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The Origin of Pattern and Polarity in the Drosophila Embryo

Review

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The development of a multicellular organism from a single egg cell requires both the determination of many cell types and the organization of these cells into an elaborate pattern (discussed elsewhere in this issue). In this review, we shall consider how much of the complexity of the pattern is already present at the beginning of this process in the fertilized egg? Since the early part of this century, embryologists have recognized that the eggs of many organisms contain localized regions of cytoplasm that direct the formation of specific parts of the embryonic pattern (Wilson, 1928). However, it is only in the case of Drosophila that the molecules responsible for these activities have been identified. Thanks largely to the use of genetic approaches, it is now known that four localized maternal signals define the basic organization and polarity of the two major embryonic axes. Thus, these signals not only specify cell states, but also provide a prepattern for subsequent development. Although Drosophila is probably unusual in the extent to which its pattern formation is controlled by maternal cues, many of the molecular processes involved have counterparts in other systems. More importantly, the characterization of these signals has also provided useful paradigms for the study of a variety of developmental phenomena, such as localized determinants, induction, and morphogen gradients. The purpose of this review is to describe our current understanding of how the four maternal signals establish positional information in the Drosophila embryo, and to discuss the molecular properties of each system.

The Drosophila egg is produced over a period of about 3½ days in the ovary of the female (Figure 1), and upon fertilization, develops extremely rapidly to form a larva after 24 hr. During the early stages of embryogenesis, the zygotic nuclei divide, without forming cells, to give rise to a syncytial blastoderm embryo (Figure 1). By 3 hr of development, the nuclei have been surrounded by cell membranes to form the cellular blastoderm, and gastrulation begins soon afterward. Although the organization of anterior–posterior and dorsal–ventral axes first becomes apparent in the region-specific cell movements of gastrulation, the basic prepattern of both axes has already been established in the syncytial blastoderm by the localized expression of zygotic pattern genes.

The anterior–posterior prepattern is formed by the spatially regulated transcription of the gap genes. While the identities of all of the gap genes that control head development are not yet completely clear, the embryo contains expression domains of *huckebein*, *tailless*, *giant*, *hunchback*, *Krüppel*, *knirps*, *giant*, *tailless*, and *huckebein*, as one moves from anterior to posterior (Knipple et al., 1985; Tautz, 1988; Mohler et al., 1989; Pankratz et al., 1989; Pignoni et al., 1990; Weigel et al., 1990). The first localized gene expression along the dorsal–ventral axis also occurs at this time, with the activation of *twist* and *snail* in the ventral nuclei of the embryo and *dpp* and *zen* in the dorsal regions (Rushlow et al., 1987a; St Johnston and Gelbart, 1987; Thisse et al., 1987; Leptin and Grunewald, 1990). All of these genes, with the exception of *dpp*, encode DNA-binding proteins that are believed to act as transcription factors, and it is the interactions between these factors and the genes that they regulate that lead to the subdivision of the anterior–posterior and dorsal–ventral axes into different regions (Rosenberg et al., 1986; Boulay et al., 1987; Padgett et al., 1987; Rushlow et al., 1987b; Tautz et al., 1987; Nauber et al., 1988; Thisse et al., 1988; Pignoni et al., 1990). The initial activation of these genes in their discrete spatial domains is controlled by the four localized maternal signals, and in this way these determinants establish the polarity and organization of both axes.

The genes discussed above are expressed in the zygotic nuclei of the embryo, but the maternal signals and the components required for their localization and function are synthesized during oogenesis. A number of genetic screens have been performed to isolate maternal-effect mutations that affect the embryonic pattern (Gans et al., 1975; Anderson and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Nüsslein-Volhard et al., 1987; Schüpbach and Wieschaus, 1989). Females that carry such mutations lay normally shaped eggs that develop into embryos with cuticular pattern defects. Several important conclusions can be drawn from the results of these screens.

First, the number of genes that are specifically involved in the establishment of positional information in the egg is quite small. About 30 genes have been identified so far, and the total number is unlikely to be much higher than this. Second, the two body axes are established independently, as mutations either affect the anterior–posterior pattern or the dorsal–ventral pattern, but never both. Third, the number of embryonic phenotypes observed is much smaller than the number of genes. This means that the genes can be assigned to four classes on the basis of which parts of the embryo they affect. The common phenotype produced by mutations in the genes of one class indicates that these genes act in a common pathway to specify a discrete part of the embryonic pattern.

One class, consisting of the dorsal group genes and *cactus*, is responsible for specifying the whole of the dorsal–ventral axis of the embryo (Figure 2). Loss-of-function mutations in the ten dorsal group genes and gain-of-function *cactus* mutations result in completely dorsalized embryos, in which all cells follow a dorsal developmental pathway (Figure 3e), while loss-of-function *cactus* muta-

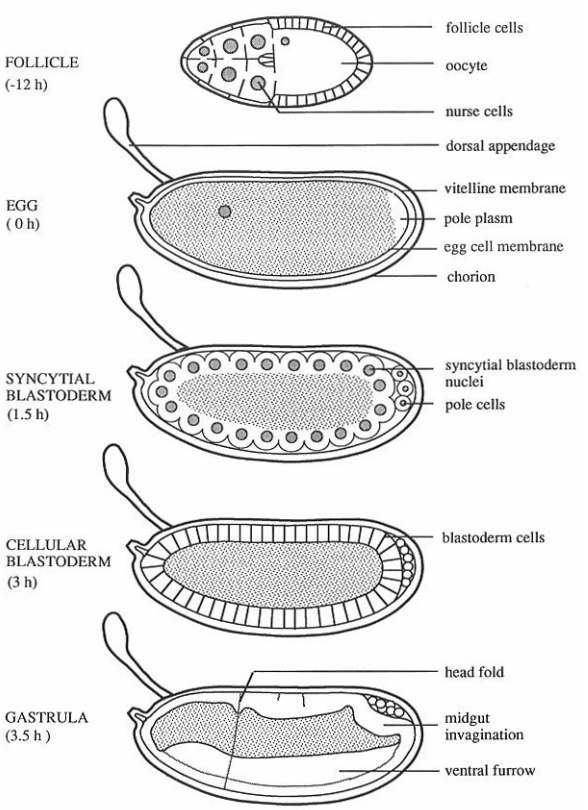

Figure 1. Oogenesis and Early Embryogenesis in Drosophila

At the beginning of oogenesis, a germline stem cell divides four times to produce 16 cells that remain connected by cytoplasmic bridges. One cell in the cluster migrates to the posterior and develops into the oocyte, while the 15 remaining cells become the anterior nurse cells. The nurse cell–oocyte complex is surrounded by somatic follicle cells, and by stage 10 of oogenesis (top panel) these cells have migrated to cover the developing oocyte. As the oocyte matures, the nurse cells contract and expel their contents into the oocyte, while the follicle cells secrete the egg coverings. Both of these cell types degenerate at the end of oogenesis. When the mature egg (second panel) is laid, it is surrounded by the vitelline membrane and the chorion, and is filled with yolky cytoplasm. The only visible specialized region of cytoplasm is the pole plasm at the posterior pole, which is yolk-free, rich in mitochondria, and contains the polar granules. After fertilization, the zygotic nuclei go through a series of rapid cleavage divisions in the interior of the egg. After nine divisions the majority of the nuclei have migrated to the cortex to form the syncytial blastoderm (middle panel). At this stage, the 3–4 nuclei that have entered the pole plasm at the posterior pole form polar buds, which will give rise to the pole cells, the precursors of the germline. The rest of the nuclei divide four more times at the surface of the egg before being surrounded by cell membranes to give rise to the ~6000 cells of the cellular blastoderm (fourth panel). Soon after cellularization is complete, gastrulation (bottom panel) begins with the invagination of the presumptive mesoderm through the ventral furrow, the formation of the posterior midgut invagination that contains the pole cells, and the appearance of the head fold.

tions and some gain-of-function dorsal group alleles lead to the development of ventralized embryos (Figure 3f) (Nüsslein-Volhard et al., 1980; Anderson et al., 1985a; Anderson and Nüsslein-Volhard, 1986; Chasan and Anderson, 1989; Schüpbach and Wieschaus, 1989; Roth et al., 1991). The specification of the anterior–posterior axis requires three classes of maternal genes: the anterior, posterior, and terminal genes (see Figure 2) (Nüsslein-Volhard et al., 1987). Mutations in the anterior class lead to a reduction or loss of head and thoracic structures, posterior group mutations cause abdominal deletions, and the terminal class (*torso* group) is required for the development of the unsegmented ends of the embryo, the acron and telson (Figures 3b–3d). To a first approximation, these three gene systems act independently and additively to define discrete parts of the pattern; the parts of the pattern removed by mutations in one system are the only regions that are unaffected when both of the other two systems are absent (Figure 4). This complementarity between the regions defined by each of the maternal signals only breaks down in the most anterior region of the embryo, the acron, which requires both the terminal and anterior systems. In the absence of the anterior signal, the terminal system directs the formation of an anterior telson, usually the posterior-most structure of the embryo.

