Determination of Anteroposterior Polarity in *Drosophila*

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The principles of pattern formation in embryogenesis can be studied in *Drosophila* by means of a powerful combination of genetic and transplantation experiments. The segmented pattern of the *Drosophila* embryo is organized by two activities localized at the anterior and posterior egg poles. Both activities exert inducing and polarizing effects on the pattern when transplanted to other egg regions. A small set of maternal genes have been identified that are required for these activities. Mutants in these genes lack either the anterior or posterior part of the segmented pattern. The unsegmented terminal embryonic regions require a third class of genes and form independently of the anterior and posterior centers.

THE FERTILIZED *Drosophila* EGG DEVELOPS IN A SHORT period of time into a hatching larva, which exhibits a highly organized segmented pattern of differentiated structures. The complexity of the larval morphology is in striking contrast to the almost homogeneous appearance of the fertilized egg cell; however, the egg cell must contain spatial cues, which guide early developmental decisions. The anteroposterior and the dorsoventral egg axes are readily defined by the shape of the egg, yet the only specialized cytoplasmic organelles known to be localized are the polar granules of the posterior pole. They are included in a distinct clear zone of "pole plasm" and incorporated in the primordial germ cells, the first cells formed in the embryo (Fig. 1a). All somatic cells are formed at 3 hours of development, when about 5000 cleavage nuclei are synchronously separated by cell membranes growing in from the egg membrane (Fig. 1b). At this first cellular state of development, the blastodermal cell layer looks almost homogeneous, yet the spatially restricted expression of embryonic segmentation genes (*1, 2*) already indicates the first steps of segmental differentiation (Fig. 1, b and c). Tracing blastodermal cells and their progeny through embryonic development has allowed the construction of fate maps, the projection of the anlagen of various larval organs and tissues onto the blastodermal sheet of cells (Fig. 1, d and e) (*3*). The segmented regions of the larval body, the thorax and abdomen, represent almost the entire length of the larva, and yet stem from a region of only about 50% of the egg length. A large region anterior to it gives rise to the derivates of the larval head and acron including anterior gut, brain, and the prominent head skeleton. These organs are involuted in the course of embryonic development and come to lie inside the thoracic region. Likewise, smaller posterior portions of the egg will give rise to the unsegmented hind end of the larva, the telson, including the posterior gut (*4*).

Alternative models have been proposed to explain the development of spatial organization in the embryo. The mosaic theory of development assumes that morphogenetic factors are distributed in the egg in a pattern isomorphic to the fate map. Each factor (or determinant) is responsible for one specific organ or tissue, which is formed at the site of its localization. In this model, a large number of different morphogenetic substances are required, which determine subsets of the pattern independently of each other. In contrast, according to gradient theories, the egg contains only a few spatial asymmetries. Pattern-generating mechanisms create concentration gradients of morphogenetic substances. Spatial complexity is determined by different concentrations of a small number of morphogens rather than by an initial molecular complexity. In this model, the molecular prepattern is quite unlike the final pattern, subsets of the pattern may not be independent of each other, and an interdependence of the specification of different egg regions would be expected (*5*). Because of the delay in cell formation during embryogenesis in *Drosophila*, embryonic induction involving cell communication—which appears to play a prime role in vertebrate development—has not been seriously considered as a primary mechanism for creating spatial diversity in the very early insect embryo.

If one generalized from the paradigm of the germline determinants, at one time a mosaic mechanism seemed most attractive to

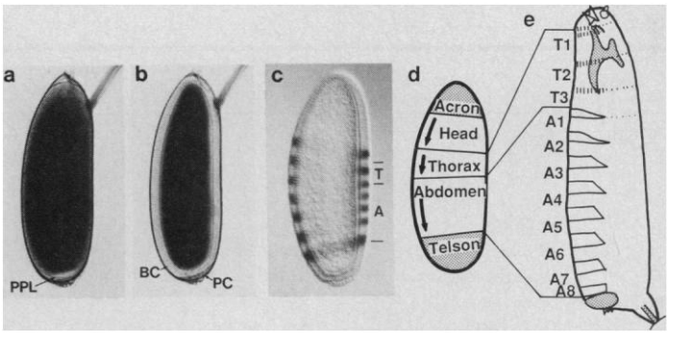

Fig. 1. Embryonic development of *Drosophila*. (**a** and **b**) Living embryo at about 1/2 hour (a) and 3 hours (b) of development. The egg is surrounded by a chorion with the micropyle marking the anterior and the dorsal appendages on the dorsal side of the egg. The *Drosophila* egg is about 500 μm long. PPL, Posterior pole plasm; BC, blastodermal cells; PC, pole cells. (**c**) Optical section through a 3-hour-old embryo stained with an antibody against the gene product of the pair-rule gene *fushi tarazu* (*1, 44*), which shows an expression pattern of seven regular stripes. The prospective regions of thorax (T) and abdomen (A) are indicated. (**d**) A schematic blastoderm fate map indicating the areas from which the various larval body regions will form. The arrows indicate the polarity of the pattern. (**e**) A schematic drawing of a wild-type larva. Some prominent landmarks for the acron and telson are shaded. For a photograph of a wild-type larva, see Fig. 4a.

