The Heidelberg Screen for Pattern Mutants of *Drosophila*: A Personal Account

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pattern formation, *Drosophila*, saturation screens, larval cuticle, embryonic lethal mutations

Abstract

In large-scale mutagenesis screens performed in 1979–1980 at the EMBL in Heidelberg, we isolated mutations affecting the pattern or structure of the larval cuticle in *Drosophila*. The 600 mutants we characterized could be assigned to 120 genes and represent the majority of such genes in the genome. These mutants subsequently provided a rich resource for understanding many fundamental developmental processes, such as the transcriptional hierarchies controlling segmentation, the establishment of cell states by signaling pathways, and the differentiation of epithelial cells. Most of the Heidelberg genes are now molecularly known, and many of them are conserved in other animals, including humans. Although the screens were initially driven entirely by curiosity, the mutants now serve as models for many human diseases. In this review, we describe the rationale of the screening procedures and provide a classification of the genes on the basis of their initial phenotypes and the subsequent molecular analyses.

Contents

INTRODUCTION

The Dawn of Developmental Genetics

By the beginning of the twentieth century, it had been recognized that the final pattern of cell differentiation in the embryo was established through a gradual process during which initially simple patterns were elaborated to achieve increasingly greater complexity. The morphological steps that produced this complexity were reproducible from one embryo to the next, but the underlying mechanisms were unknown. On the basis of the observable polarity in sea urchin and nematode eggs, the German zoologist Theodor Boveri suggested that initial cell decisions might depend on a graded distribution of some substance in the egg such that different amounts of that substance would be included in the different cells formed through cleavage (Boveri 1901). Genetic material (i.e., chromosomes), in contrast, appeared to be distributed equally among cells during cleavage. In a famous experiment, Boveri demonstrated that loss of individual chromosomes caused specific developmental defects in particular differentiation pathways (Boveri 1902; see Wilson 1925). These experiments showed that the abstract Mendelian factors, subsequently referred to as genes, were localized on individual chromosomes, and provided the foundation for the chromosomal theory of inheritance. The central ideas that emerge from Boveri's view of development are that spatial patterns are present as polar distributions of morphogenetic substances from the earliest stages, that these patterns are simple, and that the subsequent activity of genes on chromosomes builds the ultimate functional patterns in the final organism.

What these genes were and how they controlled development were unknown and beyond the reach of the technology available at the time. The strategies pursued by biological researchers after Boveri focused largely on either genetics or development. They aimed to find out more about genes and their organization on chromosomes (Morgan 1933) or to identify substances providing pattern

and polarity to the embryo. The discovery of the organizer region in the newt embryo by Boveri's student Spemann provided evidence for sequential induction of cell fate in the amphibian embryo (Spemann 1935). These observations generated great excitement but were followed by years of frustrating attempts to purify factors involved in particular developmental decisions. One major problem was the nature of assays for cell patterning available at the time. These assays generally involved the application of substances or embryonic extracts to fragments of tissue deprived of the intrinsic factor. Even when such experiments "worked," they often gave positive results with compounds that could not possibly have had a biological role. Development seemed infinitely complex and the experiments much too crude and unavoidably accompanied by unwanted side effects. Another problem in developmental biology during this period was that scientists worked on many different organisms, each chosen for a specific experimental advantage or an exciting phenomenon. A classic textbook (Kühn 1965) describes work on algae, frogs, newts, sea urchins, ascidians, daphnids, nematodes, planarians, slime molds, snails, chicken, hydra, crickets, moths, flies, midges, and grasshoppers. The individual communities were small, and the experimental approaches used were very different, such that the results were usually not comparable and did not easily lead to unifying theories of development. In the 1950s, the discovery of the double helix and the excitement of molecular biology of bacteria and bacteriophages pushed research into fields other than developmental biology and led Watson in a famous textbook (Watson 1965) to question whether "we have sufficient background at this time to attack embryology at a molecular level." Nevertheless, theoreticians, on the basis of regeneration experiments with the polyp hydra, proposed the concept of positional information (Wolpert 1969). A theory based on self-enhancement and lateral inhibition could explain the formation of stable patterns from near uniformity with plausible parameters (Gierer & Meinhardt 1972). These models assumed that complexity could arise from morphogen gradients eliciting different responses at different concentrations. But the molecular nature of such morphogens and the response to them remained elusive.

The beginning of the twentieth century also saw the rediscovery of Mendel's laws and the birth of modern genetics (Mendel 1866). Although not appreciated by most developmental biologists, genetic methods would ultimately provide a way to interfere specifically with a developing organism without causing gross disturbances. A mutation allows one to completely deplete a single component in a complex system while leaving everything else intact. Systematic mutant screens had been instrumental in identifying members of biochemical pathways in bacteria and fungi (Beadle & Tatum 1941). In bacteria, the identification of mutations in the *cis*- and *trans*-acting regulatory elements of genes played a crucial role in the biochemical isolation of transcriptional repressors (Jacob & Monod 1961). The identification of key players in the control of the cell cycle by systematic mutant screens in yeast provided another example of the powers of genetic analysis (Hartwell et al. 1970, Nurse 1975). The same strategy might also work for development. If mutations in genes controlling specific activities in the embryo could be found, it might even be possible to identify their protein products and thus gain insights into the biochemical mechanisms controlling developmental decisions.

Drosophila

In the mid-1970s, Brenner chose the nematode *Caenorhabditis elegans* to initiate studies of neural development and function (Brenner 1974). To us, however, *Drosophila* seemed the best choice for applying genetics to problems of developmental biology. The rich morphological features of the adult stages had allowed easy recognition of mutants and had made *Drosophila* the multicellular

organism in which the basic principles of chromosomal inheritance had been worked out. It has only four chromosomes and many viable marker mutants and special chromosomes that allow for the easy breeding of lethal and sterile mutations (Lindsley & Grell 1968). Although the Hadorn group had realized the importance of vital genes for development (Hadorn 1955), most of the genes studied until the mid-1970s were related to structures of the adult fly such as eye color, body pigmentation, bristles, and wing size and shape (Morgan 1933). A small number of mutations that produced alteration in the development of adult structures had also been identified. The best-characterized mutations were in homeotic genes, which cause transformation of one region of the body into another, such as antennal structures to legs or wings to halteres (Kaufmann et al. 1980, Lewis 1978). Genes such as the homeotics had fascinated scientists for a long time. Other mutants affecting pattern in the wing and color of the eyes had also been analyzed in some detail (Beadle & Ephrussi 1937). The structures of the adult fly derive from groups of cells termed imaginal discs that are set aside early in embryonic development (Gehring & Nöthiger 1973). These discs could be cultivated in the abdomen of the adult fly and, after transplantation into a larva, were able to differentiate into the cuticle structures of the respective disc (Hadorn 1963, Schubiger & Hadorn 1968). The exact relationship between these discs and the determinative events in the embryo was unclear, largely because little was known about development in the embryo. The adult cuticle structures obtained when cells from embryos were cultured using the same protocols used to culture imaginal discs suggested that a crude distinction between anterior and posterior development already existed in early embryonic stages (Chan & Gehring 1971). Clones induced by mitotic recombination indicated that blastoderm cells could still give rise to wing and leg structures within the same segment, although not to structures in adjacent segments (Wieschaus & Gehring 1976), suggesting that anterior-posterior (AP) distinctions might be sufficiently detailed to specify segments. Owing to the small size of *Drosophila* embryos and the high turgor of the eggs, testing such models experimentally seemed much more difficult than in other insect eggs such as those of *Euscelis* (Sander 1976). The famous demonstration of a localized pole-cell determinant by cytoplasmic transplantation (Illmensee & Mahowald 1974) raised hopes, however, that such determinants could also exist for somatic structures such as the imaginal discs and that procedures could be developed for their isolation.

Nevertheless, a group of scientists centered around the embryologist Donald Poulson at Yale had begun to describe embryonic development of *Drosophila* in some detail (Poulson 1937, 1950), and lethal mutants that affected embryonic development had begun to be studied (Wright 1970). The number of such mutations was small, but some of them displayed phenotypes that seemed sufficiently specific to suggest that a genetic analysis of embryonic development might be feasible.

THE HEIDELBERG SCREEN

Rationale

We set out to study the genetics of *Drosophila* development, focusing on the larva, because it is the direct result of embryogenesis and has a very different morphology than does the adult fly. This simpler, regularly segmented, headless form develops within 24 h and is endowed with a number of cuticle structures indicating pattern and polarity (**Figure 1**). Our analysis was based on the assumption that there would be two sets of genes required for larval patterning. Maternal genes would be expressed in the female during oogenesis and would provide components required for embryonic development already in the egg. Zygotic genes would supply components by transcription in the embryo (**Figure 2**). Mutations in both classes would cause lethality in the embryo and

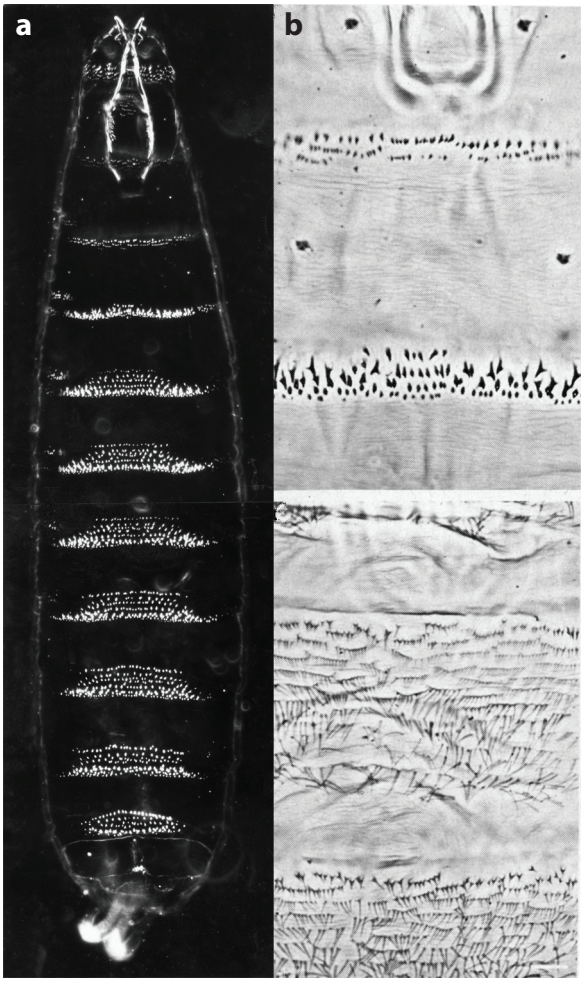


Figure 1

Cuticle preparation of a *Drosophila* first-instar larva. (*a*) Dark-field image of the ventral side. (*b*) Phase-contrast detail of the ventral (*top*) and the dorsal (*bottom*) aspect of the posterior thorax and the first abdominal segment.

display a phenotype visible in the larval structures. To understand the logic behind the process of determination and differentiation, as well as to estimate the complexity of the system and the types of decision during early development, we believed that it was necessary to find out how many genes are involved in embryonic pattern formation, whether each of these genes is unique, and what types of pattern alterations can be caused by a mutation in a single gene. Our plan therefore was to use large-scale mutagenesis experiments to identify most, if not all, genes of both classes affecting embryonic developmental decisions on the basis of visible mutant phenotypes in the larva.

To test the feasibility of using genetics to identify such genes, we first studied mutants that were available at the time. A fascinating maternal mutation, *bicaudal*, causes the formation of larvae with two rear ends in mirror-image symmetry, albeit with erratic and low penetrance (Bull 1966, Nüsslein-Volhard 1977). In a pilot screen, a new maternal mutant, *dorsal*, was discovered with very

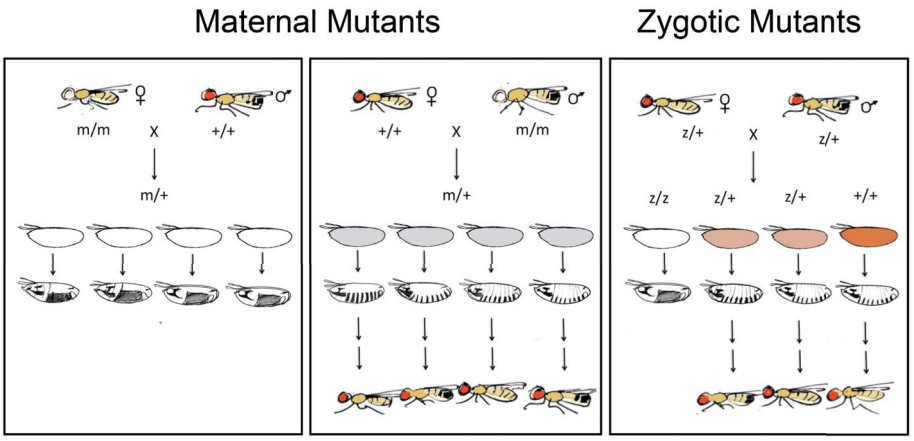


Figure 2

Genetics of embryonic patterning. Maternal and zygotic genes can be distinguished by their genetic behavior. (*Left panel*) All embryos from females that are homozygous mutant for maternally active genes are abnormal, even when crossed with wild-type males. (*Middle panel*) Although the genotype of the resultant embryos is the same as that in the reciprocal cross, in which wild-type females are crossed with mutant males, all embryos are normal. (*Right panel*) For zygotically active genes, only the homozygous one-quarter of the embryos derived from a cross between heterozygotes will be abnormal, even though all embryos develop with identical maternal contributions.

specific loss of ventral pattern elements such that the entire cuticle appears dorsalized. These mutant phenotypes suggested that the AP and dorsal-ventral (DV) axes of the egg were independently established by two gradient systems oriented at right angles to each other (Nüsslein-Volhard 1979, Nüsslein-Volhard et al. 1980). *Notch*, originally identified as a dominant mutation displaying notches in the wing, provided an example of a zygotic gene. Homozygous mutant individuals are embryonic lethal, and neural tissue had been shown to develop at the expense of epidermal tissue (Poulson 1937, 1940). When we began our experiments, virtually nothing was known about the segmentation process itself or how the number of segments was determined in any organism. We investigated existing stocks to see whether some might already contain such mutations. In this shelf screen, which included many chromosomal deficiencies that we obtained from various sources, we recognized *Krüppel*, which had been described by Gloor (1950), and fortuitously a few other mutations affecting the number or polarity of the larval segments. These findings greatly encouraged us.

