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Perspectives

Conceptual breakthroughs in developmental biology

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I
Introduction

Revising a textbook is a fascinating exercise that allows one to see quite starkly the changes that have occurred in one's discipline through the subsequent editions. As I revise a textbook that was originally published in 1985, I can see the numerous advances that have transformed the discipline of developmental biology. But even more important and much rarer than the *advances* are the true *breakthroughs*. A breakthrough is more than just an advance. For something to be a breakthrough, it must have encountered resistance – it must have *broken through* something. In thinking about breakthroughs in developmental biology, let me first construct three categories of breakthroughs. (i) *Conceptual breakthroughs*: These are critical. We rarely find things if we don't know to look for them. Conceptual breakthroughs direct our research into new areas. (ii) *Methodological breakthroughs*: Often we have the concept, but not the techniques to follow the paths opened by the concept. Methodological breakthroughs allow certain areas to be explored. (iii) *Experimental breakthroughs*: Once the technique has become available, certain experiments provide new insights into the workings of nature. As historians of biology have long acknowledged, it is rare that a single experiment creates a new paradigm. However, the weight of several experiments in the same direction can create a breakthrough into new realms.

II

This essay will look at the conceptual advances in animal developmental biology over the past fifteen years. I think that we can identify seven particularly important conceptual breakthroughs that have caused developmental biology to metamorphose into a new science.

1. Developmental biologists can indeed explain development

Fifteen years ago, embryology was what could be characterized as the only field of science that celebrated its questions more than its answers. We had the greatest problems one could imagine: How does the brain develop? How do the eyes form? How does our back develop differently than our front? How are the arteries and veins connected to the heart? But we had very few answers. The field had not changed much from 1958, when molecular biologist Sol Spiegelman chided his embryological colleagues at a meeting on development:

> I have found it difficult to avoid the conclusion that many of the investigators concerned with morphogenesis are secretly convinced that the problem is insoluble. I get the feeling that many of the intricate phenomena described are greeted with a sort of glee as if to say,"My God, this is wonderful, it is so complicated we will never understand it."
>
> It seems to me that perhaps the time has come to abandon this joyful pessimism and its attendant conviction of incomprehensible complexity. In particular, I should like to make a plea for a more optimistic view based on a belief in simplicity. The phenomena of morphogenesis can hardly be as complicated as implied by the welter of apparently unrelated observations constituting the literature of embryology.

The first indication that this joyful pessimism was over came from the laboratory of Christiane Nüsslein-Volhard. It was her laboratory that fused together embryology and genetics through the mediator of molecular biology. First, Katherine Anderson discovered a morphogenetic determinant that is an RNA. This was the mRNA for the snake protein. She was able to rescue the eggs from homozygous *snake* mothers by injecting

them with small amounts of cytoplasm from wild-type eggs. Instead of developing entirely dorsal cuticle, the dorsoventral pattern was restored. Then, in a remarkable series of experiments, this laboratory and others delineated the mechanism of anterior-posterior axis formation in *Drosophila*. The analysis of *bicoid* not only showed that a morphogen could be stored as an mRNA, but that it could be localized in one region of the cytoplasm through its 3′ untranslated region, and that a gradient of this protein could activate different genes depending upon the protein's concentration. The joyful pessimism gave way to a joyous optimism that some of the problems that had been on the books for hundreds of years could now be solved.

Interestingly, as Michael Ashburner (1993) has noted, the initial screens that detected the genes involved in anterior-posterior axis determination could have been done forty years earlier. "All this required was some standard genetics, a mutagen, and a dissecting microscope, all available in the 1930s ... It was the idea that counted." Keller (1996) has documented that this idea could not have come about until the techniques and the ethos of molecular biology enabled one to go further with the idea.