Localized Cytoplasmic Determinants

The first demonstration that the Drosophila egg contains

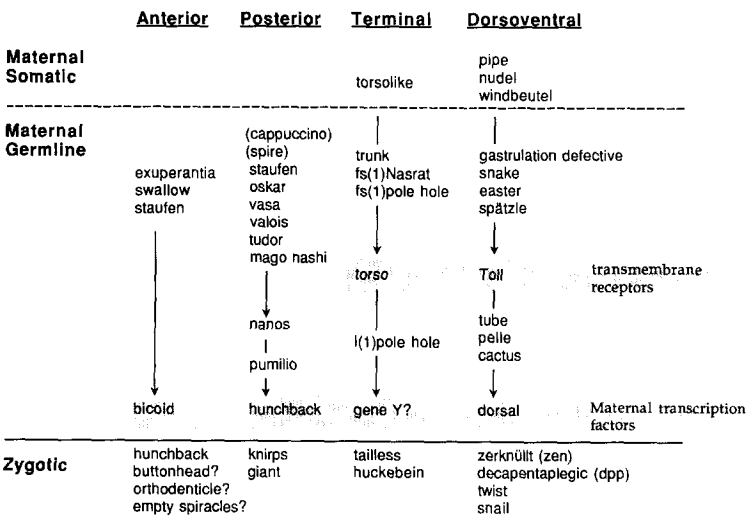

Figure 2. The Genes of the Four Maternal Systems in Drosophila

Where possible, the genes of each maternal class are shown in the order in which they are believed to act, while the lower section of the figure lists the zygotic genes that are regulated by each maternal system. Note that *staufen* is the only maternal gene that participates in two of these systems (anterior and posterior). The parentheses around *cappuccino* and *spire* indicate that these genes do not fall into the category of strict maternal-effect genes that we have used. In addition to a posterior group phenotype, mutations in these loci also affect the shape of the egg.

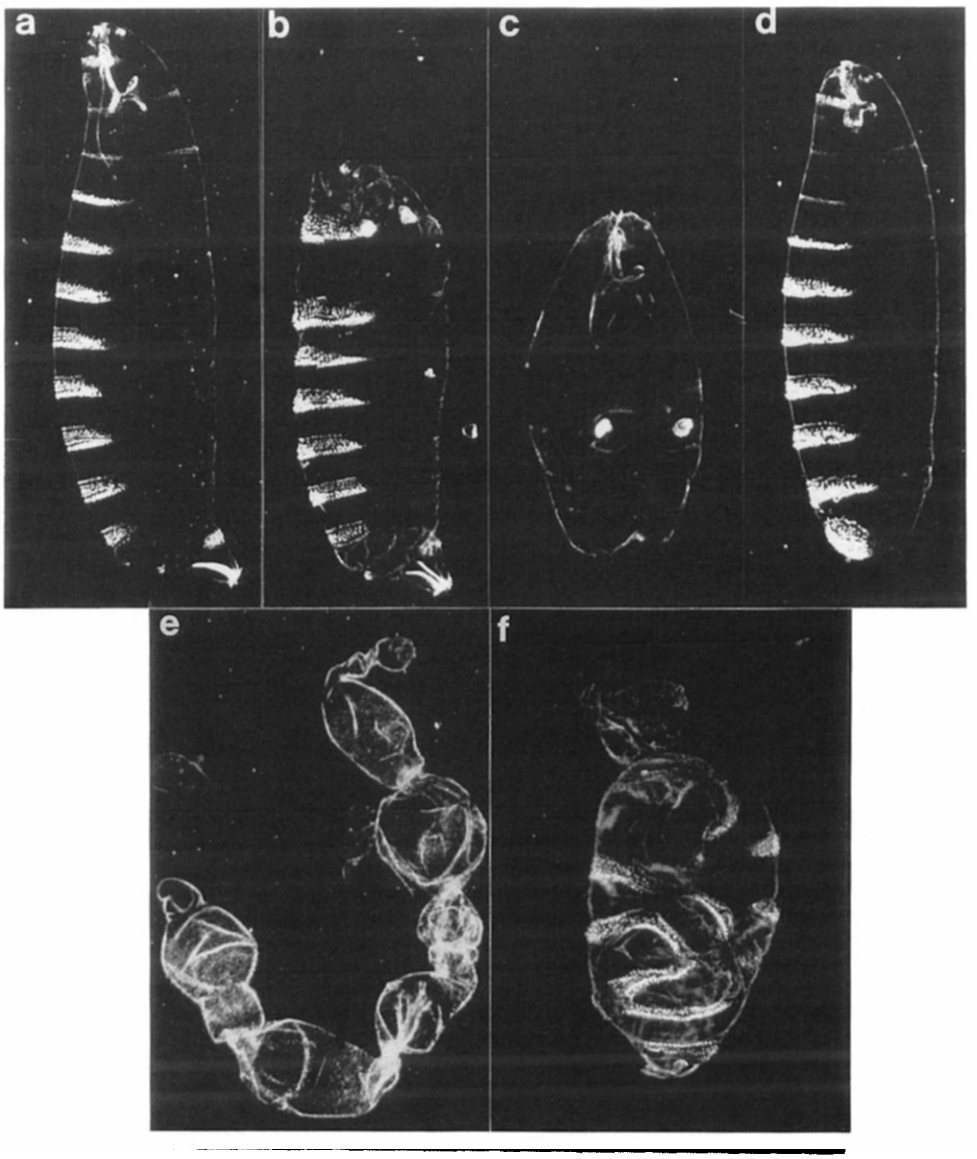

Figure 3. The Cuticular Patterns of Wild-Type and Mutant Embryos
(a) wild-type, (b) anterior (*bicoid*), (c) posterior (*oskar*), (d) terminal (*torso-like*), (e) dorsalized (*dorsal*), (f) ventralized (*cactus*).

localized cytoplasmic determinants comes from experiments in which the egg is pricked and a small amount of cytoplasm is allowed to leak out (Frohnhöfer et al., 1986; Sugiyama and Okada, 1990). Pricking at the anterior pole leads to the development of larvae with head and thoracic defects that closely resemble those produced by mutations in the anterior gene *bicoid*. When the pole plasm is removed from the posterior pole, the telson, the most posterior region of the embryo is not affected, but the resulting larvae show abdominal deletions that are very similar to those produced by posterior group mutations. These results indicate that the anterior and posterior systems produce localized signals that reside at the corresponding poles of the egg. This conclusion is supported by the results of transplantation experiments; defects produced by *bicoid* mutations can be rescued by transplanting anterior cytoplasm into the anterior pole of mutant eggs (Frohnhöfer and Nüsslein-Volhard, 1986). In a similar fashion, the transplantation of wild-type pole plasm can rescue the defects caused by posterior group mutations (Lehmann and Nüsslein-Volhard, 1986, 1987a; Manseau and Schüpbach, 1989; Lehmann and Nüsslein-Volhard, 1991). However, in this case, although the donor cytoplasm must be taken from the posterior pole, the best rescue is observed when this cytoplasm is injected into the presumptive abdominal region. Thus, the pole plasm contains a localized posterior determinant that acts more anteriorly to determine the formation of the abdomen. The pole plasm also contains a second determinant, which directs the formation of the pole cells (Illmensee and Mahowald, 1974), but