To inspect eggs from large numbers of unique fly stocks efficiently, we invented simple devices to collect eggs repeatedly and simultaneously from several stocks of flies (**Figure 3**). To inspect embryos collected on transparent agar plates, we used a special oil to make the opaque chorion transparent (Wieschaus & Nüsslein-Volhard 1986). It was quite difficult to examine the mutant phenotypes in the living embryo, so for the mutant screens, we chose a single prominent tissue of the larval body: the epidermis. The larval epidermis is covered by a secreted, extracellular cuticle that bears a number of special structures—such as denticles and hairs, sense organs, and wrinkles—arranged in a stereotypic pattern. We adopted efficient clearing protocols for the larval cuticle, allowing for the analysis of the details of its cuticle structures (Lohs-Schardin et al. 1979). The cuticle preparations made individual segments easy to detect as prominent ventral denticle belts and allowed for the analysis of other patterned features along the DV axis (**Figure 1**).

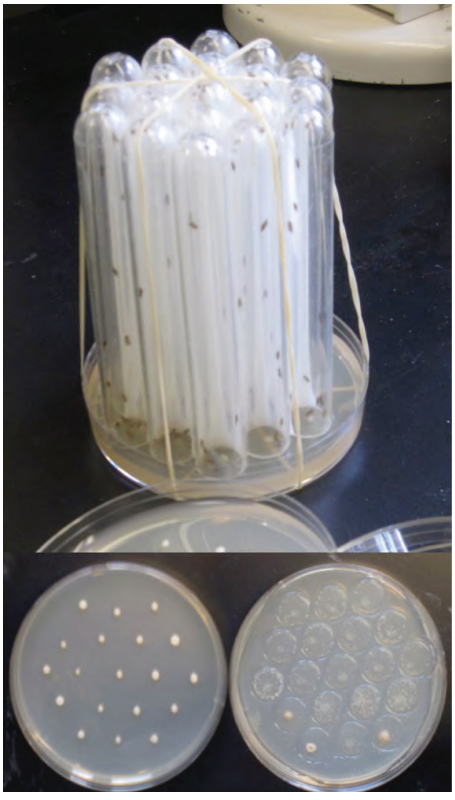


Figure 3

Replica plating egg collections from multiple mutant stocks. Flies from different mutagenized lines are transferred to tubes glued together in a block formation. Females lay eggs in defined position on yeasted apple juice agar plates. After 24 h, the normal embryos have hatched, and the unhatched mutant embryos can be collected for microscopic examination.

The integrity of the epidermis, as a coherent tissue composed of epithelial cells, is also reflected in the cuticle. Importantly, in contrast to the soft organs of the body, the cuticle stays intact for some time after the death of embryos, allowing mutant embryos to be collected over a longer time period before inspection. The anlage for the epidermis in the blastoderm stage covers a large, continuous region of the egg surface, from 15% to 65% egg length (Lohs-Schardin et al. 1979, Szabad et al. 1979) (**Figure 4**). At the anterior and posterior, cells invaginate to give rise to the head (which in dipteran insects is involuted), the gut anlagen, and the brain. Ventrally, a band of cells invaginate and develop into mesodermal structures, whereas the ventral nerve chord is derived from a lateral region that also forms ventral denticle belts. Deviations from normal that primarily affect internal organs may display a cuticle phenotype. For example, *Notch* embryos lack ventral denticle belts because the cells form instead neural tissue, and *twist* and *snail* mutants form a distorted larva owing to the lack of mesodermal invagination.

Screens aimed at saturation involve large numbers. Previous estimates suggested that *Drosophila* possesses approximately 5,000 genes that could mutate to lethality. These estimates were based on the assumption that the number of genes corresponded to the number of bands that could be visualized in the giant chromosomes present in the salivary glands and other larval organs,

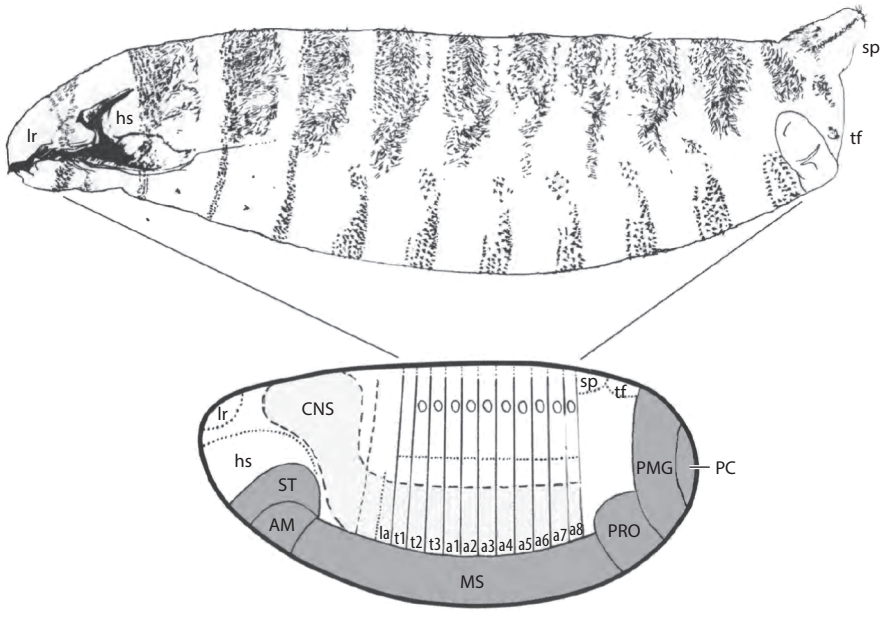


Figure 4

The relationship between the cuticle pattern of the hatching embryo and the fate map at the blastoderm stage. The primordium for the segmented epidermis represents a substantial fraction of the blastoderm and gives rise to the labial segment (la), three thoracic segments (t1 through t3), and eight abdominal segments (a1 through a8). Its pattern in cuticle preparations provides a simple assay for patterning in the earlier stages of development. Internalized structures such as the labrum (lr), head skeleton (hs), spiracles (sp), and tuft (tf) also provide useful markers for correct patterning. Mutants affecting regions of the blastoderm that give rise to soft internal tissues such as the stomodeum (ST), anterior midgut (AM), mesoderm (MS), proctodeum (PRO), posterior midgut (PMG), and pole cells (PC) can be scored only if those abnormalities have secondary consequences on the morphology of the epidermis.

and this assumption was roughly supported by the number of lethal complementation groups uncovered in chromosomal deficiencies (Judd et al. 1972). This correspondence later turned out not to be strictly true (Lefevre & Watkins 1986), but the number 5,000 provided us with a useful basis to estimate the fraction of genes that affect patterning. These genes presumably represented only a small subset of essential genes and could be distinguished from those required for more general, housekeeping functions only by direct inspection of the mutant larval patterns. Although patterning involved both maternally and zygotically supplied genes, maternal genes would be more difficult to identify, given that the screen would require an additional inbreeding generation. So we set out to do the zygotic screens first.

Mutagenesis and Scoring

The screen for embryonic patterning mutants required the establishment of inbred families of flies derived from individual males arising from mutagen-treated sperm and scoring eggs from brother-sister matings carrying the same putative mutation (**Figures 2** and **5**). Mutational frequencies were increased by feeding males a potent mutagen, EMS (ethyl methane sulfonate). The dosage we used (25 mM) induced approximately one mutation in any given gene per 1,000 treated sperm

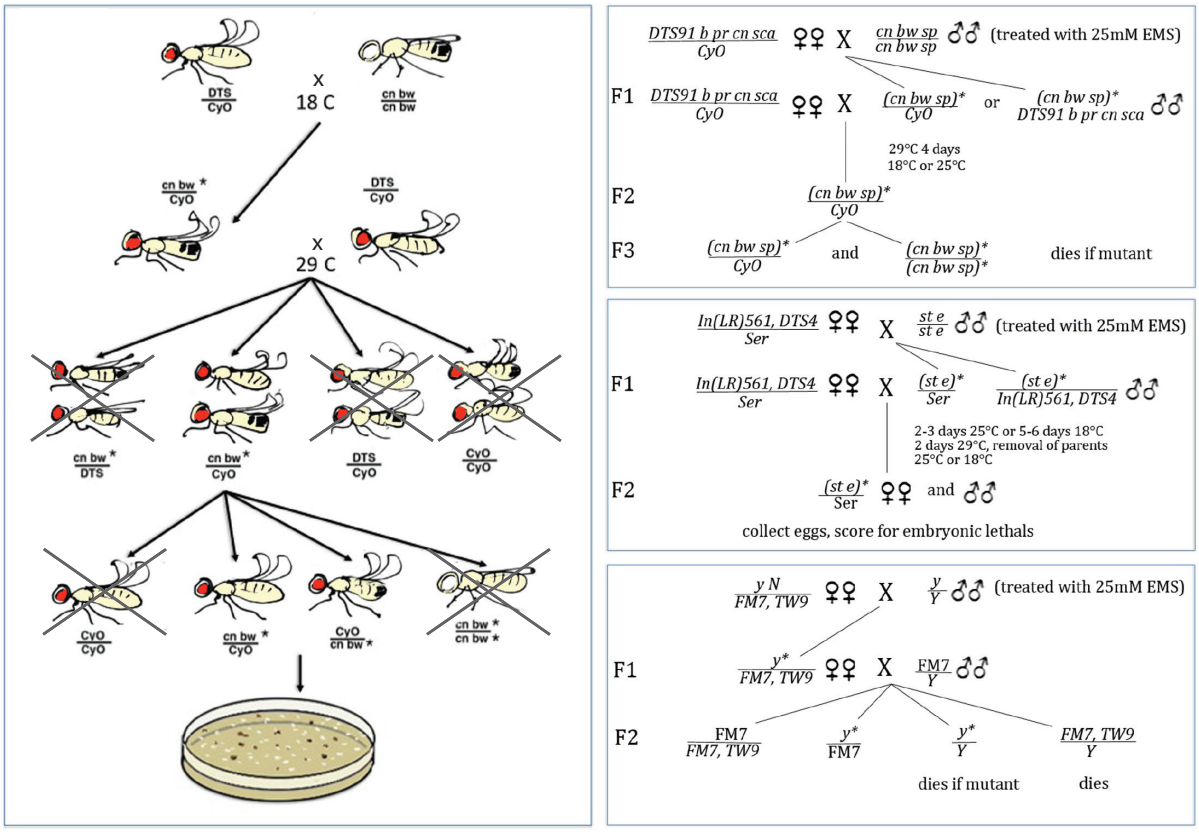


Figure 5

Crossing schemes to produce inbred lines to be tested for homozygous mutant embryos with altered patterns. The left panel provides a general schematic of the crosses, and the right panels give genetic details of the second chromosome (*top*), third chromosome (*middle*), and X chromosome (*bottom*) crosses. EMS denotes ethyl methane sulfonate.

(Lewis & Bacher 1968). With the aim of saturation, approximately five times as many test lines had to be established and screened. To avoid tedious sorting of flies, we eliminated unwanted progeny by using dominant temperature-sensitive mutations (Suzuki 1970) and by growing the adults to be used in the test generation at high temperature. After two generations of inbreeding, females and males heterozygous for the mutagenized chromosome emerged (**Figure 5**). Eggs were collected from those flies, and unhatched embryos were processed for the inspection of the cuticle. Because following all chromosomes in a single experiment was difficult, we performed separate screens for the two autosomes and the X chromosome. We tackled the second chromosome screen first, and a preliminary account of this first screen was published (Nüsslein-Volhard & Wieschaus 1980) (**Figure 6**). An invaluable technical assistant, Hildegard Kluding, participated in all screens. For the screen of the first and third chromosomes, we were joined by a postdoctoral fellow, Gerd Jürgens. Gerd is a superb geneticist who contributed substantially to the final evaluation and genetic characterization of the mutants (Jürgens et al. 1984, Nüsslein-Volhard et al. 1984, Wieschaus et al. 1984a). Gary Struhl also participated in some of the screenings.

The cuticle preparations were inspected and characterized using a checklist. We scored the following items:

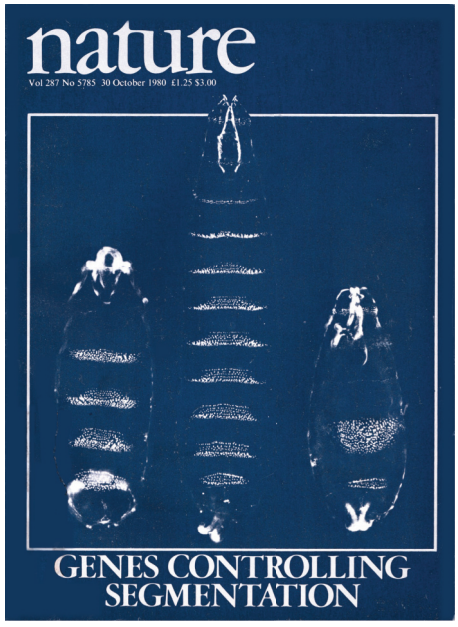


Figure 6

The altered segmentation patterns of embryos homozygous for *paired* and for *knirps*, shown flanking a wild-type pattern in the cover illustration of the *Nature* paper describing the first mutants from the mutagenesis screen (Nüsslein-Volhard & Wieschaus 1980).

Do not keep: embryonic viable, lethal normal looking, lethal BFP (brown, faint, or pimples)
Keep: cuticle differentiation, cuticle integrity, DV pattern, AP pattern, homeotic, other.

The screenings were always done by two observers simultaneously, using a discussion compound microscope equipped for dark field and phase contrast. Two observers helped us to be as unbiased as possible and reduced the danger of overlooking phenotypes due to inattention or fatigue. This setup was important, as a priori we did not know what to expect. Because the bulk of the work was the breeding of flies and because screening cuticle preparation was fun and allowed for the detection of a variety of phenotypes, our strategy was not to restrict ourselves to any particular feature, but rather to keep all mutants we could recognize as affecting any specific aspect of development in a Mendelian quarter of the eggs. Although the tested lines contained at least one lethal mutation, in the majority of egg lays, most of the embryos hatched, suggesting that transcription of the affected gene was not required during embryogenesis. In the third chromosomal screen, homozygotes for the available balancers were embryonic lethal without displaying a phenotype. Because the lethality of homozygous balancer embryos would have made the detection of new mutants difficult, we initially tested the progeny of unbalanced heterozygotes and established balanced stocks only in those lines that produced interesting embryonic phenotypes (Jürgens et al. 1984) (**Figure 5**).

