the shape of the rosette.

Drs. Christos Samakovlis (Stockholm University, Sweden) and Michael Galko (MD Anderson Cancer Center, Houston, USA) presented data based on their studies of wound healing, a process with features similar to morphogenetic processes. The Grainyhead (Grh) transcription factor controls the expression of DOPA decarboxylase (DDC) and tyrosine hydroxylase, two enzymes that participate in the formation of quinones required for crosslinking of proteins during formation of the cuticle.[24] The genes encoding these two proteins are induced in the epithelia surrounding wounds artificially applied to Drosophila embryos. The gene encoding DDC has consensus binding sites for Grh and it appears that Grh is responsible for its expression at wound sites.[25] About 100 genes carry consensus binding sites for Grainyhead, and Dr. Samakovlis described studies of one of these, *stitcher*, which is a member of the Ret family of tyrosine kinases. Grh regulates *stitcher* expression through a 2 kb intronic enhancer. Wounding leads to induction of *stitcher* expression in wild-type embryos but not in *grh* mutants. Mutations in *stitcher* lead to a delay in re-epithelialization and cuticle repair following wounding. What signals are responsible for communicating that a wound has occurred and that the wound-healing machinery should be induced? Dr. Galko showed that following the generation of a dorsal wound that creates a gap in the larval epidermis, nearby cells undergo transient dedifferentiation and, over the course of 24 hours, a disorganized epithelium migrates across the wound gap. In a screen of RNAi transgenic lines that led to inhibition of wound healing when expressed in the larval epithelium, his group identified members of the Jnk pathway, including upstream kinases and the downstream transcription factors Jun and Fos. All of these genes also participate in the morphogenetic process of dorsal closure, which shares other features of wound healing.[26] Strikingly, the PDGF/VEGF receptor, PVR, was also identified in this screen. The Drosophila genome encodes three ligands for PVR, and additional studies identified PVF1 as the essential ligand for wound healing. Tissue specific knockdown and rescue experiments led Dr. Galko to

propose a model in which wound-induced disruption of the basal lamina underlying the larval epidermis exposes epidermal cells near the wound site to PVF1 circulating in the hemolymph. This in turn leads to activation of PVR on epidermal cell membranes and the subsequent extension of migratory cell processes by those cells, and ultimately to healing of the wound site.

Several participants described studies of stem cell biology and, in particular, three talks concerned studies of stem cell fate determination and maintenance in the midgut, the Drosophila equivalent of the vertebrate small intestine. The interior surface of the midgut is lined by an epithelium made up of three cell types: enterocytes (ECs) responsible for digestion and absorption of nutrients, peptide-hormone-producing enteroendocrine (EE) cells, and intestinal stem cells (ISCs), which have recently been identified and shown to be responsible for the production of both EE cells and ECs.[27,28] Upon division, ISCs produce two daughter cells, another ISC and a cell called an enteroblast (EB), which can differentiate into either an EC or an EE. Notch is expressed in ISCs and their daughter cells, but not in differentiated ECs or EE cells. The proportion of EE cells and ECs produced by ISCs is known to be controlled by Notch signaling.[29] Benjamin Ohlstein (Columbia University Medical Center, New York, USA) and Francois Schweisguth (Institut Pasteur, Paris, France) both provided evidence indicating that Notch signaling is involved in the decision of whether the daughters of an ISC division become an ISC or an enteroblast. The daughter cell in which Notch is activated assumes the enteroblast fate, while the other retains ISC identity. Moreover, the level of Notch activation experienced by the EB determines its ultimate fate. Enteroblasts that receive a strong signal differentiate into ECs, while enteroblasts that receive a weaker signal become EE cells. Dr. Schweisguth also demonstrated that it is essential for Notch signaling to be suppressed in ISC cells. Henry Jasper (University of Rochester, USA) described studies of the relationship between aging, stress and midgut homeostasis. He described an age-related expansion of cells expressing stem cell markers, as well as dysplasia and functional degeneration of midgut epithelia in aging flies and flies subjected to oxidative stress through application of paraquat. Age-related expansion of the Escargot-expressing population of cells required components of the Jnk signaling pathway and was suppressed in flies hemizygous for a mutant copy of the gene encoding the Jnk pathway effector, Hemipterous. Finally, he showed that Jnk signaling induces ectopic Delta/Notch signaling in the midgut epithelia of aged flies. These studies have recently been published.[30] The analyses of the midgut epithelial stem cell population described by Drs. Ohlstein, Schweisguth and Jasper are made more exciting by the fact that there are many similarities between regulation of intestinal/midgut epithelial growth and differentiation in vertebrates and flies, including the major involvement of Notch signaling.[31,32]

