The fly Olympics: faster, higher and stronger answers to developmental questions

Conference on the Molecular and Developmental Biology of *Drosophila*

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The fourteenth EMBO Conference on the Molecular and Developmental Biology of *Drosophila* took place in Kolymbari, Crete, between 20 and 26 June 2004, and was organized by U. Banerjee, A. Bejsovec, S. Bray, C. Delidakis, A. Ephrussi, A. Giangrande, D. Ish-Horowicz, T. Kaufman, R. Lehmann, M. Leptin, S. Parkhurst, N. Perrimon, R. Saint, B. Shilo and J.-P. Vincent.

We dedicate this report to the memory of Edward B. Lewis (1918–2004). His work on the formation of the *Drosophila* body plan is a keystone of modern developmental biology and earned him a Nobel Prize in 1995. He was a frequent and much-valued participant in the 'Crete meeting', where his vast knowledge of fly genetics and his lively personality illuminated many discussions. He will be sorely missed.

Keywords: Crete workshop; *Drosophila* development; *Drosophila* molecular biology; EMBO meeting

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Introduction

This year, in the warm sunshine of Crete, more than 100 investigators gathered to probe some of the greatest mysteries of animal development. Two main trends became clear: first, a focus on cell biology is now answering questions of long-standing interest in developmental biology; and second, the creative application of new imaging and computational techniques is allowing ever-more challenging problems to be approached.

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Localization and integration of information

A central puzzle in development is the mechanism by which molecules achieve their precise subcellular localizations to promote local events in the cell. For example, the asymmetric localization of messenger RNAs (mRNAs), such as *bicoid* and *oskar* (*osk*) in the fly oocyte determines the future anteroposterior axis of the embryo. C. Rabouille (Utrecht, the Netherlands) has used immunoelectron microscopy to investigate the subcellular localization of these molecules at the ultrastructural level. In collaborations with I. Davis (Edinburgh, UK) and A. Ephrussi (Heidelberg, Germany), she has pioneered the use of RNA *in situ* hybridization on frozen sections to show that the localization of RNAs, including *bicoid* and *osk*, influences the intracellular distribution of their protein products. Ephrussi described her remarkable finding that *osk* mRNA localization in the oocyte requires the accurate splicing of the message during its synthesis in the nurse cells. Components of the exon-junction complex remain associated with *osk* RNA and are required for its translocation to the posterior pole of the oocyte (Hachet & Ephrussi, 2004). Another mechanism for generating polarized cells is the coordination of symmetric versus asymmetric cell divisions. R. Saint (Canberra, Australia) described a method for determining the position of the cleavage furrow in dividing cells, which might be relevant to this process. A Rho GTP-exchange factor, which is encoded by *pebble*, co-localizes with the Pavarotti kinesin-like protein and a Rho-family GTPase-activating protein at the ends of astral microtubules. This complex seems to restrict Rho activation to a cortical ring between the separating sets of chromosomes during mitosis (Saint & Somers, 2003). Even the most complicated of polarized cells, the neuron, is now yielding its secrets to explain how molecules are localized in axons so that precise axonal-guidance cues are positioned in large morphogenetic fields. Y. Hiromi (Mishima, Japan) has developed a low-density primary embryonic cell-culture system to show that isolated neurons have the intrinsic ability to subaxonally localize different molecules (Fig 1). Some of these share the same boundary, which raises the exciting possibility that there are fundamental 'compartments' in the axon.

Fig 1 | A *Drosophila* neuron in culture. The image shows a membrane-associated molecule (magenta) that is localized to a subaxonal segment. Image courtesy of T. Katsuki and Y. Hiromi (Mishima, Japan).

