Summary: A Common Language

M.P. SCOTT
Department of Developmental Biology, Beckman Center, Stanford University School of Medicine,
Stanford, California 94305

I am not ignorant of what many Learned and Inquisitive Men, both at home and abroad, especially in this last Century, have performed in the Anatomy of Animals. After all whom, if it be demanded, what is left for me to do? I Answer in the words of Seneca: (Epist. 64), Mutilum eadem restit ut operis, mutilum; restitit; nec alii Nato, post mille Saecula, procul debeat occasio, aliquid adeo adiciendi. [Much work still remains, and much will remain, nor will anyone born a thousand centuries from now be denied the opportunity to add something still.]

—Nehemiah Grew, M.D., F.R.S.,
"The Comparative Anatomy of Stomachs and Guts Begun"
Lectures of the Royal Society (1676)

My central theme in this summary is that developmental biology has come so far so fast because the mechanisms of development are turning out to be unexpectedly simple. They are not simple on an absolute scale, but certain anticipated difficulties have not come to pass. Our field now has a common language. People working on vastly different organisms share principles and facts in a way that would have been unimaginable just 10 years ago. Proteins are in families, and many families are in pathways. Underlying the vast diversity of biology we increasingly see simple commonalities. A sense of optimism and excitement pervades the field, although we have a long way to go.

PAST

Monod and Jacob, at the 26th Cold Spring Harbor Laboratory Symposium in 1961, commented, "Unfortunately, in the face of formidable technical difficulties, the study of differentiation either from the genetic or biochemical point of view has not attained a state which would allow any detailed comparison of theory with experiment... Adequate techniques of nuclear transfer, combined with systematic studies of possible inducing or repressing agents, and with the isolation of regulatory mutants, may conceivably open the way to the experimental analysis of differentiation at the genetic-biochemical level."

At the 50th Symposium, "Molecular Biology of Development," in 1985, Gerald Rubin commented, "I believe we can be optimistic about understanding the basic features of development at the molecular level, certainly before the 100th Symposium, and perhaps by the 60th." At that last Cold Spring Harbor Symposium devoted primarily to developmental biology, where did we stand? We were four years past the first reports of the Nüsslein-Volhard and Wieschaus screen—the first functional genomics project for development. Their catalog of genes affecting the epidermis was to become a catalog of genes affecting all of us. The first segmentation genes had just been cloned. Localized maternal RNA molecules were being discovered in frog eggs. Mutations had been isolated that affect early nematode development. Most of what mattered about homeobox genes was known due to Ed Lewis's half-century of work on the bithorax complex: collinearity of body and gene complex, combinatorial action, upstream regulators. The methods to get at the genes had come along at the same time as the discovery of most of the gene types. Chromosome walking, invented by David Hogness in the late 1970s, had opened the chromosomes to directed gene isolation and set in motion a flood of new projects. There was tremendous excitement, as well as substantial data, about similarities between transposons in plants, bacteria, and flies. Bulk genome analysis was to be replaced by an intimate knowledge of genes for abundant proteins, transposons, and all those mysterious genes that control development. Molecular biologists were showing that the homeotic and segmentation genes make related proteins and that fly homeotic genes are absolutely huge. The homeobox had been found in flies and had allowed the isolation of the mammalian genes. There was no evidence that these genes might have similar functions in mammals and insects and, indeed, little feeling that they do. (Rubin noted, "There is at present no evidence that any of the homeo-box-containing genes in vertebrates have a controlling function in embryonic development.") Many of the first developmental regulatory genes isolated encoded things that looked like transcription factors, but we were not sure. In situ hybridization had been invented by my thesis advisor Mary Lou Pardue, along with her thesis advisor Joe Gall, for looking at chromosomes. Thanks to Michael Akam and Walter Gehring, the technique was newly being applied to tissues, especially embryos. A first simplification had come to light: Transcription factors are often produced only in the tissues they affect. The elegant and original adapta-
tion of P elements for moving DNA into and around the genome of flies, by Gerald Rubin and Allan Spradling, was well established and being used to great benefit. Transgenic mouse methods had been developed by Ralph Brinster, Frank Costantini, Elizabeth Lacy, Erwin Wagner, Beatrice Mintz, and Frank Ruddle and were well represented at the meeting. The technique was still enough to be mentioned as part of David Baltimore’s title. Insertional mutations of mice were being isolated. Gene transfer in plants, sea urchins, nematodes, and *Dictyostelium* raised exciting prospects for progress, all since fulfilled. A small field, the cell cycle, was represented by yeast studies, mitotic factors and chromosome replication in frogs, and regulation of histone gene expression. The startling conservation of ras from mammals to yeast was discussed, and early studies of oncogene expression and function during development were presented. Gene regulation in yeast, flies, frogs, and mice was a major topic. Molecular developmental neurobiology was just getting going, with reports on neural crest gene expression, Notch (in flies only, of course; prepositional to imagine it might be elsewhere), circadian fly clocks, and insect neurogenesis. Major progress was reported in sex determination, particularly in the assembly of genetic pathways in flies and worms. Some sex determination genes had been cloned, and the existence of sex-specific RNA forms had been noted for flies, but the genetics was way ahead of the molecular biology.

