Embryo emergent: elucidating the cell biology of development

The Santa Cruz Conference on Developmental Biology 2000

Introduction

With the rapid rise in genomic information, increasing knowledge about the intricacies of cell signaling pathways, and a greater understanding of conserved gene functions, the field of developmental biology has reached a new level, where researchers face a different set of challenges. Some of these challenges and the exciting approaches being taken to surmount them were described at the Santa Cruz Conference in Developmental Biology. In this review, we highlight methodological trends and new areas of focus that emerged from the meeting.

Converging pathways, molecular complexity

At the time of the previous Santa Cruz conference in 1996, considerable progress had been made in unraveling signaling and transcriptional regulatory pathways that underlie cell fate choices. A recurring theme of the 2000 meeting was that linear pathways are an oversimplification, and that future research must take into account the many feedback loops and cross-signaling networks that link regulatory pathways throughout the cell. The complexity of cross-talk between pathways was ably demonstrated in the yeast by Ira Herskowitz (San Francisco, CA), who described the interplay between the cellular response to high osmolarity, the pheromone response and pseudohyphal growth. These seemingly distinct pathways are mediated by partially overlapping MAP kinase cascades that negatively regulate one another. For example, during the high osmolarity response, the HOG1 MAP kinase is both an activator of this pathway and an inhibitor of FUS1, a target in the pheromone pathway (O’Rourke and Herskowitz, 1998). Yeast researchers are now exploiting DNA microarray technology to assay genome-wide responses to extrinsic signals, to identify pathway components and to define epistatic relationships among key regulators. The hope is that the technology will become adaptable to more complex systems such as developing Xenopus, where evidence is also accumulating for signaling cross-talk in pattern formation of the early embryo. Janet Heasman (Cincinnati, OH) reported that chordin expression in the Xenopus organizer requires both the VegT/mesoderm-inducing pathway and the β-catenin/dorsalizing pathway. At what level the pathways converge to specify dorsal mesoderm and promote the differentiation of mesodermal cell types is still not resolved.

Another revolution in the field of signal transduction comes from the realization that the ‘lock and key’ analogy for receptor/signal interactions no longer suffices to describe what actually happens in vivo. Norbert Perrimon (Boston, MA) discussed how enzymes involved in glycosaminoglycan biosynthesis can affect different signaling pathways in surprisingly different ways (Perrimon and Bernfield, 2000). In the FGF pathway the UDP-glucose dehydrogenase encoded by sugarless and the sulfotransferase encoded by sulfateless are required to stabilize the receptor/signal interaction (co-receptor role), whereas in the Hedgehog (Hh) pathway, the heparan sulfate co-polymerase encoded by tout velu is needed to facilitate the movement of cholesterol modified Hh between cells. The distribution of extracellular Wingless is also under complex control, which as...
shown by Amy Bejsovec (Durham, NC) involves feedback from APC, a negative regulator of Wingless signaling (McCartney et al., 1999). By trapping extracellular Wingless protein on cell surfaces, the proteoglycans Dally and Dally-like also affect signal delivery (Perrimon). Other ligands, such as TGF-β family members, form complexes with proteins that modulate their activity. Work described by Michael Oelgeschlager from the DeRobertis laboratory (Los Angeles, CA) suggests that Xenopus Twisted gastrulation is an agonist that dislodges latent BMPs in BMP–chordin complexes to activate BMP signaling (Oelgeschlager et al., 2000). Ali Hemmati-Brivanlou (New York, NY) reported on another TGF-β binding protein, LTBP (latent TGF-β binding protein). Originally described as a TGF-β-masking protein, LTBP can synergize with Activin to promote mesoderm induction and thereby act as a positive regulator.

Modification of receptors is yet another strategy to modulate signaling. Vladimir Panin from Tom Vogt’s group (Princeton, NJ) reported that Fringe, a known modulator of Notch activity, catalyses the addition of N-acetylgalactosamine to Notch’s extra-cellular domain (Moloney et al., 2000). As a result, the relative affinity of Notch for its ligands and/or the proteolytic events that occur downstream of the ligand/receptor interaction could be affected. Trudi Schupbach (Princeton, NJ) showed that fine tuning of signaling can also be achieved through receptor turnover (Pai et al., 2000). During dorsal–ventral patterning of the Drosophila embryo, EGF-Receptor (EGFR) activity must be turned off ventrally to ensure specification of the most ventral cell types. This downregulation involves Cbl, a tyrosine kinase binding protein shown in other systems to promote degradation of EGFR by recruiting ubiquitin-activating and conjugating enzymes. Clearly, how ligands and receptors are presented, selectively modified, stored and degraded have become paramount issues in the field.

Nucleocentric no more!