The Drosophila eye has been a fertile source of insights about diverse biological phenomena and this meeting was no exception in that regard. Utpal Banerjee (UCLA, Los Angeles, USA) reported the result of screens for new mutations that lead to defects in cell cycle by identifying mutations that lead to defects in BrdU incorporation in homozygous mutant cells. Among the mutations identified were ones affecting Cytochrome *c* Oxidase subunit Va (CoVa), a regulatory subunit of the Complex IV of the mitochondrial electron transport chain. A variety of experiments led to the insight that decreased ATP levels in the mutant cells generated a signal that resulted in increased degradation of Cyclin E levels and consequent arrest in G_1 phase of the cell cycle. Interestingly, mutations affecting PDSW, a component of Complex I of the electron transport chain, also led to G_1 arrest. However, in this case, ATP and Cyclin E levels were normal, but there was an increase in the levels of Reactive Oxygen Species (ROS) and the Cyclin Dependent Kinase inhibitor, Dacapo. These findings point to a check-point control that links mitochondrial function with cell cycle progression. Under reduced metabolic function, increased AMP and ROS trigger signaling cascades that block Cyclin E function by a variety of mechanisms.

Dr. Benny Shilo (Weizmann Institute of Science, Rehovot, Israel) described surprising new findings regarding the regulation of Epidermal Growth Factor Receptor (EGFR) signaling in the eye. Activation of EGFR in the eye imaginal disc is mediated by the TGFα like ligand, Spitz, which is initially produced as a membrane-bound form, mSpitz. The chaperone Star is necessary for transit of mSpitz from the endoplasmic reticulum to a late compartment of the secretory pathway, where it is cleaved by the intramembrane protease Rhomboid-1, thus releasing the active ligand.[33,34] Dr. Shilo described studies of Rhomboid-3, a second Rhomboid protease that is expressed in the eye disc. EGFR signaling is activated at unusually high levels in eye discs containing Rhomboid-3 mutant cells. Moreover, Rhomboid-3 is capable of processing Star in the endoplasmic reticulum,[35] resulting in decreased transport of Spitz for activation of EGFR. Thus Rhomboid-3, unlike Rhomboid-1, acts to attenuate EGFR signaling in the eye disc, in parallel to its ability to productively generate secreted ligand.[36]

As outlined above, many of the presentations at the meeting described targeted analyses of specific biological processes. However, many other talks described investigations with a more global scope. Sarah Bray (University of Cambridge, UK) described a genome-wide analysis of mRNAs that exhibit changes in expression and genomic sequences that are bound by Su(H) within 30 minutes of activating Notch in the muscle progenitor-related cell line, DmD8. 197 genes were identified that exhibit Notch-related changes in gene expression and 262 sites of Su(H) binding were identified. Among the genes implicated as Notch targets by combining these data are mediators of cell proliferation, apoptosis and cell-cell signaling, suggesting the existence of direct cross-talk between Notch signaling and other regulatory networks. Gerold Schübiger (University of Washington, Seattle, USA) described a fascinating genome-wide screen for genes involved in regeneration of imaginal discs. This work follows from classic studies by Ernst Hadorn of transdetermination,[37] a process in which regenerating disc fragments changed from one type of disc to another. Transdetermination is rare, but its frequency can be increased by damage to a small group of cells in a so-called "weak point" in the disc. Transdetermination can also be induced through the ubiquitous expression of *wingless*. The Schübiger lab previously carried out a microarray analysis that allowed the identification of 143 genes that are upregulated in the "weak point" of the disc.[38] Three classes of genes have been identified among this group: (1) Genes that are expressed early in disc development but reactivated and required during regeneration. [e.g., the fly homologue of *augmenter of liver regeneration* (*alr*)]; (2) Genes that are normally expressed in discs but that change their expression level during regeneration (e.g., *Polycomb* and *Trithorax* group chromatin modifiers);

(3) Genes that are ordinarily not expressed in discs, and not necessary for disc development, but that are required for regeneration [e.g., *regeneration* (*rgn*) and *Matrix metalloproteinase-1* (*Mmp1*), both of which share homology to vertebrate genes involved in regeneration].