Another principal question in development asks how information from diverse genetic networks and signal-transduction pathways is integrated in the cell to produce pattern and form. A new point of connection between well-known signalling pathways has been discovered by Z. Paroush (Jerusalem, Israel). He showed that epidermal growth factor (EGF) signalling leads to the phosphorylation of Groucho, which is a global corepressor that acts with the effectors of several signalling pathways, including Notch and Wingless (Wg). Phosphorylation of Groucho attenuates its repressor function, which provides a mechanism by which the EGF pathway can antagonize Notch and other signalling cascades. M. Baylies (New York, NY, USA) presented evidence that *canoë* (*cno*) is an integration point for the Ras, Notch and Wg signalling pathways in muscle founder-cell specification. The Cno protein, which localizes to adherens junctions, shuttles between membrane-localized components of these pathways to negatively regulate their activities and thereby balance the signalling inputs that control muscle patterning. Other speakers discussed more conventional mechanisms for integrating signals at enhancer elements. R. Mann (New York, NY, USA) demonstrated that the *Distalless* (*Dll*) promoter requires both homeotic and segmentation gene input. This compartment- and segment-specific regulation restricts *Dll* expression to the thoracic segments, where it promotes the formation of the leg primordia. F. Payre (Toulouse, France) showed that the transcription-factor gene *shavenbaby* (*svb*) converts input from signalling pathways and positional cues into morphological patterns of the epidermis and cuticle, through the activation of target genes that are involved in actin, extracellular matrix and cuticle organization.

Mechanical forces and cell movement

The forces that drive cell-shape changes during development are also being identified. The fly eye, in which photoreceptor and non-neuronal support cells form precise patterns, is a useful model for morphogenic events. J. Treisman (New York, NY, USA) has discovered a role for myosin light-chain phosphatase in regulating the

position of photoreceptor cells in the eye-disc epithelium. In the absence of this phosphatase, excessive myosin activity causes photoreceptor cell bodies to drop out of the eye-disc epithelium and move towards their axon terminals. This process resembles myosin-driven retraction at the rear of migrating cells, which indicates that neurons use the phosphatase to prevent an inappropriate migratory response of the cell body (Lee & Treisman, 2004). R. Carthew (Evanston, IL, USA) has found that cone cells in each eye ommatidium (the individual units that form the compound eye) adopt shapes and assemble as if they were soap bubbles, thereby minimizing their contact with surrounding cells (Fig 2). Differential expression of the cell-adhesion molecules E- and N-cadherin is necessary for this process. Therefore, simple patterned expression of these cadherins results in a complex spatial pattern of cells due to cell-surface mechanics. R. Cagan (St Louis, MO, USA) reported that the Nephrin family members Hibris and IrreC-rst regulate patterning in the interommatidial lattice. Their interaction leads to an increase in the expression of junctional proteins, such as cadherins and catenins, which allows cells to expand physically, move into specific positional niches and establish themselves as specific cell types. Manipulating Hibris or IrreC-rst in individual cells alters their fate, which indicates that the regulation of junctions and cell morphogenesis is important for determining cell type.

Cell migration contributes markedly to tissue morphogenesis and some surprising guidance cues are being discovered in the fly embryo. D. Andrew (Baltimore, MD, USA) has found that the disaccharide trehalose might guide the posterior migration of the developing salivary gland. Enzymes that are required for trehalose biosynthesis are expressed in the tissues over which the salivary gland cells migrate, and a candidate trehalose receptor is expressed in the gland immediately before and during posterior migration. Disruption of these molecules causes defective migration, which indicates that trehalose is crucial for this process. In a different embryonic cell-migration event, R. Lehmann (New York, NY, USA) showed that a phospholipid signal might provide directional information for primordial germ cells that move from the posterior pole of the embryo to the developing somatic gonad. She has found that loss- or gain-of-function manipulations of *wunen* and *wunen-like*, which encode lipid phosphate phosphatases, disrupt the movement and survival of germ cells during this migration. Therefore, both sugars and lipids seem to provide directional cues for migrating cells in the fly.

Coordination of growth and pattern

A fundamental question in development is what coordinates the opposing forces of cell division, cell death and differentiation, to control final organ size. In the gastrulating embryo, T. Volk (Rehovot, Israel) showed that invagination of the mesodermal primordia is accompanied by cell-cycle arrest. This event is coordinated through the mesoderm-specific induction of *how*, which encodes an RNA-binding protein that downregulates mRNA levels of the cell-cycle regulator *string/cdc25*. In a screen for eye-patterning mutants, H. McNeill (London, UK) identified components of the insulin-receptor pathway, which regulates cell growth in response to nutrient conditions. Increased insulin signalling was correlated with precocious differentiation, whereas decreased signalling delayed differentiation. Cell fates remained unchanged, but tissue patterning was perturbed. Therefore, the insulin pathway regulates developmental timing to coordinate