For all chromosomes, in cases in which an anticipated quarter failed to hatch, most often the unhatched embryos looked normal. A similar number of lines showed normal morphology but poor differentiation (category BFP) or very subtle alterations. There was also a background of lines in which the frequencies of unhatched embryos were higher than 25%, and these lines often showed variable phenotypes. Many of these lines could be shown, using reciprocal crosses with wild-type

flies, to contain translocations or dominant maternal effect mutations. We were of course particularly interested in mutations whose phenotypes had discrete effects on the larval pattern, but we initially kept many lines in which the cuticle phenotypes were less informative. Our rule was that we kept mutants in which the cuticle was largely absent or very defective (e.g., *shotgun*, *bazooka*, *ghost*; see **Figure 16** below), as long as the phenotype was homogeneous and could be observed in one-quarter of the fertilized eggs. Although an individual line showed little variation in phenotype, the lines we kept in total represent a large range of different pattern alterations (see below). As the unhatched embryos were directly inspected, we also recovered mutants that did not map to the specific screened chromosome but whose phenotypes were sufficiently conspicuous to allow recognition in a small fraction of the total embryos. These mutants included several dominant maternal effect mutants such as Toll (Anderson et al. 1985) and BicD (Mohler & Wieschaus 1986) and mutants of third chromosome segmentation genes (*hedgehog*, *hairy*, *hunchback*, and *knirps*) that were isolated in the screen for the second chromosome mutants (Nüsslein-Volhard & Wieschaus 1980).

Complementation and Mapping

Once we had isolated the mutants, we set out to define genes as complementation groups mapping to one site in the genome. To identify multiple alleles, mutants were first assigned to large phenotypic classes, and within the classes smaller groups and subgroups were established on the basis of the similarity of the phenotype. Mutants within these groups were candidates for allelism, a possibility we could rapidly test by inspecting transheterozygous progeny for the production of the mutant phenotype in living embryos. This approach worked well in many instances. However, it was less successful in cases in which alleles showed different strengths of the mutant phenotype. The X chromosome presented special problems for such complementation tests, as males carrying an X-linked lethal mutation do not survive, and complementation tests depended on the availability of chromosomal duplications covering the lethality (Wieschaus et al. 1984a). Finally, mutants were mapped by recombination with visible marker mutants. In addition to recombination mapping, a set of chromosomal deficiencies covering approximately 40% of the genome was used in complementation tests to localize the gene to cytologically defined chromosomal regions. The mapping analysis indicated that the patterning genes that we identified are scattered all over the genome, with no obvious clustering of genes with similar phenotype.

In the three screens, we established a total of 26,978 families starting from single flies heterozygous for a mutagenized genome in the F1 generation. These inbred lines contained a calculated number of approximately 17,200 lethal hits (7,000 lethal hits each for the second and third chromosomes and 3,200 lethal hits for the X chromosome, which is half the size of the autosomes). Embryonic lethal mutations occurred at a frequency of 25% of lethal hits (corresponding to roughly 1,300 embryonic lethal genes in the genome). We finally kept a total of almost 600 mutants. These mutants identified 120 loci, 100 with more than one allele and 20 as single mutants that were uncovered by a chromosomal deficiency. In addition, we kept but eventually discarded many lines that either could not be assigned to complementation groups or yielded somewhat generic, difficult-to-describe heterogeneous phenotypes but otherwise fulfilled all the genetic criteria for zygotic activity. It was impossible to apply the same rigid criteria consistently, and we suspect that mutants with some subtle phenotypes were not always kept. For example, head defects were often difficult to characterize, and in the screens of the first and third chromosomes, mutants with such defects were no longer kept for practical reasons. Deficiencies and rearrangements on the tiny fourth chromosome were also inspected, and we identified a single patterning phenotype that was associated with mutations in ci^D, a previously identified complementation group on that chromosome (Hochman 1971). Several lines of evidence—such as a high average allele frequency (4–5

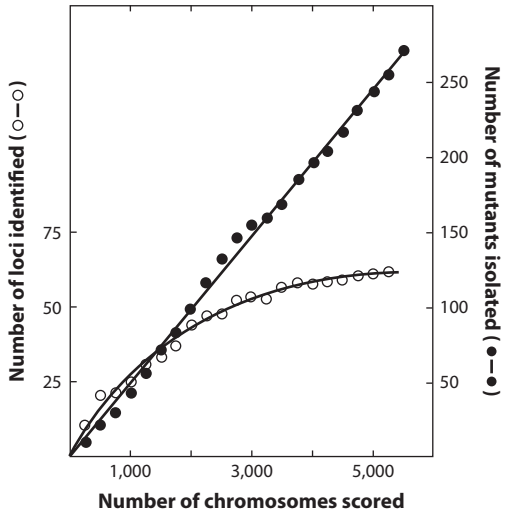


Figure 7

Saturation curve. The plot shows the number of pattern mutants isolated over the numbers of lines (chromosomes) scored (*closed circles*) as well as the numbers of new loci identified (*open circles*). From Nüsslein-Volhard et al. (1984).

alleles per locus) and the identification of point mutations matching the phenotypes of all known chromosomal deficiencies—confirmed that we had almost reached saturation in all three screens. Last but not least, by the time we reached the end of each screen, new mutants did not identify new loci and instead represented additional alleles of genes identified earlier in the same screen (**Figure 7**). A subsequent equal-sized screen of the X chromosome resulted in the identification of only one additional locus, *brinker* (Jazwinska et al. 1999; E. Wieschaus, unpublished data).

The number of 120 genes corresponds to 10% of the embryonic lethal loci and to only 2.5% of all lethal genes in the fly. This small number came as a surprise. It means that most essential genes either are dispensable for pattern determination or have roles in this process that do not require precise temporal and spatial control of gene expression. The genes represent a heterogeneous collection with different degrees of significance, value, and importance. Strikingly, not every conceivable phenotype could be found, and many phenotypes came as quite a surprise. In some instances, the pattern of the defect roughly coincided with a particular structure or organ, such as deletion of the dorsal epidermis (*schnurri*), mesodermal anlage (*twist*, *snail*), or filzkörper (*empty spiracles*). But we did not, for example, identify mutants that deleted single segments as might have been anticipated, nor did the mutant phenotypes support a model of localized determinants for individual organs or structures. In naming the new genes, we aimed for a simple description of the phenotype (*even-skipped*, *crumbs*, *faintoid*) or an association (*shotgun*, *knirps*, *slater*) and tried to avoid names suggesting an interpretation of the function of the gene.

Limits of the Heidelberg Screen

When we began the screen in 1978, we had two concerns, and both were shared by many of our colleagues during the early planning stages of the screens' design. The first concern was that development was complicated and involved thousands of genes. A mutagenesis screen not directed at a specific developmental phenomenon would produce an unmanageable number of mutations, each of which would require extensive analysis before any meaningful conclusions could be drawn.

A second and related worry was that, for the screen to be reliable, phenotypes of the dead embryos had to be informative. Removal of essential genes could have massive heterogeneous, pleiotropic effects that would obscure relevant developmental roles. The detection of a number of mutants with very striking phenotypes in our shelf screens and the isolation of mutant lines with specific phenotypes in pilot screens of the X and second chromosomes dispelled these concerns.

We, however, foresaw three reasons for our screen to miss zygotic genes with important functions in embryonic patterning. The first reason was redundancy. If the lack of one gene product can be compensated for by another gene, mutations in either gene alone might produce inconspicuous phenotypes or no phenotype at all. One example we knew about from Gerd Jürgens's at-the-time-unpublished work (Jürgens 1985) was the *Polycomb* (*Pc*) group, for which double mutants of any two members show dramatic embryonic phenotypes that are similar to those of the eponymous Pc homozygotes, in contrast to the very subtle phenotypes of single mutants. In cases in which the genes themselves are duplicated, mutants in a single gene might not produce strong enough phenotypes to be detected. Two cases within the segmentation mutants (*gooseberry* and *sloppy-paired*) turned out to have closely linked paralogs (Baumgartner et al. 1987, Grossniklaus et al. 1992), and the double mutants produce much stronger phenotypes. For practical reasons, we did not keep several mutants having subtle phenotypes or less specific effects on differentiation shared by many embryonic lethal mutations; these phenotypes and effects may have been due to the presence of paralogs in the genome. Fortunately, subsequent molecular studies indicated that in *Drosophila* the incidence of duplicated genes precluding a discovery by mutation is much smaller than in vertebrate organisms.

The second reason was haplolethality. Both copies of a gene might be required to survive to the adult stage. Although experiments with synthetic deficiencies spanning the entire genome indicated that fully penetrant haplolethal loci are rare in the *Drosophila* genome (Lindsley et al. 1972), heterozygotes for large deficiencies frequently show reduced viability. Point mutations with reduced viability in heterozygotes are less likely to be detected in a large-scale crossing scheme, and therefore our screen was potentially biased against genes with haploinsufficient effects on viability. However, heterozygotes for weaker, hypomorphic alleles at these loci survive, and our screen was thus able to identify weak alleles of genes displaying haplolethality (*decapentaplegic*) (Spencer et al. 1982) or semilethality [*Krüppel* (Gloor 1950, Wieschaus et al. 1984b) and *even-skipped* (Nüsslein-Volhard et al. 1985)].

The third reason was maternal contributions: genes transcribed during both oogenesis and embryogenesis. We relied on homozygous phenotypes and thus genes whose products needed to be supplied by transcription in the embryo. The mother initially supplies most of the RNAs and proteins present in the embryo (Davidson 1986, De Renzis et al. 2007). Such maternal gene products are generally uniformly distributed in the embryo and provide housekeeping functions required in all cell types. Maternal gene products are generally sufficient for the embryo to reach late stages with fairly normal morphology. Most of the genes we identified are not maternally transcribed, as evidenced by the analysis of clones in the germline (Lawrence et al. 1983, Nüsslein-Volhard et al. 1985, Wieschaus & Noell 1986). In cases in which maternal contributions were significant, we were able to identify mutations only when such maternal products had to be supplemented by zygotic transcription at later stages. Examples are *hunchback* (Lehmann & Nüsslein-Volhard 1987); *shotgun*/E-cadherin (Tepass et al. 1996); and *zipper*, which encodes the major cytoplasmic myosin (Young et al. 1993).

In the end, the number of genes we identified was small, and most of the phenotypes were very specific and informative. The small total number of loci was gratifying in that it allowed phenotypes to be characterized and grouped into classes and potentially developmental processes. One reason for the small number might have been that, due to our reliance on cuticle phenotypes,

mutants would have been missed if they affected only internal tissues or died early before cuticle differentiation. In the early 1990s, the Goodman lab carried out a screen scoring 13,000 lines with internal markers to detect abnormalities in the nervous system (Seeger et al. 1993). This screen identified several additional interesting loci (e.g., *robo*, *commisureless*, *single minded*) but also reemphasized how much neuronal patterning depends on genes identified in the Heidelberg screen (Doe et al. 1988). An alternative approach used compound chromosomes, deficiencies, and translocations to generate embryos lacking large chromosome regions (Leptin 1999, Merrill et al. 1988). This approach was undertaken in the hope of identifying genes that affected early embryonic morphology and that might have been missed in Heidelberg because they were relatively insensitive to chemical mutagens like EMS, or because their morphological effects did not result in a cuticle phenotype. Again, these screens were successful and identified an additional 8–10 loci that affect cellularization and gastrulation. In both of these cases, however, the yield of new loci was relatively low compared with that in the Heidelberg screen, and the Heidelberg screen continued to be a major source of material for developmental analyses throughout the 1980s and 1990s.

The number of zygotically active genes with informative embryonic phenotypes is small, given the number of tissues and structures that must be developed. The low number may be due to the fact that flies and probably most other organisms use the same genes and pathways at several times and places to establish pattern in many different tissues. It was possible to recognize their role in the earliest of the requirements by scoring the cuticle, but the phenotypes displayed there did not preclude equally important roles in other tissues.

THE GENE CLASSES

We next attempted to sort the genes into groups by using pragmatic criteria. We anticipated that similarity in phenotypes would identify genes whose products interact in development. Significantly, most genes display unique phenotypes that can be distinguished from those produced by similar genes in the same general phenotypic group. Given the degree to which the larval pattern was affected, the phenotypes suggested sequential processes of patterning the egg along the AP axis or the DV axis. We also identified mutants in which the larval body organization appeared normal but the epidermis showed specific defects, as judged by a defective cuticle. Such defects ranged from holes to various degrees of lack of structures and differentiation. For further analysis, we also inspected living embryos during development and identified those that already showed a phenotype at the onset of gastrulation.

The Anterior-Posterior Genes

Figure 8 shows a selection of 20 mutants affected in AP patterning and sorted according to the classes described in **Table 1**. Mutations affecting segment number and polarity were particularly fascinating because of the strange and unexpected pattern defects displayed in the mutant larvae. Three classes of such mutations could be distinguished (Nüsslein-Volhard & Wieschaus 1980) (**Figures 8–11**).

Figure 8

Mutants affected in AP patterning: 20 mutants of genes listed in **Table 1** represent the following classes: gap genes [*giant* (*gt*) and *Krüppel* (*Kr*)], pair rule genes [*runt* (*run*), *even-skipped* (*eve*), *odd-skipped* (*odd*), *paired* (*prd*), and *sloppy-paired* (*slp*)], segment polarity genes [*armadillo* (*arm*), *hedgehog* (*hh*), *wingless* (*wg*), and *patched* (*ptc*)], segment pattern genes [*arrow* (*arr*), *engrailed* (*en*), *lines* (*lin*), *midline* (*mid*), and *smoothened* (*smo*)], homeotic genes [*extradenticle* (*exd*)], and head genes [*buttonhead* (*btd*), *brown head* (*brh*), and *thick head* (*thi*)]. From Jürgens et al. (1984), Nüsslein-Volhard et al. (1984), and Wieschaus et al. (1984a).