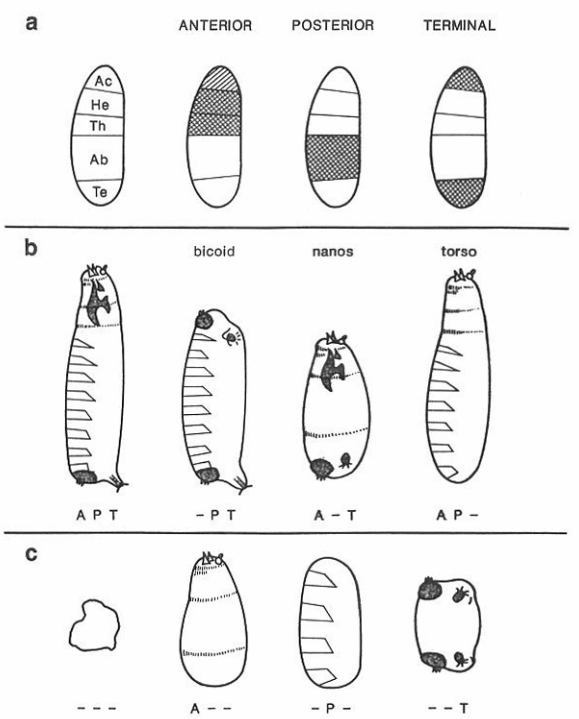

Figure 4. The Complementarity between the Parts of the Pattern Specified by the Anterior, Posterior, and Terminal Systems

(a) The wild-type blastoderm fate map and mutant fate maps showing the parts of the pattern that are absent in mutants of each class (cross-hatched). The hatched shading indicates the anterior region in *bicoid* mutants that develops into telson instead of acron. The five marked areas of the fate map show the regions of the blastoderm that will give rise to the acron (Ac), the head (He), the thorax (Th), the abdomen (Ab), and the telson (Te).

(b) A schematic representation of the final cuticle phenotypes of wild-type embryos and single mutants in each of the three systems. A, P, and T refer to the presence of the anterior, posterior, or terminal systems, respectively, while dashes indicate that this system is mutant.

(c) Final cuticular phenotypes of a triple mutant and the three possible double mutant combinations.

this signal has not yet been identified. The removal of cytoplasm from other regions of the egg does not cause specific pattern defects, nor is it possible to mimic the phenotypes engendered by mutations in the dorsal or terminal systems in this way. As described below, these maternal systems do not produce localized cytoplasmic determinants, but instead act through signals that are localized outside the egg cell.

A combination of genetic and classical embryological experiments have defined the basic properties of the four Drosophila maternal systems. One surprising aspect of these four systems is that, although they all serve to control the localized activation of zygotic pattern gene expression, at a molecular level they act through very different pathways. This is reflected in the fact that with one exception, the genes involved in the production of one maternal signal are not involved in any of the other systems (see Figure 2).

The Anterior Determinant, *bicoid*

For a maternal system to act to specify part of the embryonic pattern it needs to have two properties. First, some component of the system must be localized to provide the initial asymmetric signal. Second, this signal must directly or indirectly lead to the production of an active transcription factor that regulates zygotic target genes. In the case of the anterior system, both of these roles are performed by the products of the gene *bicoid*.

bicoid RNA is localized in the cytoplasm at the anterior pole of the egg (Figure 5A), and is translated after fertilization to produce an anterior to posterior concentration gradient of bicoid protein that extends over the anterior two-thirds of the embryo (Figure 5B) (Frigerio et al., 1986; Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988a; St Johnston et al., 1989). This gradient is believed to arise from diffusion from the anterior source coupled to a uniform rate of degradation. Two lines of evidence demonstrate that the bicoid protein gradient is sufficient to determine the polarity and pattern of the anterior half of the embryo. As the number of maternal copies of *bicoid* is increased, more RNA and protein are produced, resulting in an expansion of the bicoid protein gradient toward the posterior. This change in the shape of the gradient produces a corresponding change in the anterior fate map, as monitored by the positions of gap and pair-rule gene expression domains at the blastoderm stage and the position of the head fold at gastrulation (Driever and Nüsslein-Volhard, 1988b; Struhl et al., 1989). A more dramatic demonstration is provided by RNA injection experiments (Figure 6) (Driever et al., 1990). Injection of in vitro synthesized RNA into other positions in the embryo results in a protein gradient that directs the formation of ectopic head and thoracic structures, with the most anterior pattern elements forming closest to the site of injection. These results show that anterior structures form in regions with high concentrations of bicoid, while lower concentrations lead to the development of more posterior pattern elements. In this way the shape of the gradient defines the polarity of the anterior pattern.

The presence of a homeodomain within the bicoid protein suggests that bicoid is a sequence-specific DNA-binding protein that determines the anterior pattern by directly regulating zygotic target genes (Frigerio et al., 1986; Berleth et al., 1988). One target is the gap gene *hunchback*, which is required for the development of the thorax and part of the head, and is first transcribed at the syncytial blastoderm stage in a large anterior domain that extends to about 50% egg length (see Figure 5C) (Bender et al., 1987; Lehmann and Nüsslein-Volhard, 1987b; Tautz et al., 1987). This early expression is dependent on the bicoid protein gradient, as it does not form in *bicoid* mutant embryos and it expands posteriorly when the *bicoid* gene dosage is increased (Figures 7a–7d) (Schröder et al., 1988; Tautz, 1988; Struhl et al., 1989). There are a number of bicoid-binding sites in the *hunchback* upstream region, including three strong and three weak sites in the 300 bp immediately 5′ to the major start site of zygotic transcription (Driever and Nüsslein-Volhard, 1989a). This region of *hunchback* can direct the bicoid-dependent expression of a reporter gene in the anterior half of the embryo, indicating that bicoid acts as a transcriptional activator of *hunchback* (Schröder et al., 1988; Struhl et al., 1989; Driever and