Many people turning to the molecular biology of development for new opportunities had been intellectually raised on beautiful bacterial genetics. The special elegance of yeast mating type, arguably among the best understood genetic hierarchies in any organism, had extended sophisticated gene regulation to a eukaryote. The recognition of a relationship between bacterial repressors, mating-type repressors, and the homeodomain provided a satisfying unification of development with gene regulation in single-celled organisms. New techniques used in bacteria could be applied, and lacZ and other gene fusions were beginning to be applied to developmental problems.

What was missing in 1985, Rubin commented, “The interactions that occur between cells to execute morphogenesis await our attention.” The dearth of signaling studies at that meeting is really striking. Cell-cell interactions were discussed in the context of *Dictyostelium* cAMP signaling and *Myzococcus*; the only higher eukaryotic signaling topic was hormonal signaling. In the 1987 meeting, about half of the talks dealt with signaling.

There was another difference between the earlier meeting and this one that cannot easily be seen by reading papers. The 1985 meeting encompassed several groups of scientists whose work seemed far apart. They were striving to understand each other. Sometimes they did not understand—sometimes they did not strive. There were strong sentiments that animal development could not be unified and that the “principles” being learned from bacteria, yeast, and small animals with easy genetics would eventually be eclipsed by biology more directly relevant to humans. I think that no one anticipated the actual outcome: that all the work being done on different organisms would be linked in ways that make everyone’s efforts important. Within a few years of that 1985 meeting, we all had to be developmental biologists, rather than frog developmental biologists, mouse developmental biologists, or invertebrate developmental biologists. This transition is incomplete, but has tremendous momentum. In a sense, there are no model organisms because the information learned is so directly applicable. We have a common language.

Mike Levine’s tongue-in-cheek principle of a “statute of limitations” (see Zhou et al.), established during this 1997 meeting, stated that rediscoveries of results first reported more than 10 years ago could be described as new findings. In fact, the reality of this year’s meeting has been an increasingly deep appreciation of seminal biological observations made over a period of centuries, with references to Leonardo da Vinci’s illustrations of lungs, Ramon y Cajal’s neuroanatomy, Cabry’s 1887 experiments on ascidian mosaicism, and the rich biological findings of the present century that we still strive to explain at a molecular and cellular level. The old experiments can be approached once again, with new markers, pure genes, and pure proteins.

**PRESENT**

There are not very many kinds of proteins, and that has made all the difference in developmental biology. This is probably not a false result, because it is based on many years of extensive and varied genetic screens as well as other methods of gene discovery, like transducing viruses and disease genes. About one-third of the talks at this 1997 meeting focused on signals of just four types: Wnt, Hedgehog, TGF-β, and FGF, each of them at work in many contexts. Add in a dozen classes of transcription factors, a similar number of types of cell surface constituents, and machinery for transcription, translation, transport, and the cytoskeleton, and you would be well on your way to building a complex animal. It did not have to be this way. With perhaps 12,000 genes in the fly genome and perhaps 100,000 in a mammalian genome, there was room for far more complexity. Most of the proteins fall into families, and a quick look at a new protein sequence often tells you where to look in the cell—sometimes even where to look in the embryo. We always hope to discover new and exciting types of regulators, but a great many of them turn out to be in familiar groups. If we had to cope with hundreds of truly distinct signals our work would be very much harder. Instead, learning from fly genetics that β-catenin is involved in the Wnt pathway leads to a feast of good information coming from vertebrate systems, and eventually a direct connection with human colon cancer. Eddy De Robertis (De Robertis et al.) quoted Balzac (1842): “There is only one animal.”