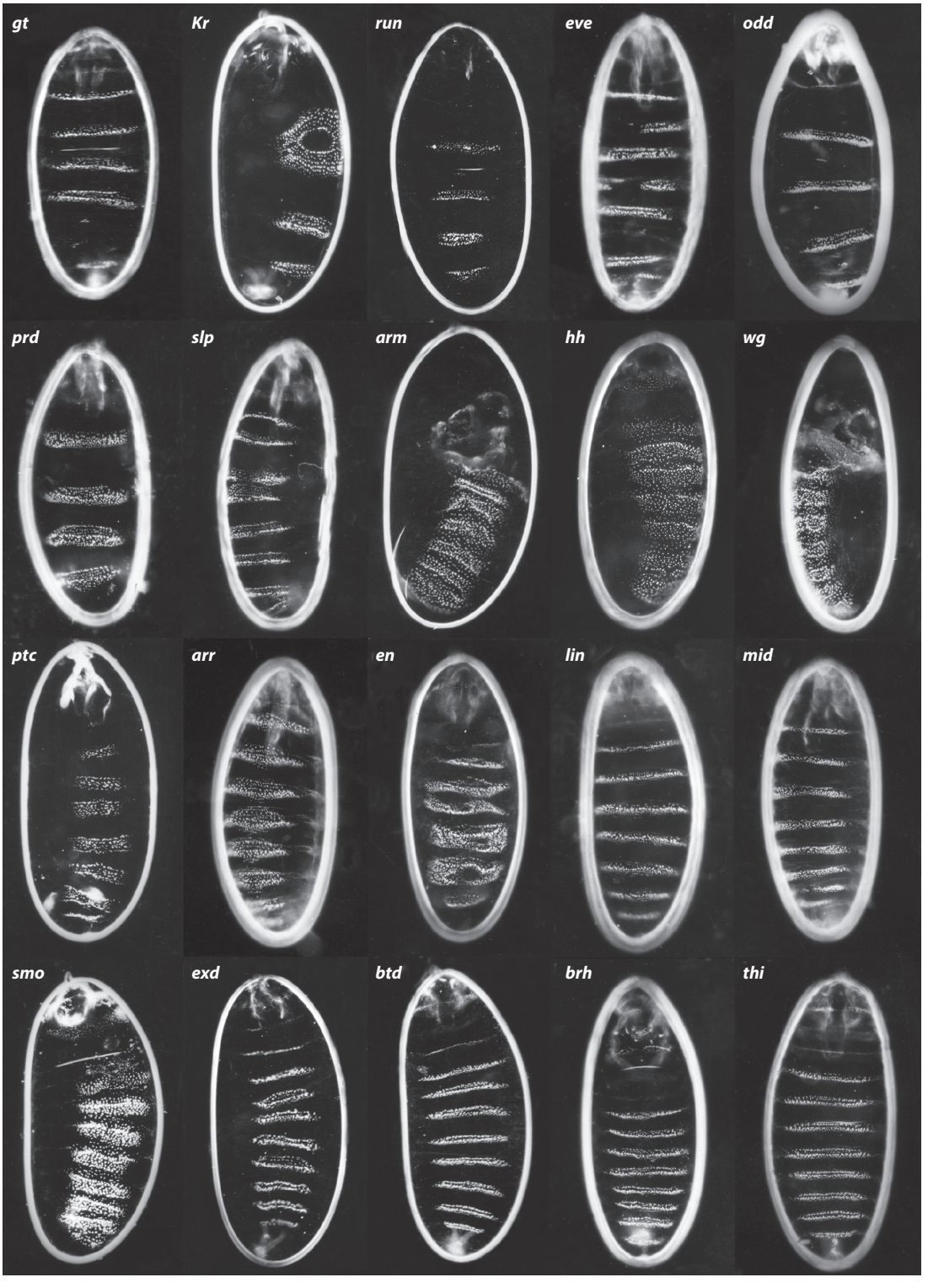


Table 1 Genes affecting anterior-posterior patterning

Class	Locus	Name	Band[b]	Phenotype	Biological process	Protein type	Human ortholog(s)[c]	Human diseases (examples)
Gap	*gt*	*giant*	3A3	A5–A7 deletion, head defect	Transcription	Beta zip transcription factor	—	—
	hb	*hunchback*	85A5	Gnathal segments, thorax deleted	Transcription	Zinc finger transcription factor	—	Ikzf1
	kni	*Knirps*	77E3	A1–A7 fused	Transcription	Zinc finger transcription factor	—	—
	Kr	*Krüppel*[a]	60F5	Anterior abdomen, thorax deleted, mirror of A6	Transcription	Zinc finger transcription factor	BCL6	—
	tll	*tailless*	100A6	A8 and telson deleted, head defect	Transcription	Steroid receptor transcription factor	NR2E1	—
Pair rule	*eve*	*even-skipped*	46C10	Lawn of denticles, in weak phenotype denticle bands of T1, T3, A2, A4, A6, A8 deleted	Transcription	Homeodomain transcription factor	EVX1, EVX2	—
	ftz	*fushi tarazu*[a]	84A6	Denticle bands of T2, A1, A3, A5, A7 deleted	Transcription	Homeodomain transcription factor	—	—
	h	*hairy*[a]	66D8	Denticle bands of T1, T3, A2, A4, A6, A8 deleted	Transcription	Helix-loop-helix transcription factor	HES1, HES4	—
	odd	*odd-skipped*	24A1	Denticle bands of T2, A1, A3, A5, A7 deleted	Transcription	Zinc finger transcription factor	OSR2, OSR1	—
	opa	*odd-paired*	82D8	Denticle bands of T2, A1, A3, A5, A7 deleted	Transcription	Zinc finger transcription factor	ZIC2, ZIC5, ZIC3	Holoprosencephaly 5
	prd	*paired*	33C3	Denticle bands of T1, T3, A2, A4, A6, A8 deleted	Transcription	Homeodomain paired box transcription factor	PAX3/5/6/7/8	Waardenburg syndrome

	run	*runt*	19E1	Segment boundaries of T1, T3, A2, A4, A6, A8 duplicated	Transcription	p53/RUNT-type transcription factor	RUNX1/2	Metaphyseal dysplasia
	slp	*sloppy-paired*	24C6	Like paired, smaller deletions	Transcription	Forkhead domain transcription factor	FOXG1	Rett syndrome
Segment polarity	*arm*	*armadillo*	2B14	Denticle band duplication	Cell signaling	Transcriptional cofactor	β-Catenin, CTNNB1	Hepatocellular ovarian cancer
	ci	*cubitus*[a]	102A3	Denticle band duplication	Transcription	Gli/zinc finger transcription factor (Hh pathway)	Gli	Glioblastoma, basal cell carcinoma, medulloblastoma
	gsb	*gooseberry*	60F2	Denticle band duplication	Transcription	Homeodomain paired box transcription factor	PAX3/5/6/7/8	Waardenburg syndrome, type 1 leukemia
	hh	*hedgehog* (*bar-3*[a])	94E1	Lawn of denticles	Cell signaling	Secreted ligand	SHH, sonic hedgehog	Solitary median maxillary central incisor
	ptc	*patched* (*tufted*[a])	44D5	Segment border duplication	Cell signaling	Cell surface receptor (Hh pathway)	PTCH2	Basal cell carcinoma
	wg	*wingless*[a]	27F2	Lawn of denticles	Cell signaling	Secreted ligand	WNT1	Osteogenesis imperfecta
Segment pattern	*arr*	*arrow*	50A9	Enlarged denticle bands	Cell signaling	Cell surface receptor (Wnt pathway)	LRP5	Osteoporosis-pseudoglioma syndrome
	en	*engrailed*[a]	47F17	Anterior denticle bands deleted, pair rule fusions	Transcription	Homeodomain transcription factor (Hh pathway)	EN1	
	lin	*lines*	44F6	Anterior denticle bands deleted	Transcription	Transcriptional cofactor (Wnt pathway)	LINS1	Mental retardation-27 (MRT27)
	mid	*midline*	25E2	Anterior denticle bands deleted	Transcription	T-box transcription factor	TBX10	MRT27

(*Continued*)

Table 1 (*Continued*)

Class	Locus	Name	Band[b]	Phenotype	Biological process	Protein type	Human ortholog(s)[c]	Human diseases (examples)
	nkd	*naked* (*naked cuticle*)	75F2	No denticle bands	Cell signaling	Intracellular signal processing (Wnt pathway)	NKD1/2	—
	otd	*orthodenticle*	7F10	Posteriorly pointing denticles	Transcription	Homeodomain transcription factor	OTX1	—
	smo	*smooth* (*smoothened*)	21B7	Segment pattern, enlarged denticle bands	Cell signaling	GPCR like (Hh pathway)	SMO	Basal cell carcinoma, medulloblastoma
	upd	*unpaired*	17A5	Irregular segmentation; T3, A5, A8 defective	Cell signaling	Secreted signal	—	—
Homeotic	*ant*	*Antennapedia*[a]	84B	Homeotic; T2, T3→T1	Transcription	Homeodomain transcription factor	HOXA6	—
	bxd	*bithoraxoid*[a] (*Ubx*)	89E1	Homeotic, A1→T3	Transcription	Homeodomain transcription factor	—	—
	exd	*extradenticle*	14A5	Homeotic; T2, T3→T1	Transcription	Homeodomain transcription cofactor	PBX2	—
	hth	*homothorax*	86C1	Homeotic; T1→T2, T3	Transcription	Homeodomain transcription factor	MEIS2	—
	Scr	*Sex combs reduced*[a]	84A5	Homeotic, labium larger than maxilla, T1→T2	Transcription	Homeodomain transcription factor	HOXA6	—
Homeotic Pc group	*Asx*	*Additional sex combs*[a]	51A5	Posterior transformation	Chromatin remodeling	Polycomb protein	ASXL1/2/3	Bainbridge-Ropers syndrome, Bohring-Opitz syndrome
	Pc	*Polycomb*[a]	78C6	Posterior transformation	Chromatin remodeling	Chromodomain protein	—	—
	Psc	*Posterior sex combs*[a]	49E6	Posterior transformation	Chromatin remodeling	Zinc finger transcription factor, ring type	Hsap/PCGF2	—

Head defect	*bhe*	*broad head*	ND	Broad head	ND	ND	—	—
	brh	*brown head*	ND	Broad head	ND	ND	—	—
	btd	*buttonhead*	8F10	Broad head	Transcription	Zinc finger transcription factor	SP1/3/5	—
	cli	*clift* (*eyes absent*[a])	26E1	Broad head	Transcription	Transcriptional coactivator and phosphatase	EYA4	Deafness, autosomal cardiomyopathy, CMD1J
	cra	*crack*	ND	Broad head	ND	ND	—	—
	fkh	*forkhead*	98D5	Head skeleton forked, telson defect	Transcription	Forkhead domain transcription factor	FOXB1, FOXC1	Iridogoniodysgenesis type 1, Axenfeld-Rieger syndrome type 3
	lea	*leak* (*robo2*)	22A1	Broad head	Cell signaling	Cell surface receptor	Robo-2	—
	sal	*spalt*	32F1	Broad head	Transcription	Zinc finger transcription factor	—	—
	sli	*slit*	52C9	Broad head	Cell signaling	Secreted signal	SLIT1/2/3	—
	thi	*thick head*	ND	Broad head	ND	ND	—	

[a]Allele previously known. [b]Mutants mapping at band positions 1–20: Wieschaus et al. (1984a), mutants mapping at band positions 21–60: Nüsslein-Volhard et al. (1984), mutants mapping at band positions 61–100: Jürgens et al. (1984). [c]Incomplete; em dashes indicate no homologs identified. ND denotes not determined.

Figure 9

Gap and pair rule mutants: gap mutants *Krüppel* (*Kr*) (strong phenotype), *hunchback* (*hb*) (weak phenotype), *odd-skipped* (*odd*) (strong phenotype), and *even-skipped* (*eve*) [weak (eve^{ID}) and strong (eve^{RR13}) phenotypes]. The ventral aspects of mutant larvae were dissected out of the vitelline membrane.

First, there were five gap gene mutants (*giant*, *hunchback*, *knirps*, *Krüppel*, and *tailless*) that caused large deletions in embryonic regions in a gene-specific manner. Mutants of the eight pair rule genes showed pattern deletions in every other segment. Surprisingly, mutants for each of these eight genes showed a distinct frame of deletions, skipping specific elements in the even- or odd-numbered denticle bands and adjacent naked cuticle (*even-skipped*, *odd-skipped*, *hairy*, *fushi tarazu*, *runt*) or displaying characteristic pairwise fusions of the denticle bands (*paired*, *odd-paired*, *sloppy-paired*) (**Figures 8**, **10**, and **11**). The size of the maximum deleted region is characteristically different for null alleles in each gene, ranging from the deletion of a small portion of the denticle band (*odd-skipped*) to almost an entire segment equivalent, resulting in an unsegmented lawn of cuticle (*even-skipped*) (**Figures 9** and **11**).

The third class of mutants, segment polarity mutants, displayed deletions associated with duplications in each segment. In this class, phenotypes of duplicated denticle bands in four of the five genes (*hedgehog*, *armadillo*, *gooseberry*, *wingless*) were quite similar, whereas one phenotype (*patched*) was distinct from the other phenotypes (**Figures 10** and **12**).