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explain the patterning of the somatic tissue in the *Drosophila* embryo. However, spectacular pattern duplications can be created in insect embryos by quite simple manipulations, which strongly suggests the involvement of more dynamic mechanisms with the self-organizing properties of gradient models. As we shall discuss in this article, neither a mosaic mechanism nor a gradient mechanism in its extreme form is the single most important mechanism for patterning in early insect development. Evidence from experimental embryology as well as from developmental genetics indicates that there is prelocalization, not of mosaic determinants but rather of two activity centers that act as sources for morphogenetic gradients. These gradients in turn organize the pattern and polarity of the embryo.

Classical Embryology: Two Polar Centers

To elucidate the essential features of the system providing spatial information in the egg, one must obtain the following information: (i) the number of components (factors) specifically involved in pattern formation, (ii) the spatial distribution of these factors, (iii) the relations between the pattern and the factors, and (iv) the type of interaction or interdependence of different factors. The general approach to gathering this kind of data is to experimentally manipulate the egg cell such that an altered pattern results. Ideally, one would like to eliminate, one by one, each factor, and study the pattern formed in its absence. Replacement of the factors at the normal or an ectopic position in the egg yields further important information on their function and properties.

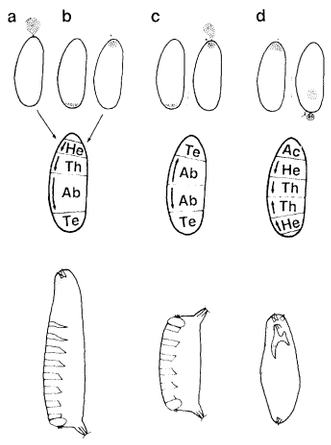

Fig. 2. Experimental manipulation of wild-type embryos. The upper row indicates the experimental procedure, the second row, the fate maps of the operated embryos, and the lower row, schematic drawings of the resulting larvae. A photograph of a typical embryo treated according to procedure (c) is shown in Fig. 5c. Ac, acron; He, head; Th, thorax; Ab, abdomen; Te, telson. Procedures are as follows: (**a**) Anterior pricking, (**b**) transplantation of posterior pole plasm to the anterior, (**c**) transplantation of posterior plasm to the anterior combined with leakage of anterior plasm, and (**d**) transplantation of anterior plasm to the posterior.

Despite several technical limitations in manipulating the fate of a system as delicately balanced and complex as the insect egg, experiments of classical embryology have provided much insight into the system properties of insect embryonic pattern formation (*6*). Two organizing centers with long-range influences on the pattern were discovered, although the methods used have not yet provided assays for the identification of morphogenetic substances. Sander mechanically separated the egg content of the leafhopper *Euscelis* into two parts by constriction, and transposed posterior pole plasm to other regions of the same egg. His elegant set of experiments led to the conclusion that an activity localized in the pole plasm exerts graded long-range effects on polarity and pattern. A counteracting influence of the anterior egg region was inferred. He formulated a double-gradient model to interpret his results (*7*).

More direct evidence for an anterior organizing activity was provided by studies on chironomid midges by Yajima (*8*) and later by Kalthoff (*9*). Treatment of the anterior pole by various (destructive) means resulted in a reorganization of the pattern in the anterior half of the egg and the formation of mirror-image duplications of the posterior pattern. Kalthoff interpreted his findings in terms of localized anterior determinants, which are required for head and thorax formation and also inhibit abdomen formation. The sensitivity to ultraviolet irradiation and ribonuclease treatment suggested that the anterior determinants were ribonucleoproteins. In a gradient model developed for insect development on the basis of Sander's and Kalthoff's experiments, Meinhardt suggested that the minimal requirements could be fulfilled by one gradient with a high point at the posterior pole and that it is not necessary to assume an anterior

Table 1. Maternal genes affecting the anteroposterior pattern of the *Drosophila* embryo. Genes that have been identified by maternal effect mutations with little or no effect on zygotic viability (*20*). Some of them, in a number of alleles, show additional phenotypic traits other than the one mentioned here (+). In one case at least (o), zygotic lethal alleles have been isolated. It is possible that some of the genes as yet unmarked turn out to have other phenotypic traits or later functions.

Class	Gene	Map position*	Phenotype	Remarks	References
Anterior	*bicoid* (*bcd*)	84A	Deletion of head and thorax, acron transformed to telson	Localized activity at anterior pole	*24, 27*
	exuperantia (*exu*)	57E	Weak anterior deletions	Affect *bcd*$^+$ localization	*23, 29*
	swallow (*swa*)$^+$	5E			*16, 28*
	bicaudal (*bic*)	49DE	Large anterior deletion and duplication of posterior abdomen and telson	Low penetrance	*30*
	Bicaudal-D (*BicD*)$^+$	36C		Anterior duplication of posterior activity	*31*
	Bicaudal-C (*BicC*)$^+$	35DE		Recessive female sterile	*31*
Posterior	*oskar* (*osk*)	85A	Deletion of abdomen (excluding telson) and pole plasm	Localization of posterior activity in posterior pole plasm	*24, 32*
	vasa (*vas*)$^+$	35C			*23, 26*
	tudor (*tud*)	57B–D		As above	*23, 43*
	staufen (*stau*)$^+$	55A–F		As above, also anterior defect	*23, 26*
	valois (*val*)$^+$	38A–E		Localization of posterior activity in posterior pole plasm	*23, 26*
	nanos (*nos*)	92A	Deletion of abdomen, pole plasm present	Localized activity?	*26*
	pumilio (*pum*)^o	85C		Signal transmission?	*33*
Terminal	*torso* (*tor*)	43E	Deletion of acron and telson		*23*
	trunk (*trk*)	31A–C			*23*
	torsolike (*tsl*)	93E			*14*
	fs(1) polehole [*fs(1) ph*]$^+$	5CD		Also female sterile alleles	*22*
	fs(1) Nasrat [*fs(1) N*]$^+$	2AB			*34*

*Cytological mapping.