We have new tools, too. Since the previous meeting, we have knockout mice thanks to Mario Cappechi and others, enhancer traps from the Gehring lab, the use of Flp recombinase for making clones lacking gene function from Kent Golic and Susan Lindquist, the use of Flp to make clones of cells expressing a gene from Gary Struhl,
and the use of GAL4 for spatially restricted ectopic gene expression from Norbert Perrimon. An assortment of the rich array of zebrafish mutants was described for us by Driver et al. There are many and varied better ways of screening for differentially expressed genes. In the analysis of mice lacking FGF8 function (Lewandoski et al.), we saw advanced uses of Cre/Lox and Flp combined. Enhancer and gene traps have been used massively in Arabidopsis genetics to create a large array of useful mutants (Moore et al.; R. Martienssen, unpubl.), and viral mutagenesis in fish is allowing much more rapid gene isolation (Amsterdam et al.). Positional cloning is practical in the zebrafish (A.F. Schier, unpubl.). Many of these methods depend on transposons, a fitting tribute to Barbara McClintock and Cold Spring Harbor Laboratory.

Transcription in Development

Nathans described the complex features of expression and function for the Brn3 family of transcription factors. Each member of the family is differently expressed in neurons of the auditory, somatosensory, or optic systems. The presence of the same transcription factor in, for example, ganglion cells and auditory neurons may hint at functional parallel between the cells previously viewed as entirely different. What looks different to us may not be so different.

Kenyon et al. showed how the beautifully precise pulsations of mab3 (Hox) expression are regulated by Wnt signaling. They also demonstrated that when mab3 is off, it's for a reason, and when it's on, it's for a reason. This work brings Hox gene functions to a new level of precision and opens the way to understanding the kinetics of their functions.

Levine (Zhou et al.) reported studies of transcriptional regulation in response to the dorsoventral regulator Toll, which was produced in activated form at the anterior of the embryo. This established compartmentalized transcription of dorsoventral regulators along the anteroposterior axis. The success of this experiment shows how firm is the understanding of the triggering influences. The roles of insultators and competition between enhancers for a promoter were described in reference to adjacent promoters in Hox complexes and engineered competitions. Toll was prominent again in a description of parallels between regulation of dorsoventral patterning and immunity (Wu and Anderson). Downstream events in the pathway are involved in immunity both in flies and mammals.

Homeobox genes play a prominent role in development in plants, as they do in fungi and animals. We learned of Knail, a homeobox gene that affects meristem cell division and fate (R. Martienssen, pers. comm.). A plethora of homeobox genes in plants also provides a link to the animalia and protista.

At late stages of neurogenesis, large domains of the brain are laid out by a series of regulators, many of which are now known. They include Hox and Fgf in the hindbrain; Engrailed, Wnt, and Pax in the midbrain; and Lim1 (Wakamiya et al.), Otx, and Emx in the forebrain. Differential effects of one transcription factor are often attained by input from signaling pathways (Kolm and Sive). Rowitch (Rowitch et al.) and Aizawa (Matsuo et al.) described experiments manipulating or destroying these components, and a clear hierarchy is beginning to take shape. What functions are regulated by these signals and transcription factors further downstream is unknown.

The hindbrain is a prime location for studying regulation of Hox genes (Nonechov et al.). Kreisler, a MAE class transcription factor expressed early in the hindbrain, is an activator of Hox genes in rhombomere 5. The integration of the Kreisler-binding site with an Ets-binding site provides the complete pattern, with the Ets-binding site restraining where Kreisler is able to activate its Hox target. Hoxh2 is regulated by Krox20 in rhombomeres 3 and 5, but by Hoxb1 in rhombomere 4, and so the complete pattern is built up. Pufferfish Hox complexes show distinctive changes compared to mammalian complexes; the conservation of sequences will lead us to a rich set of eis regulatory elements.

In muscle development the main emphasis was also on transcription (Baylies et al.). twist in flies is the "master of the universe," with the amount of Twist controlling whether cells will form somatic musculature, and high-level Twist converting ectoderm to muscle. Differentiation of the muscle requires the fly version of Mef2. Remarkably, each muscle derives from a founder myoblast that is determined by a process involving inhibition of other cells from following the same path. The founder then recruits fusion partners until an adequate population is built up.