Members of a fourth class of mutations affecting AP pattern display pattern alterations in each segment, such as enlarged (*arrow*, *smoothened*) or reduced (*engrailed*, *lines*, *orthodenticle*) denticle bands (**Figures 8** and **13**). In *engrailed*, the anterior portion of the denticle band in each segment is deleted, and in addition, there is a pairwise fusion of denticle bands (**Figures 8** and **11**), explaining why this gene was initially classified as pair rule (Nüsslein-Volhard & Wieschaus 1980). The possibility of grouping genes into classes suggested to us—without knowledge of their molecular function—that the segmented pattern was sequentially established. Initially, large unique regions were specified that guided the establishment of a first periodic pattern with double-segment periodicity. This pattern in turn was subdivided into fields of individual segments, each with its own pattern and polarity (**Figure 10**). Finally, and independently of the

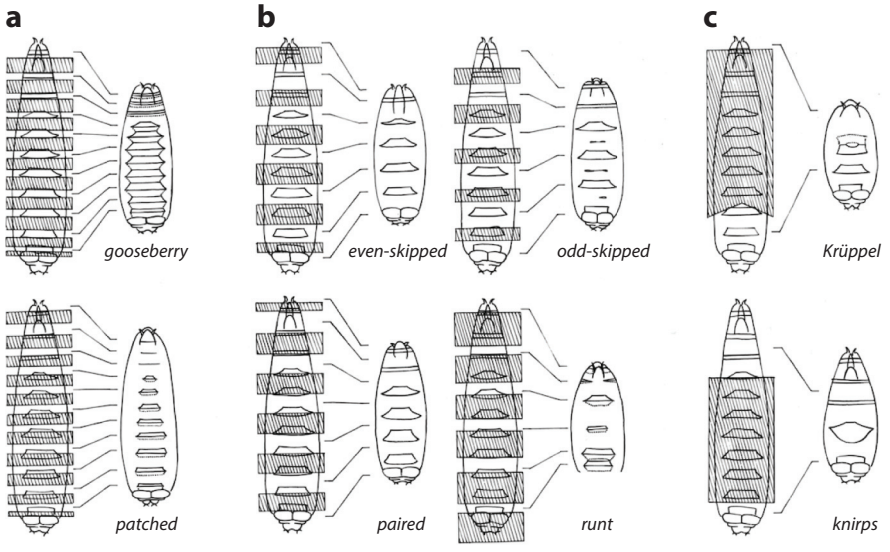


Figure 10

Pattern deletions in an embryo homozygous mutant for genes (*a*) in the segment polarity class (*gooseberry* and *patched*), (*b*) in the pair rule class (*even-skipped*, *odd-skipped*, *paired*, and *runt*), and (*c*) in the gap gene class (*Krüppel* and *knirps*). Modified from Nüsslein-Volhard & Wieschaus (1980).

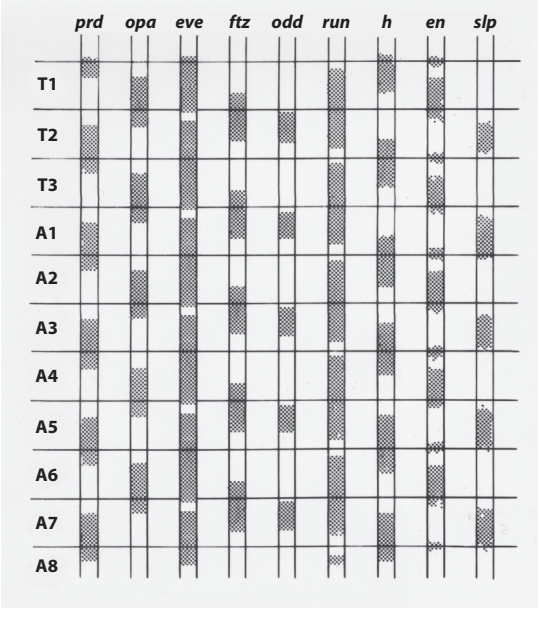


Figure 11

Schematic representation of deletion patterns in pair rule mutants. The shaded areas indicate the regions lost in the mutant patterns of strong alleles. Gene abbreviations (from *top left* to *top right*): *prd*, *paired*; *opa*, *odd-paired*; *eve*, *even-skipped*; *ftz*, *fushi tarazu*; *odd*, *odd-skipped*; *run*, *runt*; *h*, *hairy*; *en*, *engrailed*; *slp*, *sloppy-paired*.

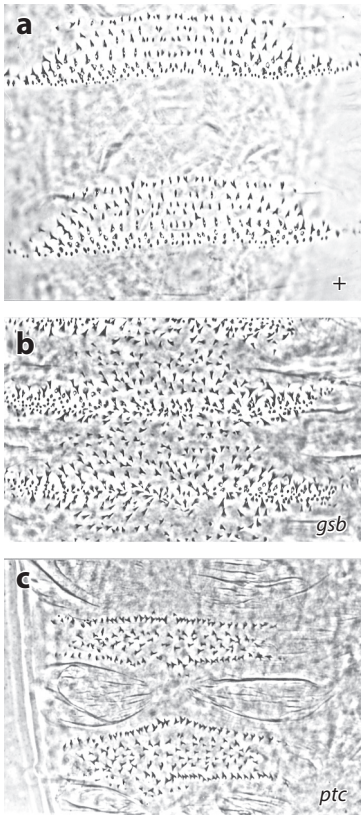


Figure 12

Segment polarity mutants: details from the ventral anterior abdomen of (*a*) wild-type (+), (*b*) *gooseberry* (*gsb*), and (*c*) *patched* (*ptc*) larvae (phase contrast).

establishment of a correct segmented pattern [as we could show by the construction of double mutants with *Ubx* (Nüsslein-Volhard & Wieschaus 1980)], the character of each segment was determined by the action of the homeotic genes.

Members of the final group of AP patterning mutants, the homeotic mutants, are affected in the identities of segments without affecting segmental pattern. Because morphological differences between segments in the embryo are not as striking as those in the adult (Lewis 1978), such homeotic mutations cause only subtle alterations in the larval segment pattern. Such transformations were useful, however, because they often represented the null phenotype of the gene. Examples are the *bithoraxoid* mutant, in which the first abdominal segment seems to be transformed into thorax, and the *Antennapedia* mutant, in which all three thoracic segments (T1–T3) look like the first thoracic segment (T1). Neither allele displayed the adult phenotypes previously described for dominant alleles in these genes. Our screen identified mutations in two new homeotic genes (*extradenticle* and *homothorax*) displaying transformations visible in the shape of the denticle bands (**Figure 13**). We also identified mutations in members of the Pc group (*Polycomb*, *Additional sex combs*, and *Posterior sex combs*).

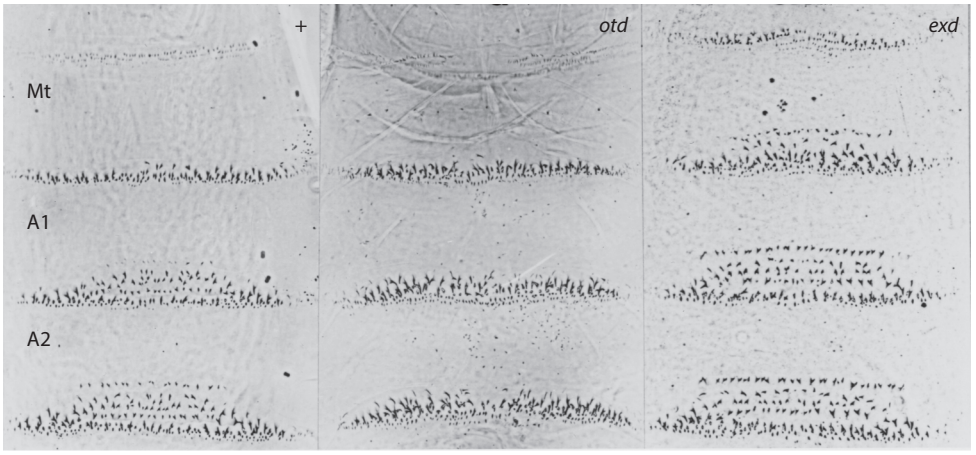


Figure 13

Segment pattern and homeotic mutants: posterior thorax and anterior abdomen of *orthodenticle* (*otd*) (abdominal denticle belts reduced) and *extradenticle* (*exd*) (posterior transformations). Other abbreviations: A, abdominal segment; Mt, metathorax. From Wieschaus et al. (1984a).

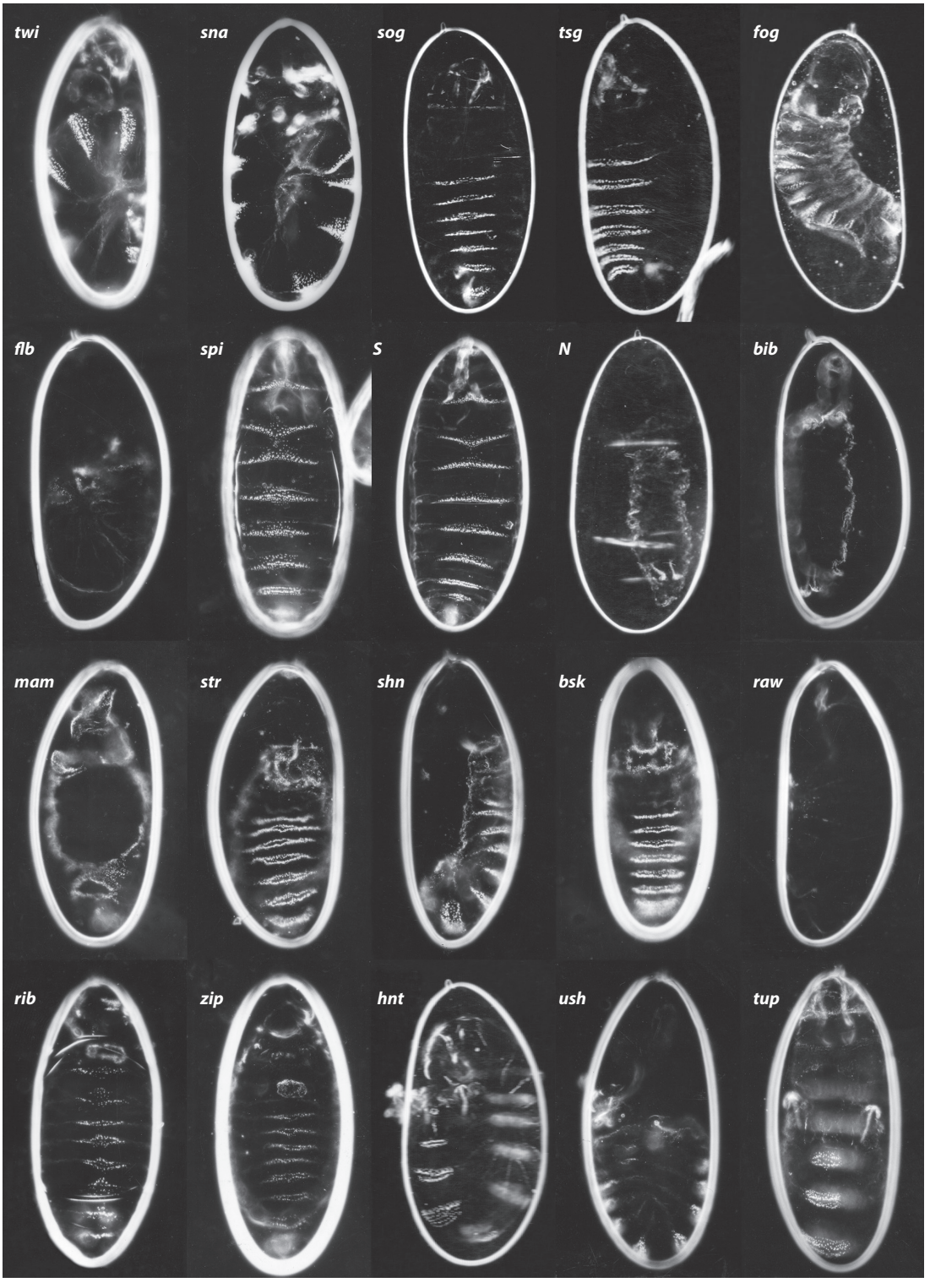
As discussed above, head mutants without additional features were difficult to characterize further. Two of them, *forkhead* and *buttonhead*, displayed a more conspicuous head skeleton, with *forkhead* showing posterior defects as well.

The Dorsal-Ventral Genes

Figure 14 shows a selection of 20 mutants affected in DV patterning and sorted according to the classes described in **Table 2**. The most obvious early morphogenetic movements in the fly embryo involve cells of the mesoderm and the amnioserosa that are established along the embryonic DV axis during gastrulation. Upon inspection of the development of living mutant embryos, we identified seven loci affecting gastrulation movements (**Table 2**).

In *twist* and *snail* mutants, the ventral furrow fails to form, the ventral denticle bands are reduced, and the larvae are twisted in the egg case (**Figure 15**). This dorsalized phenotype is indistinguishable from the dominant phenotype of the maternal gene *dorsal*, suggesting that *twist* and *snail* act immediately downstream of *dorsal* (Simpson 1983). Mutants in four genes of the gastrulation group (*decapentaplegic*, *tolloid*, *screw*, and *shrew*) display enlarged denticle bands at the expense of dorsal cuticle (reminiscent of ventralized maternal phenotypes of *Toll* and *cactus*) (Arora & Nüsslein-Volhard 1992) (**Figures 14** and **15**). In other mutants, germ band extension is abnormal or delayed, or folds form in the germ band. In these latter mutants (*folded gastrulation*, *short gastrulation*, and *twisted gastrulation*), the cuticle displays various defects (**Figure 14**). Five genes in a second group (the *spitz* group) do not affect early gastrulation movements but share a phenotype in which the median part of the denticle band in each segment is deleted and the head skeleton is pointed (**Figure 15**). In the strongest member of this group, *faint little ball*, this phenotype is observed only in weak alleles, whereas in the amorphic condition all ventral cuticle is absent (**Figures 14** and **15**).

Mutations in the *Notch* gene had previously been shown to produce a neuralized phenotype (Poulson 1940) in which the ventral epidermis is transformed into neural tissue. In cuticle



preparations, only a shield of dorsal cuticle is preserved, and the ventral denticle belts are completely absent (**Figure 14**). We found this easily detected phenotype in mutants of five different loci (*Notch*, *Delta*, *neuralized*, *mastermind*, *big brain*) (Lehmann et al. 1983) (**Table 2**). In another class of mutants, the dorsal cuticle is absent (*schnurri*, *slater/thickvein*), the cuticle is dorsally open (*basket*, *raw*, *ribbon*, *zipper*) or has dorsal slits or holes, or the larvae are curled up and anterior and posterior parts are joined laterally (the U-shaped group: *hindsight*, *u-shaped*) (**Figure 14**). Although there are characteristic differences between the phenotypes, it was not possible on the basis of the phenotype alone to distinguish defects in the mechanics of dorsal closure, actual patterning phenotypes in the dorsal hypodermis, and more general cellular failures. For these reasons, our classification rests on the most general morphological features as well as on subsequent analyses.

The Epidermis Genes

A large fraction of our mutants displayed phenotypes suggesting a normal arrangement of the embryonic anlagen. However, the cellular properties of the epidermis (or the cuticle shed by the epidermis) were affected. **Figure 16** shows a selection of 20 mutants sorted according to the classes described in **Table 3**. In several mutants affecting cell polarity (*bazooka*, *stardust*, *crumbs*), the cuticle had small or larger holes, depending on the strength of alleles. Others (*string*, *three rows*) had no or a reduced number of enlarged denticles resulting from fewer but larger hypodermal cells. A strange group of mutants (*retroactive*, *krotzkopf verkehrt*, *knickkopf*) showed a combination of strongly reduced head skeleton, weak denticles, and hyperactivity in the mutant larvae, which often turned around in the egg case such that the tail end would be at the anterior egg pole; these mutants and a couple of others were affected in cuticle synthesis and organization.