2. The core of development consists of paracrine factors, transcription factors, and the signal transduction apparatus between them

Developmental biology is a science of arrows. In a search for specific transcription factors which began with the operon model of Jacob and Monod, numerous trans-regulatory factors and the enhancers and promoters to which they bind were elucidated. In addition, the paracrine factors—the TGFs, BMPs, Hedgehogs, FGFs, and Wnts—slowly emerged from the "soluble filtrates" that were seen to induce differentiation. Inducers were the paracrine ligands and competence was the ability to receive and process the signal from the bound receptor. Between the paracrine factors acting at the cell surface and the transcription factors working within the nucleus were the signal transduction cascades, pathways initially delineated by oncologists who were interested in cell division. We now have complex circuits not only within cells but between cells. The biochemistry of the embryo has become intercellular as well as intracellular.

The delineation of the pathways linking paracrine factors, signal transduction cascades, and transcription factors was also a triumph of molecular biology. The techniques of biochemistry were not adequate to isolate and purify the minute amounts of labile paracrine factors or transcription factors. The key advance was to isolate the mRNA rather than the protein. Before the use of molecular probes, I can think of only one selective transcription factor that had been characterized- the androgen receptor.

3. Homologous genes and pathways exist between distantly related phyla

This is an important breakthrough, for the predominating concept had been set forth by people as illustrious as Theodosius Dobzhansky and Ernst Mayr. As Mayr concluded in 1966, "... the search for homologous genes is quite futile except in very close relatives." Embryologists agreed. Each organ was seen to develop very differently from any other organ. This notion was destroyed by the discovery of the *Hox* genes in vertebrates. We now have remarkable homologues. *Tinman* is used in both the flies and vertebrates to make hearts, *Pax6* is used in both flies and vertebrates to make eyes. The *fringe*, *hedgehog*, and *serrate* proteins are used to generate limb patterns in both vertebrates and arthropods (Shubin *et al* 1997).

In addition to homologous genes, there are also homologous pathways (see Gilbert 1996; Gilbert *et al* 1996). One of the first to be noticed was the RAS pathway which is used in the construction of the *Caenorhabditis elegans* vulva, the *Drosophila* seventh photoreceptor, and the division pathway in mammalian skin. We have also seen that the Wnt-hedgehog pathway first elucidated in *Drosophila* is also conserved gene-for-gene in the vertebrates. These two paracrine factors interact within the disc to specify the proximal/distal, dorsal/ventral, and anterior/posterior axes. The same molecules that specify these axes in the eye also specify them in the leg and wing discs. So we have a serial process homology. Moreover, the same pathway exists in vertebrates. Every member of the pathway in insects has a homologue in the vertebrate embryo, and the same interactions that transmit the *Drosophila* Wingless signal to the nucleus through armadillo and pangolin protein are seen in the vertebrates, wherein the Wnt signal is manifest in the entry of β-catenin and Lef-1 into the nucleus. The genes are the same and the protein interactions are the same. Only the readout is changed from tissue to tissue and from species to species. Interestingly, the same Wnt/hedgehog interactions seen in producing the fly limbs are seen in the interactions that are involved in the morphogenesis of vertebrate limbs. If a vertebrate hedgehog protein (which is usually synthesized only in the posterior mesoderm) is expressed anteriorly, the limb develops a mirror-image duplication. This is the same phenomenon that occurs when hedgehog protein is induced to form in the anterior portion of the fly wing disc (see Ingham 1994). Similarly, the Rel-protein pathway is used for dorsal-ventral patterning in the *Drosophila* blastoderm and for immunocyte function in both flies and mammals (figure 1; see Shelton and Wasserman