The development of the heart is of direct clinical relevance; Harvey pointed out that 1/100 live births brings with it some type of heart anomaly. The heart arises from paired mesodermal precursors that migrate to the ventral midline and fuse. Schultheiss (Schultheiss and Lassar) described two signal sources involved in this process, one unknown source from the anterior endoderm, and BMP2 from the neural plate and notochord. The growth of the heart provides another remarkable case of evolutionary conservation, with both the Tinman/Nkx2.5 and the MEF2 transcription factors revealing common origins of the pump. The eHand and dHand bHLH proteins were a major subject this year, with elimination of dHand function in knockout mice leading to deletion of much of one of the ventricles (Lin et al.). Left-right asymmetry is prominent in multiple tissues, but most of all in the heart, and a large number of markers have been found to be asymmetrically expressed in heart precursors in a transcription and signaling cascade. The Nodal-related Xenopus gene Xnr1 is a signaling component that is able to effect situs inversus after injection into frog embryos (C. Wright, unpubl.); misexpression of Nodal in chicks has the same effect (Kengaku et al.). Evidence for the involvement of activins upstream of Nodal was provided by experiments in which endogenous activin-related molecules were inhibited by follistatin (Kengaku et al.), so we are moving from what could be to what is. eHand and a secreted cytokine called ANF may be important for the asymmetry (Biben et al.). The MEF2 family of regulators, four genes in mice, have all been knocked out and show the redundancy of some of the genes (Q. Lin et al.).
The effects of MEF2C are especially dramatic, with the right ventricle deleted and a failure of looping morphogenesis.

Nodal has also been implicated in primitive endoderm functions that appear to be crucial for determining anteriority in early vertebrate embryos (Varlet et al.; Thomas et al.).

**Signals and Morphogens**

Stuhl (pers. comm.) described elegant experiments using the *Drosophila* abdomen to learn how Hedgehog and other signals control bristle polarity in the epidermis. The most important finding was that both graded signaling and signal relays seem to be involved, each one affecting a different readout of the initiating signal.

Jessell (Ericson et al.) described new work on dosage responses to Hedgehog signals in the neural tube. At least five different roles for Sonic hedgehog were observed depending on signal concentration, and only twofold changes were needed.

Fgf signaling analyzed with knockout mice employed some of the most advanced manipulations of endogenous mammalian genes (Lewandoski et al.; Rossant et al.). Alleles were created with partial function or specific splice forms eliminated, a tour de force of hard work. FGF8 has been implicated in cell migration events that are necessary for proper gastrulation. The cells are unable to move from the primitive streak (Lewandoski et al.). This may be a parallel with *C. elegans* FGF functions, which can also affect migration. Later roles for FGF8 were ascertained by doing tissue-specific gene elimination with Crel/Cre, revealing severe limb defects. A hypomorphic allele revealed the loss of the midhindbrain region (Lewandoski et al.). FGF receptor 1 has a role in controlling cell behavior in the primitive streak and is necessary for proper anteroposterior vertebral patterning, suggesting a role in activation or maintenance of Hox expression (Rossant et al.).

BMP signals play roles in many processes, among which are formation of the cartilage primordium, cartilage differentiation, and limb patterning by cell death. Their receptors can be dedicated to different purposes, raising the question of how downstream events take different directions (Zou et al.). But often BMPs have to be stopped in their tracks. The discovery of antagonistic activities in signaling has been highlighted here, in part for their role in Spemann’s organizer. We had seen from Tam’s experiments (Tam et al.) some special properties of the mouse organizer, as it can reappear after being deleted. A zebrafish mutant, now called *chordino*, has a substantially ventralized embryo (De Robertis et al.), in keeping with the view of Chordin as a ventralizer. The Noggin protein, a BMP antagonist, has been eliminated from the mouse genome, and the consequences include defects in skeletal development, chondrogenesis, and joint formation (R. Harland, unpubl.).

Wnts battling FzRs, and chordins fighting TGF-βs, give us a new image of the checks and balances that produce animals. Why might these antagonistic activities have evolved originally? How about War!! One amusing idea is that antagonistic secreted proteins originally evolved not to coordinate growth and pattern, but as weapons. A protein produced by one organism would nullify a signal essential to the life or success of a competitor, like jamming enemy radio communications. How would the genes ever end up in the same animal? The most severe competition is usually between organisms that are related and occupy similar niches. A first step would be to evolve a variant signal, the second to produce an antagonist specific to the competitor, and the third to produce an antagonist to the home signal and use it in a well-regulated manner. Lobbing chordin at the enemy to dorsalize him—a colorful world of microbes.

**Guidance Systems**

Signaling systems were prominent in the investigations of branching morphogenesis. The initial determination of cell fates in the fly trachea primordia is key because no more cells are created as a vast network of tubes forms. Tracheless, a bHLH protein, initially determines the cells. The different types of cells depend on a battle between Dpp (a BMP2/4 relative) signaling and influences of fly EGF signaling. Outgrowth and branching of the trachea in flies is guided by FGF signals at one stage and by hypoxia in target tissues at a later stage (an exciting parallel to mammalian lung development, and some of the same genes are involved) (Shilo et al.). The tip cells that branch must prevent adjacent cells from doing so, and they do this by blocking the response to FGF while mysteriously being resistant to the blocking signal themselves (Krasnow). Sprouty, a cell surface protein which does this, may be a general regulator limiting FGF function, as a mouse homolog is expressed near FGF8 at multiple locations. The Sprouty-FGF relationship is reminiscent of the BMP-Chordin relationship, although in the former case the molecular mechanism is still to be learned.