Other mutants (*faint*, *unpigmented*, *pale*, *faintoid*, *dopadecarboxylase*) displayed unpigmented denticles and mouth parts, suggesting that the melanin pathway was affected. The denticles and hairs are abnormal in *crinkled*, *shavenoid*, and *shavenbaby* mutants (**Figure 17**). Other mutants failed to form a cuticle, although characteristic remnants of the internal organs were still present. Members of the halloween group—*phantom*, *shade*, *spook*, *shadow*, *disembodied*, and *shroud*—shared a very similar phenotype that was distinct from that of *ghost* and *haunted*.

MOLECULAR BIOLOGY

The mutagenesis experiments in Heidelberg were carried out with the primary aim of understanding the logic and complexity of embryonic pattern formation in terms of its genetics and the phenotypes. So little was known about these areas that at the time we did not yet think in molecular terms and (despite one of us having been trained as a biochemist) had not the slightest ideas or hypotheses about the nature of the gene products. When we discovered the segmentation gene hierarchy, it immediately became obvious that it would be very exciting to know the

Figure 14

Mutants affected in dorsal-ventral patterning: 20 mutants of genes listed in **Table 2** represent the following classes: gastrulation dorsalized group [*twist* (*twi*) and *snail* (*sna*)], gastrulation *decapentaplegic* group [*short gastrulation* (*sog*), *twisted gastrulation* (*tsg*), and *folded gastrulation* (*fog*)], *spitz* group [*faint little ball* (*flb*), *spitz* (*spi*), and *Star* (*S*)], neuralized mutants [*Notch* (*N*), *big brain* (*bib*), and *mastermind* (*mam*)], dorsal pattern mutants [*slater* (*str*)*/thickvein*, *schnurri* (*shn*), and *basket* (*bsk*)], dorsal closure group [*raw*, *ribbon* (*rib*), and *zipper* (*zip*)], and *u-shaped* group [*hindsight* (*hnt*), *u-shaped* (*ush*), and *tail up* (*tup*)]. From Nüsslein-Volhard et al. (1984) and Wieschaus et al. (1984a).

Table 2 Genes affecting gastrulation and dorsal-ventral patterning

Class	Locus	Name	Band[b]	Phenotype	Biological process	Protein type	Human ortholog(s)	Human diseases[c]
Gastrulation, dorsalized	*sna*	*snail*	35D2	Dorsalized, no ventral furrow	Transcription	Zinc finger transcription factor	SNAI2/3	Piebald trait, Waardenburg syndrome
	twi	*twist*	59C1	Dorsalized, no ventral furrow	Transcription	Helix-loop-helix transcription factor	TWIST1/2	Robinow-Sorauf syndrome, Saethre-Chotzen syndrome
Gastrulation, decapenta-plegic group (BMP)	*dpp*	*decapentaplegic*[a]	22F1	Ventralized	Cell signaling	Secreted ligand	TGFβ, BMP2/4	Brachydactyly type A2, hemochromatosis type 1, microphthalmia
	fog	*folded gastrulation*	20E2	Germ band folded, ventral holes	Cell signaling	Secreted factor	—	—
	scw	*screw* [*l(2)IG76*]	38A	Ventralized, posterior end pulled in	Cell signaling	Secreted ligand	BMP5	—
	sog	*short gastrulation*	13E1	Short germ band extension	Cell signaling	Ligand processing	CHRD	—
	srw	*shrew*	64A12	Ventralized, posterior end pulled in	Cell signaling	BMP signaling	TWSG1	—
	tld	*tolloid*	96A19	Ventralized, posterior end pulled in	Cell signaling	Metalloprotease	TLL1/2, BMP1	Atrial septal defect 6, osteogenesis imperfecta
	tsg	*twisted gastrulation*	11A1	Posterior end pulled in	Cell signaling	Secreted factor	TWSG1	—
Spitz group (EGF)	*flb*	*faint little ball* (*torpedo*, *EGFR*)	57E9	Ventral cuticle deleted, head pointed	Cell signaling	Epidermal growth factor receptor	EGFR	Adenocarcinoma of lung, non–small cell lung cancer
	pnt	*pointed*	94E10	Midventral deletion, head pointed	Transcription	ETS transcription factor	ETS1/2	—

	rho	*rhomboid* (*veinlet*)	62A2	Midventral deletion, head pointed	Cell signaling	Ligand processing (spitz)	RHBDL2	—
	spi	*spitz*	37F2	Midventral deletion, head pointed	Cell signaling	Secreted ligand: EGF	EGF	Hypomagnesemia 4
	S	*Star*[a]	21E4	Midventral deletion, head pointed	Cell signaling	EGFR chaperone	—	—
Neuralized	*bib*	*big brain*	30F5	Ventral cuticle absent, neuralized	Cell signaling	MIP/aquaporin family channel	—	—
	Dl	*Delta*[a]	92A1	Ventral cuticle absent, neuralized	Cell signaling	Cell surface ligand	DLL1/2	Adams-Oliver syndrome 6
	E(spl)	*enhancer of split*[a]	96F10	Ventral cuticle absent, neuralized	Transcription	Helix-loop-helix transcription factor	—	—
	mam	*mastermind*	50C23	Ventral cuticle absent, neuralized	Transcription	Transcriptional coactivator	MAML1	—
	N	*Notch*[a]	3C7	Ventral cuticle absent, neuralized	Cell signaling	Cell surface receptor	NOTCH1/2/3	Aortic valve disease, Alagille syndrome 2
	neu	*neuralized*	85C2	Ventral cuticle absent, neuralized	Cell signaling	E3 ubiquitin ligase	NEURL1B	—
Dorsal pattern	*pnr*	*pannier*	89B9	Dorsal cuticle absent	Transcription	Zinc finger transcription factor	GATA2	Emberger syndrome, immunodeficiency 21
	put	*punt*	88C9	Dorsal open	Cell signaling	Ser/Thr protein kinase receptor (dpp pathway)	TGFBR2	Loeys-Dietz syndrome 2, hereditary nonpolyposis 6
	shn	*schnurri*	47D6	Dorsal cuticle absent	Transcription	Zinc finger transcription factor	HIVEP1/2/3	—
	str	*slater* (*thickveins*[a])	25D1	Dorsal cuticle absent	Cell signaling	Ser/Thr protein kinase receptor (dpp pathway)	Activin receptor ACVR1	Fibrodysplasia ossificans progressiva
Dorsal closure	*aop*	*anterior open*	22D1	Dorsal anterior deletion	Transcription	ETS transcription factor	—	—
	IA109	*anterior open*	46E4	Dorsal anterior deletion	Transcription	JUN	—	—
	bsk	*basket*	31B1	Dorsal anterior deletion	Cell signaling	JUN kinase	MAPK9	—

(*Continued*)

Table 2 (*Continued*)

Class	Locus	Name	Band[b]	Phenotype	Biological process	Protein type	Human ortholog(s)	Human diseases[c]
	cno	*canoe*	82F4	Dorsal open	Cell adhesion	Ras GTPase actin-binding protein	MLLT4	—
	kay	*kayak*	99B10	Dorsal open	Transcription	Beta zip transcription factor	—	—
	raw	*raw*	29E4	Dorsal open, cuticle poorly differentiated	Cell signaling	Antagonist of JUN kinase pathway	—	—
	rib	*ribbon*	56C6	Ventral denticle bands narrow, dorsal open	Transcription	Zinc finger transcription factor	—	—
	yrt	*yurt*	87E11	Dorsal posterior open	Cytoskeleton	Band 4.1–like protein	EPB41L5	—
	zip	*zipper*	60E12	Head and dorsal closure defective, hole in ventral thorax	Cytoskeleton	Nonmuscle myosin II	MYH9 (myosin heavy chain 9)	Deafness, autosomal dominant 17; Epstein syndrome
U-shaped group	*hnt*	*hindsight* (*pebbled*)	4C10	No shortening, dorsal closure lateral	Transcription	Zinc finger transcription factor	RREB1	—
	spt	*serpent*	89E1	Defect in shortening posterior abdomen	Transcription	Zinc finger transcription factor	—	—
	tup	*tailup* (*islet*)	37B1	Defect in shortening, broad head	Transcription	LIM homeodomain transcription factor	Islet (ISL1/2)	—
	ush	*u-shaped*	21D1	No shortening, dorsal closure lateral	Transcription	Zinc finger transcription factor	—	—

[a]Allele previously known. [b]Mutants mapping at band positions 1–20: Wieschaus et al. (1984a), mutants mapping at band positions 21–60: Nüsslein-Volhard et al. (1984), mutants mapping at band positions 61–100: Jürgens et al. (1984). [c]Incomplete; em dashes indicate no homologs identified.

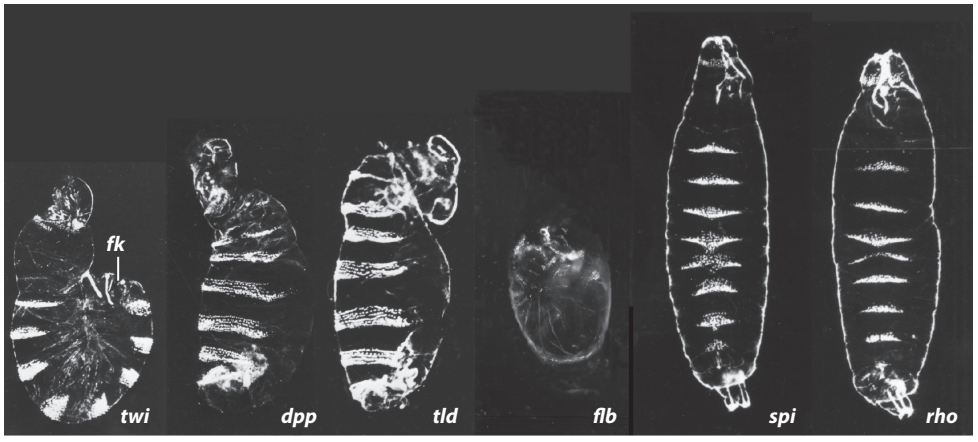


Figure 15

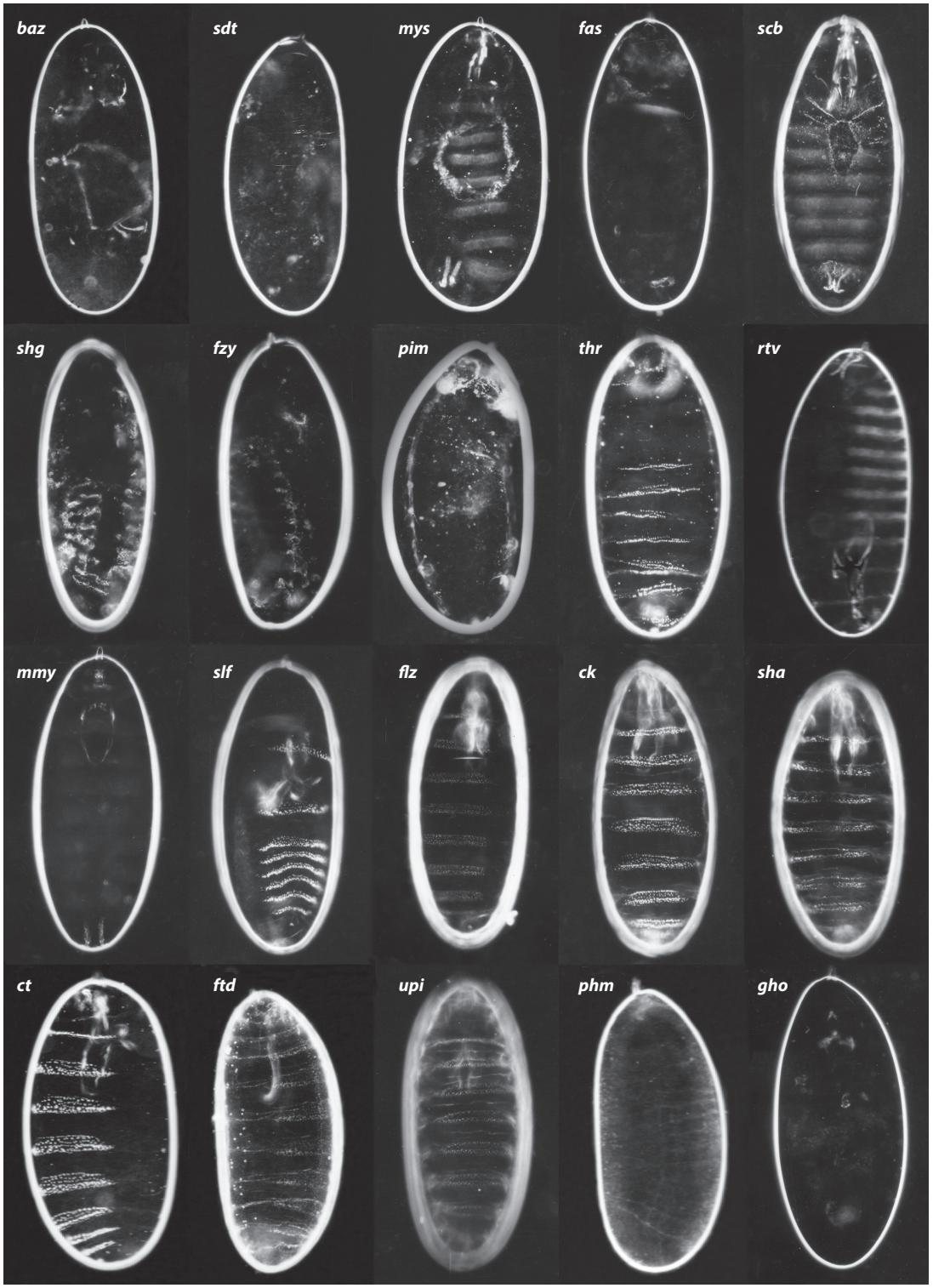
Gastrulation and ventral pattern mutants: gastrulation dorsalized *twist* (*twi*) and gastrulation ventralized *decapentaplegic* (*dpp*) (weak allele) and *tolloid* (*tld*); *spitz* group *faint little ball* (*flb*), *spitz* (*spi*), and *rhomboid* (*rho*). The ventral aspects of mutant larvae dissected out of the vitelline membrane. The vitelline membrane of the *flb* embryo was removed by Photoshop. From Arora & Nüsslein-Volhard (1992) and Mayer & Nüsslein-Volhard (1988).

nature of the proteins they encoded and where they were present in the embryo. These were the early days of molecular biology. The advent of recombinant DNA technologies and positional cloning allowed for the identification of the genes. The banding patterns observed in giant salivary gland chromosomes of *Drosophila* were especially useful in these early molecular analyses because they allowed mutants to be assigned to visible bands and thus to physical positions on chromosomes.