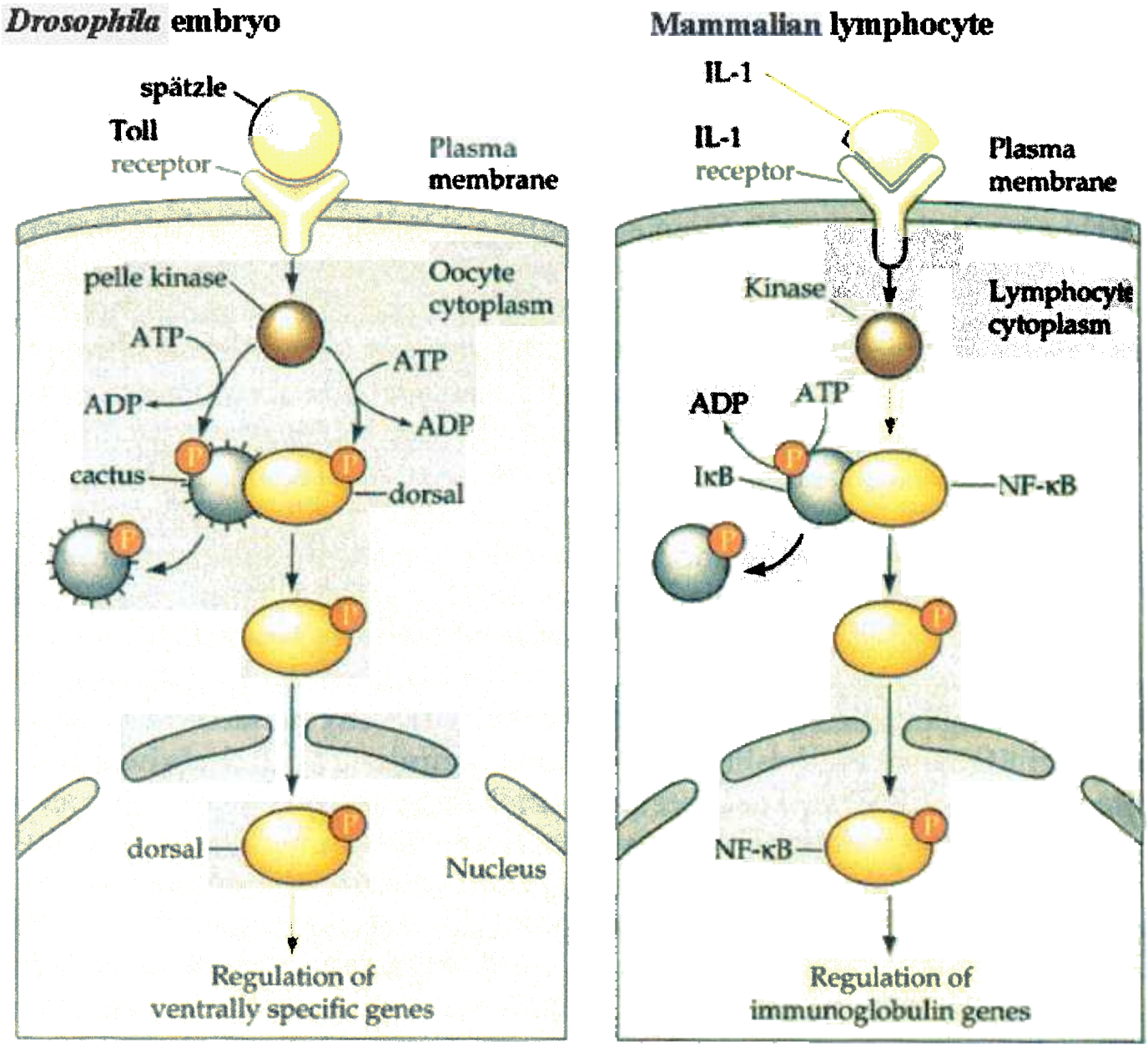

Figure 1. Homologous developmental pathway involving Rel proteins. The pathway is homologous protein for protein. In *Drosophila*, the binding of the ligand to the cell membrane receptor activates the dorsal protein which can enter the nucleus to become a transcription factor. In vertebrate B-lymphocytes, the binding of a homologous ligand to a homologous receptor activates the NF-κB protein to enter the nucleus to become a transcription factor. (After Gilbert 1997)

1993). Like structural homologies, homologies of process show homologous structures arranged in homologous orientations.

Homologies of process should not be confused with analogies. Whereas analogy indicates common function (as in the locomotor function of insect and human legs), homology indicates common structural origin. Insect legs and vertebrate legs are analogous as they come from different embryological structures and are made of different materials arranged in different ways. Homologous pathways can be used to construct several different organs, such as the RAS pathway in the nematode vulva and *Drosophila* photoreceptor 7. This does not mean that the photoreceptor and the vulva cells are in any way homologous. Rather, the homologous pathways are informational cassettes that can be used to mediate intercellular interactions in a variety of cells.