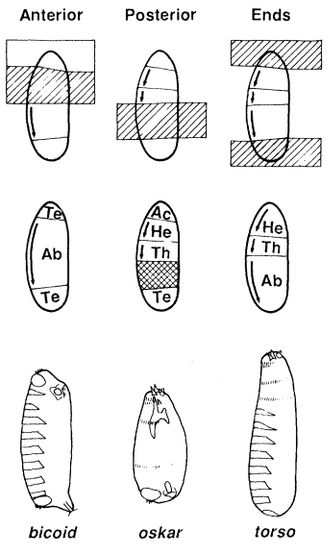

Fig. 3. The three phenotypic classes. In the upper row, the normal origin of the regions deleted in mutant embryos is indicated by the hatched rectangles. In *bicoid*, the acron is transformed into a telson, indicated by the open rectangle. The second row shows the corresponding changes in the fate maps of the remaining areas of the embryo, while the lower row gives schematic drawings of the phenotypes.

center and gradient for the explanation of most of the data (*10*). However, transplantation experiments were not performed in these insects and therefore the spectacular phenotypes created remained open to more than one interpretation.

Illmensee and Mahowald studied the determination of the germline in the *Drosophila* embryo and demonstrated the localization of one or more factors in the posterior pole plasm required for pole cell formation (*11*). In recent experiments, we studied the fate of the somatic tissues after removal and transplantation of cytoplasm in *Drosophila* embryos (*12*). Our results are consistent with the results obtained in other insects. Similar results have been obtained by S. Sugiyama and M. Okada (*13*).

Two Localized Centers of Activity in the *Drosophila* Egg

Removal of cytoplasm (5 to 10% of the egg volume) from the anterior or posterior pole, but not from other egg regions, has distinct effects on the resulting embryonic pattern. After anterior pricking, the acrons and heads of the embryos were absent or much reduced, and the segmented pattern was shifted toward the anterior (Fig. 2a). When the posterior pole plasm was removed, the abdominal region, but significantly not the most posterior somatic region (the telson) was frequently defective (*12*). This experiment suggests that factors are localized at the anterior and posterior egg pole that are required at a distance for the development of particular embryonic regions. To test whether these factors indeed have inductive properties, we transplanted anterior and posterior polar cytoplasm to other egg regions. When posterior pole plasm was transplanted to the anterior pole, phenotypes indistinguishable from those obtained after removal of anterior plasm were obtained (Fig. 2b). Head formation was more or less reduced, which suggests that the posterior factors inhibit the anterior ones. If the anterior factors are removed at the time of transplantation, the posterior plasm can induce a complete posterior abdominal end with reversed polarity at the anterior, resulting in a bicaudal phenotype (Fig. 2c).

Comparable results were obtained by transplanting anterior cytoplasm, which can suppress posterior development. In this case, suppression was most effective when the target site was the abdominal region and not the posterior egg pole. This suggests that the anterior activity does not directly interfere with the posterior center but, more likely, with a signal emanating from it or its target. Inductive properties can also be demonstrated for the anterior cytoplasm, although large volumes must be transplanted for optimal effects. In these cases, embryos with head and thorax structures at both ends may form (Fig. 2d) (*14*). In summary, these experiments support the following conclusions. (i) The anterior polar cytoplasm harbors an activity required for anterior development and the posterior pole plasm contains an activity required for posterior development. (ii) Both activities exert long-range effects on the pattern. (iii) The anterior and posterior activities inhibit each other. (iv) Most importantly, both activities have inductive properties and can reorganize pattern and polarity when transplanted to ectopic egg regions.

Despite the striking phenotypes obtained, the experimental approach described above could not be used to elucidate the number and function of components present in these centers. Activities that are not localized, or that become depleted simultaneously with others, remain undetected. To overcome these difficulties, a genetic analysis of the informational content of the egg was required. Mutations in genes that encode molecules with morphogenetic functions should be recognizable by a phenotype in which the spatial arrangement of embryonic primordia is altered. Because the informational content of the egg cell is established during oogenesis under the control of the maternal genome, mutants are of the maternal effect type: the phenotype of the embryo—an altered pattern—depends on the genotype of the female that produced the egg rather than on that of the embryo itself.

The Isolation of Maternal Effect Mutants

Classical *Drosophila* genetics was focused on the genetics of the adult fly, and only recently have systematic searches for embryonic mutants been carried out (*15–17*). Efficient methods for analyzing embryonic patterns allow a detailed description of embryonic phenotypes (*18*). In the next section, the phenotypes and properties of maternal genes affecting the egg cell content and specifically the anteroposterior pattern will be described. Genes affecting egg morphology as well as embryonic pattern (*19*) and genes required for general cellular differentiation and cell formation have not been included. The limitations of the screens are such that genes with a lesser degree of temporal specificity may not be included in the sample if they also cause zygotic lethality (*20, 21*).

