EMBO WORKSHOP REPORT

Flies over Crete: *Drosophila* Molecular Biology Kolymbari, Crete, July 12–18, 1998

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Introduction

For the past 20 years, leading scientists studying a broad range of aspects of *Drosophila* genetics and development meet every two years at the remote and magical spot of Kolymbari, off the north-west coast of Crete. These meetings have always been 'trend setters' in the field due to the intense discussions and open exchange of ideas, prompted by the sheer beauty and isolation of the place and the informal atmosphere it fosters. The workshop is unique among *Drosophila* meetings in that it covers a broad range of topics and yet maintains a small forum. It has been interesting to follow the change in emphasis in these meetings over the years, from discussions on heat-shock response to gene cloning and expression, and finally towards a more comprehensive biological analysis of genes and pathways determining embryonic and post-embryonic development. A clear trend can be observed, from more descriptive molecular studies towards the biological understanding of the context in which individual genes and entire pathways function. It is therefore always exciting to identify new themes that come up in these meetings and are likely to shape future work in the field.

It is impossible to provide a comprehensive review of a meeting which included 124 talks and covers an extremely wide range of topics in developmental and cell biology, neurogenesis, organogenesis, gene expression and chromosome structure. This review will therefore highlight only some of the exciting themes of the meeting; those pertaining to signaling, transcription and technical innovations.

Broad inhibition of signaling

A central motif emerging from the recent meeting was the description of a plethora of mechanisms which inhibit morphogenetic signaling pathways. Past efforts were concerned with the capacity of diffusible morphogenes to reach and activate their targets many cell diameters from the source. It now seems that a variety of mechanisms are involved in executing the opposite task, namely acting to restrict and reduce the overall level of signaling, making it harder for patterning molecules to induce their respective targets and thus restricting the expansion of morphogen gradients. This mechanism can be regarded as a constitutive one, attenuating signaling uniformly, and may be compared to the elevation of the water level around an island that will determine the extent of its exposed

pinnacles (i.e. the domains which respond to peaks of signaling), an appropriate comparison for a meeting held in Crete.

Steve Cohen (EMBL, Heidelberg, Germany) described the role of Nubbin, a POU-domain protein expressed in the wing pouch that restricts Notch activity and allows it to induce Wingless (Wg) expression only in the wing margin, where Notch signaling is maximal and can overcome the repression by Nubbin. Nubbin-binding sites on Notch-target genes have been identified, so Nubbin may be repressing the activity of Su(H) triggered by Notch.

Another broad repressive activity operating at the level of transcription was described for the *Drosophila* TCF/Pangolin transcription factor, representing the nuclear target for activation by the Wg pathway. Amy Bejsovec (Northwestern University, Evanston, IL), in collaboration with Mark Peifer (University of North Carolina, NC), presented genetic and molecular evidence that TCF is associated with Groucho (a universal transcriptional repressor, see below). As a rule, TCF is associated with Groucho in an inhibitory complex, and only in response to high levels of Wg signaling can the activator Armadillo replace or overcome the Groucho effect, and TCF can induce its target genes. Another component involved in TCF repression was described by Mariann Bienz (MRC, Cambridge, UK). The *Drosophila* CBP/p300 protein (which was previously identified as an activator of transcription with histone acetylase activity) associates with TCF and functions, in this context, as a repressor. In mutants for the locus (*nejire*), expression of the *Ubx* midgut enhancer expands on both sides of the Wg source. Acetylation of TCF by CBP at the N-terminal domain (which is responsible for Armadillo binding) was demonstrated, suggesting that CBP activity reduces Armadillo-binding to TCF. CBP may be involved in transcriptional repression in other contexts, as Hiroshi Akimaru (Tsukuba Life Science Center, Japan) described a requirement for the protein to assist Dorsal in repressing genes like *zen*, in the ventral and lateral parts of the embryo. Another possible chromatin associated protein is Eyelid (Jessica Treisman, NYU, NY), which appears to antagonize Wingless signaling.

The champion general repressor of transcription is clearly Groucho, originally identified as a member of the Notch pathway and previously described to be involved in segmentation, sex determination, neurogenesis and terminal patterning. Additional facets of Groucho activity were presented. Al Courey (UCLA, Los Angeles, CA) described a requirement for Groucho in converting the Dorsal activator into a repressor of target genes. As Dorsal is a transcriptional activator of several promoters, simple binding of Groucho to Dorsal can not sufficiently explain repression specificity. It is probable that in promoters undergoing repression by Dorsal, additional DNA-binding

factors which are also in contact with Groucho provide the right context for negating gene expression. At the termini of the early embryo, Groucho functions at several successive steps. First, it uniformly represses expression of genes like *tailless* and *huckebein*, and this repression is relieved only at the termini where high levels of Torso signaling take place. Subsequently, Groucho associates with Huckebein, to repress the transcription of *snail* at the termini (Ze'ev Paroush, Hebrew University, Jerusalem). Additional putative transcriptional repressors were described by Susan Parkhurst, Hutchison Cancer Center, Seattle, WA). Using the Hairy protein, which played a major role in the original identification of Groucho's repressive properties, to identify associated proteins, a *Drosophila* homolog of a vertebrate tumor suppressor gene that interacts with Adenovirus E1a protein (CtBP) and a RING finger protein with similarity to BRCA1 were identified.