However, there may be some "deep homologies" wherein homologous pathways create the same structures in very distinct phyla and suggest that nature only figured

out how to make the structures once. One of these "deep homologies" involves the nervous system. Here, chordin secreted by the organizer of amphibian embryos binds to and blocks the action of BMP-4. This prevents the ectoderm from expressing the genes that specify the cells to become epidermal. Similarly, in the ventral surface of the fly embryo, the arthropod homologue of chordin (the short-gastrulation protein; sog) blocks the lateralizing effects of its BMP-4 homologue (the decapentaplegic protein) to allow its ectoderm to become neural. These molecules can even substitute for their homologues. Chordin mRNA will cause neural formation in flies; injection of sog into *Xenopus* causes ectopic notochord and CNS. By blocking BMP, chordin specifies the ectoderm to be neural, whether it be dorsal in the frog or ventral in the fly (for review and references, see De Robertis and Sasai 1996; Gilbert 1997).

Jonathan Bard (personal communication) has joked about a time traveller going back to a developmental biology meeting in the early 1980s:

> In that 1983 meeting, there would have been no sessions on genes because there were no interesting genes to talk about. Several sociological factors would have impressed our time traveller: first, how few people were at the meeting, second, their friendliness, and third, their lack of real interest in one another's work. In fact the last two were closely related, since work on one organism had almost no relevance to that of another, there was no sense of competition. Today, it is different: There is a slight downside to living in this age of wonderment. Then, we did'nt have to know much of anything outside our own little area, today we have to know almost too much. We have to remember the details of the ontogeny of a dozen animals.

The fact that so many genes and developmental pathways are homologous between organisms means that we have to know about research done on other creatures. The paired-box genes in flies are important in mice. The neurogenic genes in *Drosophila* are active in the vertebrate nervous system, too. Even within an organism, the same genes or gene family members are being used to construct several organs. In 1983, a person working on limb development didn't have to know about what was happening in dental or renal research. Now they do. One colleague of mine told me that he subscribes to the journal *Neuron* so that he can find out what's going on in kidney development.

4. Modularity is an integral part of the developmental process

A corollary of these homologies of process is that they occur in certain bounded regions. As Bonner (1988) noted, modularity is associated with "gene nets" that can participate in many different aspects of development. Indeed development occurs through discrete and interacting modules (Riedl 1978; Gilbert *et al* 1996; Wagner 1996; Raff 1997). In development, such modules include morphogenetic fields (such as those described for the limb or eye), imaginal discs, cell lineages such as the inner cell mass or trophoblast, insect parasegments, and vertebrate organ rudiments. Modular units allow different parts of the body to change without interfering with other functions.

The fundamental principle of modularity allows for three processes to alter development: dissociation, duplication and divergence, and co-option (Raff 1997). Since the modules are on all levels from molecular to organismal, it is not surprising that one sees these principles operating on all levels of development. Dissociation allows one module to change without affecting other modules. This permits heterochrony, wherein one module can change its temporal expression relative to the other modules of the embryo. Dissociation also permits allometry, wherein different parts of the organism grow at different rates. The principle of duplication and divergence is also seen as morphogenetic fields produce variations on a theme from an ancestral form. Different sizes and shapes of teeth are created by modifications of a basic theme, and the hindlimb is subtly distinguished from the forelimb. Lastly, modularity allows co-option such that a portion of the agnathan gill arches could give rise to jaws, and that this same module could later give rise to the mammalian middle ear cartilage (see Gould 1990).

The limb field, the heart field, the eye field, the primary induction field are all being re-established and put on a molecular basis. The discovery of the paracrine factors and their signalling cascades have made it clear that there is an intercellular as well as an intracellular biochemistry. Moreover, the same pathways being used to develop the organism are also used to maintain it. Thus, many of these pathways had been elucidated by oncologists and physiologists who were interested in tissue interactions in adults. The idea that physiological regulatory systems would be constructed from the fields and pathways that originally had been used for development was first proposed on theoretical grounds by Robertson and Cohen (1972). Now we know that the RAS pathway, the Wnt pathway, the JAK/STAT pathway, the IP3 pathway and numerous others are used for both the development and the maintenance of tissue structure. Abnormalities of these pathways in adults can produce tumours. It seems that just as the cell is the unit of physiological structure and function, the morphogenetic field may be the unit of ontological structure and function, not only the gene.