A significant trend is that developmental biologists’ interests are shifting away from gene regulation toward the cellular machinery powering developmental change. As pointed out by speakers working in invertebrate and vertebrate model systems, and articulated by Mike Levine (Berkeley, CA), ‘the challenge is to fit complex transcriptional regulatory networks to the nuts and bolts of cellular morphogenesis.’ Toward this goal, Levine undertook a molecular screen to identify genes controlling cell movements that shape the Ciona intestinalis notochord. Bill Smith (Santa Barbara, CA) is using a mutagenesis screen cleverly adapted to another ascidian species to recover mutations that disrupt notochord extension (Moody et al., 1999). Richard Harland (Berkeley, CA) discussed related cell movements in the frog embryo. Labeling membranes with green fluorescent protein (GFP) revealed that cells undergo polarized behaviors to perform the movements of convergence and extension. This polarization depends on Xenopus Dishevelled, implicating a pathway equivalent to the Drosophila planar cell polarity signaling cascade in vertebrate gastrulation (Wallingford et al. 2000).

Several other groups reported on the utility of GFP to characterize the behavior of subcellular structures during development. For example, using a tubulin–GFP fusion, Allan Spradling (Baltimore, MD) described how the fusome, an asymmetric membrane-rich organelle essential for oogenesis in Drosophila, influences the polarization of the microtubule network in early germ cells. (Grieder et al., 2000). Complementary experiments are also revealing a role for the fusome in organizing mitochondrial inheritance during oogenesis. Adapting similar visual strategies to mouse embryology is the goal of Scott Fraser (Pasadena, CA), who is devising several non-invasive imaging techniques suitable for vertebrate systems.

Departure from a nucleocentric view was further evidenced by the closer focus on activities occurring at the edges of cells. Chris Doe (Eugene, OR) working in Drosophila and Michel Labouesse (Illkirch, France) in Caenorhabditis elegans described a group of cortically-enriched proteins (Lethal Giant Larvae, Disc Large and Scribble) that regulate asymmetric protein localization in cells with distinct apical/basal polarity (Legouis et al., 2000). Mutations in Lethal giant larvae can be suppressed by reducing the level of myosin II, revealing a negative interaction between these cortical proteins and the actomyosin network (Peng et al., 2000). Eric Wieschaus (Princeton, NJ) reported on another protein on the edge, Null0, which is required for the formation of specialized basal junctions during cellularization of the Drosophila blastoderm. Null0 must be degraded before gastrulation, as its prolonged expression blocks the formation of mature apical adherens junctions between cells (Hunter and Wieschaus, 2000). Thus, developmental biologists are characterizing molecules involved in basic cellular processes as they probe how cells modify their shape and contacts with neighbors in the context of the growing embryo.

Genetics gets green and goes genomic

Genetic screens are still the mainstay for developmental analyses, as mutations continue to implicate unanticipated proteins and biochemical processes. Arnon Rosenthal (South San Francisco, CA) reported that the zebrafish foggy locus, first identified because mutants produce extra serotonergic neurons and fewer dopaminergic neurons in the hypothalamus, encodes a highly conserved regulator of transcriptional elongation. How exactly this protein works to determine classes of neurons in the brain is unclear, although the mutation was found to disrupt a repressive activity within the transcriptional elongation machinery. Didier Stainier (San Francisco, CA) showed that the zebrafish mutation, miles apart, which produces cardia bifida due to migratory defects of the bilateral heart primordia, encodes a receptor for the lipid sphingosine-1-phosphate (Kupperman et al., 2000). Whether lipid-mediated signals are required for cell polarity or for migration of the myocardial epithelium remains to be determined. Another heart mutant, heart and soul, is defective for an atypical protein kinase that was previously implicated in the formation of tight junctions in mammalian cells and in asymmetric cell division in C. elegans. New players acting in well-studied pathways are also being discovered. Using a forward genetic approach in the mouse (Anderson, 2000), Kathryn Anderson (New York, NY) identified several mutations that interfere with Hedgehog signaling, two of which likely define new modifiers of the Ih pathway.

While the power of the classical genetic approach remains undisputed, the criteria for mutagenesis screens are changing, In
particular, an increased understanding of the underlying cell biology is allowing investigators to design focused screens that target the mechanism of interest more directly. Genetic dissection of intricate cellular processes in Drosophila, such as the formation and branching of tracheal tubes (Mark Krasnow, Stanford, CA) or of neuromuscular synapses (Graeme Davis, San Francisco, CA) is expected to bring us closer to the basis of lung branching structure and synaptic physiology in humans. As is the case in studies of subcellular organization, GFP labeling of proteins is proving to be an important breakthrough for discovering subtle mutant phenotypes at a high resolution. For instance, a synaptobrevin–GFP fusion enabled Yishi Jin (Santa Cruz, CA) to paint nerve endings green and recover C. elegans mutants with abnormal synapses (Zhen et al., 2000).

