# Fly developments: an introspective

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The EMBO Workshop on the Molecular and Developmental Biology of *Drosophila* took place on June 23–29, 2002 at the Orthodox Academy of Crete, Greece, for the 13th time since its inception in 1978. As has been customary in these meetings, cutting-edge and mostly unpublished research was presented in a broad range of topics. With the completion of the genome sequence, new insight is beginning to emerge from the use of global approaches to examine the regulatory pathways governing multicellular processes, and such approaches were evident in a number of talks. However, from the majority of the presentations, it became clear that fresh insight and exciting advances are also being gained by looking inward, to the inner workings of cells. The presentations were punctuated by the addition of new components to established pathways, as well as the joining of previously unlinked pathways and processes. The most-prominent topics were developmental decisions and patterning, although studies on gene expression, cell biology, neurobiology and immunology were also included. Finally, progress on the annotation of the genome and on a number of functional genomic projects was reported. The large quantity and high quality of research presented during this exciting week prohibits reporting on a fair cross-section of the talks in a limited space, so we will (somewhat arbitrarily) limit ourselves to a selected number of topics.

### Intracellular trafficking and signaling

A number of major signal transduction pathways have been identified that are required for proper patterning and development throughout metazoa. In the past, the focus has been at the membrane to determine the components and the general flow of signal transduction through these pathways. With the general outline of these pathways now in place, many talks highlighted new components or new pathway events such as intracellular trafficking required to correctly localize signaling components. New components discussed were proteins, but also lipids or sugar chains, since proper glycosylation or lipid modification is crucial in many signaling pathways. Intracellular trafficking again focused not only on proteins but also on other macromolecules with a major emphasis on RNA. Localization of mRNAs in the large *Drosophila* oocyte has long been a focus of developmental genetics. This has now been extended to smaller cells, such as neuroblasts and polarized epithelia. **Henry Krause** (University of Toronto) showed that correct protein localization and gradient formation by the morphogen Wingless (Wg) critically depends on the apical localization of *wg* mRNA.

In the fly ovary, signal transduction has long been known to be important for axis generation. Communication between the oocyte and its surrounding somatic cells is mediated by the *gurken/torpedo* (EGFR) signaling pathway. Localization of the TGF$\alpha$-like ligand Gurken to specific subdomains of the oocyte membrane is required for this signaling and the subsequent establishment of both egg and embryo polarity. **Siegfried Roth** (University of Köln) showed that *cornichon* (*cni*), a gene required for localization of Gurken to the oocyte surface, is a member of a conserved family of proteins implicated in CopII-mediated transport. Roth showed that Cni binds directly to both the extracellular domain of Gurken and to the CopII subunit Sec23, leading them to propose a role for Cni in recruiting Gurken into vesicles as it leaves the oocyte endoplasmic reticulum.

The transforming growth factor-beta (TGF$\beta$) signaling family encompasses a group of extracellular proteins that are important developmental regulators in both vertebrates and invertebrates. Signaling by *decapentaplegic* (*dpp*), a fly TGF$\beta$ molecule, has been implicated in many developmental processes involved in growth and patterning. It has been proposed that Dpp functions as a long-range morphogen, forming a gradient of activity that can affect patterning events up to 30 cells away from the signaling source. **Marcos González-Gaitán** (Max Planck Institute, Dresden) presented evidence for forming the Dpp morphogen gradient via vesicular trafficking through the cells. Using a Dpp–GFP fusion protein, he showed that Dpp is released into the extracellular space, where, however, its diffusion is restricted. Dpp is then subjected to dynamin-dependent and clathrin-mediated endocytosis, allowing it to move in a non-directional fashion cell by cell.

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Intracellular trafficking was also shown to be important for cell-type differentiation and migration in the Central and Peripheral Nervous System (CNS and PNS). Asymmetric cell divisions in neural precursors have been shown to require a number of asymmetrically localized determinants, including Numb. Numb has been shown to act by downregulating signaling through the transmembrane receptor Notch. In screens for downstream components of Numb signaling, **Juergen Knoblich**'s laboratory (Institute of Molecular Pathology, Vienna) identified the endocytic protein α-adaptin, whose role is to recruit transmembrane cargo molecules into clathrin-coated pits. The asymmetric segregation of α-adaptin, together with Numb during cell division, and Numb's direct binding to both α-adaptin and Notch, have led them to propose that Numb acts by polarizing endocytosis of Notch during the asymmetric cell divisions.