Careful estimates based on the allele frequencies indicate that the majority of the genes specifically affecting the informational content of the egg cell for embryonic pattern formation should now have been identified (*22–24*). Summarizing the results from several laboratories, a number of important conclusions may be drawn: (i) The number of genes is small and probably does not exceed 40, about 30 of which are known (less than 1% of the genome). (ii) Groups of genes share similar or identical phenotypes so that the number of different phenotypes caused by "lack-of-function" mutations is most likely not more than ten. (iii) The majority of genes affect either the anteroposterior or the dorsoventral pattern of the embryo. (iv) In general, the pattern of large egg regions or even the entire axis of the embryo is affected by single point mutations.

These data tell us that the informational content of the egg cell for spatial pattern formation is of limited complexity. The maximum number of components with morphogenetic properties is far below that of the different structures formed during embryonic development at different egg regions. Further, the elimination of a single gene product can cause a deletion of structures normally formed from large regions of the egg. The organization of the embryonic pattern apparently relies on a small set of pattern-forming systems. Tentatively, these systems may be defined by groups of genes with

identical or related phenotypes. They act independently to organize (perhaps in the form of gradients) the pattern along large stretches of the anteroposterior or dorsoventral (*25*) axes.

Genes of the Anteroposterior Pattern

Of the 30 maternal effect genes with a clear function in embryonic pattern formation, 18 affect the anteroposterior pattern. The genes can be classified by the resulting phenotypes into groups that affect (i) the anterior, (ii) the posterior, or (iii) the terminal regions of the egg (Table 1, Fig. 3). The prototypes of the three classes, *bicoid, oskar,* and *torso*, define the entire fate map in three almost nonoverlapping deletion patterns (Fig. 3). In *bicoid* emryos, head and thorax are missing. The terminal anterior region, the acron, is transformed to the telson (Fig. 4b). In *oskar* embryos the entire abdomen and the posterior pole plasm are missing. The telson is present in *oskar* as in all other posterior-group mutants (Fig. 4c). Finally, mutations in *torso* eliminate the two unsegmented terminal regions, the acron and the telson (Fig. 4d). The complementarity of the three phenotypes is illustrated in double-mutant combinations: *bcd osk* double-mutant embryos consist of just two telsons arranged in mirror-image symmetry, and all segmented areas of the larva are lacking (Fig. 5a). The *torso* mutants, in combination with *oskar* or *bicoid* cause embryos to form segmented anterior or posterior patterns, respectively, lacking both terminal regions. Finally, in triple mutant embryos, no differentiated structures can be discerned in the cuticle (Fig. 5b) (*14, 26*).

The Anterior Pattern

bicoid (*bcd*) (*27*): Embryos of strong alleles lack head and thorax entirely, whereas weak alleles cause smaller anterior deletions. These embryos resemble those obtained by removing the anterior cytoplasm from wild-type embryos (Fig. 2a). That *bicoid* embryos are indeed defective in an activity normally localized at the anterior pole has been shown in transplantation experiments (Fig. 6). A normal pattern of head and thorax can be formed in mutant embryos if cytoplasm from the anterior tip of wild-type eggs is transplanted into the anterior tip of mutant eggs. Cytoplasm from more posterior regions is not active (Fig. 6b).

The degree of anteriorness induced (whether thorax only or thorax and head structures are formed) is dependent on the amount of cytoplasm transplanted. The activity can also be influenced by the number of bcd^+ gene copies in the donor mothers; the most active cytoplasm is obtained from eggs of females with a bcd^+ duplication, whereas heterozygous females produce eggs with much less active cytoplasm, and no activity can be detected in bcd^- embryos. This dosage dependence suggests that the quality of the structure formed is determined by the quantity of the *bcd* gene product delivered at the injection site (*27*).

The most striking feature of bcd^+ activity is the ability to induce the formation of anterior structures in other regions of the embryo (*27*). When anterior wild-type cytoplasm is transplanted to the middle of *bcd* mutant embryos, head structures may develop in nonterminal positions, with two flanking thoracic fields of mirror-image polarity (Figs. 5f and 6c). Transplantation of anterior cytoplasm to the posterior pole can induce thorax formation there as well as cause polarity reversal of large regions of the embryo, for example, an inverted sequence of abdominal segments (Figs. 5e and 6d). The anterior inductive activity is strictly dependent on bcd^+ wild-type copies in the females producing the donor eggs. As outlined above, the anterior activity can also induce anterior structures in posterior regions of wild-type embryos, although with somewhat lower efficiency (*14*).

exuperantia and *swallow*: The maternal genes *exu* (*23*) and *swa* (*28*) have phenotypes that superficially resemble those of weak *bcd* alleles. The anteriormost embryonic structures are lacking, but, in contrast to *bcd*, the posterior head and the thoracic regions are enlarged rather than reduced in size (Fig. 7, a, b, and c). Several lines of evidence suggest that the *exu* and *swa* genes are required for the proper anterior localization of bcd^+ activity (*29*). In *exu* and *swa* embryos, bcd^+ activity is much reduced at the anterior pole, and is instead present (in low amounts) in more posterior egg regions than normal. This altered distribution of bcd^+ activity results in a deletion of the anteriormost structures (because they require higher bcd^+ concentrations), and an extension of subterminal anterior regions (because a low level of bcd^+ activity is present in larger than normal regions) (Fig. 7c). We postulate that during oogenesis the swa^+ and exu^+ gene products anchor the bcd^+ product at the anterior end of the oocyte where it remains localized throughout early development.