5. Changes in development are responsible for major evolutionary changes

This is one of the most important events in biology and in contemporary science. Goldschmidt (1940) said that evolution consists of inherited changes in the patterns of development. We now have evidence that major phenomena of evolution do indeed correlate with heredi-table changes in development. For example, Averof and Patel (1997) have shown that the pattern of *Ubx* and *abd-A* Hox gene expression correlates with the presence or absence of the modification of thoracic limbs into feeding maxillipeds. These maxillipeds form only where the genes are not active. The repression of these genes in the anterior trunk segments correlates with the changes in segment specification into gnathal-like appendages. A similar correlation of Hox gene expression with morphology comes from vertebrates, where the distinction between cervical and thoracic vertebrae as well as the distinction between thoracic and lumbar vertebrae is mediated by Hox genes (Gaunt 1994; Burke *et al* 1995). One of the most interesting correlations is the difference in *Hox* gene regulation between fins and limbs. It appears that the autopod may be an evolutionarily novel structure that was permitted to form by the expression of *Hoxd-11* and *Hoxd-13* (figure 2; see Shubin *et al* 1997).

But Hox genes need not be differently expressed to have effects on evolution. The downstream genes can also be different, such that *Ubx* expression gives halteres in flies and hindwings in butterflies. Thus, development, genetics, and evolution are being reunited. This is an enormous conceptual breakthrough. It is a far distance from when Dobzhansky (1951) could declare, "Evolution is a change in the genetic composition of populations. The study of the mechanisms of evolution falls within the province of population genetics."

Rather, there is now a new developmentally oriented concept of the gene in evolution. As Dobzhansky (1964) said, "Nothing in biology makes sense except in the light of evolution," and the nature of the gene is no exception. In the *population* genetic model of evolution, one extrapolated from microevolution to macroevolution. The gene was measured by differences in the allelic

