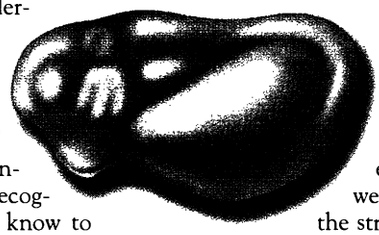
tenna of the fly into a leg? It may well be that no general principles are involved in the control of morphogenesis and cell differentiation. Even so, we do not yet have an example where we understand in detail the development of a single adult organ. We remain largely ignorant of timing mechanisms and how the size of different structures is controlled. It also has to be recognized that we do not yet know to what extent the principles of animal development apply to plants, although recent progress has been dramatic, and genes have been identified that control the identity of floral structures (*12*).



How many genes control development—as distinct from providing the housekeeping functions of the cell? The answer is not known, but one can guess. Analysis of early insect development suggests that only about 100 genes are involved in controlling patterning during early development. And in the nematode at least 50 genes are known that control vulva development (*13*). If one thinks of, say, 100 genes for each multicellular structure in the adult, then 50 different structures in *Drosophila* would require 5000 genes. For mammals, for which there are some 350 distinct cell types, tens of thousands of genes might be needed. Understanding the function of so many genes is made even more difficult by cases of apparent redundancy. That is, it is possible to knock out certain genes in mice without there being any obvious effect on the phenotype. It is likely that true redundancy is illusory and merely reflects the failure to provide the correct test for an altered phenotype. It may thus be very difficult to work out the true function of such genes.

Will the egg be computable? That is, given a total description of the fertilized egg—the total DNA sequence and the location of all proteins and RNA—could one predict how the embryo will develop? This is a formidable task, for it implies that in computing the embryo, it may be necessary to compute the behavior of all the constituent cells. It may, however, be feasible if a level of complexity of description of cell behavior can be chosen that is adequate to account for development but that does not require each cell's detailed behavior to be taken into account.

An analogy to some of these problems is found in the analysis of protein folding, which seems a much simpler problem, but where it may not be possible to work out the final structure from the sequence information by using first principles. Rather, the solution will come from homology (*14*). As with protein folding, homologies drawn from an extensive database could provide the best basis for making predictions about development. It is not unreasonable to think that enough will eventually be known to program a computer and simulate some aspects of development. We will, however, understand much more than we can predict. For example, if a mutation were introduced that altered the structure of a single protein, it is unlikely that it will be possible to predict its consequences.

So what will the next 20 years bring? Undoubtedly powerful new techniques will be invented that will enable us to understand the details of gene action and the biochemistry and biophysics of cell behavior. Working out the detailed action of all those genes, proteins, interactions, and kinases will be a hard slog and often tedious. It is unlikely that any new general principles will be discovered. However, the current excitement will continue as we come to understand the detailed mechanisms, and as more and more similarities between apparently different developmental systems emerge. Almost certainly there will be new ways of integrating particular aspects of development, and so we will learn, for example, the logic underlying the apparently varied mechanisms for generating periodic structures and the reasons for the variety of mechanisms for setting up the axes in early development. We can also look forward to great progress in the area of evolution and development. We may then see the solution to grand problems like how basic body plans emerged, how they are conserved, and the origin of developmental novelty. We will thus come to understand how development constrains and directs the form of all multicellular organisms.

References

1. L. Wolpert, *Biol. J. Linn. Soc.* **39**, 109 (1989); L. Wolpert, *Development* (suppl.), in press.
2. P. Lawrence, *The Making of a Fly* (Blackwell, Oxford, 1992).
3. _____ and G. Morata, *Cell* **78**, 181 (1994).
4. T. Grunwald and G. M. Rubin, *ibid.* **68**, 271 (1992).
5. J. M. W. Slack, P. W. H. Holland, C. F. Graham, *Nature* **361**, 490 (1993); C. Kenyon, *Cell* **78**, 175 (1994); R. Krumlauf, *ibid.*, p. 191.
6. K. Basler and G. Struhl, *Nature* **368**, 208 (1994); R. D. Riddle, R. L. Johnson, E. Laufer, C. Tabin, *Cell* **75**, 1401 (1993).
7. R. E. Keller, J. Shih, C. Domingo, *Development* (suppl.), p. 81 (1992).
8. E. N. Olsen, *Genes Dev.* **4**, 1454 (1991).
9. J. Slack, *Curr. Biol.* **4**, 116 (1994).
10. L. Nieswander, S. Jeffreys, G. Martin, C. Tickle, *Nature*, in press.
11. L. Wolpert, *Development* (suppl.), p. 3 (1989).
12. D. Weigel and E. M. Meyerowitz, *Cell* **78**. 203 (1994).
13. H. R. Horvitz and P. W. Sternberg, *Nature* **351**, 535 (1991).
14. C. Chothia, *ibid.* **357**, 543 (1992).