bicaudal: The bicaudal phenotype (Figs. 2c and 5c) differs from the *bcd*, *exu*, and *swa* phenotypes in that the anterior deletion is accompanied by a duplication of posterior abdominal pattern. In symmetric bicaudal embryos, the anterior deletion is much larger

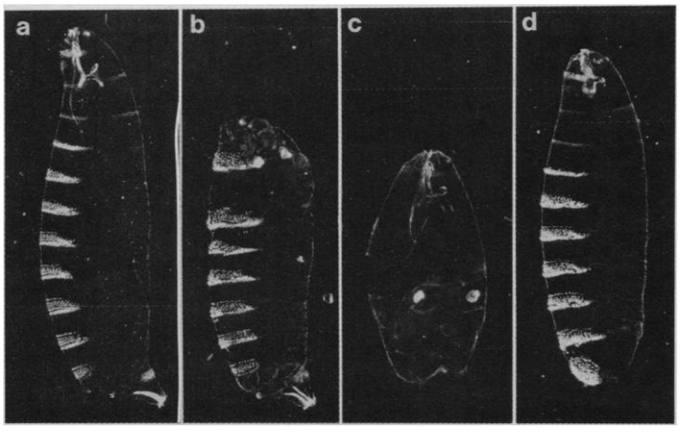

Fig. 4. The cuticular patterns of wild-type and mutant embryos: (**a**) wild-type, (**b**) *bicoid*, (**c**) *oskar*, and (**d**) *torsolike*. Dark-field photographs of cuticular preparations (*18*). The length of the wild-type larva in (a) is about 1 mm.

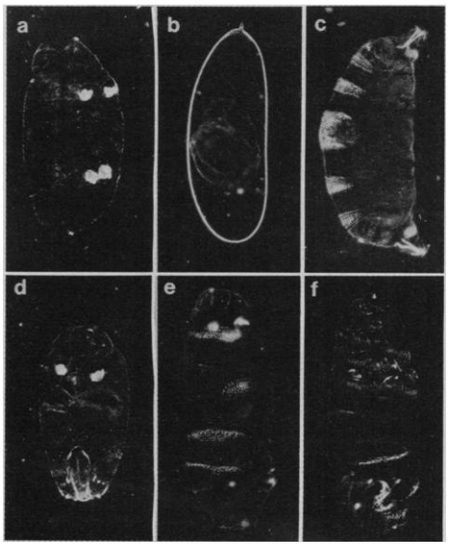

Fig. 5. The cuticular patterns of experimental animals; (**a**) *bcd osk* double mutant; (**b**) *bcd osk tsl* triple mutant. The vitelline membrane has not been removed from the embryo. (**c**) Bicaudal embryo made from a wild-type embryo according to the procedure in Fig. 2c. (**d**) Reversed "oskar" made from a *bcd osk* according to the procedure in Fig. 6g. (**e**) Embryo from experiment of Fig. 6d. (**f**) Embryo from experiment of Fig. 6c.

than that observed in *bcd* embryos, including a large portion of the anterior abdomen (*30*).

The bicaudal phenotype is caused by mutations in at least three genes (Table 1) (*31*). None of the *bicaudal* mutations causes a uniform and consistent phenotype: a whole spectrum of varying degrees of anterior deletions is exhibited in embryos produced from individual females. Further, in at least two of the three *bicaudal* genes that have been characterized (*BicD* and *BicC*) the bicaudal embryo is not the lack-of-function phenotype (*31*). This suggests that the genes do not directly code for morphogenetic substances.

The phenocopies described above (Figs. 2c and 5c) suggest that in mutant embryos there is a reduction of anterior (bcd^+) activity coupled to the ectopic presence of posterior activity at the anterior pole (*12*). In the case of *BicD*, the presence of posterior activity at the anterior pole could be demonstrated in transplantation experiments. Furthermore, genetic evidence indicates that the primary effect of the *BicD* mutation is on the distribution of posterior activity rather than on the anterior bcd^+ activity (*26, 32*).

The Posterior Pattern

The phenotypes of mutants of the genes of the posterior center are more homogeneous than those of the anterior center. Seven maternal genes have been identified that are required for the development of the abdomen (Figs. 3 and 4c). Five of these genes have an additional phenotypic trait: they lack the posterior pole plasm including the polar granules. As a consequence, no pole cells are formed in mutant embryos (Table 1). Abdominal segmentation can be restored in all posterior mutants by transplantation of posterior pole plasm from wild-type embryos (*26, 32*). The mutant embryos, however, respond best to transplantation when the activity is transplanted into the prospective abdominal region and not, as might be expected, to the posterior pole of mutant embryos. In contrast to the anterior bcd^+ activity, the posterior activity does not significantly influence the pattern when transplanted to regions anterior to the abdomen, and a slight posteriorization is all that results when pole plasm is transplanted to the anterior tip of mutant or wild-type embryos (*12, 32*). However, when the inhibitory effect of bcd^+ on posterior development is eliminated, for example, in bcd^- embryos as recipients, the posterior pole plasm exerts a strong inducing activity, and complete posterior ends with reversed polarity are formed at the anterior (Fig. 6e) (*14*). This indicates that the activity localized in the posterior pole plasm also has organizing and polarizing influences on the pattern.