Peter Cherbas (Indiana University, Bloomington, USA) described experiments designed to examine the characteristics of Drosophila tissue culture lines based on their patterns of transcription. The Drosophila Genomics Resource Center collection currently contains 108 cell lines representing embryo-derived cell lines from *D. melanogaster* and other Drosophila species, cell lines derived from the CNS and cell lines derived from imaginal discs (https://dgrc.cgb.indiana.edu/cells/). Dr. Cherbas has been examining the basal and ecdysone-induced transcriptome of a selection of these cell lines that have been derived from various tissues. The patterns of transcription differ widely between the various cell lines. However, despite the fact that these are immortalized cell lines, the cells exhibit patterns of transcription that are consistent with expectations based on their source. In fact, the patterns of transcription exhibited by several of the wing disc-derived cell lines enabled the identification of the particular region of the disc whose spatial markers they express. With regard to the ecdysone effects (after 5 hrs of ecdysone treatment), among the various cell lines the average number of genes exhibiting a change of at least two fold was about 200, most of which were cell line specific, and about half of which represented decreases in expression. Fewer than 20 genes exhibited ecdysone responses in all cell lines. One of the intriguing observations to come from these studies was that all ribosomal protein-encoding genes exhibit a small decrease in expression (of about 10%), in response to ecdysone. Whether or not this change in expression is meaningful remains to be determined.

Susan Celniker (Lawrence Berkeley National Laboratory, Berkeley, USA) reported on studies designed to generate a comprehensive map of gene expression during embryogenesis. Using cDNAs to generate probes for whole mount in situ hybridization to embryos, the expression patterns of 6,400 genes have been determined so far. Images of expression patterns have been converted into "virtual" embryos enabling comparison and clustering of genes based on their expression at various times or in various tissues. In this way, 1,881 genes expressed early in embryogenesis (Stages 4–6) have been grouped into 39 clusters representing distinct patterns of expression, thus reducing the complexity of the system for further analysis. Manolis Kellis (MIT, Cambridge, USA) and Michael Eisen (University of California, Berkeley, USA) described studies that extend beyond *D. melanogaster* to other members of the genus Drosophila and to other species of flies. Dr. Kellis described some of the results of an extensive bioinformatics comparison of the genomes of the 12 sequenced Drosophila species as a means of identifying and confirming protein-coding genes, RNA genes, microRNAs and regulatory motifs. In addition to the identification of many new candidate regulatory motifs conserved in promoter and enhancer regions of genes in the various species, these studies also uncovered a number of unexpected genomic features such as examples of stop codon readthrough in the case of a large number of neuronal protein-encoding genes and instances of codon frame-shifting in the expression of some proteins. Dr. Eisen also described comparative genomic studies, in this case of variation in the structure of early embryonic gene enhancers present in the 12 genomes and in the orthologous genes in more distantly related fly species (families Sepsidae and Tephritidae). Surprisingly, despite extensive sequence divergence and binding site rearrangement between Drosophila, sepsid and tephritid non-coding DNA, enhancer regions from sepsid and tephritid genes were capable of recapitulating appropriate patterns of transcription in Drosophila. These studies have led Dr. Eisen to suggest that small, highly conserved 20–30 bp sequences containing pairs of adjacent or overlapping factor binding sites represent functional "minimodules" that combine to form enhancers in the orthologous genes of the various species.

Norbert Perrimon (Harvard Medical School, HHMI, Boston, USA) described a strategy for comprehensive, systems-based analysis of signal transduction pathways, using the Insulin and EGF receptor signaling pathways as examples. The analysis begins with tandem affinity purification (TAP)[39] of more than 20 tagged, known components of the pathway isolated from ligand-stimulated S2 tissue culture cells. Affinity-purified material is then subjected to quantitative mass spectrometric (MS) analysis. These analyses are then followed by functional studies in which RNAi is used to perturb TAP/MS-identified components in cell culture, using microarrays to examine the transcriptome and the phosphorylation state of known pathway effectors at various times following ligand stimulation. Dr. Perrimon demonstrated that this type of analysis could lead to the identification of new components as well as insights into the dynamic properties of the signaling pathways. Brian Oliver (NIDDK, NIH, Bethesda, USA) began his talk by pointing out that while genome sequencing, transcriptional profiling and proteomics have enhanced our ability to identify genes and pathways that correlate with various processes, determining the function of specific gene products remains a rate-limiting step. He went on to show how an integrated system that studies the effects of both RNAi and small compounds can accelerate this process, using lipid droplet storage as a model system. Among the results presented, he used RNAi to show that members of the COPI transport complex and ARF1 are required to limit lipid droplet storage and that inhibition of COPI function by small molecules such as ExoI and Brefeldin A mimic the lipid storage effect of COPI knockouts. He also pointed out that the National Institutes of Health Chemical Genomics Center (NCGC) operates a program to aid biomedical researchers in carrying out screens of small molecules (http://www.ncgc.nih.gov/about/mission.html).