Of Flies and Fishes

Christiane Nüsslein-Volhard

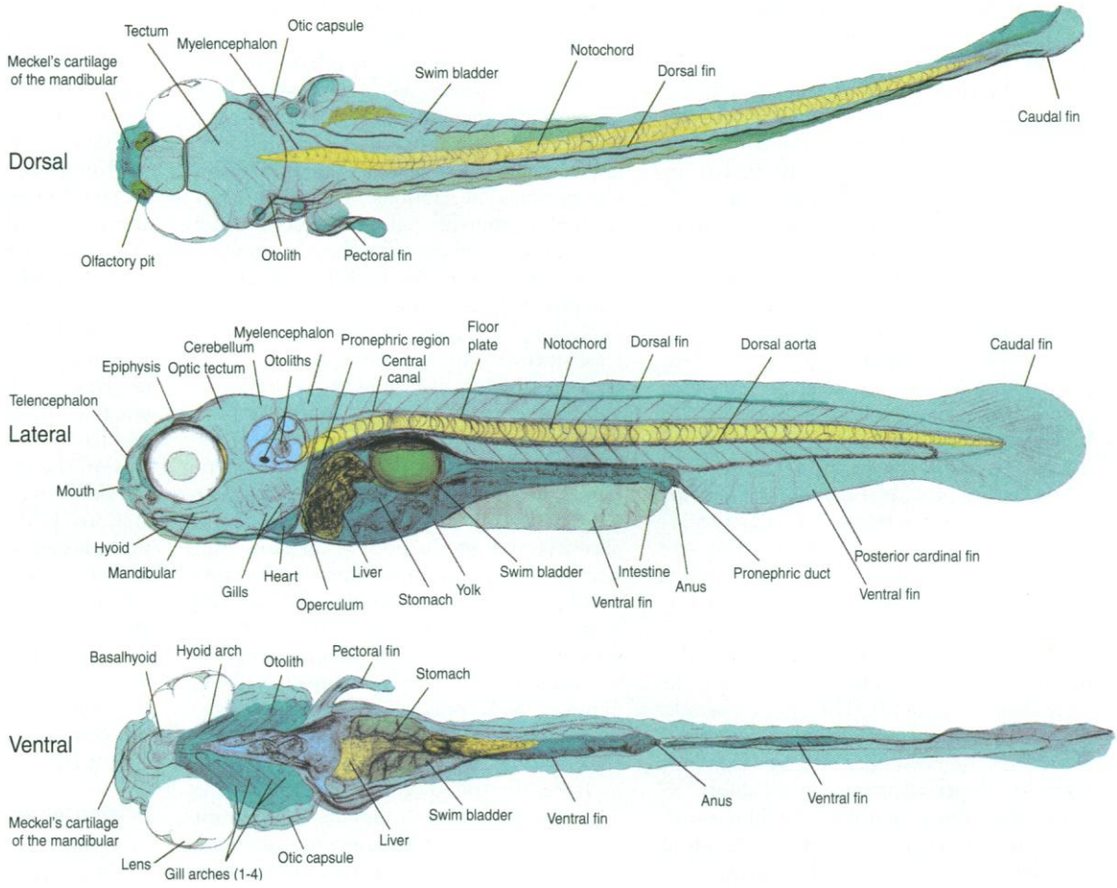
In vertebrates, the single most successful approach for identifying genes of importance in development is based on the surprising finding that important control genes, or at least stretches of sequences of control genes, are conserved through evolution. Thus, for many genes discovered in the invertebrate model systems *Drosophila melanogaster* and *Caenorhabditis elegans*, "homologs" in frog, mouse, and chicken have been identified, and their functions in vertebrate organisms tested with loss-of-function mutations made by embryonic stem (ES) cell–mediated homologous recombination (*1*). Mouse genes with similarity to selected *Drosophila* genes frequently show severe loss-of-function phenotypes. This result is in contrast to what one generally finds for biochemically characterized vertebrate proteins, where in many instances the loss-of-function phenotype shows little or no visible abnormality in the development or patterning of the animal.