The spatial requirements and properties of the posterior activity suggest that a source localized in the pole plasm produces a spreading factor that is responsible for the organizing properties of the posterior activity (*32*). Mutants of the five genes that also affect the pole plasm are source-deficient and therefore no activity can be detected in mutant embryos (*26*). Of the two genes that do not affect the pole plasm (*nanos* and *pumilio*), *pumilio* embryos prove to have posterior activity; when pole plasm within mutant *pumilio* embryos is shifted experimentally to the abdominal region, abdominal segmentation is partially restored. This and other lines of evidence suggest that *pumilio* is required for the release and transmission of the posterior signal (*33*). Finally, mutations in the gene *nanos* result in normal-appearing pole plasm, which can support the development of functional pole cells; however, no posterior activity can be detected in transplantation tests (*26*) (Fig. 7d). *nanos* is therefore the best candidate for a gene directly responsible for the posterior signal.

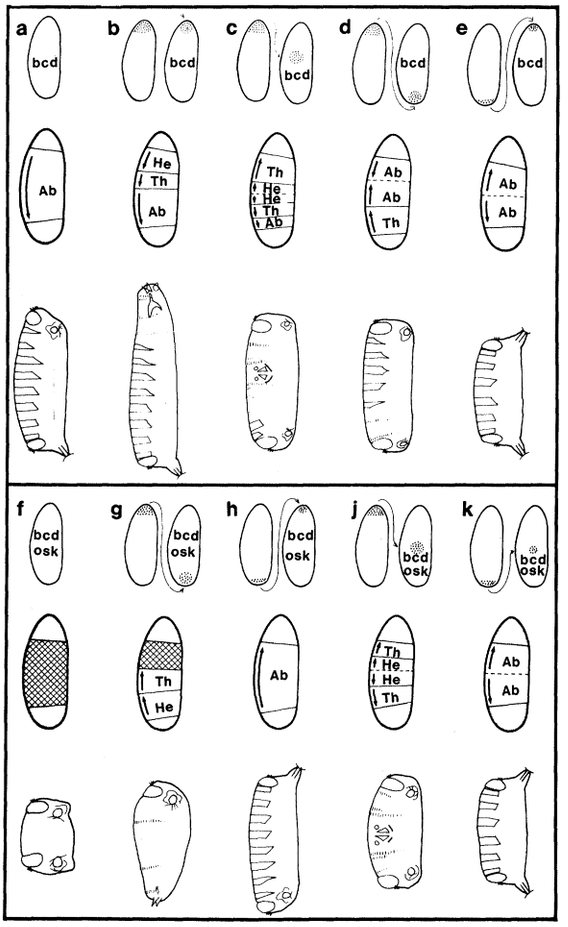

Fig. 6. Transplantation experiments with *bicoid* embryos (**a** to **e**) and *bicoid-oskar* embryos (**f** to **k**) as recipients. In each panel, from top to bottom, the experimental design, the resulting fate map, and the final differentiated pattern are illustrated. The donor embryos were from wild-type females or from females with an extra bcd^+ gene copy (b to d, g, and j).

The Terminal Regions

Whereas the mutant phenotypes of the first two classes of genes can be "phenocopied" by manipulating the polar plasms of wild-type embryos, the phenotype of *torso* and the other members of this class is unexpected (*23*). The terminal class of genes is required for the formation of the unsegmented anterior and posterior regions (acron and telson) of the embryo. Mutant larvae reveal anterior and posterior deletions, and the posterior abdominal anlage expands towards the pole at the expense of the telson anlage including the posterior gut primordia (*34*). Pole plasm is present in these embryos, and transplantation experiments reveal that anterior and posterior activity is normal (*14, 26*).

The dependence of telson formation on the terminal class of genes rather than on those of the posterior center was somewhat misleading for the early interpretation of the mutant phenotypes of posterior-group genes. The similarity of the abdominal phenotype to that of the zygotic gap gene *knirps* (*1*) and the effect on the pole cells of most of the genes initially made it difficult to recognize their role in organizing the posterior pattern (*35*). Similarly, the presence of a telson at the anterior of *bcd* embryos suggested a posterior duplication and a much stronger relation to bicaudal embryos than we currently believe to be true. In our present view the anterior telson duplication is explained as a transformation of the anterior terminal region into a posterior one in the absence of bcd^+.

Interaction Between the Anterior and Posterior Activity

Several lines of evidence indicate that the activities localized at the anterior and posterior egg pole are necessary and sufficient for the establishment of polarity in the *Drosophila* embryo. The inhibitory interactions between the anterior and posterior activities observed in the transplantation experiments may play a stabilizing role in normal development. Elimination of the anterior activity in *bcd*$^-$ embryos facilitates the formation of a bipolar pattern—a bicaudal embryo—in transplantation experiments. Likewise, double-headed embryos can be induced by transplanting anterior cytoplasm to the posterior of a mutant of the posterior class. In double mutants of *bicoid* and *oskar*, polarity is no longer detectable, and in transplantation experiments, head structures can be induced at the posterior end and abdomens at the anterior without encountering inhibitory effects residing in the recipient embryo (Fig. 6, g and h) (*14*).

