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Genetic control of Development in Flies and Fishes

par Christiane Nüsslein-Volhard
Max- Planck-Institute for Developmental Biology, Tübingen, Allemagne

The process of embryonic development, with its highly ordered increase in complexity accompanied by perfect reproducibility, is controlled by a subset of the animal's genes. To identify the genes that are required for the development of complexity, pattern and shape and to understand their functions is the focus of my research.

As a child, I loved animals and plants. We had a large garden, and I kept pets. At school I had excellent teachers in Mathematics and Biology, and I knew at the age of 12 or so that I wanted to be a natural researcher.

I studied biology, physics and chemistry, got a diploma in biochemistry and did my graduate work on bacterial RNA polymerase at the Max-Planck-Institute in Tübingen. When I was searching for my own research topic, the problem of morphogenesis began to fascinate me. At our institute the group of Alfred Gierer was establishing hydra as an experimental system with the aim at isolating factors controlling morphogenesis in this simple animal. They developed a gradient theory of pattern formation based on lateral inhibition. However, morphogens and gradients were still elusive. Development seemed infinitely complex and the experiments much too crude and unavoidably accompanied with unwanted side effects. At the same time, Friedrich Bonhoeffers laboratory did a systematic search for mutants affecting DNA replication in E coli during which the DNA polymerase that is the in vivo replicating enzyme was identified as product of the DNA E gene. This convinced me that the same strategy might also work for development. If mutations in genes encoding morphogens could be found, it might even be possible to identify their protein products and thus understand the molecular nature and mode of action of morphogens.

In the mid-1970s, Drosophila seemed the best choice for applying genetics to problems of developmental biology. It was the model organism in which the basic principles of chromosomal inheritance had been worked out. A small number of mutations had been identified that caused alteration in development. In the early 70ties, the first maternal mutants in Drosophila were isolated and transplantation experiments in Drosophila embryos had revealed the existence of cytoplasmic determinants. I did my postdoctoral research in the laboratory of Walter Gehring at the Biozentrum in Basel and Klaus Sander at the University of Freiburg. My aim was to screen for maternal mutants with defects in embryonic patterning,

and use transplantation of wild type cytoplasm as an assay for the isolation of morphogens. During my postdoctoral time I learned Drosophila genetics and developed methods allowing large scale screening of mutations affecting embryonic patterning. I isolated the mutant *dorsal*, and, together with Pedro Santamaria, showed that the transplantation of wild type cytoplasm could indeed rescue the mutant phenotype.

My first independent position was at the EMBL in Heidelberg, were I shared a lab with Eric Wieschaus, whom I had met in Basel and who at that time collaborated with the lab of Madeleine Gans in Gif sur Yvette on the role of soma and germ line in maternal mutants. Eric and my common interest concerned segmentation of the Drosophila embryo. At the time, virtually nothing was known about segmentation and the determination of segment number in any organism. We set out to systematically screen for mutations affecting segment number and polarity. For the mutant screens, we chose a single dominant tissue of the larval body, the skin. By the end of embryonic development, the skin is covered by a cuticle that bears a great number of special structures such as denticles and hairs, sense organs and wrinkles, that are arranged in a stereotyped pattern. This pattern makes individual segments easy to detect and allows analysis of other structural features as well. Mutations that affect patterning could be distinguished from those required for more general, housekeeping, functions by direct inspection of the larval cuticle patterns of mutant embryos. In our saturation screen we screened embryos from 20 000 inbred lines and identified a total of 120 genes with essential and important function in the development of the pattern of the larva, representing 2.5 % of all genes mutating to lethality in Drosophila. We identified 20 genes affecting segmentation. Three classes could be distinguished suggesting that the segmented pattern was established sequentially. Initially, large unique regions were specified that guided the establishment of a first periodic pattern with double segment periodicity. This in turn would be subdivided into fields of individual segments, each with its pattern and polarity. This model was confirmed by subsequent molecular analysis of the gene products. This had become possible in the mean time by the development of recombinant DNA technology. Within the next years, many of the segmentation genes were cloned in several laboratories. It was found that many encode transcription factors. By and large, the early fate map of the embryo is established by a series of transcription factors distributed as molecular prepatterns. By regulating each other, these patterns become refined until the molecular pattern directly determines the morphological pattern.

From the EMBL I went to the Friedrich Miescher Laboratory of the Max-Planck- Gesellschaft in Tübingen and run an independent junior research group until 1985, when I was appointed Director of the MPI for Developmental Biology in Tübingen, the position I am still holding today. At the FML, we focused on maternal genes determining the informational content of the egg cell controlling the patterning of the embryo. We and others, notably the laboratory of Mme Gans in Gif sur Yvette, and Eric Wieschaus and Trudi Schüpbachs Laboratory in Princeton, discovered about 30 maternally required genes that are involved in axis determination. The antero-posterior axis is determined independently from the dorso-ventral axis, as mutations affect either the one or the other, never both. We found bicoid and oskar, and the dorsal-group genes. In transplantation experiments we found that oskar and bicoid were required to form two organizing centers with long range activity localized at the anterior and posterior of the egg. Bicoid encodes a transcription factor that is produced at the anterior tip of the egg from a prelocalized mRNA and spreads posteriorly to form a gradient. Artificially changing the gradient results in a shift of the pattern. This demonstrated that Bicoid acts as a morphogen determining the pattern in a concentration dependent manner. We also unraveled the signaling systems determining the dorsoventral axis, with 11 components

including Toll as membrane bound receptor and spätzle as extracellular ligand. Also in this case a morphogenetic gradient of a transcription factor, Dorsal, determines different regions along the dorsoventral axis, however the gradient is formed by differential uptake of the Dorsal protein into the blastoderm nuclei. Many excellent scientists have contributed to these findings, and I would like to mention Ruth Lehmann, Hans Georg Frohnhöfer, Wolfgang Driever and Daniel St Johnston who worked on the patterning along the antero-posterior axis, and Kathryn Anderson, Dave Stein and Siegfried Roth for their contribution to the understanding of the DV- Axis.

Drosophila has rather special properties. It is in many respects very different from vertebrate animals. Therefore it was not clear *a priori* to what extent the results obtained in *Drosophila* could be generalized, and how much we could learn from them for an understanding of the development of vertebrates. When George Streisinger established the zebra fish, a small freshwater cyprinid, as a genetic model organism to study development, I got fascinated by this animal. Zebrafish has transparent embryos, which are rapidly developing and thus ideally suited for in vivo imaging. We performed, this time as a collaborative project of 12 scientists, a large scale mutagenesis experiment in which we isolated 1200 mutants and defined over 300 novel genes. In this and subsequent screens we collected mutants affecting many aspects of early development such as gastrulation, segmentation, muscle formation, development of brain, heart, liver, skin, fin and sensory organs, but also the morphology of the adult animals. Work in my laboratory focused on traits that are distinct between insects and vertebrates. We are investigating processes of cell- and tissue migration, the development of the skin and its organs, and neural crest derived structures. Our present projects with special emphasis on the genes required for the development of the beautiful pigment pattern of the adult fishes should contribute to an understanding of the genetic basis of morphological evolution.