Because we made the mutants from our screen available to the scientific community before publication, many of them were identified and sequenced and their expression patterns characterized in the years that followed. The discovery of the P-element and its use in mutagenesis and genetic transformation (Rubin & Spradling 1982) provided another advantage, speeding up the exploration of our collection. The first genes derived from the Heidelberg screens were cloned and published by 1984–1985. By 1995, 75 of the 120 genes had been identified; by 2002, 95. As we write this review, only 5 of the loci (see **Tables 1** and **3**) have yet to be unambiguously matched to sequences in the *Drosophila* genome.

Transcriptional Control

The first attempts at cloning *Drosophila* patterning genes by chromosomal walking were successful in cloning the homeotic genes *Ultrabithorax* and *Antennapedia* (Bender et al. 1983, Garber et al. 1983, Scott et al. 1983). These studies identified the first transcription factors and resulted in the discovery of the homeobox, a DNA-binding domain with homology to many genes in all animals (Laughon & Scott 1984, McGinnis et al. 1984, Scott & Weiner 1984). The first segmentation gene cloned [*fushi tarazu* (Hafen et al. 1984)], also encoding a homeobox gene, was not one of our early discoveries (Jürgens et al. 1984), but its location in the *Antennapedia* complex made it easily accessible by chromosomal walking. Next, the gap gene *Krüppel* was cloned using DNA



dissected from polytene chromosome band 60F (Preiss et al. 1985). *Krüppel*, like two other gap genes, *hunchback* (Tautz et al. 1987) and *knirps* (Nauber et al. 1988), encodes a transcription factor of a second class: the zinc finger DNA-binding proteins (Rosenberg et al. 1986). The first gene in the segment polarity/pattern class to be cloned (*engrailed*) was also shown to encode a homeobox transcription factor (Kuner et al. 1985, Poole et al. 1985). By this point, in situ hybridization techniques had been developed that allowed the location of transcripts in the embryo to be determined. The expression of *fushi tarazu* and *hairy* in seven stripes (Hafen et al. 1984, Ingham et al. 1985), the single domain of *Krüppel* (Knipple et al. 1985), and the 14 *engrailed* stripes (DiNardo et al. 1985, Fjose et al. 1985, Kornberg et al. 1985) provided amazing proof of the segmentation hierarchy we had proposed in the Nüsslein-Volhard & Wieschaus (1980) paper.

Many of the early-acting genes, in particular the segmentation genes of the gap and pair rule class, were found to encode transcription factors (**Table 1**). The expression of these genes in blastoderm-stage embryos generally corresponded to the pattern of deletions observed in the mutants (**Figure 10**). The transcripts of gap genes were expressed in unique large regions (Capovilla et al. 1992, Knipple et al. 1985, Tautz et al. 1987), those of pair rule genes in 7 stripes with different genes having slightly different registers (Frasch & Levine 1987, Gergen & Butler 1988, Hafen et al. 1984, Ingham et al. 1985, McDonald et al. 1986) (**Figure 11**) and segment polarity/pattern transcripts in >14 stripes (Baker 1987, Riggleman et al. 1990, Stanojevic et al. 1991, Tabata et al. 1992). In genetic experiments involving double-mutant combinations, some of these genes were shown to regulate others within their group or to function downstream, and molecular data confirmed this finding (Howard & Ingham 1986, Ingham et al. 1988, Jäckle et al. 1986). For example, each of the seven stripes of the pair rule genes *even-skipped*, *runt*, and *hairy* is determined by a combination of different gap genes, whereas others depend on the activity of these primary pair rule genes (Frasch & Levine 1987, Hiromi & Gehring 1987, Howard & Struhl 1990; see, however, Schroeder et al. 2011). The early fate map along the AP axis of the embryo is generally established by a series of transcription factors that provide molecular prepatterns. By regulating each other, these patterns become refined until the molecular pattern directly determines the morphological pattern of the structures developing in the embryo as monitored by the cuticle pattern of denticles and hairs. The spatial control of downstream genes occurs via concentration-dependent enhancement or inhibition of their transcription as well as by combinations of two or more transcription factors (reviewed in Lawrence 1992 and Pankratz & Jäckle 1993).

Early-expressed genes that control the ventral pattern (*twist*, *snail*) (Boulay et al. 1987, Leptin 1991, Thisse et al. 1988) encode transcription factors whose expression domains directly correspond to the pattern of shape changes occurring in the presumptive mesoderm during gastrulation (Leptin & Grunewald 1990). Later-expressed genes controlling lateral and dorsal pattern and dorsal closure (*schnurri*, *pannier*, *kayak*, *u-shaped*, *hindsight*) also encode transcription factors (Arora et al. 1995, Frank & Rushlow 1996, Ramain et al. 1993), as do several head genes such as *forkhead* and *spalt* (Weigel et al. 1989) (**Table 2**).

Figure 16

Mutants affected in epidermal structure and integrity: 20 mutants of genes listed in **Table 3** represent the following classes: cell polarity [*bazooka* (*baz*), *stardust* (*sdt*)], cell adhesion [*myospheroid* (*mys*), *faint sausage* (*fas*), *scab* (*scb*), and *shotgun* (*shg*)], cell number [*fizzy* (*fzy*), *pimples* (*pim*), and *three rows* (*thr*)], chitin [*retroactive* (*rtv*), *mummy* (*mmy*), and *schlaff* (*slf*)], denticles [*filzig* (*flz*), *crinkled* (*ck*), and *shavenoid* (*sha*)], sense organs, tracheae [*cut* (*ct*)], pigmentation [*faintoid* (*ftd*) and *unpigmented* (*upi*)], halloween group [*phantom* (*phm*)], and secretion [*ghost* (*gho*)]. From Nüsslein-Volhard et al. (1984) and Wieschaus et al. (1984a).

Table 3 Genes affecting epidermal structure and integrity

Class	Locus	Name[a]	Band[b]	Phenotype	Biological process(es)	Protein type	Human ortholog(s)	Human disease[c]
Cell polarity	*crb*	*crumbs*	95F10	Many small holes	Apical basal cell polarity	Crumbs protein family	CRB1/2	Retinitis pigmentosa
	baz	*bazooka*	15F1	Large dorsal and ventral holes in cuticle	Apical basal cell polarity	Par-3-like protein	PARD3B	—
	sdt	*stardust*	7D17	Cuticle almost absent, small remnants	Apical basal cell polarity	Membrane-serine protein kinase	MPP5, CASK	Mental retardation microcephaly
Cell adhesion	*fas*	*faint sausage*	50B6	Poorly differentiated cuticle and head	Cell adhesion	Immunoglobulin superfamily	—	—
	mys	*myospheroid*[a]	7D5	Dorsal closure defective	Cell adhesion	Integrin receptor	ITGB3/4/8	Epidermolysis bullosa
	scb	*scab*	51E10	Middorsal hole	Cell adhesion	Integrin alpha subunit	ITGA4	—
	shg	*shotgun*	57B15	Many small holes in cuticle	Cell adhesion	E-Cadherin	CDH20	—
Cell number	*fzy*	*fizzy*	35F1	Ventral cuticle degenerated	Cell cycle, cytokinesis	APC-Cdc20 complex activity	CDC20	—
	pbl	*pebble*	66A18	Ball of naked cuticle	Cell cycle, cytokinesis	RhoGEF	ECT2	—
	pim	*pimples*	31D10	Poorly differentiated cuticle and head	Cell cycle, cytokinesis	Securin-like protein	—	—
	stg	*string*	99A5	Few rows of denticles	Cell cycle, cytokinesis	Cdc25	CDC25A	—
	thr	*three rows*	54F3	Few rows of denticles	Cell cycle, cytokinesis	Separase regulation	—	—

Cuticle	*grh*	*grainy head I(M)45, Elf1*	54E10	Head skeleton glassy, cuticle thin	Transcription	CP2-type transcription factor	LBP-1, CP2	—
	kkv	*krotzkopf verkehrt*	83A1	Cuticle and denticles thin; head skeleton reduced	Chitin synthesis	Chitin synthase	—	—
	knk	*knickkopf*	85F13	Cuticle and denticles thin, head skeleton reduced	Chitin synthesis organization	GPI-anchored protein	—	—
	mmy	*mummy*	26D7	Undifferentiated	Chitin synthesis	*N*-Acetylglucosamine diphosphorylase	UAP1	—
	rtv	*retroactive*	10A8	Cuticle and denticles thin, head skeleton reduced	Chitin synthesis organization	Membrane-anchored extracellular protein	—	—
	slf	*schlaff*	25C	Collapsed larval body	Cuticle organization	Secreted protein	—	—
Denticles, hairs	*ck*	*crinkled*[a]	35B8	Denticles thick and forked, hairs fused, bristles	Cytoskeleton	Myosin VIIB	MYO7A/B	Usher syndrome type I
	flz	*filzig*	45-46D	Denticles and hairs grainy	Cuticle structure	Secreted enzyme	—	—
	sha	*shavenoid*	47F2	Denticles sparse, hairs short, trichomes absent	Cytoskeleton	Actin interaction protein	—	—
	svb	*shavenbaby* (*ovo*)	4E2	Denticles, hairs	Transcription	Zinc finger transcription factor	OVOL1/2/3	—
	ems	*empty spiracles*	88A2	No filzkörper, no antennae, head open	Transcription	Homeodomain transcription factor	EMX2	Schizencephaly
	gra	*grain*	84F1	Filzkörper not elongated, head defect	Transcription	Zinc finger transcription factor	GATA4	Testicular anomalies, ventricular septal defects
	trh	*trachealess*	61C1	No tracheae, no filzkörper	Transcription	Helix-loop-helix transcription factor	NPAS1/3	—

(*Continued*)

Table 3 (*Continued*)

Class	Locus	Name[a]	Band[b]	Phenotype	Biological process(es)	Protein type	Human ortholog(s)	Human disease[c]
Pigmentation	*ddc*	*dopadecarboxylase*[a]	37C1	Unpigmented cuticle and head skeleton	Melanin/serotonin pathway	Dopa decarboxylase	HDC, DDC	Gilles de la Tourette syndrome
	fai	*faint*	ND	Unpigmented cuticle and head skeleton	ND	ND	ND	ND
	ftd	*faintoid* (*quicksilver*)	10F1	Unpigmented cuticle and head skeleton	Melanin/serotonin pathway	Copper ion transporter	ATP7	Menkes disease
	ple	*pale*	65C3	Unpigmented cuticle and head skeleton	Melanin/serotonin pathway	Tyrosine hydroxylase	PAH, TH	Segawa syndrome, phenylketonuria
	upi	*unpigmented* (*Punch*)	57C7	Unpigmented cuticle and head skeleton	Melanin/serotonin pathway	GTP cyclohydrolase	GCH1	Dystonia, Dopa responsive; hyperphenylalaninemia
Halloween group	*dib*	*disembodied*	64A5	Undifferentiated cuticle and head	Ecdysone biosynthesis	Cytochrome P450 family	—	—
	phm	*phantom*	17D1	Undifferentiated cuticle and head	Ecdysone biosynthesis	Cytochrome P450 family	—	—
	sad	*shadow*	87A1	Undifferentiated cuticle and head	Ecdysone biosynthesis	Cytochrome P450 family	—	—
	shd	*shade*	70E4	Undifferentiated cuticle and head	Ecdysone biosynthesis	Cytochrome P450 family	—	—
	spo	*spook*	64D5	Undifferentiated cuticle and head	Ecdysone biosynthesis	Cytochrome P450 family	—	—
	sro	*shroud*	99C1	Undifferentiated cuticle and head	Ecdysone biosynthesis	Short-chain dehydrogenase	—	—
Secretion	*gho*	*ghost*	22D4	Undifferentiated cuticle and head	Secretion pathway	COP II ER-to-Golgi transport	Sec 24CD	Cole-Carpenter syndrome 2
	hau	*haunted*	83B7	Undifferentiated cuticle and head	Secretion pathway	β-COP ER-to-Golgi transport	Sec 23A	Craniolenticulosutural dysplasia

[a]Allele previously known. [b]Mutants mapping at band positions 1–20: Wieschaus et al. (1984a), mutants mapping at band positions 21–60: Nüsslein-Volhard et al. (1984), mutants mapping at band positions 61–100: Jürgens et al. (1984). [c]Incomplete; em dashes indicate no homologs identified. ND denotes not determined.

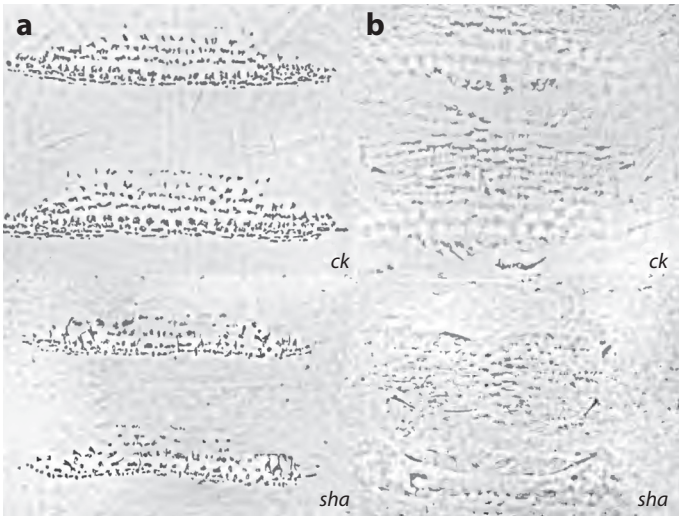


Figure 17

Denticle and hair mutants: (*a*) ventral and (*b*) dorsal aspects of anterior abdomen of *crinkled* (*ck*) (*top*) and *shavenoid* (*sha*) (*bottom*) (phase contrast). For wild type, see **Figures 1** and **12.**

Because the Heidelberg mutations produced phenotypes in homozygous embryos, but not in their heterozygous siblings, our screen identified genes that must be transcribed in the embryo. The pattern of that transcription must depend on some pattern that we assumed was present in the egg prior to its fertilization. Our screen could not identify such maternally acting genes, and in Heidelberg we established the genetic strategies of the screens subsequently carried out. These screens had to allow stocks to be carried through the multiple generations required to produce homozygous females that could be tested for maternal effects on embryonic patterning. The screens for maternal mutants carried out in Tübingen and Princeton led to the identification of approximately 30 genes involved in axis determination (Anderson & Nüsslein-Volhard 1984; Nüsslein-Volhard 1991; Nüsslein-Volhard et al. 1987; Schüpbach & Wieschaus 1986, 1989) that operate upstream of the zygotic genes we identified in Heidelberg. The principal morphogen gradients determining the egg axes, encoded by *bicoid*, *nanos*, *torso*, and *dorsal*, control the spatial patterns of expression of the transcription factors of the first embryonic prepattern, the gap genes, and the gastrulation genes such as *twist*, *snail*, and *zerknüllt* (St. Johnston & Nüsslein-Volhard 1992).