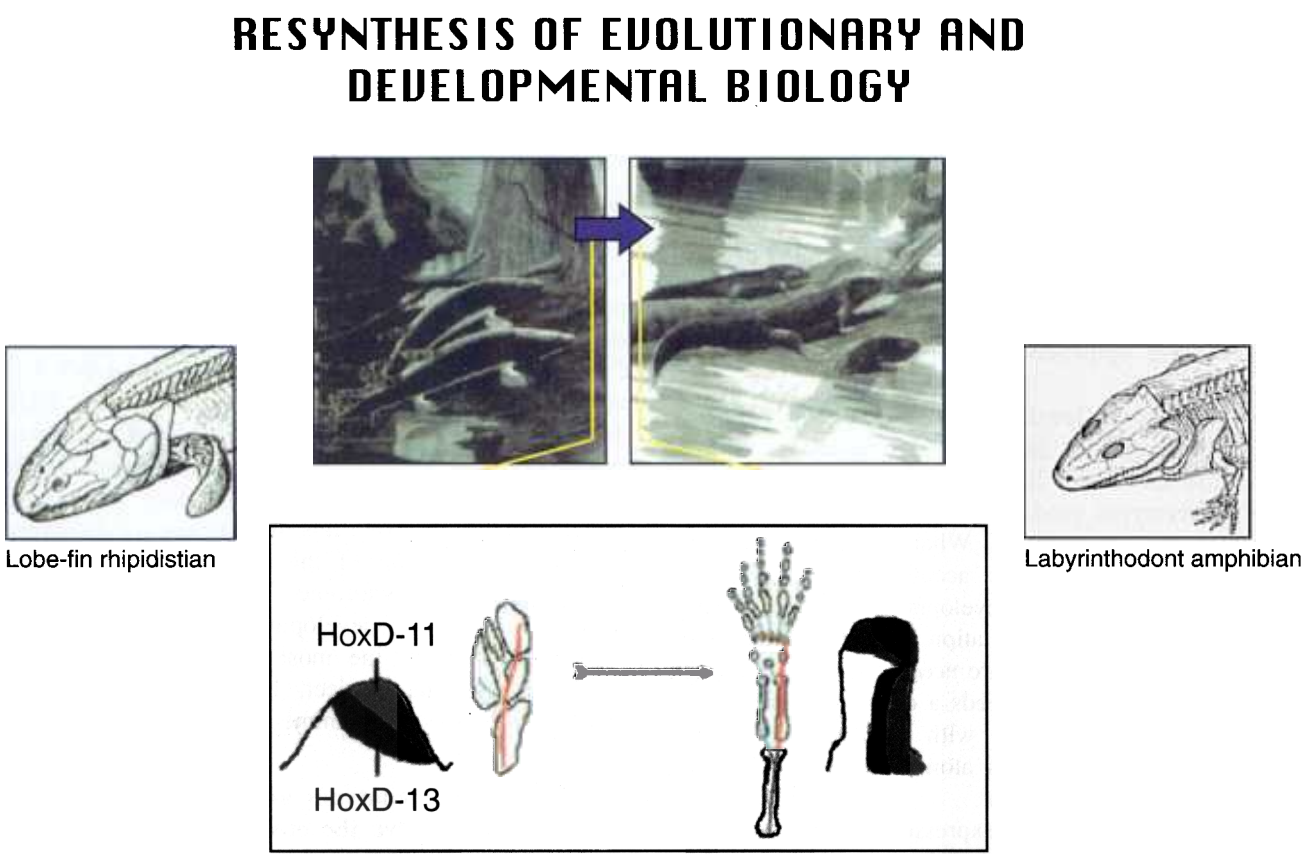

Figure 2. Integration of developmental biology and evolution: the transition between fins and limbs. This change correlates with the inversion of *Hoxd-11* and *Hoxd-13* transcription patterns. The above picture is a reconstruction of what might have occurred during the transition from fish to amphibian. (From several sources)

Table 1. Differences in the evolutionary roles of genes in the classical, population genetics model of evolution and the developmental genetics model of evolution.

The changing roles of genes in explaining evolution	
Classical genes	*Developmental genes*
Abstraction → globin	Defined DNA sequences
Manifest by differences	Manifest by homologies
Explains natural selection	Explains phylogeny
Allelic coding regions of structural proteins	Regulatory regions of developmental factors
Expressed in adults competing for reproductive advantage	Expressed in developing embryos and larvae
Individual action as autonomous agent	Action as part of pathway of context-dependent genes

coding region, and it was of value to the adult who was competing for reproductive success. In the new synthesis of *developmental* genetics and evolution, macroevolutionary phylogenies are measured by the similarities of genes, the important part of the gene is the regulatory region, and these genes are manifest in the construction of the embryo rather than in the differential fitness of the adult. Moreover, the developmental genetic gene is not an autonomous player. It is part of a larger pathway or network (table 1).

This idea was first sketched out by Conrad Hal Waddington (1953) when he noted that natural selection can work at two very different levels. The traditional one concerned the elimination of adult phenotypes (''normative selection'') while the less studied mode of selection (''stabilizing selection'') eliminated those individuals whose epigenetic systems of interactions were not stable.

This changes the levels of explanation in evolutionary biology. Whereas for the population geneticist, it was adequate to know that XX karyotypes produced females and XY karyotypes produced males, that is not sufficient for developmental biologists. What is mechanism in the classical population genetic account of evolution has become correlation in the developmental genetic account of evolution. Both the population genetic and the developmental genetic accounts are needed. The breakthrough is the realization that one needs a developmental genetic account of evolution along with a population genetic account. Population genetics alone will not explain the processes of macroevolution.

J B S Haldane (1953) expressed a sense of these things to come, using a wonderful analogy: ''The current instar of the evolutionary theory may be defined by such books as those of Huxley, Simpson, Dobzhansky, Mayr and Stebbins. We are certainly not ready for a new moult, but signs of new organs are perhaps visible.'' This recognition of the new emerging within the old ''points forward to a broader synthesis in the future.'' We have now broken through the old integument, and the new organs of a broader, developmentally-oriented synthesis are being constructed.