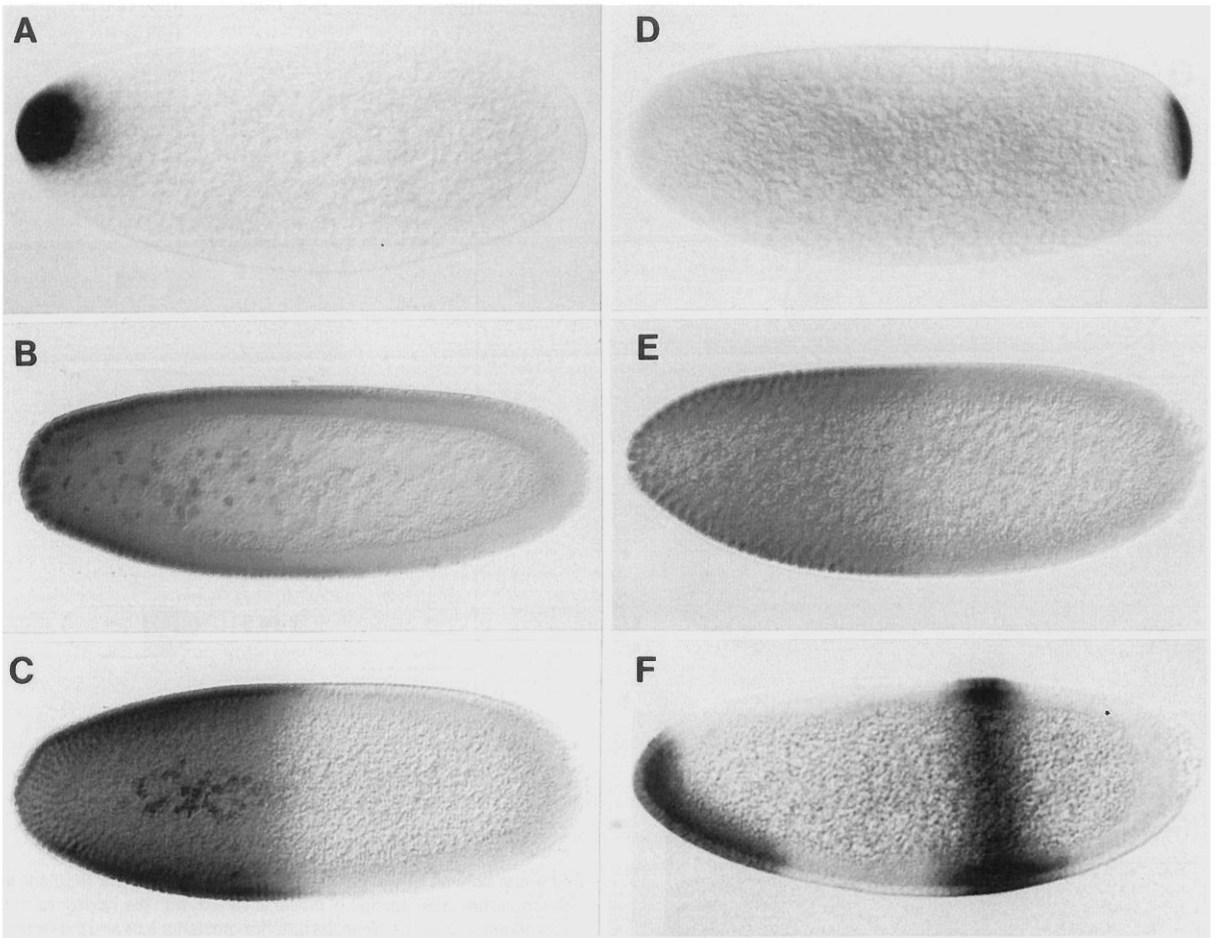

Figure 5. The Distribution of the Localized Maternal RNAs, Transcription Factors, and Target Gene Expression in the Anterior and Posterior Systems
(A–C) The anterior system. (A) *bicoid* RNA. (B) The bicoid protein gradient. (C) The zygotic *hunchback* expression domain.
(D–E) The posterior system. (D) *nanos* RNA. (E) hunchback protein translated from maternal *hunchback* RNA. (F) *knirps* expression (only the posterior domain of *knirps* expression is dependent upon the posterior system).

Nüsslein-Volhard, 1989b). It is unlikely that bicoid requires any specific cofactors to activate transcription, since these *hunchback* sequences are also able to mediate the bicoid-dependent activation of reporter genes in both yeast and Drosophila tissue culture cells (Driever and Nüsslein-Volhard, 1989a; Struhl et al., 1989).

Since *hunchback* is only required for the development of part of the region defined by the anterior system, the bicoid protein gradient must also regulate other zygotic target genes. Indeed, the dependence of the anterior fate map upon the shape of the protein gradient indicates that bicoid acts as a morphogen, with different threshold concentrations defining a number of anterior positions. A model for how this may occur is suggested by analysis of the ability of different bicoid-binding sites to direct the embryonic activation of a basic promoter (Driever and Nüsslein-Volhard, 1989b). Four tandem copies of a high affinity bicoid-binding site direct expression in a large anterior domain that is approximately the same size as the wild-type *hunchback* domain (Figure 7e). In contrast, four low affinity sites direct expression in a much smaller anterior region (Figure 7f). Adding extra binding sites to either construct leads to a large increase in the level of expression, but only causes a slight posterior shift in the extent of the domain. Thus, promoters with low affinity bicoid-binding sites require high concentrations of protein to be activated and are therefore expressed in small anterior regions, while high affinity sites can bind bicoid at lower concentrations and direct expression in larger domains. Although there is still some doubt whether the threshold concentration for activation by bicoid depends solely on the affinity of the bicoid-binding sites in the genes that it normally regulates (Struhl et al., 1989), it is clear that the bicoid gradient can activate target genes in anterior domains of various sizes. In this manner, the smooth protein gradient can be converted into a number of discrete domains of zygotic gene expression, which define several anterior positional values.

The model proposed above requires the existence of at least one additional zygotic gap gene (gene X in Figure 9; Driever and Nüsslein-Volhard, 1989a), which is directly regulated by bicoid and which is expressed in a smaller

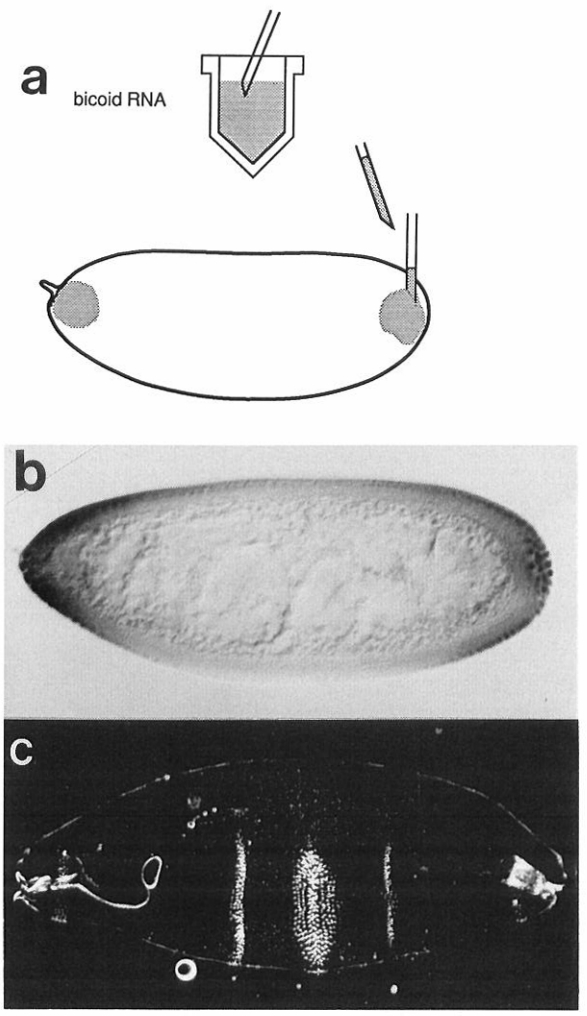

Figure 6. Injected *bicoid* RNA Can Induce a Second Anterior Pattern

(a) Experimental design. In vitro transcribed *bicoid* RNA is injected into the posterior end of wild-type embryos.

(b) The resulting double gradient of bicoid protein.

(c) The cuticular pattern of the dicephalic embryos that develop.

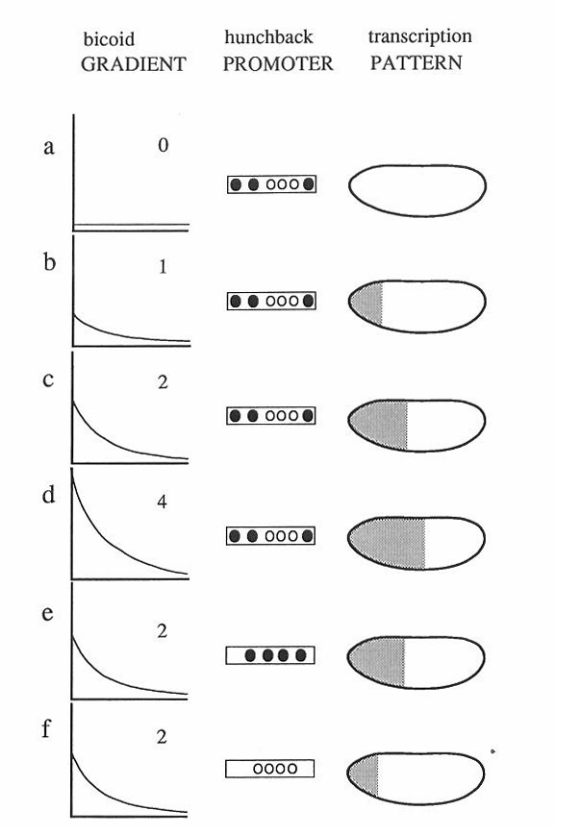

Figure 7. The Spatial Regulation of Zygotic Transcription by the bicoid Protein Gradient

The first column shows the shape of the bicoid protein gradient as the maternal gene dosage of *bicoid* is varied, with the horizontal axis representing distance along the anterior–posterior axis and the vertical axis showing the concentration of bicoid protein. The second column shows the bicoid-binding sites in the regulatory region of *hunchback* (a–d) or two reporter gene constructs (e–f). The closed circles indicate high affinity binding sites, and the open circles, low affinity sites. The third column shows the size of the anterior expression domain of a gene with the bicoid-binding sites shown in column 2, in the presence of the gradient shown in column 1. (a) No maternal copies of *bicoid*, wild-type *hunchback*. (b) One maternal copy of *bicoid*, wild-type *hunchback*. (c) Two maternal copies of *bicoid* (normal) wild-type *hunchback*. (d) Four maternal copies of *bicoid*, wild-type *hunchback*. (e) Two maternal copies of *bicoid*, reporter gene construct containing four high affinity bicoid-binding sites. (f) Two maternal copies of *bicoid*, reporter gene construct containing four low affinity bicoid-binding sites.

anterior domain than *hunchback*. No gene has been proven to fulfil both these criteria, but there are several promising candidates. Mutations in *orthodenticle*, *empty spiracles*, and *buttonhead* delete partially overlapping, adjacent regions of the head, just anterior to the region affected by *hunchback* (Dalton et al., 1989; Cohen and Jurgens, 1990; Finkelstein and Perrimon, 1990). Each of these genes is required very early in development, as the mutants alter the expression patterns of other zygotic pattern genes at the cellular blastoderm stage. Thus, in terms of their phenotypes and their positions in the zygotic hierarchy, these loci behave like gap genes. *empty spiracles* and *orthodenticle* encode homeodomain proteins that are first expressed in a single stripe near the anterior end of the syncytial blastoderm embryo (Dalton et al., 1989; Finkelstein and Perrimon, 1990). Furthermore, the positions of these stripes depend upon the bicoid gradient, since both shift posteriorly when extra maternal copies of *bicoid* are present. Another possible target for *bicoid* regulation is the anterior domain of *giant* expression, whose position is also dependent on the shape of the bicoid protein gradient (Eldon and Pirrotta, 1991; Kraut and Levine, 1991a). However, it still remains to be shown that bicoid protein binds to the regulatory regions of any of these genes to activate their transcription directly. Without knowing how many genes are regulated by the bicoid gradient, it is not possible at present to determine how many different threshold concentrations of bicoid are used to determine the anterior pattern.