Restricted inhibition

The above mechanisms represent ubiquitous repressive systems. Another class of inhibitors involves elements that carry spatial or temporal information, and thus shape morphogen gradients in an instructive manner. Brinker represents a novel protein which has antagonistic interactions with the Dpp pathway (Siegfried Roth, Max Planck Institute, Tübingen, Germany, and Chris Rushlow, NYU, NY). Brinker is expressed in a complementary pattern to Dpp at several stages, including the early embryo, wing disc and ovary. This pattern is maintained by mutual antagonism in the expression of the two genes, thus sharpening the borders of Dpp activity. The questions of where and how Brinker intersects with the Dpp pathway remain open.

Activation of the EGF receptor pathway in neighboring cells is used to recruit these cells to follow the same developmental pathway as the founder cells. Two patterning inhibitors associated with this pathway were described. In the R8 eye photoreceptor cell and the embryonic chordotonal organs, cells expressing Atonal provide a source for activation of the EGF receptor pathway in neighboring cells, culminating in the induction of the ETS domain transcription factor Pointed P2. It is necessary to block the activity of Pointed P2 in these source cells, and this is achieved by expression of a protein termed Edl in the same cells (Yasushi Hiromi, National Institute of Genetics, Mishima, Japan). Edl acts as an antagonist of Pointed P2; it contains a 'Pointed domain' found in Pointed P2, but does not include the ETS DNA-binding domain. Edl binds Pointed P2, preventing its activity as a transcriptional activator. It seems that Pointed P2 carries out two opposing activities, i.e. repressing the expression of *rhomboid* (which is essential for triggering the EGF receptor pathway) and inducing response genes. By inhibiting Pointed P2, Edl thus increases *rhomboid* expression and represses the induction of Pointed target genes in the Atonal-expressing cells.

Another inhibitor of the same pathway is Argos, a secreted protein with an EGF domain, acting in many tissues. In the ovary, the EGF receptor, expressed in the follicle cells, is activated in a broad region in the dorsal

anterior corner of the egg chamber lying above the oocyte nucleus. In contrast, *argos* is induced by the pathway only in a narrow stripe immediately above the nucleus, where maximal levels of receptor activation take place. As a result of inducing the inhibitor Argos, signaling is reduced in this central region. This splits the single peak of EGFR signaling into two peaks, which specify the position of the dorsal appendages (Matthew Freeman, MRC, Cambridge, UK). In this case Argos inhibits signaling close to its source; in other instances, such as the eye or embryonic ectoderm, where Argos and an activating ligand of the pathway (Spitz) emanate from the same focus, Argos functions to reduce overall signaling, thus affecting primarily cells that are distant from the signaling source.

Novel signaling components

While no novel signaling pathways were described in the meeting, several new components and regulatory interactions in the known pathways were reported. Gerry Rubin (University of California at Berkeley, Berkeley, CA) presented two new components in receptor tyrosine kinase signaling, identified by suppressor screens in the eye. PTP-ER is a protein tyrosine phosphatase that dephosphorylates MAP kinase, and appears to be negatively regulated by MAP kinase phosphorylation. Another component is Cnk, an adaptor protein that associates with Raf, and may target it to cell-cell contact regions.

No *Drosophila* meeting is complete without detailed discussion of the Notch signaling pathway. Kuzbanian (Kuz), a metalloprotease, was previously shown to be an essential component in Notch signaling. It now appears that a cardinal molecule modified by Kuz is Delta (Dl), a Notch ligand (Spyros Artavanis-Tsakonas, Yale University, CT), in collaboration with Tian Xu (Yale University, CT). Genetic interactions point to a more direct interaction between Kuz and Dl rather than between Kuz and Notch. The appearance of a cleaved extracellular Dl fragment in S2 cells was noted, and shown to be absent following induction of a dominant-negative Kuz or addition of metalloprotease inhibitors. This fragment, which can bind Notch-expressing cells, is also missing in *kuz* mutant embryos. These observations may change our view of the Notch pathway, in the sense that diffusible Dl signals could be capable of affecting not only the cells immediately adjacent to the Dl-expressing cells.

Notch not only directs individual cell fates, but also in collaboration with other genes can influence implementation of entire developmental programs leading to the formation of appendages (Walter Gehring, Biozentrum, Basel, Switzerland). Normally, activation of Notch in the eye imaginal disc is responsible for induction of the genes *eyeless* and *twin of eyeless*, which are at the top of the hierarchy of eye development, as demonstrated by the induction of ectopic eyes following their misexpression. Combinations of activated Notch with other proteins can induce different appendages. For example, in association with Antennapedia, ectopic wings and legs are induced on the head.