Genetic and biochemical approaches have both contributed substantially to discovering the basis for axon guidance. The netrins, acting over long distances both to attract and to repulse, emerged from both nematode genetics and remarkable biochemical successes. Tessier-Lavigne (Leonardo et al.) described different receptors and how their different activities may lead a growth cone rushing toward or away from the nervous system midline. A battery of surface proteins with immunoglobulin-like domains and fibronectin-like domains has emerged from fly genetic studies reported by Goodman et al. A key finding in this arena is that the proteins are localized to specific parts of the cell surface, a phenomenon reminiscent of synaptogenesis itself. S.L. Zipursky (unpubl.) described the role of the Cdc42/Dcc signal transduction system in guiding neuronal targeting in the visual system. This connection with cytoskeletal regulators was to me a particularly interesting step beyond the cell surface, beginning to lead us to the changes in cell shape and behavior that must surely underlie targeting. The cadherins also play a role in axon patterning, as revealed by *Drosophila* mutants (Takeichi et al.).
The argonauts of cells, the neural crest cells, are guided in their migration by Eph-class ligands made in the somites. Repulsion from Eph channels the moving cells. Crest cells are guided in their differentiation by transcription factors and signals, and Anderson et al. reported how transcription factors can make crest cells competent to see BMP signals and take on the fate of autonomic nerves. As the cells move to their targets, they become exposed to BMP, and the resulting induction of MASH1 transcription factor appears to make the cells still more responsive to BMP signals in a positive feedback loop.

Signal Transduction

This past year has equipped us with long-sought receptors for two major signaling families, Wnt and Ihh. The Drosophila Frizzled protein, studied for many years for its role in controlling bristle patterning, has a new family member, Frizzled2, and both can bind Wnt signals (Nuss et al.), as can related proteins in frogs (R.T. Moon, unpubl.). Perrimon et al. described the involvement of hep- aran sulfate proteoglycan receptors in the Wg pathway, and he suggested the existence of a co-receptor that acts together with Fz2. Unity with the worm came from the finding of a mannos transporter implicated in Wg processing. This same transporter type was described by Horvitz (Herman and Horvitz) as being important for epithelial invagination. A debate over which of two transmembrane proteins, Patched and Smoothened, was most likely to be involved in Hh reception was decided in favor of using both. One (Ptc) appears to bind the protein and the other (Smoo) to transduce the signal when released from the negative influence of Ptc. However, the molecular mechanics of this process are far from understood.

Rubin et al. showed the genetic pathway involving ras, derived from screens of 80,000 flies. Cascades and feedback, antagonistic functions, are evident in this pathway as well.

Hedgehog proteins are tethered through a remarkable modification, by cleaving the signal into two parts and attaching a cholesterol molecule to the amino-terminal signalling part. Mutations eliminating Sonic hedgehog function cause holoprosencephaly, as do inhibitors of cholesterol metabolism (Beachy et al.). Scott Johnson and Scott) described the involvement of a kinase-related protein in the Hedgehog signal transduction pathway. Hedgehog signaling has many roles, among them regulation of different muscle types in the zebrafish (Quirik et al.). A Sonic hedgehog mutation in the fish (Rauch et al.) causes altered somites and shortened pectoral fins; additional defects seen in the mouse knockout, such as cyclopia, may be eliminated in the mouse due to the action of other Hedgehog family members. An array of other fish mutants holds great promise for being related to other Hedgehog-signaling components.

Cascades of transcription factors and signal transduction systems in plants use components distinctly related to animal regulators. No direct homologies with animal developmental mechanisms are apparent as yet, but 12 years from now… A kinase has been implicated in meristem patterning, where it coordinates the process of cell division in different cell layers (Meyerowitz). We also learned about how few genes can be sufficient for dramatic morphological changes in plant breeding and therefore presumably evolution, such as from teosinte to modern corn (Doebley and Wang).

Cell Biology and Development

The increased focus on cell biology is part of a general trend away from pure developmental biology and to specific molecular biology of the cell questions. For example, fly sex determination became a study of RNA splicing, fly chromosome dosage compensation a study of chromatin, eye development a study of the ras pathway, segment polarity genes a study of Wnt and Hedgehog signal transduction, pair-rule genes a study of combinatorial actions of transcription factors, Hedgehog processing a study of cholesterol modification of proteins, and so on.