Cell Signaling Pathways

Patterning within individual insect segments has long been known from experimental manipulations to depend on cell communication (Lawrence et al. 1972, Wigglesworth 1972). Our screen identified lethal alleles in many components of the major cell signaling pathways that pattern the fly embryo. Many of these pathways play significant roles in the development and disease of vertebrate organisms as well. In recent years, names of the vertebrate pathways have sometimes been adopted in flies; examples are the EGF pathway for the Spitz group, Wnt for the Wingless group, and BMP/TGFβ for the Decapentaplegic pathway. Among the genes affecting the AP

pattern (**Table 1**), several of the segment polarity and segment pattern class encode members of two signaling pathways, Hedgehog (*hedgehog*, *patched*, *smoothened*) and Wnt (*wingless*, *armadillo*, *arrow*), which shape the individual segments (Hooper & Scott 1989, Ingham & McMahon 2001, Nakano et al. 1989, Nusse & Varmus 1992, Peifer & Wieschaus 1990, Riggleman et al. 1990, Sanson et al. 1999, Tabata et al. 1992). The pattern along the DV axis depends on two signaling systems, BMP (*Decapentaplegic/BMP*, *tolloid*, *screw*, *shrew*, *short gastrulation*, *folded gastrulation*, *twisted gastrulation*, *schnurri*, *slater/thickveins*, *punt*) (Costa et al. 1994, Dale et al. 1992, Ferguson & Anderson 1992, François et al. 1994, Harden 2002, Mason et al. 1994, O'Connor et al. 2006, Ruberte et al. 1995, Shimell et al. 1991) and EGF (*faint little ball/EGFR*, *spitz/EGF*, *rhomboid*, *Star*, *pointed*) (Bier et al. 1990, Mayer & Nüsslein-Volhard 1988, Price et al. 1989, Raz & Shilo 1993, Rutledge et al. 1992, Schejter & Shilo 1989) (**Table 2**). Neurogenesis involves the Notch pathway (*Notch*, *Delta*, *neuralized*, *big brain*, *mastermind*) (Campos-Ortega 1993, Fehon et al. 1990). Components of the same pathway could often be recognized on the basis of their common phenotype in fly embryos as described above.

Alleles in some of these pathways had been previously identified on the basis of their dosage effects on adult morphology (*Notch*, *Delta*, *Star*) or partial loss-of-function alleles that were homozygous viable in adults (e.g., *wingless*, *engrailed*, *hairy*, *thickveins*, *decapentaplegic*). Because the partial loss-of-function phenotypes produced by such mutations in adults were often variable, it was not obvious how these genes related to each other. Recognizing similarities in phenotype was potentially more straightforward for us, given that screens based on embryonic lethality are more likely to yield genetic null alleles. In some cases, however, classification was still difficult without further molecular analyses. Components of the same pathway might have opposite (complementary) phenotypes when one product acted negatively on another (as, e.g., in the case of Hedgehog and Patched), and it was not possible to distinguish members of the Hedgehog and Wingless pathways on the basis of subtle differences in the denticle lawns (see **Figure 8**).

Whereas the mutations discovered in the Heidelberg screen often identified the founding member of a pathway and provided anchors for future research, only through subsequent studies in many labs using genetic mosaics, ectopic expression, and epistasis experiments were other components of these pathways identified and the way in which individual components interact with each other elucidated. A crucial step in filling in missing genes in the pathways was the identification of components supplied not only by zygotic transcription in the embryo, but also during oogenesis. For most pathways, these components represent the majority of the genes in each pathway and are supplied to the embryo by the mother. They cannot, however, be identified by conventional maternal effect screens, because they result in lethality once the maternal supplies are used up, and homozygous females are lethal. Their identification required production of germ line clones, which was accomplished in pioneering studies initiated by Norbert Perrimon (Perrimon et al. 1989), as well as in suppressor and enhancer screens (Raftery et al. 1995).

Cellular Differentiation

Because our screen was based on examination of the embryonic cuticle, we expected that, in addition to identifying patterning mutations, we would identify genes whose expression was required for epidermal development and differentiation (**Table 3**). One group of mutations (e.g., *crumbs*, *bazooka*, *stardust*) affected gene products essential for apical basal cell polarity (Bachmann et al. 2001, Tepass et al. 1990, Wodarz et al. 1999), and another group of mutations (*shotgun*, *canoe*) affected cell adhesion (Tepass et al. 1996). Mutations in cell cycle components (*string*, *pebble*, *fizzy*, *three rows*) (Edgar & O'Farrell 1990, Lehner 1992, Sigrist et al. 1995) could be identified due

to the failure of epidermal cells to complete the normal postblastoderm mitotic cycles. Despite the smaller number of epidermal cells in such mutants, these embryos showed globally normal segmentation, but the reduced number of epidermal cells produced fewer or no denticles. Mutations in the final differentiation stages of the epidermis were detected due to failure of the embryo to make normal cuticles. Mutants with altered denticle, hair, and bristle morphology (*crinkled*, *shavenoid*) (Bejsovec & Chao 2012, Kiehart et al. 2004) were affected in cytoskeletal proteins. Several genes displayed reduced filzkörper, terminal structures of the tracheae, suggesting that the tracheal system was affected. Although we did not keep mutant lines with broad heterogeneous defects in final morphology, three classes of mutant phenotypes were sufficiently consistent to define central steps in epidermal differentiation. All three groups are defined by enzymatic activities. Mutants in one group (*krotzkopf verkehrt/chitin synthase*, *knickkopf*, *retroactive*) define major steps in chitin synthesis and organization (Moussian et al. 2005, 2006), and mutants in the second group (*dopadecarboxylase*, *pale*, *faintoid*) are involved in a pigmentation pathway that is associated with melanin and catecholamine synthesis. Some mutants in the halloween group (*phantom*, *shroud*, *shade*, *spook*) define steps in the biosynthesis of ecdysone, which is required for final epidermal differentiation (Gilbert 2004), whereas other members of this group (*ghost* and *haunted*) encode members of secretion pathways (Norum et al. 2010).

IMPACT OF THE SCREEN

In the early 1980s, when our mutants became available, transcriptional control was a hot topic due to the analysis of gene control in bacteria and yeast. The scientific community quickly recognized that segmentation genes provided an approach to unraveling transcriptional hierarchies, and *Drosophila* became the center of topical biological research. Soon it became the best-known multicellular model organism and, together with the nematode *C. elegans*, resulted in the acceptance of genetic model organisms for biological research. The discovery of homology between key players in development throughout the animal kingdom—which was made possible by the molecular analysis of many of the *Drosophila* genes, first the homeotic gene complexes (Duboule & Dollé 1989, Graham et al. 1989) but perhaps foremost the members of the conserved signaling pathways such as Notch, Hedgehog, Wnt, EGF, and BMP—underscored the usefulness of studying *Drosophila* as a model for development and even human disease.

In the early 1980s, embryological studies of the mouse and the development of mouse embryonic stem (ES) cells had just begun. ES cells allowing for the selection of rare recombination events that modified genes in the mouse (reverse genetics) opened up numerous possibilities for investigating the genetics of vertebrates. It soon became obvious that the most successful strategy for the identification of important regulatory genes in mice was to clone the *Drosophila* homologs. The powerful techniques being developed in mouse for embryological investigation profited greatly from this identification of relevant genes for analyses. Performing mutagenesis screens in mice remains a challenge. By contrast, zebrafish, a vertebrate model system, is amenable to a genetic approach, and systematic screens have led to the identification of more than 400 genes affecting the patterning of the fish larva (see Nüsslein-Volhard 2012). Interestingly, most genes affecting gastrulation in zebrafish have homologs in *Drosophila*, albeit with reversed effects on the DV axis: *swirl* and *snailhouse/BMP2* and *-4* cause dorsalization, whereas *chordino/short gastrulation* embryos are ventralized. In contrast, segmentation in vertebrate embryos, reflected in the formation of the metameric somite pattern, occurs differently than in *Drosophila*. The Hedgehog pathway (*sonic you/hedgehog*) is involved in shaping the somites, and mutants affected in somite formation (*beamter*, *after eight*, *deadly seven*) encode Delta and Notch homologs that act in conjunction with

a segmentation clock that subdivides the AP axis into metameric units (Holley et al. 2002, van Eeden et al. 1996). Only one *Drosophila* segmentation gene, a *hairy* homolog (*her-1*), participates in the segmentation clock (Holley et al. 2002).

In many respects, *Drosophila* development is quite special, and *Drosophila* therefore may not serve as a general model for other organisms. Early *Drosophila* development is characterized by an initial period in which the cleavage nuclei are not separated by cell membranes. This syncytial stage allows the hierarchy of transcription factor prepatterns to develop; these processes do not have parallels in early vertebrate development. The establishment of the embryonic axes by localized determinants in the form of RNA or extracellular cues is not even conserved in closely related insect species. As discussed above, the subdivision of the vertebrate embryo into metameric units occurs through a process very different from that in *Drosophila*. In contrast, all intercellular signaling pathways are conserved, and research in *Drosophila* has been instrumental in discovering many components that underlie growth and differentiation in various developmental processes. These pathways participate in the structuring of organs and tissues in all metazoans. Several genes discovered in our screen have homologs causing congenital diseases in humans or are human oncogenes. They have thus been instrumental in developing therapies for the associated human diseases (**Tables 1**, **2**, and **3**). The first drug, Vismodegib, as an inhibitor of the Hedgehog signaling pathway was recently clinically approved. We also learned many fundamental principles of cell biology in higher organisms, given that cellular properties such as cell polarity, the cell cycle, and the cytoskeleton are highly conserved.

When we carried out our screen, these considerations were far from our thoughts (**Figure 18**). We did not choose *Drosophila* as a model organism; in contrast, our screen pushed it to become one. Our aim was to investigate an animal that is sufficiently complex but at the same time simple enough to be able to understand as many facets of its properties and development as possible. The big leap was, on the one hand, that we chose the cuticle pattern of the larva instead of that of the adult fly and, on the other hand, that we sought to solve problems of embryology by using a genetic approach for which *Drosophila* was very well suited. We thought of our work not as having potential medical relevance, but as providing a comprehensive contribution to understanding the living world. The combination of embryology and genetics as an interdisciplinary approach made this aim feasible. The later discovery of the grand homologies—that an ancient metazoan common ancestor already contained most of the genes of the basic developmental tool kit (summarized in Carroll et al. 2004)—came as a big surprise and immediately upgraded the impact of our work. In retrospect, it is hard to understand why we were so surprised by this discovery, given that we are faithful Darwinians. But perhaps the history of developmental biology, stressing the differences more than the commonalities, had made the idea of comparing frogs with flies or worms seem far fetched. In fact, in the pregenetic days of developmental biology, the way in which development was described greatly depended on the methods applied to examine it (Kühn 1965). Frog people seemed never to speak about genes, whereas fly people rarely used the term factor. Comparing factors between organisms became possible only when their respective genomic sequences were known. The fact that spatial patterns of gene expression are more similar than the morphologies in different organisms further supported a general synthesis spanning the development of all animal species. In the coming years, the advent of next-generation sequencing and the application of the CRISPR/Cas9 technology for reverse genetics will open up the possibility of investigating many more organisms in better detail. The striking experimental advantages of *Drosophila*, not least its short life cycle and external development and the many genetic tricks and technologies possible in this organism, will keep it at the forefront of biomedical research.

Figure 18

Eric Wieschaus and Christiane Nüsslein-Volhard in 1979, at the time of the mutagenesis screen.

THE AUTHORS

Eric Wieschaus initially wanted to become an artist, got interested in embryology as an undergraduate at the University of Notre Dame, and was introduced to *Drosophila* embryos as a student in the lab of Donald Poulson at Yale University. He did his thesis in 1974 with Walter Gehring on clonal analysis, mapping the early development of imaginal discs in *Drosophila*. He moved with Gehring from Yale to Basel, Switzerland, where he participated in the lab's isolation of maternal mutants and developed the use of pole cell transplantation to test germ line versus soma contributions to embryonic development, using mutants from the Gehring and Gans groups. He then did a postdoc with Rolf Nöthiger in Zürich, Switzerland, working on *Drosophila* germ line development and sex determination.

Christiane Nüsslein-Volhard studied biology, physics, and biochemistry and did a thesis in molecular biology, isolating RNA polymerase–binding sites on phage DNA in Tübingen, Germany, in 1973. She got interested in development through the work on *Hydra* with Alfred Gierer and the Gierer-Meinhardt gradient model. The large-scale mutagenesis screen in *Escherichia coli* done in Tübingen, resulting in the identification of the true DNA-replicating enzyme, induced her to work on a genetically tractable organism to identify morphogens. She started to work on *Drosophila* in 1975 as a postdoc with Gehring in Basel, where she discovered *dorsal*. During a postdoctoral year in Freiburg, Germany, with Margit Schardin, she performed the blastoderm fate map by using laser ablations.

Both researchers have been independent group leaders, sharing a lab at the EMBL in Heidelberg, Germany, from March 1978 to March 1981. Wieschaus then went to Princeton and Nüsslein-Volhard to Tübingen.

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