Why do many *Drosophila* genes make a fortune in vertebrate embryology? To answer this question, a brief review of the way in which they were identified in *Drosophila* is necessary. In flies, mutants were systematically sought; single genes essential for embryonic pattern formation were identified by virtue of their loss-of-function phenotype. Such saturation screens were possible principally because *Drosophila* is so ideal for genetic research. In particular, the small number of chromosomes, and the existence of giant chromosomes of the salivary glands provided a unique physical measure for the numbers of genes and the analysis of chromosomal aberrations. *Drosophila* has about 6000 "essential" genes, of which 5000 mutate to lethality (roughly one-third each are embryonic, larval, or

The author is at the Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany.

pupal lethal) and about 1000 to sterility. The total number of transcription units is approximately 20,000.

Embryology is not *Drosophila*'s greatest strength, but given the advantages of *Drosophila* genetics, it is still a better than adequate system. The external larval cuticle provides good landmarks of position and polarity, but the internal organs are harder to score without special fixing and staining procedures. In large-scale screens, mutants affecting the pattern of the larval cuticle in a specific and often unique manner were isolated. They defined no more than about 120 genes required in the zygote after fertilization (*2–4*). Among those are the segmentation genes such as *engrailed*, *wingless*, and *hedgehog*, but also genes affecting gastrulation (*twist*), head formation (*forkhead*), neurogenesis (*Notch*), and the cell cycle (*string*), and many whose role in development has not yet been elucidated in molecular terms. In similar screens for maternal-effect mutations, less than 50 genes have been found that are required during oogenesis for patterning the embryo. These include *bicoid*, *dorsal*, *nanos*, and *torso* (*5–7*). Although in these screens mutations exclusively affecting the internal organs could generally not be identified, it is likely that the total number of genes with indispensible and specific roles in embryonic development and pattern formation is not much larger than 200—about 3% of the essential genes (1% of the transcription units). This means that, also in *Drosophila*, mutations in the vast majority of genes cause no significant alteration in patterning.



The 5-day-old zebrafish. The developing fish still displays the larval pigment pattern and shape, but soon will be able to swim and feed. Many organs and structures are clearly visible and can be scored in the living animal without fixing and staining. [Image manipulation by Susan Nowoslawski]

Are most genes in the fly not relevant for development or are the screens not suitable to identify them? Probably both. Many genes encode structural proteins that are required in many cell types, so their loss of function does not result in a specific, interpretable phenotype. Rather, homozygous embryos, larvae, or pupae die without visible or distinct symptoms. Other genes may function in several processes at different times in development, so only the earliest function can be identified by mutation because mutant individuals do not live long enough to reveal the requirement for the gene in a subsequent process. This means that later functions (for example, wing and leg formation, organization of the brain, and oogenesis) are underscored in mutant screens. In other instances, gene pairs or gene families partly or completely share functions in development, again precluding their identification in mutant screens. A few cases of pairs of genes with partly overlapping functions—such as *sloppy paired* and *gooseberry*—have been found fortuitously. In other cases, mutant alleles that affected just one and not the other function of a gene made discovery possible (*cactus* and *torpedo*). In genetic mosaics, genes with late functions, such as maternal contribution to embryonic development, were identified in special screens (*8*). However, despite the high degree of saturation in both the zygotic and maternal screens, it is likely that a substantial fraction of genes with important functions in *Drosophila* pattern formation have so far escaped identification in mutant searches because of partial redundancy or multiple functions.