6. The resynthesis of medical genetics and medical embryology is necessary for the explanation of congenital malformations

In the entire field of genetics, medical genetics is the most classical area. It retains the gene mapping programme and until recently was little more than descriptive analysis. Similarly, medical embryology is probably the most classical of all the subfields of developmental biology. Until recently, it consisted of the descriptions of normal and abnormal anatomy. The rapprochement of medical genetics and medical embryology may be the most meaningful of all the syntheses between developmental biology and genetics. Inborn errors of metabolism used to refer entirely to the genes encoding metabolic and structural proteins. However, the discovery of paracrine factors, transcription factors, and their signalling pathways has been coupled with candidate gene mapping to produce a new biochemistry of human development. A paradigm of this technique is analysis of the FGFR3 gene whose gain of function mutations cause thanatophoric dwarfism and achondroplasia (figure 3, see Muenke and Schell 1995).

After identifying developmentally important genes in flies, frogs, fish, and mice, we can see if the human homologue maps to a region whose mutations have a particularly appropriate phenotype. In this manner, we have identified genes for transcription factors such as SRY (aberrant male sex determination), Pax6 (aniridia), Twist (Saethre-Chotzen craniosynostoses), and WT-1 (renal agenesis), paracrine factors such as GDNF (Hirschsprung syndrome), and putative signal transduction proteins such as RET (Hirschsprung syndrome), FGFR3 (achondroplasia/thanatophoric dwarfism) and tabby (anhydrotic dysplasia). In this manner, we are discovering not only the location of the mutation but the pathophysiology of the syndrome. In one of those strange paradoxes of science, developmental biology, which has contributed some of the most substantial criticisms of the human genome project, has become the leading beneficiary of this programme.

7. Important phenotypes are not encoded by the genome and involve the environmental regulation of gene expression

The integration of developmental biology and ecology is the most recent conceptual breakthrough, and there

are two routes leading up to a fascinating new area—developmental ecology. The first involves the mechanisms of teratogenesis. What do teratogens do? A few years ago we had no idea. Now, it seems, we are beginning to have some candidates. Alcohol, for instance, appears to inhibit the homotypic adhesion of the L1 cell adhesion molecule in the brain as well as interfering with msx2 expression. Valproic acid appears to inhibit Pax1-induced somite formation (Barnes *et al* 1996).

The second path to developmental ecology involves the analysis of life history strategies. We have come to realize that our model systems have several things in common. Our model systems have converged on several features: small size, rapid sexual maturity, large litter production, early separation of germline and soma, and most importantly, the ability to develop in the laboratory (see Bolker 1995). That is to say, our organisms are selected for their ability to develop without environmental cues. However, in the real world, many, if not most, organisms are finely tuned to their environments. Some cases are spectacular—temperature dependent sex determination in reptiles, the ability of fish to change their sex depending upon the density of males in the population, the predator-dependent changes in morphology in numerous species from cnidarians to frogs, the diet-dependent polyphenism of *Nemoria* which allows it to look like a seed case in the spring and a twig in the summer, and many others. We are dealing here with what might be called Tertiary Induction—induction from outside the organism. Last year, we had our first indication of what genetic steps might be occurring. Developmental biologist Sean Carroll teamed up with ecologist Paul Brakefield to analyse the seasonal polymorphism of the *Bycyclus* butterfly eyespots (Brakefield *et al* 1996). The low temperature morph does not have an eyespot and blends into the dry leaf litter of the season. The high temperature morph is an active flier whose eyespots deflect predators. The high temperatures allow the maintenance and expansion of *distal-less* transcription which organizes the eyespot. In the absence of *distal-less* maintenance, the eyespots fail to enlarge. The proximate causes of life history strategies remain almost completely unknown, and this will almost certainly be a major research programme in the near future.