The Posterior Determinant

Although superficially similar, the posterior system differs in several major respects from the anterior. First, while the

initial localized signal at the posterior pole is a maternal RNA, the product of this RNA does not regulate zygotic gene expression directly. Instead, the posterior determinant acts by preventing the translation of a transcription factor encoded by an ubiquitous maternal RNA. Second, unlike bicoid, which plays an instructive role in anterior pattern formation, the posterior signal only plays a permissive role.

The pole plasm at the posterior pole contains two localized signals: the posterior determinant, which controls the development of the abdomen, and a second signal that directs the formation of the pole cells. Mutations in all posterior group genes result in abdominal deletions, but the majority of these genes (*cappuccino*, *spire*, *staufen*, *oskar*, *vasa*, *valois*, *tudor*, and *mago nashi*) are also required for the formation of the pole plasm, with its characteristic polar granules, and for pole cell development (Mahowald, 1968; Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Lehmann and Nüsslein-Volhard, 1987a; Manseau and Schüpbach, 1989; Boswell et al., 1991). Mutations in this class of posterior group gene cause their abdominal phenotype not by preventing the production of the posterior determinant, but by failing to localize this signal to the posterior pole (Sander and Lehmann, 1988; Lehmann and Nüsslein-Volhard, 1991). These genes are all required for the localization and stepwise assembly of the polar granules during oogenesis, and the formation of these granules seems to be a prerequisite for the localization of the posterior determinant.

The two remaining posterior group genes, *nanos* and *pumilio*, are specifically involved in the determination of the abdomen and are not required for the formation of polar granules or pole cells (Lehmann and Nüsslein-Volhard, 1987a, 1991). No posterior determinant activity is detectable at any stage in *nanos* mutant ovaries or eggs, indicating that *nanos* is required for the synthesis of this signal (Lehmann and Nüsslein-Volhard, 1991). In fact, *nanos* actually encodes the posterior determinant, and *nanos* RNA is highly concentrated in the pole plasm of the freshly laid egg (see Figure 5D) (Wang and Lehmann, 1991). Injection of in vitro synthesized *nanos* RNA into the abdominal region of mutant embryos restores normal abdomen development, while the injection of a control RNA containing a frame shift mutation has no effect on the phenotype. Furthermore, the wild-type RNA can also rescue the abdominal deletions produced by all other posterior group mutations. Since *nanos* activity is required in the region where the abdomen will form, while the RNA is localized at the posterior pole, either nanos protein, or some activity dependent on nanos, must move to this more anterior region. The distribution of the *nanos* product is not yet known, but the simplest model would be that the protein diffuses from its posterior source to form a posterior–anterior gradient in a similar way to that in which the bicoid gradient forms. *pumilio* mutations seem to prevent sufficient nanos activity from reaching the presumptive abdominal region, as mutant embryos do not form complete abdomens, although they have posterior determinant activity in their pole plasm (Lehmann and Nüsslein-Volhard, 1987a). It is possible that the *pumilio* product facilitates the anterior movement of nanos. Alternatively, pumilio could enhance the amount of nanos activity produced at the posterior pole.

The gap genes *knirps* and *giant* are both required for the formation of the abdominal pattern and are expressed in adjacent domains in the syncytial blastoderm embryo, in the region where the abdomen will develop (Mohler et al., 1989; Pankratz et al., 1989). This expression is dependent on *nanos*, since neither domain is formed in posterior group mutant embryos (Rothe et al., 1989; Eldon and Pirrotta, 1991; Kraut and Levine, 1991a). However, although *nanos* provides the localized signal that initiates abdomen formation, it does not directly regulate these zygotic gap genes. Instead, *nanos* appears to prevent the expression of a transcriptional repressor encoded by maternal *hunchback* RNA. We have already described how the bicoid gradient activates zygotic *hunchback* expression in the anterior of the embryo. *hunchback* is also transcribed during oogenesis to give rise to a maternal transcript that is uniformly distributed in the mature egg (Schröder et al., 1988; Tautz, 1988; Tautz and Pfeifle, 1989). During the cleavage stages of embryogenesis, this maternal RNA is degraded in the posterior half of the embryo, and hunchback protein, which is first synthesized during this period, shows a similar distribution (see Figure 5E) (Tautz, 1988; Tautz and Pfeifle, 1989). In *nanos* mutants, both the RNA and the protein are present throughout the embryo, indicating that an early function of *nanos* is to repress maternal hunchback expression in posterior regions. Using two different experimental approaches, Hülskamp et al. (1989) and Struhl (1989) have caused the ectopic expression of hunchback protein in the posterior half of the embryo, in the presence of wild-type *nanos* activity. In both cases, this posterior hunchback protein blocks the formation of the abdomen and results in a phenotype that is very similar to that produced by *nanos* mutations (Hülskamp et al., 1989; Struhl, 1989). Since hunchback contains six zinc finger domains and binds DNA, it is likely that it prevents abdomen formation by directly repressing the expression of *knirps* and *giant* (Tautz et al., 1987; Stanojević et al., 1989; Treisman and Desplan, 1989). These results indicate that an essential role of *nanos* is to prevent the posterior expression of *hunchback*. In fact, this is the only role that *nanos* plays in the determination of the abdomen. Using mitotic recombination to generate *hunchback* mutant germline clones, it has been possible to produce embryos that lack both maternal *hunchback* activity and *nanos* activity (Hülskamp et al., 1989; Irish et al., 1989; Struhl, 1989). These embryos develop normal abdomens and give rise to fertile adults. Thus, in the absence of maternal *hunchback*, *nanos* is not required for normal development.

The protein sequence of *nanos* shows no strong similarities with other known proteins and therefore does not suggest how the nanos product might regulate the expression of *hunchback*. However, since *nanos* activity affects the distribution of both maternal *hunchback* RNA and hunchback protein, it probably acts at the level of the RNA (Tautz, 1988; Tautz and Pfeifle, 1989). This has now been confirmed by the identification of a short sequence that occurs

twice in the 3′ untranslated region (3′UTR) of the *hunchback* transcript that is required for nanos regulation (Wharton and Struhl, 1991). The presence of both copies of this sequence is sufficient to confer *nanos*-dependent posterior repression on a heterologous transcript. Ectopic *nanos* activity seems to suppress anterior development by a similar mechanism. When *nanos* RNA is injected into the anterior of the egg, or is mislocalized there genetically by *Bicaudal-D* mutations, the bicoid protein gradient never forms (Wharton and Struhl, 1989; Wang and Lehmann, 1991). *bicoid* RNA is degraded prematurely in these eggs, but it is still present at the time that the protein would normally be translated. The 3′UTR of *bicoid* RNA contains a similar sequence to the two found in *hunchback* RNA, and this suggests that *nanos* regulates the expression of both of these transcripts by preventing their translation, and that the degradation of these RNAs is probably a consequence of this translational control (Wharton and Struhl, 1991).