Certain experimental systems originally employed for global patterning studies are now being used for studies at the cellular level. This is a trend that seems particularly promising. Notable examples are the study of epithelial invagination in the worm vulva (Herman and Horvitz) and Drosophila oogenesis (Spradling et al.; Rongo et al.; Markussen et al.). Epithelial invagination requires a set of proteins involved in glycosylation. A screen for mutations affecting germ cell migration led to the identification of the Zfh1 transcription factor as important (Rongo et al.). Together with Timman, it is required for the interaction between pole cells and the mesoderm into which they move. The fusome, a structure connecting the developing oocytes that is derived from mitotic spindles, appears to set up a transport system and may be involved in storing, modifying, or delivering molecules such as cyclins that control cell fates by controlling the cell cycle (Spradling et al.). The fusome is part of the system for producing “the equivalent of an 8-lb baby every three hours,” (Daunting as this may have seemed to the XX members of the audience, the length of Drosophila sperm exceeds the length of the adult, giving pause to the rest of the audience.) Localized substances within the oocyte govern fates in the anteroposterior axis and regulate determination of germ cell identity. The regulation of oskar, one of these regulators, has been particularly interesting. The 3' UTR of oskar is required to bring the RNA to the posterior pole. The bruno protein is required to block translation of oskar mRNA before it reaches the posterior pole, and Ephrussi (Markussen et al.) reports the identification of a new protein, p50, that may be required for translation after the mRNA reaches the pole.

The 3' UTR has also been implicated in worm sex determination, in particular in the regulation of the decision to make sperm versus oocytes in the germ line. Two genes that act on the relevant UTR, mgo1 and mgo5, both encode RNA-binding proteins with DEAD box motifs (Puoti et al.). The proteins are also related to the Pumilio protein previously implicated in related functions in flies. RNA localization, a major theme of the meeting, emerged again in the study of a fish wnt homolog that appears to be involved in germ cell determination (Amsterdam et
Asymmetry

The talks on bacteria and yeast reminded us why these are the best experimental organisms. We would have been even more humbled by a phage talk. The “lowest life forms” were described by Hofmeister and Losick, who presented three mechanisms of asymmetric protein activity in *B. subtilis*. In an asymmetric division that creates a small spore, one sigma factor becomes active exclusively in the spore due to the mysterious activity of a membrane-bound protein localized to the septum between the two parts of the cell. A second sigma factor is activated only in the larger mother cell in two ways: (1) elimination of the protein from the forespore and (2) processing of the protein by a membrane-bound protease that mediates a signal across the septum from the forespore.

Asymmetry in yeast budding is inherited from the previous budding event, although the information about the previous division is interpreted according to the genotype of the cell (Herskowitz). Two GTPase systems control local assembly of the cytoskeleton for budding. Another form of asymmetry is the activation of HO in the daughter, but not mother, cell after division. A localized RNA, encoding an HO repressor, is found in the distal daughter cell. The budding site inheritance system controls localization of the RNA.

Proceeding to plants, we were reminded that *Fucus* responds to light with oriented growth. Remarkably, it can remember the direction of light for hours (Quatrano). The establishment of a cortical growth site allows asymmetric development, but how the light signal is transduced is not yet clear. The cortical site is established by microfilament-mediated movement of vesicles to the site.

Asymmetric cell division is a frequent event in biology and is especially prevalent in the fly nervous system. Several localized proteins (Prospero, Numb, Inscutable, and A3) and at least one localized RNA (*inscutable*) are involved. The localization of the RNA is separable from the localization of the protein (Knoblich et al.). A3 is a component critical for localizing Prospero protein, a transcription factor that spends part of its time hanging on to the membrane to end up in the right nucleus. Numb can also bind to A3. Numb, part of the output of the system and localized independently of Prospero, may act by binding to Notch and biasing cell-cell communication events to control cell fates. Chai et al. reported the exciting finding that a protein previously known to be involved in RNA localization during embryogenesis, Staufen, binds to *inscutable* RNA, and *staufen* mutants affect *inscutable* RNA localization. Unity in biochemistry.