INTEGRATION OF DEVELOPMENTAL BIOLOGY AND MEDICINE: FGF RECEPTOR-3 AND THANATOPHORIC DYSPLASIA

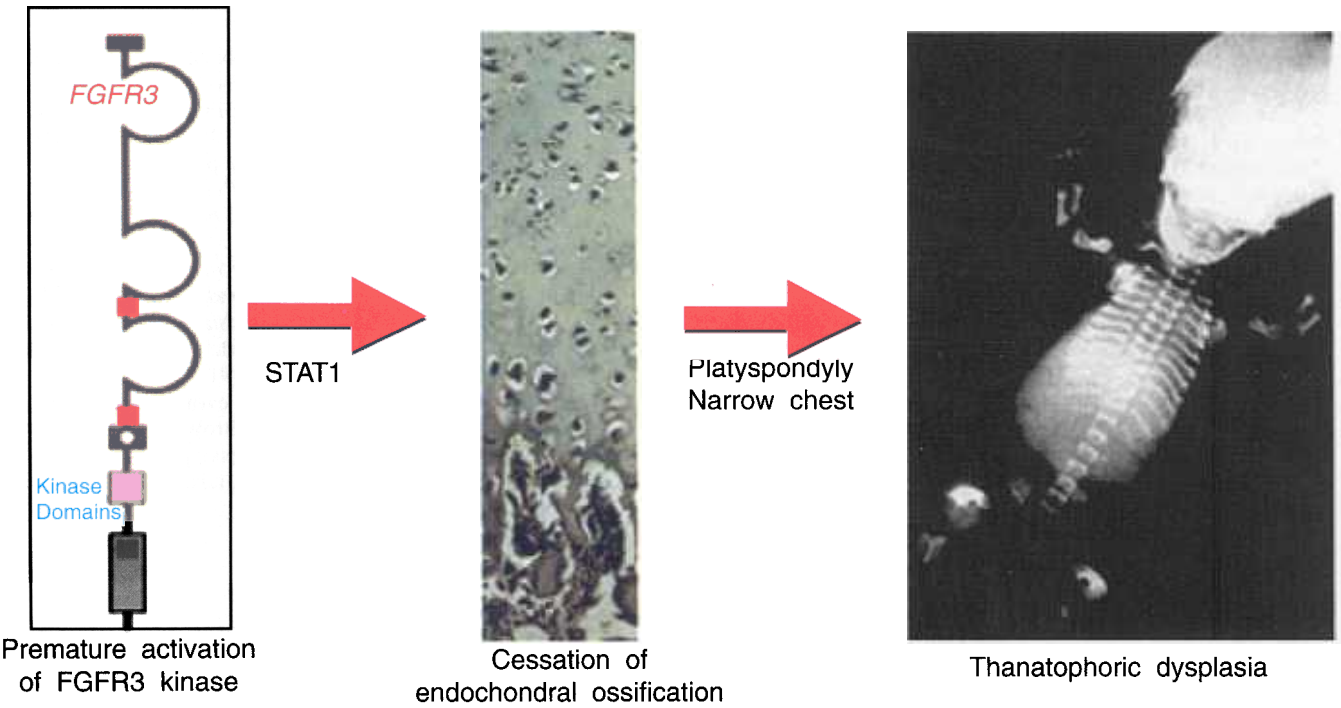

Figure 3. Integration of developmental biology and medicine: the pathogenesis of thanatophoric dwarfism. Gene mutation in fibroblast growth factor receptor 3 prematurely activates the STAT pathway in the developing chondrocyte. This leads to the cessation of chondrocyte growth and the elimination of the growth plate. The resulting infant has insufficient rib cartilage as well as poor bone growth in limbs. (From several sources)

III

In 1894, Wilhelm Roux, in the founding document of Entwickelungsmechanik, predicted that once embryology was put on a physiological basis, it could return to the rest of biology and become its centre, integrating physiology, anatomy, inheritance, and evolution:

> The specific processes of life are bound to the form and structure of its substrata. Hence, *developmental mechanics* as the science of the causes of these formations will sometime constitute *the common basis of all other biological disciplines* and, *in continual symbiosis with these*, play a prominent part in the solutions of the problems of life. (Italics in original)

A century later, developmental biology is beginning to fulfill that prophecy and assume that central position that Roux expected it to enjoy.

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