Several presentations outlined technological advances being applied to Drosophila research. Gerry Rubin (HHMI Janelia Farm, Ashburn, VA, USA) described efforts to use genomics in the development of new tools for the study of neuroanatomy and neurogenetics in the fly. He emphasized that cells and the groupings that they form, rather than genes, are the functional units of the nervous system and there is a need for tools that can interrogate cells and the neuronal circuits that they comprise, to elucidate their roles in nervous system function. The goal of the Rubin group has been to generate a large collection of transgenic lines that reproducibly express Gal4 in distinct small subsets of cells in the adult brain, which will allow the cell-specific expression of various gene products such as toxins and ion channel proteins, or of marker proteins that can be visualized. As of June 2008, starting with DNA fragments from the flanking non-coding and intronic regions of 930 selected genes, 4849 plasmids ready for genomic insertion by bacteriophage ϕC31 integrase[40,41] have been generated, from which 3049 transgenic fly lines have

been produced. The expression patterns of 600 of these integrated constructs have been imaged. As an additional test of this approach, 44 DNA fragments from four previously characterized genes were inserted into the vector and then introduced into flies and their patterns of expression examined. Over 80% of the fragments from these four genes led to expression in the brain in patterns that, on average, contain fewer than 100 cells. Based on these results, Dr. Rubin suggests that the Drosophila genome contains over 50,000 enhancers and that the regulatory elements of genes are made up of multiple enhancers that each drive expression in a distinct set of cells at particular stages of development. The results of these studies have recently been published.[42]

Hugo Bellen (Baylor College of Medicine, HHMI, Houston, USA) described a variety of new technological advances being developed in his laboratory. One of these was the construction of two libraries of genomic DNA fragments contained within P[acman][43] bacterial artificial chromosomes. The two different libraries have inserts of average length 20 kb and 80 kb, respectively. In principle, these artificial chromosomes can be introduced into the Drosophila genome by either P-element-mediated transposition or by ϕC31 integrase-mediated site-specific recombination. 80% of Drosophila genes are sufficiently small to be included within a DNA clone in the 20 kb library. The 80 kb library, which contains 36,864 end-sequenced clones representing 12-fold coverage of the genome, will enable the introduction of larger segments of DNA into the genome than has previously been practical using P-element-mediated transgenesis and should allow rescue of genes too large to be fully contained within genomic DNA fragments cloned in the 20 kb library. Moreover, the P[acman] clones facilitate various approaches for the alteration of DNA inserts in *E. coli* by recombineering.[44]

Experiments employing bacterial artificial chromosomes of the type described above were reported by Dietmar Schmucker (Harvard Medical School, Dana Farber Cancer Institute, Boston, USA), who has been carrying out a structure/function analysis of the gene encoding the Drosophila orthologue of Down syndrome cell adhesion molecule (Dscam).[45] Dscam is widely expressed in the nervous system, mainly in growth cones, axons and dendrites, where it participates in the generation of neuronal connectivity. The *Dscam* gene is about 60 kb in length and has the potential to produce 38,016 distinct protein isoforms by alternative splicing. Each isoform is made up of 24 exons, 20 of which are common to all isoforms and four of which are variable. Exons 4, 6, 9 and 17 are represented by 12, 48, 33 and 2 variants, respectively. The particular combinations of variants of exons 4, 6 and 9 determine the sequence and structure of three of the nine extracellular, immunoglobulin domain repeats present in the protein, which presumably contribute to binding specificity. As an approach to examining the requirement for sequence and structural diversity in Dscam for neuronal connectivity, Dr. Schmucker first generated a *Dscam* null mutation. Using a BAC carrying the *Dscam* gene, a modified variant that contain a single variant of exon 6 was created and introduced into the null mutant flies with the observation that no connectivity was generated by neurons in the absence of diversity for exon 6.

An example of the application of the ϕC31 integrase system was reported by Francois Karch (University of Geneva, Switzerland), who has used it to dissect the regulatory elements of the *Abd-B* gene of the Bithorax Complex. A 60 kb regulatory region downstream of *Abd-B* is divided into four regulatory domains designated *iab-5*, *iab-6*, *iab-7* and *iab-8*, which direct *Abd-B* expression in abdominal segments 5, 6, 7 and 8, respectively.[46] Dr. Karch described the generation of flies in which *iab-6* had been deleted and replaced by an attP target site for ϕC31 integrase. Flies homozygous for the mutation exhibit a homeotic transformation of A6 into A5. Starting with a 19 kb genomic region containing the intact *iab-6* region, which rescues the deletion mutant phenotype, Dr. Karch's group has been using recombineering in bacteria to generate overlapping deficiencies for systematic ϕC31/attB-mediated reintroduction into the mutant flies. One integrant, carrying a deletion of a segment of 927 bp that lacks binding sites for gap and pair rule proteins, behaves like a deletion of the *iab-6* region, indicating the importance of that small region of *iab-6* and the power of this approach for examining gene regulatory elements.