Evolution

The single most amazing advance during the past 12 years is, of course, the recognition of great similarities among animals that at first glance seem entirely different. The list of similarities is increasing all the time. Currently, there is evidence for the following: anteroposterior body plan controlled by Hox genes (Cappelletti; Kenyon et al.; J. Kim et al.), dorsoventral body plan controlled by TGF-β signals and their antagonists (De Robertis et al.; R. Harland, C. Wright; both unpub.), heart development controlled by Tinman/Nkx2.5 (Biben et al.) and MEF2 (Lin et al.), eye development controlled by Pax6/eyeless/aniridia, CNS midline regulation of axon extension (Leonardo et al.; Goodman et al.), branching morphogenesis of lung and trachea (Krasnow; Shilo et al.), limb dorsoventral and distal-proximal patterning (Schwabe et al.; Kengaku et al.), limb anteroposterior patterning (Kengaku et al.), and Toll signaling in insect and mammalian immunity. In fairness, I must note apparent differences as well. No role for FGF in the outgrowth of insect limbs has been noted, in contrast to the vertebrate scenario.

We are increasingly aware that biological structure is stable, because it replicates, whereas geological structure is transient. Our meeting took place on a pile of gravel called Long Island, swept by glaciers off the surface of Maine within times measured in tens of thousands of years. The mosquitoes outside have scarcely changed in tens of millions of years, unfortunately.

The picture is therefore of commitment of certain gene systems to certain developmental purposes. When preformationists were retreating from their fully irrational stance to one slightly less so, they proposed that there was no complete homunculus, but instead each body part of the progeny inherited a representative bit from the corresponding parental organ. We now face a corrected version of that idea, of gene sets appropriate to certain tissues being inherited as working packages.

We learned that certain tooth structures are thought to have evolved more than 20 times despite the remarkable complexity of signaling proteins in the signaling center called the enamel knot (Thesleff and Jernvall). Eyes were thought to evolve more than 40 times. The idea is that selective pressure caused the same outcome on separate occasions. Another view seems more likely to me. The genetic program for a structure may have evolved once, and then was stashed away intact (perhaps used for other purposes?), only to become reactivated later. With this view, intermediate forms need not have retained all the structures common to their ancestors and descendants.

Hox complexes in anteroposterior patterning are not too hard to accept because the ancestors presumably had heads and tails and fronts and backs, but believing that our appendages evolved from a creature whose other progeny became insects or squids has been hard to believe. The whole appendage gene program may have existed for a primordial purpose that need not have been anything very fancy, for example, a simple outgrowth. Then as vertebrates or insects evolved, the easiest way to form an outgrowth was to recruit the set of gene systems intact and initiate them at a localized spot on the body (Kengaku et al.).

Following through this reasoning, I wonder whether we can dispense with the outgrowth altogether and think
back to a still more primitive situation when the set of gene systems was integrated for another purpose altogether. We could even imagine events in the single cell, such as a shape change, requiring a certain genetic program. In speculating about this, we would be helped if we knew much more about the use of, for example, Wnt, Hh, FGF, and TGF-β signals in very simple organisms. In the same way that yeast mating-type studies have been directly informative in thinking about Hox genes, understanding how higher animal types of signals are used in single-celled organisms, or organisms with relatively few cells, might help us to understand how a sponge uses the genes that we use to form arms. Are organs related to organelles?

**FUTURE**

Developmental biology has at least two practical uses: (1) for learning how animals and plants grow in order to make it possible to change how they grow or regenerate and (2) for “functioning,” i.e., taking novel proteins such as one important for a disease and finding out how they relate to other proteins and pathways.

Developmental biology has yet to make any serious impact on medicine, but this is rapidly changing. In the short term, the identification of inherited human disease genes has brought to light the involvement of familiar regulators of development. Putting them into genetic pathways and developmental contexts will permit the design of new therapies and diagnostics. Oncogenes and tumor suppressors, such as Ras (Rubin et al.) and Patched (Johnson and Scott), are now being viewed as development regulators gone awry. Their normal roles will be helpful in understanding their evil roles.

Regeneration has not been a major focus in recent years, but as normal development is better understood, the right questions will be asked about how complex structures can be reformed or healed. The current tests of bone morphogenetic proteins for helping fractures heal may seem, in years to come, the crudest beginning. The seemingly miraculous reformation of amphibian limbs may be extended to other tissues: to rebuilding a damaged spinal cord, making insulin from a regenerated pancreas, or repairing a heart or liver. These prospects are exciting.

The human genome project is bringing a new importance to work on animal development. Flies, frogs, and worms may well become test organisms for placing a mystery gene into some sort of conceptual framework. Rubin’s description (Rubin et al.) of *kuzbanian* provides an example. A newly found gene was quickly recognized as belonging to the Notch pathway. The right biochemical tests (cleavage of Notch) could be immediately designed from this knowledge and the protein’s sequence, which revealed a metalloprotease. “Model” organisms will become increasingly important for this sort of purpose; it is easy to imagine standardized ways of placing “new” proteins into established pathways, a sort of aptitude test for genes.