The surprising result that *nanos* is not required in the absence of maternal *hunchback* raises several important questions. First, if *nanos* is not supplying an instructive signal, how is the abdominal pattern generated? The answer to this question lies in the long-range interactions between the gap genes. In maternal *hunchback nanos* double mutant eggs, the anterior and terminal signals are still present and lead to the activation of zygotic *hunchback* in the anterior of the embryo and the terminal gap genes at both ends. hunchback protein is distributed in a gradient extending into the posterior half of the embryo that can specify the anterior and posterior borders of *Krüppel* expression and the anterior border of the *knirps* domain (Gaul and Jäckle, 1989; Stanojević et al., 1989; Hülskamp et al., 1990). It is likely that the protein product of the terminal gap gene *tailless* also forms a gradient from the posterior of the embryo that specifies the posterior borders of *knirps* and *giant* expression (Pankratz et al., 1989; Pignoni et al., 1990; Eldon and Pirrotta, 1991; Kraut and Levine, 1991a). The correct positioning of the gap gene expression domains is further refined by regulatory interactions between *Krüppel*, *knirps*, and *giant* (Pankratz et al., 1989; Eldon and Pirrotta, 1991; Kraut and Levine, 1991a, 1991b). In this way, the initial asymmetric signals produced by the anterior and terminal systems, the secondary gradients of gap gene products and the interactions between the gap genes are sufficient to define the order of expression of the abdominal genes *Krüppel*, *knirps*, and *giant*, without an instructive signal from the posterior determinant.

Since the abdomen forms normally in the absence of maternal *hunchback* and *nanos*, it is hard to imagine why this system has evolved. Although it acts by a very different mechanism, the posterior determinant restricts maternal *hunchback* expression to the same anterior region as that in which bicoid activates zygotic *hunchback* (see Figures 5C and 5E). Thus, the anterior and posterior determinants both define the anterior region of the embryo in the same way. It is possible that the posterior system is the more primitive of the two and originally subdivided the anterior–posterior axis on its own, while *bicoid* evolved more recently and took over this role. This does not explain why a functional posterior system has survived, despite the presence of *bicoid*. One possibility is that the existence of maternal *hunchback* and *nanos* allows the anterior expression of hunchback protein to begin slightly earlier and increases the amount of protein produced; these two effects may help to speed up embryogenesis and make pattern formation a more error-free process. *nanos* has also evolved the ability to regulate bicoid translation, which is more difficult to explain, as nanos is restricted to the posterior half of the wild-type embryo, while *bicoid* RNA is localized to the anterior pole.

The Terminal System

As is the case for the anterior and posterior systems, the defects produced by several terminal and dorsal group mutations can be rescued by cytoplasmic transplantations or RNA injections (Santamaria and Nüsslein-Volhard, 1983; Anderson and Nüsslein-Volhard, 1984; Anderson et al., 1985b; Müller-Holtkamp et al., 1985; Seifert et al., 1987; Klingler et al., 1988; Strecker et al., 1989). However, these results differ in two important respects. In general, the rescuing activities are not localized within the donor eggs, and the polarity of the resulting patterns is not affected by the site of transplantation into the recipient embryos. There are two partial exceptions to these generalizations (see below), but they do not alter the basic conclusion that the terminal and dorsoventral systems do not produce cytoplasmic determinants that are localized in the unfertilized egg. Instead these two systems share a number of features that indicate that they provide positional information to the egg by a quite different mechanism. In both the terminal and dorsal groups, at least one gene is required in the somatic cells of the female, and not in the germline (Stevens et al., 1990; Stein et al., 1991). These genes are most probably expressed in the somatic follicle cells that surround the developing oocyte. In addition, one of the germline genes in each system encodes a transmembrane protein that is uniformly distributed in the egg membrane and that shows homology to other cell surface receptor proteins (Hashimoto et al., 1988; Sprenger et al., 1989). Gain-of-function alleles have been recovered in both of these putative receptor genes and result in the opposite phenotype to loss-of-function mutations (Anderson et al., 1985a; Klingler et al., 1988). These alleles are believed to produce mutant receptors that are constitutively active everywhere in the egg.

Although our information on the pathway for either system is incomplete, drawing on data from each, one can propose the following scheme. In each system, one of the genes that is required in the soma is active in a subpopulation of the somatic follicle cells, leading to the production of a localized signal that is deposited outside the egg in the vitelline membrane or in the perivitelline space, a fluid-filled region between this membrane and the egg membrane. After fertilization, the asymmetric signal provided by the follicle cells causes the release of a localized ligand that binds to the receptors in the egg membrane. The receptors then transmit this signal to the inside of the egg, activating a signal transduction pathway that results in

the local activation of a transcription factor that regulates zygotic pattern gene expression.

In the terminal system, the product of the gene *torso* probably acts as a receptor for an extracellular signal that is produced at the two poles of the egg. The *torso* protein sequence contains an N-terminal signal peptide, a putative transmembrane domain, and a C-terminal region that show significant homology to the tyrosine kinase domains of other receptors (Sprenger et al., 1989). This structure strongly suggests that *torso* encodes a transmembrane receptor tyrosine kinase. Although *torso* RNA is synthesized during oogenesis, the protein is not translated until after fertilization, when it localizes to the egg membrane (Casanova and Struhl, 1989). As predicted by experiments showing that *torso*-rescuing activity is not localized along the anterior–posterior axis, both the RNA and protein show a uniform distribution (Klingler et al., 1988; Casanova and Struhl, 1989; Strecker et al., 1989).

In addition to loss-of-function *torso* mutations, which cause a typical terminal group phenotype (see Figure 3d), there exist three gain-of-function alleles that have the opposite effect (Klingler et al., 1988; Strecker et al., 1988). Embryos laid by mutant females develop normal terminal structures but have defects in the segmented regions of the pattern (Klingler et al., 1988). The gain-of-function segmentation defects are suppressed in embryos that are also mutant for the terminal gap genes, *tailless* and *huckebein*, the zygotic targets for the terminal system (Klingler et al., 1988; Strecker et al., 1988; Weigel et al., 1990). This indicates that these phenotypes are most probably due to the ectopic expression of *tailless* and *huckebein* in the middle of the embryo, which leads to the repression of central gap genes such as *Krüppel*. It is believed that the gain-of-function *torso* alleles encode mutant receptors with constitutive tyrosine kinase activity independent of the binding of ligand.

The existence of *torso* gain-of-function mutations has made it possible to determine which of the other maternal *torso* group genes act upstream of *torso* in the production of the ligand and which act downstream in the signal transduction pathway inside the egg. In double mutant combinations, mutations in the upstream genes should have no effect upon the ligand-independent gain-of-function phenotype, but those in downstream genes should suppress this phenotype and should instead cause the loss of terminal structures. These experiments have placed *torso-like*, *trunk*, *fs(1)Nasrat*, and *fs(1)pole hole* upstream of *torso* and *l(1)pole hole* downstream (Ambrosio et al., 1989; Stevens et al., 1990). *l(1)pole hole* is the Drosophila homolog (*D-raf*) of the vertebrate proto-oncogene *c-raf*, which encodes a serine/threonine kinase (Nishida et al., 1988; Ambrosio et al., 1989). *c-raf* has been implicated in the signal transduction pathways of a number of vertebrate receptor tyrosine kinases, but its exact role is unclear (reviewed by Li et al., 1991; Rapp, 1991). The demonstration that mutations in *D-raf* cause terminal pattern deletions and suppress the *torso* gain-of-function phenotype indicates that the raf kinase is an essential component of the *torso* signal transduction pathway. It is likely that there are still several genes that act downstream of *torso* that have not yet been identified, including the transcription factor at the end of this pathway (gene Y in Figures 2 and 9) that activates *tailless* and *huckebein* in the terminal regions of the embryo.

The genes upstream from *torso* are responsible for the production of the localized extracellular ligand at the two ends of the egg. Since the developing oocyte is surrounded by about 1000 somatic follicle cells, the simplest way that such a ligand might be localized is if it is produced by terminal subpopulations of these cells. Germline clones of *trunk*, *fs(1)Nasrat*, and *fs(1)pole hole* give rise to embryos that display a typical *torso* group phenotype, indicating that these genes are required in the nurse cell–oocyte complex (Perrimon and Gans, 1983; Schüpbach and Wieschaus, 1986). In contrast, *torso-like* is required in the soma and not in the germline (Stevens et al., 1990). Stevens et al. (1990) have produced follicle cell mosaics that are mutant for *torso-like* and have found that *torso-like* clones of only 6–30 cells surrounding the posterior pole of the egg can produce a terminal phenotype at just the posterior end of the resulting embryo. Thus, *torso-like* is specifically required in the terminal follicle cells, strongly suggesting that these cells produce the localized terminal signal. It is not known whether *torso-like* or the three germline-dependent upstream genes actually encode the ligand for *torso*. *torso-like* may produce an inactive ligand that is anchored in the vitelline membrane until after fertilization when it is released by the activities of the germline genes. Alternatively, the follicle cell signal may lead to the local activation of one of the germline products, which then acts as a ligand for *torso*.