In *Drosophila*, it is very difficult to estimate the numbers of genes with redundant functions. Homologous recombination is not available for flies, and a mutation in a cloned gene in general can only be easily identified if it has an essential phenotype, such as lethality or sterility. Although it is not at all clear why certain genes are present in multiple copies and others only once, or whether genes with unique functions are more "important" than redundant genes, research has largely concentrated on genes with unique and indispensible functions. They are easier to work with, and valuable information obtained from the loss-of-function mutations can be fully exploited. On the other hand it means that a gene identified on the basis of the protein product alone is likely to be one of the many in which the mutant phenotype does not yield much useful information. The strong selection by a mutant screen for genes with distinct and specific functions in embryonic development cannot efficiently be applied in the mouse, so the conservation of sequences, and often function, between these species is tremendously beneficial to vertebrate embryology.

Although the information that may be gained from *Drosophila* genes in vertebrate development has yet to be fully exploited,

the cloning-by-homology approach clearly has limitations. First, loss-of-function alleles can only be made for previously cloned genes. Therefore, genes that escaped detection in the fly cannot be found in the vertebrate this way. Because internal organs have not been systematically scored in *Drosophila*, this is a very important consideration. Most important, the concentration on fly homologs represents a strong bias toward conserved functions; functions specific to vertebrates go undetected.

The best way of finding out what we have missed is to apply the mutational approach that was so successful in *Drosophila* to a vertebrate organism. Because of the high degree of homology between genes of mouse, frog, chicken, or fish, a gene from one organism provides easy access to its homolog in another vertebrate. From gastrulation onward, the early development of vertebrates is remarkably conserved, and in the one instance where mutations in homologous genes are available in both fish (*no tail*) and mouse (*T* or *brachyury*), the phenotypes suggest identical function in early development (*9*). Therefore, the only essential requirements for the organism of choice are the possibility of large-scale mutagenesis experiments and the ability to clone the genes identified by mutations. The classical experimental organisms—frog and chicken—are not suited for genetic research because of their large space requirements and long generation time. The mouse has the longest tradition in genetic research. Elegant and uniquely powerful methods are available for generating transgenic and knockout mice with ES-cell technology. However, because of its intrauterine development and small litter size, the mouse is not well suited for screening of embryonic mutants.

So we have turned to the zebrafish, *Danio rerio*. The great property of this organism is its embryonic development rather than its genetics. In a mating, hundreds of eggs are produced and the clear embryos develop synchronously in the completely transparent eggs. Division and migration of individual cells can be followed in the living embryo through gastrulation and primary organogenesis. After 24 hours the major events have already taken place—somitogenesis and the formation of brain, eye, ear, and notochord. In the following days, the various organs differentiate fully, the embryo begins to pigment, and a small larva starts swimming and feeding on the sixth day after fertilization (see figure). Because the embryos are transparent, elegant lineage tracing and transplantation experiments can be performed (*10*).

The zebrafish was selected as an experimental system by the late George Streisinger who, with his collaborators at the University of Oregon, explored its potential for genetic research. In many respects, zebrafish genetics is inferior to that of *Drosophila*. *Danio rerio* has a rather long generation time of 2 to 4 months, many and as yet uncharacterized chromosomes, and so far very few adult visible markers or chromosomal aberrations. It is also inferior to mice in that ES-cell techniques are not yet available. On the other hand, a number of valuable genetic methods have been developed that cannot be used in flies or mice (*11*). Haploid embryos can be raised at least until hatching. Although in haploids there is a high background of abnormal development, this trick facilitates experiments such as mutant screens where space is a problem, as well as mapping experiments. From haploid embryos, homozygous diploids can be made and raised to adults, allowing the production of isogenic fish strains (*11*). Most important, the freezing of zebrafish sperm is possible. This method is crucial for keeping large numbers of mutant lines. A small number of intriguing visible adult and embryonic mutants have been obtained with γ-ray mutagenesis and haploid screens in the Oregon labs, showing the mutability of the fish's genome (*12–15*).