Neurons within the CNS extend axons that grow in length and change direction in response to attractant and repellent cues. Whether or not an axon crosses the midline depends on its recognition of the midline repellent Slit by the receptor Roundabout (Robo). Downregulation of Robo is required for the growth cone to ignore Slit and cross the midline. In a surprising turn of events, **Barry Dickson** (Institute Molecular Pathology, Vienna) reported that the level of Robo present on the growth cone is a result of the intracellular sorting of Robo by Commissureless (Comm), a novel transmembrane protein. For axons to cross the midline, Comm must bind to Robo thereby diverting it from the secretory pathway to late endosomes and lysosomes for degradation. **Guy Tear** (King's College, London) presented evidence that this sorting event could be mediated by Nedd4, an E3 Ubiquitin Ligase that binds to Comm. Tear showed that Nedd4 is required for Comm ubiquitination and its subsequent targeting to late endosomes.

## Planar polarity

Tissue and organ morphogenesis require populations of cells to co-organize, often involving alterations of individual cell polarity along some or all of its axes. Studies of the precise polarization patterns of hairs, bristles, and ommatidia in flies have identified a number of the players and a general outline of the regulatory mechanisms governing the planar cell polarity (PCP) signaling pathway. Regulation of the PCP signaling pathway for hairs has been proposed to be governed at three levels. The first step, mediated by the atypical cadherin Fat, involves global signaling cues across whole tissues yielding directional information to individual cells. The second step, mediated by the serpentine transmembrane receptor Frizzled (Fz), interprets the global directional cues to produce subcellular asymmetries. The third step is tissue specific, requiring specific factors to read the subcellular asymmetries and translate them into the appropriate morphological outcomes.

Work presented by **Jeff Axelrod** (Stanford University, Palo Alto) focused on the manner in which high fidelity is achieved for PCP signaling in wing epithelia. Polarity in the wing is disrupted when large clones of cells lacking Fat are generated. Axelrod proposed that local cell alignment by the Fz mechanism works in both small and large Fat clones. In the small clones, it is sufficient to generate normal polarity. However, in the large clones, it is not robust enough to traverse the large spans of tissue that lack the global signal, and therefore cell alignment wanders off course. These results led him to conclude that the high fidelity observed in wing epithelial polarity is the result of interplay between the first two steps of the PCP signaling pathway.

The process by which Fat controls the global signaling cues of PCP is largely unknown. **Helen McNeill** (Cancer Research UK, London) reported that Atrophin (Atro) is a Fat-interacting protein. Atro is a transcriptional repressor that has been linked to neurodegenerative disease in humans. Consistent with their finding that Atro binds directly to the cytoplasmic domain of Fat, McNeill showed that Atro is present in both the nucleus and cytoplasm. *atro* mutations show strong genetic interactions with *fat* and their data suggest that *fat* acts through *atro* to induce a planar polarity morphogen.

A global cue to establish planar polarity is likely to be a secreted factor, but such factors have so far eluded identification. **Peter Lawrence** (MRC-LMB, Cambridge) postulated that the vector of a gradient of a secreted factor could impart planar polarity information to a field of cells. Using clonal analysis in the abdomen, he showed that neither Hh nor Wg, both important gradient-forming morphogens in abdominal segment patterning, seem to be the cell polarizing factor. Instead, the secreted protein Fj, which is expressed at the front of each anterior abdominal compartment, and might spread forwards and backwards into the two compartments, giving opposing slopes, is a likely candidate for such a factor. *fj* genetically interacts with the cadherin-like molecules encoded by *fat* and *dachsous* and together they affect planar polarity, in opposite ways, in both anterior and posterior abdominal compartments.