The Dorsoventral System

The dorsoventral pathway is the most complex of the four maternal systems in Drosophila, since it requires the largest number of genes and specifies positional information along the whole of the dorsoventral axis. However, the basic features of this pathway are very similar to those of the terminal system. An initial ventral signal outside the egg leads to the production of a localized ligand for a receptor in the egg membrane. The localized activation of the receptor then initiates a signal transduction pathway inside the egg that culminates in the spatial regulation of zygotic gene expression. Like *torso*, the dorsal group gene *Toll* encodes a transmembrane protein that is believed to act as the receptor for a localized external signal (Hashimoto et al., 1988, 1991). The Toll protein sequence contains an N-terminal signal peptide, a putative transmembrane domain, and two distinct regions of homology to other genes. The extracellular portion of the protein contains two blocks of leucine-rich repeats followed by cysteine-containing domains that are similar to those found in a number of other receptors, including both the α and β chains of the human platelet glycoprotein Ib (a receptor for von-Willebrand factor and thrombin), while a stretch of 217 amino acids in the intracellular domain of Toll shares 26% amino acid identity with the intracellular portion of the interleukin-1 receptor (Lopez et al., 1988; McFarland et al., 1989; Keith and Gay, 1990; Vicente et al., 1990; Soppet et al., 1991; Squinto et al., 1991; Gay and Keith, 1991; Schneider et al., 1991). These homologies strongly support a model in which Toll acts as a receptor

for a localized external ligand, and transduces this signal to the interior of the egg. Consistent with this view, Toll is expressed everywhere in the egg membrane at the syncytial blastoderm stage, the time at which the upstream signal is known to be active (Hashimoto et al., 1991).

While *Toll* loss-of-function mutations produce a dorsalized phenotype, dominant gain-of-function alleles result in the development of embryos that are ventralized (Anderson et al., 1985a; Schneider et al., 1991). Several of these mutations are believed to produce Toll protein with constitutive activity that is partially independent of the extracellular signal (Schneider et al., 1991). Using the same approach described for the terminal system, these gain-of-function alleles can be used to position the other dorsal group genes relative to *Toll* in the pathway: *gastrulation-defective*, *pipe*, *nudel*, *windbeutel*, *snake*, *easter*, and *spätzle* all function upstream of *Toll* in the production of the localized signal; *tube*, *pelle*, and *dorsal* (*dl*) act downstream, in the signal transduction pathway inside the egg (Anderson et al., 1985a; K. V. Anderson, personal communication).

Genetic experiments have suggested that the product of the gene *dorsal* lies at the end of the signal transduction pathway that begins when *Toll* is activated on the ventral side of the embryo. Only *dorsal* mutations produce a dorsalized phenotype in double mutants with loss-of-function, ventralizing *cactus* alleles (Roth et al., 1991). Thus, *dorsal* functions downstream of *cactus*, while all other dorsal group genes do not. Second, only in the case of *dorsal* can a localized rescuing activity be found in transplantation experiments, although this localization only appears at the syncytial blastoderm stage (Santamaria and Nüsslein-Volhard, 1983). Finally, unlike all other dorsal group genes, loss-of-function *dorsal* mutations show a dominant effect (Nüsslein-Volhard et al., 1980). At 29°C, *dl*/+ females produce embryos that do not develop mesoderm, showing that the determination of the most ventral cell fates in the embryo requires higher levels of *dorsal* activity than the lateral and dorsal regions.

The exact role of *dorsal* has only become evident as a result of molecular experiments. These show that the ventral signal transmitted by *Toll* regulates the differential nuclear localization of dorsal protein. The resulting gradient of dorsal protein in the nuclei then controls zygotic gene expression in a concentration-dependent manner. Both *dorsal* RNA and protein are synthesized during oogenesis and are uniformly distributed in the cytoplasm of the freshly laid egg (Steward et al., 1988; Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). When the zygotic nuclei reach the cortex of the egg after the ninth cleavage division, the protein becomes highly concentrated in the nuclei on the ventral side of the embryo and is depleted from the ventral cytoplasm (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). At more lateral levels, the nuclear and cytoplasmic concentrations of dorsal are approximately equal, while the protein is excluded from the nuclei on the dorsal side. The formation of the concentration gradient of dorsal in the syncytial blastoderm nuclei seems to be entirely regulated at the level of nuclear localization, since a uniform distribution of protein is observed when the nuclear membranes break down during mitosis (Roth et al., 1989).

Using different combinations of mutants in the genes that act upstream of *dorsal*, it is possible to produce a wide range of dorsalized, lateralized, and ventralized phenotypes. In all cases, the distribution of dorsal in the nuclei correlates perfectly with the expression patterns of the zygotic dorsoventral genes and the final cuticular phenotype (Roth et al., 1989). For example, in loss-of-function dorsal group mutations, dorsal protein is excluded from the nuclei at all positions around the dorsoventral axis, the dorsal zygotic genes *zen* and *dpp* are expressed everywhere while the ventral genes *twist* and *snail* are not expressed, and all the cells adopt a dorsal fate (Figures 8i–8m). In the strongest ventralizing mutants, the converse is seen. dorsal protein localizes to all of the nuclei, which leads to the repression of *zen* and *dpp* and the activation of *twist* and *snail* all around the circumference of the embryo, and all cells follow a ventral pathway of development (Figures 8a–8d). Finally, in mutant combinations that produce a lateralized phenotype, dorsal protein is evenly distributed between the nuclei and the cytoplasm, and neither the dorsal nor ventral zygotic genes are expressed in the main part of the embryo (Figures 8e–8h). These observations provide strong evidence that the nuclear concentration of dorsal determines the dorsoventral pattern by controlling the expression of the zygotic genes.

The sequence of *dorsal* indicates that it is likely to encode a transcription factor. The N-terminal half of the protein shares approximately 50% amino acid identity with the N-terminal portions of the proto-oncogene *c-rel* and the p50 and p65 subunits of the transcription factor, NF-κB, and this region of similarity includes both the DNA-binding and dimerization domains of these proteins (Steward, 1987; Ghosh et al., 1990; Kieran et al., 1990; Nolan et al., 1991; Ruben et al., 1991). More direct evidence that dorsal acts as a transcription factor comes from studies on the zygotic genes that are regulated by the dorsal protein gradient. The dorsal-dependent repression of *zen* expression in the ventral and lateral regions of the embryo requires a repression element in the *zen* promoter that contains a number of dorsal-binding sites (Doyle et al., 1989; Ip et al., 1991). The upstream sequences that control the ventral expression of *twist* also contain two clusters of dorsal-binding sites, and these regions can mediate the dorsal-dependent activation of transcription in tissue culture cells (Thisse et al., 1991; Jiang et al., 1991; Pan et al., 1991). Interestingly, dorsal protein binds more strongly to the sites in the *zen* repression element in vitro than it does to sites in the *twist* upstream region (Thisse et al., 1991; Jiang et al., 1991). Therefore, dorsal may control the spatial domains of zygotic gene expression in a similar way to that proposed for bicoid. Genes like *zen* with high affinity dorsal-binding sites can bind dorsal protein when it is present at the low concentrations found in lateral nuclei, while higher concentrations are needed to bind to the lower affinity sites of genes such as *twist*, and this will restrict the binding to the ventral nuclei of the embryo. One important additional property of dorsal is that it seems to function as both a transcriptional activator and repressor, activating