Fig 2 | Minimizing surface contacts. A four-unit soap-bubble cluster (left) and the four-cone cell cluster in a cobalt-sulphide stained ommatidium (right) show similar packing properties. Image courtesy of T. Hayashi and R. Carthew (Evanston, IL, USA).

growth and differentiation during eye development. Cell competition has an important role in determining the size of a growing organ. L. Johnston (New York, NY, USA) showed that local expression of the growth-promoting gene *Myc* causes neighbouring cells to engage in competitive interactions in the wing disc. Cells with less *Myc* are at a disadvantage and die through Hid-induced apoptosis. Cell competition and apoptosis thereby prevent overgrowth and ensure that an appropriate wing size is reached at the end of development (de la Cova *et al*, 2004). G. Morata (Madrid, Spain) showed that apoptosing cells in the wing disc activate the ectopic production of Wg and the decapentaplegic (Dpp) protein, and that artificially blocking apoptosis allows these cells to persist and create gross developmental aberrations. He postulated that during normal endogenous apoptosis, the transient activation of Wg and Dpp might compensate for cell loss by promoting cell proliferation in the surrounding tissue. These talks highlighted the complex interplay between cells that drive tissue-wide organizational processes.

New tools for dissecting complex processes

The fly also promises to lead the way in solving perhaps the most challenging developmental problem of all: how neurons find their appropriate synaptic targets to establish proper connectivity in the brain. This complex system is now being analysed using ingenious approaches and powerful techniques. D. Schmucker (Boston, MA, USA) discussed a cell–cell recognition molecule, the hypervariable cell-surface receptor Down syndrome cell-adhesion molecule (Dscam), which is required for axonal targeting. Tandem arrays of alternative exons allow the generation of approximately 38,000 Dscam isoforms through alternative splicing. Using single-cell labelling techniques and genetic manipulation, Schmucker has shown that reducing the splicing choices by 40% results in specific wiring defects. This provides the first functional evidence for the importance of molecular diversity in neuronal receptors and might be a means of deciphering the molecular-recognition code that underlies synaptic specificity. C. Desplan (New York, NY, USA) has characterized the specification of neuronal cell type in the fly eye to clarify the molecular basis of colour vision. He has shown that the *spalt* gene directs the inner-versus-outer photoreceptor cell decision, whereas the *prospero* gene controls distinctions between the inner photoreceptors (Cook *et al*, 2003). Connections of these various

classes of photoreceptor with optic-lobe neurons are being examined using mosaic analysis with a repressible cell marker (MARCM), which can label single neurons in the brain. B. Hassan (Leuven, Belgium) discussed his use of the dorsal-cluster neurons (DCNs), with their reiterative pattern of 12 parallel axons in the optic lobe, to examine axon extension and retraction. Using the Gal4-upstream activating sequence (UAS) system to disrupt signalling pathways, such as c-Jun N-terminal kinase (JNK) and Ras, he showed that a complex network of interactions controls the extension, retraction and stabilization of axons independently of their guidance. A. Brand (Cambridge, UK) has discovered that stem cells can regenerate in the embryonic central nervous system (CNS). Ventral midline cells, which divide only once before differentiating into neurons or glia, remain physically connected to their sister cells. However, a midline cell can be induced to divide again if its sibling cell is destroyed. Remarkably, this extra cell division generates a new midline cell that differentiates appropriately to replace the damaged cell. These findings indicate that the fly can serve as a model to study cellular regeneration in the vertebrate CNS.

Even seemingly impenetrable genetic networks are yielding to clever computational approaches. U. Gaul (New York, NY, USA) described a thermodynamics-based algorithm, Ahab, which she has developed to study transcription control in the segmentation gene network (Schroeder *et al*, 2004). Predicted control elements consistently reproduced endogenous gene-expression patterns. Furthermore, a comparison of binding-site composition across all known control elements established basic composition rules that underlie the mode of action of maternal and gap transcription factors. J. Axelrod (Stanford, CA, USA) has used a mathematical model of planar-cell polarity (PCP) signalling, on the basis of experimentally identified signalling proteins and their interactions, to show that network logic can account for the full range of PCP phenotypes, including the ability of mutant cell clones to perturb the polarity of neighbouring cells. B. Shilo (Rehovot, Israel) has used experimental and computational approaches to study how the embryonic ventral ectoderm translates a graded activation of the EGF pathway into sharp boundaries. Working with N. Barkai (Rehovot, Israel), he monitored the degradation of the Yan transcriptional repressor and proposed a mechanism of zero-order hypersensitivity, on the basis of a reversible shift of a substrate between two alternative states. Moving to the dorsal surface, M. O'Connor (Minneapolis, MN, USA) examined the production of the bone morphogenetic protein (BMP) signalling gradient, which controls dorso-ventral patterning. He used computational analysis to show that the use of heterodimeric BMPs (Dpp–Scw), modulators (Sog–Tsg) and receptors (Tkv–Sax) can produce the observed spatially restricted BMP distribution in the developing embryo. S. Small (New York, NY, USA) used a position-weight matrix to assess gap-protein-binding sites in the *eve* promoter and found that enhancer sensitivity is determined by the ‘strength’ of clusters of repressor sites (Clyde *et al*, 2003). Regionalization of the early embryo depends on this system of repressive interactions, which allow several transcriptional domains to be established simultaneously.