It was assumed that the major molecules transducing the signals from activated receptor tyrosine kinases had been identified, until the work of Markus Affolter (Biozentrum, Basel, Switzerland, in collaboration with Maria

Leptin, Köln University, Germany) presented a new surprise. The *dof* gene (downstream of EGF) is essential for signaling by both *Drosophila* FGF receptors, Breathless and Heartless. It encodes a novel adaptor protein that may be phosphorylated by the receptor and serve as a docking site for Grb2 and other proteins. Dof is expressed only in the tissues where activity of FGF receptors is required, i.e. trachea and mesoderm. The fact that Dof is absolutely necessary and specific for FGF receptor signaling may provide another tier of regulation for restricting or localizing the activities of different receptor tyrosine kinases that are activated in overlapping cell populations.

While all receptor tyrosine kinases studied so far in *Drosophila* were dedicated to morphogenesis or cell division, Ernst Hafen (Zurich University, Switzerland) described a new function for the *Drosophila* insulin receptor cascade. Mutations in the insulin receptor or in the downstream component IRS1, result in smaller flies having smaller cells which are normally patterned (hence the IRS1 locus is termed *chico*). This pathway may thus adjust cell size after completion of the morphogenetic processes, according to the level and availability of metabolic sources, which could induce different levels of the still unknown activating ligand(s).

Morphogenetic cell movements

New approaches to study how activation of signaling pathways is translated to morphogenetic cell movements have been described. In the cluster of 16 germ cells, the future oocyte is always located at the posterior pole of the egg chamber, raising the issue of the mechanism responsible for this localization, which in turn will allow the oocyte to be in contact with the posterior follicle cells and trigger activation of the EGF receptor. It turns out that the follicle cells on the posterior side of the germline cyst transiently upregulate the expression of the adhesion molecule E-Cadherin. In parallel and independently, the cell which subsequently will become the oocyte also increases its Cadherin-dependent adhesiveness. Homophilic interactions between the two cell layers, thus position the future oocyte at the posterior end of the cyst (Daniel St Johnston, Wellcome/CRC, Cambridge, UK).

The role of Hedgehog signaling in controlling differential cell affinities was presented by Peter Lawrence (MRC, Cambridge, UK) and Seth Blair (University of Wisconsin, Madison, WI). Marked clones of wild-type cells in segments or adult appendages are 'happy', as reflected by their capacity to mix with neighboring cells and form wiggly borders. Mutant clones that do not resemble their neighbors in terms of reception of the Hedgehog signal (for example *smoothened*-mutant cells just anterior to the A/P compartment boundary, which lack Hedgehog reception in a region of high Hedgehog signaling) are 'unhappy', forming a tight cluster with their kin, and generate straight and minimal borders with their neighbors. Such cells react by moving away from the A/P boundary region, either anteriorly, where there is less reception of Hedgehog signal (abdomen), or into the posterior compartment, where Hedgehog reception is absent (wing). This illustrates the potential of a morphogen to change cell affinities and subsequent patterning.

Innovative techniques

Many of the quantum jumps in the *Drosophila* field are based on innovative techniques. It is impossible to imagine what the field would look like today in the absence of P elements or the Gal4/UAS inductive system. Pernille North (EMBL, Heidelberg, Germany) presented a modification of the UAS vectors utilizing the promoter of the P transposase, which now allows them to be induced in the germline, thus opening the door to misexpression in this tissue which was previously refractive to it. In many instances it is desirable to activate Gal4 and its target genes only in a restricted population of cells, in order to carry out lineage tracing, induce cell death or other processes. Jon Minden (Carnegie Mellon University, Pittsburgh, PA) described the injection of caged Gal4-VP16 protein into early embryos. Local UV activation of the protein can induce targeted gene expression in a small population of cells, for example the dorsal head cells giving rise to the optic lobe. Norbert Perrimon (Harvard, Boston, MA) presented the development of a temperature-sensitive version of Intein, a protein catalyzing its own protein splicing. By inserting this version of the Intein within the coding region of transgenes, it may be possible to inactivate or activate Intein splicing by temperature shifts, and hence the activity of these proteins. This may create a temperature-sensitive allele for any given gene. Finally, Andrea Brand (Wellcome/CRC, Cambridge, UK) and Carl Thummel (University of Utah, UT) presented the use of GFP to follow planes of cell division in the embryo or metamorphosis in living pupae, respectively.

The progress in the *Drosophila* genome sequencing project and in the annotation of accumulating data is likely to change many of our experimental approaches, by allowing rapid cloning of genes or identification of mutations, and more global examination of gene families. In two years, at the next Crete meeting, the genome sequencing should be nearing completion (Gerry Rubin, University of California at Berkeley, Berkeley, CA), gene knockouts for a majority of genes will have been generated (Alan Spradling, Carnegie Institute, Baltimore, MD) and cDNA probe arrays to monitor differential gene expression (David Hogness, Stanford, CA) will be widely utilized. With more powerful tools to address old problems such as cell polarity, cell migration and establishment of cell and tissue identity, we should be able not only to accumulate much more data, but also to obtain a deeper understanding of these central and still enigmatic processes.