With All This Progress, We Still Have a Superficial Understanding

The underlying principles still evade us. We have an understanding of the instruments but not the orchestra. Why is FGF used in one instance and TGF-β in another? Was it history that gave us these choices, or is there logic to the application of certain protein types to certain purposes?

There is room for substantial technical advances. We do not have easy ways of identifying genes regulated by transcription factors. We do not know nearly enough about the inheritance of determined cell fates, or what makes cells responsive to a signal at one moment and refractory the next. We do not know how to reanimate embryonic programs. We are at the very beginning of applying developmental biology to human disease genes to create new treatments, or to other human needs such as dealing with pathogen resistance, pest control, and parasitology. We need to invent more ways to make dominant negative mutations, one of the best chances to overcome redundancy in vertebrate genomes. We had some good examples in this meeting, such as dominant negative BMP receptors (Zou et al.; C. Wright, unpubl.) and fusion of an Engrailed repression domain to the Lmx1 homedomain (Schwab et al.). We lack good methods for observing evolution in progress, although the rapidity of evolution that can be observed in field biology studies gives me optimism on this count (see, e.g., *The Beak of the Finch: A Story of Evolution in Our Time* [1994, Knopf, New York] by Jonathan Weiner, reviewing the work of Peter and Rosemary Grant).

How do signals move through tissues or between cells? Are there facilitating, as well as obstructive, factors? At this meeting, receptors were proposed as both facilitators and limitors to ligand movement. Roel Nusse (Nusse et al.) described experiments suggesting facilitation of Wingless movement by its receptor, Frizzled2, and G. Struhl (pers. comm.) described how the Patched receptor can restrict movement of its ligand, Hedgehog. In addition to these factors, extracellular matrix and transcytosis were mentioned as relevant to the movement of ligands through tissue. One practical value of better understanding how molecules move would be to allow the design of targeted ligands with altered signaling range.

We need methods for looking at protein activities in space and time, providing information formally equivalent to knowing when and where genes are transcribed or where proteins are located. We had one nice example of this at the meeting from Benny Shilo (Shilo et al.): the detection of active MAP kinase with an antibody specific to the activated form.

We need to look at more animals. The wonders of nature provide tools as well as aesthetic pleasure in science. The butterfly patterning discussed by Carroll (J. Kim et al.) provides one example. Early development is really diverse, even within the vertebrates. What can be done to understand the biological meaning of the differences and the mechanisms used to generate those differences? Very often, interesting molecular biology is revealed by inter-
esting biology, for example in antibody diversity in the immune system. R. Beddington (Thomas et al.) pointed out the potential value of looking at armadillo development, where four primitive streaks form in one embryo. The major hurdle to be overcome is that no one wants to do all the basic gene characterization for a whole new system, so shortcuts must be found.

With all of our optimism derived from the past dozen years, we must remember the remarkable complexity of the Organ of Corti shown to us by Nathans (Xiang et al.). He mentioned that whenever he feels we are really making progress, a look at the Organ of Corti is humbling. We have a very long way to go in understanding how global regulators of the sorts discussed here control cell morphology.

A Common Language

In groping for analogies that may help us to think about the common heritage of developmental mechanisms, I turned to linguistics. Here, I had heard, a revolution had come from the proposition that languages with seemingly nothing in common in fact had related underlying structures. This seems exactly like our awakening in developmental biology. The linguistic thesis, as I understand it, is that languages all have certain syntactical rules in common because language is constrained by the structure and organization of the brain. I referred to Noam Chomsky’s more accessible writings (Reflections on Language [1975, Random House, New York]) to learn more and was startled to find that he had used developmental biology as an analogy for his ideas about language. He wrote:

... “human cognitive systems, when seriously investigated, prove to be no less marvelous and intricate than the physical structures which develop in the life of the organism.”

“The idea of regarding the growth of language as analogous to the development of a bodily organ is therefore quite natural and plausible.”

“It is a curious fact about the intellectual history of the past few centuries that physical and mental development have been approached in quite different ways. No one would take seriously the proposal that the human organism learns through experience to have arms rather than wings.”

“More intriguing to me at least is the possibility that by studying language we may discover abstract principles that are universal by biological necessity and not mere historical accident, that derive from mental characteristics of the species.”

If Chomsky is right, and language takes its structure from the working structure of the brain, then we are talking about one and the same thing: Genes that organize brain development organize language. Commonalities in language reflect common paths of development. Language becomes a phenotype of the genes we have discussed here. Let’s find out. In the meantime, we can share and enjoy the new common language of developmental biologists.

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