Recently the methodology for raising and keeping large numbers of mutant lines safely and with little maintenance has been developed. The chemical mutagen ethylnitrosourea induces point mutations with high efficiency (*16*, *17*). In pilot screens, a number of mutants have been obtained following a straightforward inbreeding scheme and the scoring of diploid homozygous embryos for visible and specific aberrations from normal development. In contrast to *Drosophila*, where markers or balancer chromosomes in general restrict screening to one of the three major chromosomes, in the fish the entire genome is screened at once. This, in a way, is even more efficient than the fly screens, although only mutants with a visible phenotype (that is, only a small fraction of all essential genes) can be identified. Presently, screens aimed at near saturation are being carried out in two laboratories (the laboratory of W. Driever, Massachusetts General Hospital and Harvard University, Boston, Massachusetts, USA, and my laboratory at the Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany). These screens are the equivalent in fish of the screens in *Drosophila* for zygotic embryonic mutants (*2–4*); maternal screens are still beyond reach. As the transparency of the embryo allows the scoring of many internal organs in considerable detail (see figure), a broader range of mutants is detected, and the number of genes they define is probably larger than in *Drosophila*. So far, the scoring criterion has been a distinct and specific phenotype visible under a stereomicroscope in 25% of the embryos or larvae from a cross between heterozygous fish, but more special screens with particular assays are feasible. For example, in the laboratory of F. Bonhoeffer at the Max-Planck-Institut in Tübingen, mutations affecting the retinotectal projection have been isolated by anterograde labeling of axons, as part of the Tübingen large-scale screen.

The identification of a gene by a point mutation does not immediately allow molecular cloning and analysis of the corresponding gene, as does insertional mutagenesis. Although elegant in principle, in practice, insertional mutagenesis has the problem of low efficiency and often strong bias. In the fish, it is still impracticable, despite encouraging reports on retroviral integrations (*18*). However, in two laboratories, genome maps of the zebrafish are being produced with both random-amplified polymorphic DNA (RAPD) markers (*19*) and simple sequence repeats (SSRs) (*20*, *21*). It is expected that the density of markers will soon allow very high resolution mapping and positional cloning with appropriate libraries. And then, the fish will almost fly.

References and Notes

1. S. L. Mansour, K. R. Thomas, M. R. Capecchi, *Nature* **336**, 348 (1988).
2. C. Nüsslein-Volhard, E. Wieschaus, H. Kluding; *Roux's Arch. Dev. Biol.* **193**, 267 (1984).
3. E. Wieschaus, C. Nüsslein-Volhard, G. Jurgens, *ibid.*, p. 296.
4. G. Jurgens, E. Wieschaus, C. Nüsslein-Volhard, *ibid.*, p. 283.
5. K. V. Anderson and C. Nüsslein-Volhard, in *Gametogenesis and the Early Embryo* (Liss, New York, 1986), p. 177.
6. C. Nüsslein-Volhard, H. G. Frohnhöfer, R. Lehmann, *Science* **238**, 1675 (1987).
7. T. Schupbach and E. Wieschaus, *Genetics* **121**, 101 (1989).
8. N. Perrimon, L. Engstrom, A. P. Mahowald, *Dev. Biol.* **105**, 404 (1984).
9. S. Schulte-Merker, F. van Eeden, M. E. Halpern, C. B. Kimmel, C. Nüsslein-Volhard, *Development* **120**, 1009 (1994).
10. C. Kimmel, *Trends Genet.* **5**, 283 (1989).
11. G. Streisinger, C. Walker, N. Dower, D. Knauber, F. Singer, *Nature* **291**, 293 (1981).
12. C. Walker and G. Streisinger, *Genetics* **103**, 125 (1983).
13. C. B. Kimmel, D. A. Kane, C. Walker, R. W. Warga, M. B. Rothman, *Nature* **337**, 358 (1989).
14. K. Hatta, C. B. Kimmel, R. K. Ho, C. Walker, *ibid.* **350**, 339 (1991).
15. M. E. Halpern, R. K. Ho, C. Walker, C. B. Kimmel, *Cell* **75**, 1 (1993).
16. L. Solnica-Krezel, A. F. Schier, W. Driever, *Genetics* **136**, 1401 (1994).
17. M. Mullins, M. Hammerschmidt, P. Haffter, C. Nüsslein-Volhard, *Curr. Biol.* **4**, 189 (1994).
18. S. Lin *et al.*, *Science* **265**, 666 (1994).
19. J. H. Postlethwait *et al.*, *ibid.*, **264**, 699 (1994).
20. D. J. Goff *et al.*, *Genomics* **14**, 200 (1992).
21. H. Jacobs, personal communication.

22. I thank N. Hopkins, M. Leptin, G. Jurgens, F. Bonhoeffer, and M. Mullins for critical reading of the manuscript and many helpful suggestions.