Hugo Bellen also described a new plan to generate insertional mutations in all Drosophila genes using the transposable element Minos,[47] which exhibits a less biased pattern of transposition than that of P-element and Piggybac transposons, which have been used in the generation of previous collections of insertion mutants. They have also generated a Minos gene trap vector element (MIMIC) that contains two inverted ϕC31 attP sites, which enable the use of recombinase-mediated cassette exchange[48] to replace the stretch of DNA between the attP sites by any DNA flanked by ϕC31 attB sites. In one potential application, using the appropriate gene-trapping cassette, it should be possible to direct the expression of a protein of interest in the pattern defined by the particular MIMIC site of insertion. 500 MIMIC element insertions in the fly genome have been generated so far.

Computational biology comprised a far larger part of the meeting than was usual in the past, indicative of a more general trend to incorporate quantitative analysis into developmental genetics research. Bassem Hassan (Katholeike Universiteit, Leuven, Belgium) presented a quite provocative talk that introduced the concept of 'systems genetics' or, more colloquially, 'genetomics'. He began with the assertion that forward genetic screens typically involve random mutagenesis of a whole chromosome or the entire genome, and are uninformed by the large body of information about genes that exists in various databases. He presented a software tool called HighFly (http://med.kuleuven.be/cme-mg/lng/HighFly) that amalgamates this database information to predict the degree of relatedness of all genes versus a particular query gene. The purpose of this tool is therefore to predict candidate genes that may interact with a query gene, and these candidate genes can then be tested experimentally using standard genetic techniques. He compared the results of a random deficiency-kit screen for modifiers of *atonal*, followed by screening for the individual modifier genes, and found that all positive genes within the 12 positive deficiencies ranked in the top 10% of HighFly predictions. This suggests that HighFly has the potential to streamline modifier screens by determining the priority of candidate genes to be identified.

An interesting question was posed by Steve Cohen (TEMASEK Life Sciences Laboratory, Singapore) about the biological relevance of computationally-predicted miRNA binding sites. He argued that few predicted sites are demonstrably important from genetic analysis. To support this contention, he discussed his previously published work on *miR-278*, which regulates *expanded* mRNA,[49] *miR-8*, which regulates *atrophin* mRNA,[50] and *miR-14*, which regulates the mRNA

encoding the Ecdysone Receptor.[51] Despite computational predictions of 188–692 targets for these microRNAs, overexpression of the single known target mRNA phenocopies mutation of the corresponding microRNA in all three cases. How this apparent conundrum will be resolved was the topic of a good deal of discussion.

On the final day of the meeting, several of the participants and a few special guests (Michael Ashburner, Juan Modolell and Yoshiki Hotta) took to the lectern and shared some reflections upon their memories of 15 previous meetings held here. We heard about some major scientific advances first reported at this meeting. We learned that in earlier times, travel to Crete was not as dependable as it currently is and that there were occasions during which participants found themselves stranded on the mainland during the first few days of the meeting. We were told of one year (1978) when political events on Crete led to an occupation of the Orthodox Academy by protesters holding a sit-in, leading to some worry on the part of the organizers that it would not be possible for the meeting to be held. These worries were not realized, as a deal was brokered, and the protesters vacated the Academy to enable the meeting to be held. Upon completion of the meeting, the Academy was reoccupied by the protesters. The various recollections were at some times humorous, at other moments moving.

Unique features of this conference are the warm relationships and profound comradery that have developed between many of the scientific participants and the Academy itself. In a world where meetings are usually held in character-free hotels or conference centers, it is gratifying to convene in the home of people of inquiry who, like the scientists, have dedicated their lives toward seeking a deeper understanding of the natural world. In past years, the meeting program often included a cultural event that shared some of Crete's rich traditions with participants. The banquet and Cretan folk dancing on the last night remains a meeting staple, as does Peter Lawrence's 30-year quest to observe ascalaphid Neuropterans in their natural abode. While Peter's endeavor has never achieved its stated goal, it has accomplished much more through cementing friendships among Drosophila scientists and deepening their appreciation for the natural beauty of Crete's highland interior. There is clearly much yet to be learned through the study of our chosen model system, and the affection of the community for this wonderful conference should ensure that Kolymbari remains an important venue for the dissemination of exciting new breakthroughs in the fly field for many years to come.

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