Innovative tools are also being developed for systematic genome-wide analysis of gene function. Proof of the old adage ‘you are what you eat’ comes from C. elegans laboratories capitalizing on the ‘feeding’ method devised by Lisa Timmons and Andy Fire (Timmons et al., 2000). Specific gene function is obliterated after worms dine on bacteria engineered to produce the appropriate double-stranded RNA (RNAi or RNA-mediated interference). Julie Ahringer (Cambridge, UK) is creating a veritable feeding frenzy in efforts to survey the loss-of-function phenotypes of all ~19 000 ORFs predicted by the C. elegans sequencing project. To gain more insight into the RNAi phenomenon itself, Craig Mello (Worcester, MA) has isolated mutants resistant to ingested dsRNA. Intriguingly, some of the same mutants remain sensitive to dsRNA introduced by injection.

Bill Skarnes (Berkeley, CA) reported that the ‘secretory trap’ insertionional mutagenesis scheme, designed to target all membrane or secreted proteins in the mouse, has paid off with the recovery of a number of interesting loci. One encodes a potential new Wnt receptor that is structurally related to the receptor for low-density lipoprotein (Pinson et al., 2000). Technology for single gene inactivation has also increased in sophistication, with the combined use of Flp and Cre recombinases to create hypomorphic or tissue-specific null alleles. Such chromosomal constructs allow the functional dissection of signals such as FGF8 (Gail Martin, San Francisco, CA), that are involved in multiple processes, or such as Nodal (Elizabeth Robertson, Boston, MA), that have complicated spatial and temporal modes of action in the early embryo.

Development diversifies

Another advantage of the new molecular genetic tools is that developmental biologists are no longer confined to the realm of the embryo but are reaching out to tackle some of the ‘big questions’ in biology. Issues such as specification and the basis for morphological change, sexual orientation, neuronal discrimination, synaptic plasticity and the regulation of life stages are but a few being actively explored. In some cases, this may require a bold move away from the usual model systems, as illustrated by David Kingsley (Stanford, CA), studying the remarkable diversity of the bony elements of stickleback fishes. Isolated fish populations are well-suited for the study of the genetic and environmental forces underlying morphological variation, which, ideally, will reveal general principles that have been at work in vertebrate skeletal evolution. With the framework of a stickleback genetic map in hand, the identification of polymorphisms linked to a wide array of physical and behavioral traits holds promise for future gene identification.

For other uncharted areas, however, model systems continue to deliver results and an entry to related processes in other species. Genetic analysis of the internal clock that controls the timing of developmental stages has progressed in C. elegans. Gary Ruvken (Boston, MA) described recent work on let-7, a small RNA that regulates the transition from larva to adult, in part, by inhibiting translation of proteins that promote larval cell fates (Reinhart et al., 2000). The discovery of temporally regulated fly and human let-7 orthologues leads to the tantalizing idea that conserved mechanisms may very well coordinate the timing of developmental stages as diverse as larval instars and human puberty. Comparative approaches have also begun to pay off in the sex determination field, where genes orthologous to the regulators of male development mab-3 (C. elegans) and double sex (Drosophila) are turning up in birds, turtles and mice. David Zarkower (Minneapolis, MN) indicated that mab-3 is required in C. elegans for males to remain attracted to the opposite sex (Yi et al., 2000), suggesting that insights into the regulation of sexual behavior may soon be an added bonus. Control of life span, another big question well-served by model systems, was discussed by Cynthia Kenyon (San Francisco, CA), who proposed that the integration of sensory inputs from the environment and internal inputs from the germline modulates the rate of aging in C. elegans (**Hsin and Kenyon, 1999; Apfeld and Kenyon, 2000). Finally, Cori Bargmann (San Francisco, CA) addressed the topic of neuronal diversification in olfaction. She showed how a novel signaling pathway akin to lateral inhibition (but involving calcium signaling rather than Notch) leads to the differential expression of odorant receptors (Troemel et al., 1999), potentially allowing bilaterally symmetric neurons to smell differently.

Conclusion

A strong message from the year 2000 Santa Cruz meeting is that, while developmental biologists are still entranced by the exquisite pattern of the embryo, new directions are emerging for attacking both smaller and bigger issues. In the millennial crystal ball, one foresees that by the next Santa Cruz meeting the divide between the cell biologist and the embryologist will dissolve, as we gain a greater appreciation of the roles of subcellular trafficking, cyto-architectural remodeling and organelle function in shaping developmental events. As anticipated by Eric Wieschaus (Princeton, NJ), ‘if we could understand what genes do in a cell biological context, we will come a long way in understanding development.’ To fulfill the mandate, directed genetic screens will take on even higher levels of sophistication with tools for visualizing cellular detail, as well as for monitoring tissue interactions and cell physiology in situ, leading to surprising discoveries about organ form and function. Finally, inroads will be made into biological problems previously thought to be too complex or even intractable, from the growing interchange between genetic/genomic strategies and molecular lessons first learned from the developing embryo.
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