Although formally the two activities appear to have symmetrical (reciprocal) properties, there are a number of significant differences between the anterior and posterior activities. Nonreciprocity is seen in the embryonic fate map that results after the elimination, by mutation, of one center. The elimination of the anterior activity in *bcd*$^-$ embryos causes a spread of the posterior pattern toward the anterior [see, for example, the expression pattern of the segmentation gene *fushi tarazu* (Fig. 7, a and b)]. Thus, the anterior activity is required not only for the formation of the anterior pattern but also for keeping the posterior pattern in check. For the posterior activity, however, a similar function is not observed. In mutants of the posterior class, the anterior pattern forms almost uninfluenced at the normal position, only the thoracic anlagen are enlarged (Fig. 7d) (*26*, *29*).

The differences between the two activities are not restricted to the degree of mutual inhibition. Whereas both exert long-range organizing effects, which are probably best explained by a source and signal mechanism (see below), the posterior activity appears to have more dynamic self-organizing properties than the anterior. This is best illustrated in the relation between the distribution of the activity and the resulting pattern in transplantation experiments. In the case of *bcd*$^+$ activity, the "anterior peak" usually forms at the site of injection. The degree of anteriorness reached at the injection site appears to be directly dependent on the amount (or concentration) of *bcd*$^+$ activity, and incomplete anterior patterns lack the anteriormost pattern elements (acron, or acron and head). In contrast, when the posterior activity is injected into nonterminal positions, it either has no effect at all, or it induces a "posterior peak" but this is formed always at an egg pole. Further, the artificial posterior peaks generally include the posteriormost structures. Whenever incomplete posterior patterns are formed, it is the nonterminal structures that are lacking. When *bcd osk* embryos (polarity-neutral recipients) are injected with anterior plasm in the middle, they form the anteriormost structures at nonterminal sites, and these may be thorax or head structures, depending on the concentration of anterior activity reached in the recipient embryo (Fig. 6j). In contrast, posterior activity frequently induces bicaudal embryos with two posterior peaks, one at either pole and with striking symmetry (Fig. 6k). The occasional unipolar embryos resulting in such an experiment have their posterior ends at the anterior or the posterior pole, but not in the middle (*14*).

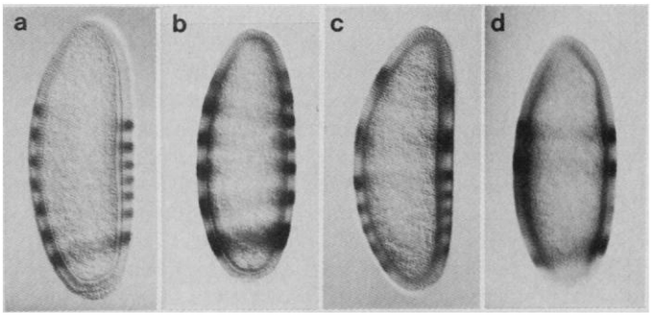

Fig. 7. Fate maps of wild-type and mutant embryos as revealed by the expression pattern of the pair-rule gene *fushi tarazu* (*ftz*). (**a**) In the wild type, the seven stripes cover a region giving rise to the posterior head segments, the thorax, and the entire abdomen. The posteriormost stripe marks the anterior rim of the telson anlage (see Fig. 1). (**b**) *ftz*-expression in a *bcd* embryo of moderate allelic strength (*bcd*$^{2-13}$) (*26*). All seven stripes are present, but the pattern is enlarged and spread toward the anterior. (**c**) *exu*. The distances between the anterior three stripes are about three times as large as in the wild-type, whereas the posterior stripes are crowded. (**d**) *nanos*. Diffuse expression of stripes 3 to 6 correlates with the lack of abdominal segmentation. The anterior two stripes are larger than normal.

The Organization of the Pattern by an Anterior and a Posterior Gradient

Both activities, as defined in transplantation tests, are localized to the anterior or posterior pole of wild-type embryos. Yet they have long-range organizing influences that determine the polarity and sequence of the segments in the neighborhood of the transplantable activity. This and other properties are best explained by invoking gradient mechanisms. It is likely that the activities represent sources of morphogenetic substances spreading away from the source and, through their regionally different concentrations, determine the sequence of different qualities observed in the harmonious and regular patterns formed. The identification of genes responsible for the activities allows a molecular investigation of these assumptions. However, we would like to point out that in the absence of functional assays like mutant phenotypes and transplantation tests, many properties of morphogenetic activities must remain undetected.

The gene *bicoid* has recently been cloned and sequenced. It codes for a messenger RNA (mRNA) that is localized in the anterior cytoplasm of wild-type embryos (*36*). This suggests that it is the *bcd* mRNA that is the major active component in our transplantation experiments. As described above, genetic and transplantation studies had suggested that two additional genes of the anterior class, *exu* and *swa*, are involved in the localization of the *bcd* activity to the anterior pole (*29*). In embryos from *exu* or *swa* females the *bcd*$^+$ mRNA is initially nearly homogeneously distributed (*36*). In normal embryos, the localized mRNA may serve as a source for the *bcd* protein, which spreads by diffusion. A constant decay rate coupled to a suitable rate of synthesis and rate of diffusion would provide a simple molecular mechanism producing a stable concentration gradient of the *bcd* protein throughout the egg.