PCP in the eye is established through distinct fate specifications of the initially symmetric R3 and R4 photoreceptors. Work from **Marek Mlodzik**'s laboratory (Mount Sinai, New York) described the circuitous interactions between the prospective R3 and R4 photoreceptors leading to proper PCP establishment. They reported that an increase in Fz signaling specifies R3 fate. Expression of the transmembrane ligand Delta (Dl) in R3 is triggered by Fz via the Jun-kinase (JNK) pathway and then activates the Notch signaling pathway in R4. Notch specifies R4 fate and upregulates expression of the atypical cadherin Flamingo (Fmi). Fmi expression in R4 blocks the JNK pathway and thus represses Dl, preventing Notch signaling in R3, thereby maintaining the initial asymmetry in Fz signaling required to establish the R3 fate.

### Genomics approaches and resources

With the completion of the *Drosophila melanogaster* genome sequence, the complexities of annotation have led to a call for the sequencing of additional *Drosophila* species, as well as other insects. Comparison of the *melanogaster* sequence with that of additional species is expected to enhance the accuracy of the current *Drosophila melanogaster* genome annotations, particularly in providing verification of predicted open reading frames and identification of conserved regulatory elements/structural domains within the genome. **Hugo Bellen** (Baylor College of Medicine, Houston) reported that sequencing of the *Drosophila pseudoobscura* genome under the directorship of Richard Gibbs at Baylor's Human Genome Sequencing Center is underway and expected to be finished by Spring 2003. For updated information see: http://www. hgsc.bcm.tmc.edu/projects/drosophila. **Fotis Kafatos** (EMBL, Heidelberg) provided an update of the *Anopheles gambiae* (mosquito) sequencing (completed) and annotation (underway) projects. For more information and additional links, see: http://flybase.bio.indiana.edu:82/.data/news/announcements/Apopheles_Genome.html.

To take full advantage of the power of *Drosophila* genetics in the post-genomic era, generating comprehensive sets of mutations is essential. **Michael Ashburner** (University of Cambridge, Cambridge) described the efforts of the Drosdel Consortium (http://131.111.146.35/~pseq/drosdel/ddindex. html) to create a second generation deletion kit. The current collection of deficiency-containing stocks is widely used in sensitized suppressor/enhancer screens; however, it suffers from incomplete coverage and genetic background differences. The new deletion kit is being generated on an isogenic genetic background using pairs of modified FRT-containing P-elements in *trans* that when exposed to FLP recombinase generate a deletion (and its reciprocal duplication) of the sequences between the two elements. Another approach for the systematic generation of deletions was described by **William Gelbart** (Harvard University, Cambridge) and is based on the local hopping of a *Hobo* transposon, followed by intra-chromosomal recombination between the new copy and the original one.

The most comprehensive gene disruption project so far has been jointly undertaken by the Bellen, Rubin, and Spradling laboratories and **Hugo Bellen** (Baylor College of Medicine, Houston) presented a progress report. The aim of this project is to create a P-element insertion in most fly genes. Two and a half thousand new strains have already been added to the one thousand strains previously available from the Bloomington Stock Center, with their ultimate goal to obtain ten thousand insertion strains, or a P-element insertion in 75% of all the fly genes. The new strains are being made available to the community as they are generated. For more information see: http://flypush.imgen.bcm.tmc.edu/pscreen.

Correlating information from all of these functional genomics projects and making them readily accessible is a daunting undertaking. **William Gelbart** (Harvard University, Cambridge), representing the FlyBase Consortium, emphasized the Consortium's goal of ascribing all attachable biological information to the DNA sequence. As he announced, Release 3 annotations of the *Drosophila melanogaster* genome have now been made public since August 2002. Gelbart discussed the challenges of keeping all of the information on FlyBase current and accessible, particularly stressing their need for the community to flag errors and send updates to FlyBase. FlyBase can be viewed at: http://flybase.bio.indiana.edu:82).

If one is to summarize the direction of *Drosophila* research as evidenced from this meeting, the overriding theme is the traditional reliance on multidisciplinarity in order to approach biological problems. Global (genetic and now genomic) approaches have brought into the limelight new factors and new interactions in the regulation of cellular events in *Drosophila* biology. Microscopic (cellular, mosaic and biochemical) analysis has provided in-depth insight on the function of these new factors, gradually building toward a molecular understanding of this complex multicellular organism. There are still large gaps in our knowledge, as evidenced for instance by the large number of predicted genes with no known function. In keeping with tradition in the *Drosophila* field, the continuing cross-fertilization between global and microscopic approaches will no doubt yield many exciting new findings in the years to come.