New techniques have identified target sites for the transcription factors that control development. K. White (New Haven, CT, USA) has taken a whole-genome approach to define developmentally regulated genes. By mapping transcription factors to their *in vivo*

binding sites in the genome, he has identified the direct targets of developmental control factors, such as the pair-rule homeodomain proteins and the ecdysone receptor. Biochemical approaches also continue to be central to the search for targets. S. Parkhurst (Seattle, WA, USA) has used a new chromatin-profiling method, DamID, in combination with polytene-chromosome staining. She identified direct transcriptional targets for the basic helix-loop-helix (bHLH) repressor Hairy, which is important in embryonic patterning, as well as for three of its cofactors: Groucho, CtBP and Sir2. Some targets point to roles for Hairy in growth control and morphogenesis, which must be coordinately regulated with pattern formation (Bianchi-Frias *et al*, 2004). S. Cohen (Heidelberg, Germany) has been searching for a different kind of developmental target: complementary sequences for microRNAs. He has found more than 100 of these small non-coding RNAs and is now identifying the genes that they regulate post-transcriptionally. He also described new computational techniques and experimental validation methods and showed that predicted target sites tend to be conserved in other insects, which allows the rapid elimination of false positives. The genome sequences of *Drosophila pseudoobscura* and *Anopheles* have proven to be invaluable for confirming the biological relevance of the results obtained by computational analysis.

Conservation of developmental processes

An evolutionary approach has also provided insight into the functional significance of mRNA targeting during development. D. Ish-Horowicz (London, UK) discussed his work on the apical localization of pair-rule gene transcripts, which encode transcription factors that are required for insect segmentation. RNA-localization signals in these transcripts are evolutionarily labile in dipteran embryos: their apical localization correlates with a rapid rate of development and with the apical positioning of nuclei in blastoderm embryos. This proximity of mRNA to the nucleus facilitates the uptake of the translated protein, which might increase the reliability of segmentation in rapidly developing species, such as *Drosophila* (Bullock *et al*, 2004). Another interesting insight into conserved developmental processes was discussed by S. Roth (Cologne, Germany). Although the Toll/nuclear factor- κ B signalling cascade is required to induce ventral cell fates in *Drosophila* and *Tribolium*, the regulation and function of the target genes in the cascade differ between these two organisms. In *Tribolium*, the homeobox gene *zerknüllt*, which is a dorso-ventral patterning gene in *Drosophila*, is involved in antero-posterior patterning. This indicates that the ancestral state for Zen function is antero-posterior specification, which is a more conventional role for this *Hox* gene that is located in the Antennapedia complex. The future looks bright for making functional comparisons among different species, according to M. Averof (Heraklion, Greece). He has developed new tools for carrying out genetic manipulations in non-model organisms. For example, the *Minos* transposable element now efficiently transforms *Tribolium* (Pavlopoulos *et al*, 2004) and the crustacean *Parhyale hawaiiensis* to yield stably transformed lines that carry *lacZ* reporters and gene-misexpression cassettes. Such techniques will eventually allow us to determine how genetic changes account for the diverse morphologies that we see among different animal species, which is the ultimate test for our understanding of developmental processes.

In summary, this meeting showcased the power of the fly as a model system for solving the mysteries of development. The unique combinations of cell biological, genetic, molecular and computational tools that are now available to fly researchers promise rapid advances in our understanding of how the linear DNA sequence of the genome is converted into the three-dimensional form of a functional organism.

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