A general gradient model based on a source producing a spreading morphogen is also suitable for the posterior center. However, the significant differences between the properties of the anterior and posterior system discussed above, in particular the independence of the final pattern on the initial distribution of the activity, suggest a more elaborate mechanism with self-organizing potential. A mechanism based on lateral inhibition and involving auto- and cross-catalysis between two antagonistic substances has been proposed which, in computer simulation, can provide the patterns observed (*9*, *37*). On the other hand, it is also possible that many of the apparent differences between the anterior and posterior systems reflect asymmetrical properties of the zygotic genes responding to the spatial cues provided by the primary gradients rather than to the gradient systems themselves.

The Mode of Action of the Two Centers

As described, the informational content of the egg cell that determines embryonic polarity is relatively simple compared to the complexity of the final differentiated pattern. Two morphogens localized at the anterior and posterior egg poles produce signals that spread toward the other pole. This molecular prepattern of two opposing gradients is quite unlike the final fate map. It must be interpreted and elaborated in the course of embryonic development to yield the regular segmented pattern of the larva. Targets for the gradient signals are, in all probability, the gap genes, a group of segmentation genes that are expressed (essentially) in large unique subregions of the embryo (*1, 2*). The phenotypes and domains of expression of the gap gene *hunchback* (*38, 39*) suggest that it is directly under the control of *bicoid*. By analogy, a likely target of the posterior signal is the gap gene *knirps*. An attractive model for further subdivision involves inductive interactions between different gap genes, guiding the expression pattern of pair rule genes (*40, 41*). The presence of a homeo box sequence (*42*) in the *bcd* coding region suggests a direct role in transcriptional control. However, the mechanisms by which different concentrations of the *bcd* protein control the expression pattern of different segmentation genes are still subject to speculation.

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4. In this article we use the terms acron and telson not in the strict definition as the terminal regions lacking coelomic cavity and neuromer. The situation of the segmental origin of the anterior and posterior ends in *Drosophila* is complex, as the apparent unsegmented regions include derivatives of originally segmented anlagen [G. Jürgens, R. Lehmann, M. Schardin, C. Nüsslein-Volhard, *Wilhelm Roux's Arch. Dev. Biol.* **195**, 359 (1986); G. Jürgens, *ibid.* **196**, 141 (1987)]. Such a more complete "telson" is readily defined by the mutant phenotypes of *bicoid, oskar*, and *torso*. It includes all structures posterior to the 8th abdominal segment (posterior gut, malpighian tubules, and derivatives of segments 9, 10, and 11) as well as the filzkörper and spiracles, derivatives of the dorsal portion of the 8th abdominal segment. The acron is more difficult to define, and the term is used here by analogy for the region at the anterior lacking in the *torso*-group mutants. It includes the labrum and large portions of the cephalopharyngeal skeleton. Antennomaxillary complex, cirri, and mouthhooks are not part of the "acron."
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19. For example, the mutant *dicephalic* [M. Lohs-Schardin, *Wilhelm Roux's Arch. Dev. Biol.* **191**, 28 (1982)] sometimes causes double-headed embryos, among a spectrum of other embryonic phenotypes. In mutant embryos, the egg shape also shows an anterior duplication, and the phenotype can be traced back to a bipolar arrangement of the nurse cells at the anterior and posterior of the oocyte.
20. There is no a priori reason why molecules used in early pattern formation should not be used again at later times in development. We further assume that a large number of mundane metabolites, enzymes, and other molecules participate in the early pattern-forming process. A distinction between "pure" pattern mutants and those with additional or more general functions is therefore to a certain degree arbitrary. The criterion of time-specific requirement (maternal effect alleles with no or little effect on viability) has proven to be quite successful in identifying important genes in the process. It is more difficult to assess the role of lethal genes in early maternal processes. In experiments involving germline mosaics, it has been found that a number of lethals also show rather specific maternal effects on the pattern. These phenotypes, however, rarely are as discrete as those of pure maternal effect mutants [N. Perrimon and A. P. Mahowald, *Dev. Biol.* **118**, 587 (1987)]. In contrast to the majority of lethal genes, the lethal genes that have a clear effect on embryonic pattern only rarely are required during oogenesis as well, while most of them are strictly zygotically expressed [E. Wieschaus and M. Noell, *Wilhelm Roux's Arch. Dev. Biol.* **195**, 63 (1986)] or their maternal expression is not required for survival of the embryos [*hunchback* (*38*)].
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45. We thank our colleagues in this laboratory for critical and stimulating discussion and comments on the manuscript. The constructive criticism of W. Birchmeier, F. Bonhoeffer, D. Ish-Horowicz, H. Meinhardt, K. Sander, H. Schnabel, R. Schnabel, T. Schüpbach, and G. Struhl helped substantially to make the text readable as well as to sharpen some of our views. We thank R. Groemke-Lutz for the photographs. H. Tearle for the drawings, and D. Kaiser for secretarial assistance.