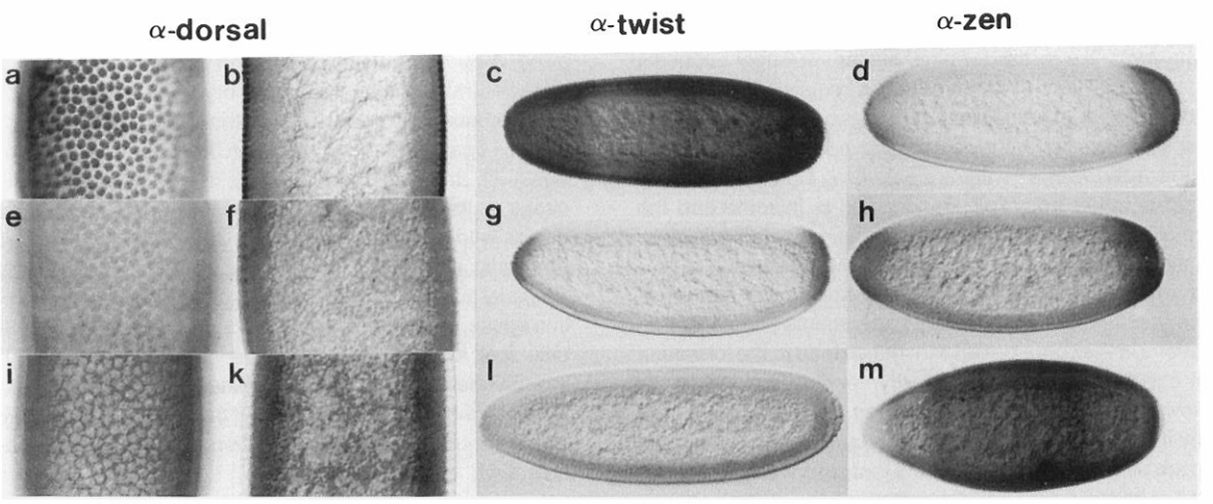

Figure 8. The Regulation of Zygotic Transcription by the Nuclear Concentration of dorsal Protein

The uniform distribution of dorsal protein and the resulting expression of the zygotic target genes, *twist* and *zen*, are shown for three apolar phenotypes.

(a–d) V0, completely ventralized embryos (*cactus*^{A2}; *Toll*^{rm9}). dorsal protein is localized to all of the nuclei, resulting in the expression of twist and the repression of zen protein, all around the egg circumference. (Note that the poles of the egg behave differently in several of these stainings, because of the influence of the terminal system.)

(e–h) L1, lateralized at a ventrolateral level (*Toll*^{rm9}). dorsal protein is present at equal concentrations in the nuclei and the cytoplasm, and neither twist nor zen is expressed.

(i–m) D0, completely dorsalized (*Toll*^{5BRE}/Deletion). dorsal protein is excluded from all of the nuclei, twist is not activated, and zen is expressed everywhere.

(a), (e), and (i) show surface views of the middle part of syncytial blastoderm embryos stained with an antibody against dorsal protein; (b), (f), and (k) show optical midsections of the same embryos; (c), (g), and (l) show optical midsections of cellular blastoderm embryos stained with an anti-twist antibody; (d), (h), and (m) show optical midsections of cellular blastoderm embryos stained with an anti-zen antibody. Data from Roth et al. (1989).

twist ventrally and repressing *zen* in the ventral and lateral nuclei. At present, we have no information on why the binding of dorsal can have these two opposite effects, but this probably depends on the context of the binding sites and on the other factors that bind in the region (Pan et al., 1991).

The homology between *dorsal* and the p50 and p65 subunits of NF-κB is especially intriguing since the activity of NF-κB is also regulated at the level of nuclear localization. In several cell types NF-κB is found in the cytoplasm in an inactive form in which p65 is bound to IκB (Baeuerle and Baltimore, 1988a, 1988b; Nolan et al., 1991). Upon activation of the cells by a number of signals, IκB is released from the complex, allowing the active NF-κB to translocate to the nucleus. There is strong evidence that *cactus* performs a homologous function to IκB in the Drosophila dorsoventral system (Roth et al., 1991). The sequence of *cactus* is actually very similar to that of IκB (R. Geisler and C. N.-V., unpublished data), and *cactus* loss-of-function mutations produce an increase in dorsal nuclear localization, resulting in a ventralized phenotype, which one would expect if *cactus* encodes a cytoplasmic anchor for dorsal protein. In vitro experiments have shown that the phosphorylation of IκB by cellular kinases can activate NF-κB (Ghosh and Baltimore, 1990). While a similar process may occur in the dorsoventral pathway, it is unlikely that the ventral activation of dorsal nuclear localization occurs solely through the modification of cactus. The ventralized embryos produced by even the strongest *cactus* mutations are still polar, and although more dorsal protein localizes to the nuclei, the ventral nuclei still contain higher levels of protein than those on the dorsal side (Roth et al., 1991). It is possible that none of these mutations completely abolishes *cactus* activity, but it seems more likely that the release of dorsal protein from cactus inhibition is insufficient to produce the highest levels of dorsal nuclear localization. This suggests a model in which the dorsal group genes activate dorsal nuclear localization independently of *cactus*, perhaps by modifying dorsal directly. It is interesting to note that one of the signals that can lead to the activation of NF-κB is interleukin-1 (Shirakawa and Mizel, 1989; Shirakawa et al., 1989). As mentioned above, the interleukin-1 receptor is homologous to Toll, raising the possibility that both receptors use similar intracellular signaling pathways to regulate nuclear localization. The two dorsal group genes *tube* and *pelle* are required for the transmission of this activating signal in Drosophila, but although *tube* has been cloned, its sequence does not suggest what role it may play (Letsou et al., 1991).

The discovery that *Toll* encodes a transmembrane receptor that is localized in the egg membrane has led to the hypothesis that dorsoventral polarity is induced from outside the egg by a localized ligand for Toll, and that the production of this external signal depends upon the seven dorsal group genes that act upstream from *Toll* in the hierarchy. Two recent results have provided strong support for this model (Stein et al., 1991). First, three of the upstream dorsal group genes—*pipe*, *nudel*, and *windbeutel*—are re-

quired in the soma and not in the germline. The similarity with *torso-like* in the terminal system strongly suggests that at least one of these genes is required in a specific population of ventral follicle cells. Second, transplantation experiments have shown that the perivitelline fluid that surrounds the egg contains a polarizing activity. When this fluid is taken from *Toll*$^-$ donors and is injected into the perivitelline space of *pipe*, *nudel*, or *windbeutel* recipients, it induces the formation of ventral pattern elements. Furthermore, the site of injection determines the polarity of the resulting embryos. These experiments show that mutations in the soma-dependent genes lead to the formation of eggs with no intrinsic polarity, lending support to the view that these genes are required for the production of the initial asymmetric signal. No polarizing activity is found in the perivitelline fluid of donors that carry a wild-type copy of *Toll*, but wild-type *Toll* is required in the recipients. These observations suggest that the rescuing activity corresponds to the Toll ligand. In the presence of wild-type *Toll*, the ligand will bind to the Toll protein in the egg membrane of the donors and will therefore no longer be free in the perivitelline fluid for transplantation.

The four other genes upstream of *Toll* are germline dependent (Seifert et al., 1987; Konrad et al., 1988; Stein et al., 1991). While *gastrulation-defective* cannot be rescued by cytoplasmic transplantation and has a temperature-sensitive period that begins during oogenesis, *easter*, *snake*, and *spätzle* can be rescued by the injection of RNA or cytoplasm into mutant embryos (Anderson and Nüsslein-Volhard, 1984; Seifert et al., 1987). This indicates that the activities of these genes are not required until after fertilization. *snake* and *easter* have been cloned and their sequences indicate that they both encode serine proteases that are probably secreted as inactive precursors (DeLotto and Spierer, 1986; Chasan and Anderson, 1989). It is likely that *spätzle* also encodes a product that is secreted into the perivitelline space, since the dorsalization produced by mutants in any of these three genes can be rescued by the transplantation of perivitelline fluid (Stein and Nüsslein-Volhard, 1992). Similar transplantation experiments have shown that *easter*, *snake*, and *spätzle* are required for the production of the polarizing activity that is believed to correspond to the ligand for Toll. For example, perivitelline fluid from *easter Toll* donors cannot generate polarity in *pipe* recipients, even though there is no Toll protein present in the donors to sequester any ligand produced.

While all the evidence so far supports the idea that the ventral follicle cells provide the initial asymmetric signal in the dorsoventral pathway, the relationship between this signal, the germline-dependent activities, and the Toll ligand remains unclear. The expression of the somatic genes in the ventral follicle cells could result in the synthesis of a localized inactive ligand for Toll that is released after fertilization as a result of the activities of the germline-dependent genes, perhaps by proteolysis. Alternatively, the follicle cell signal may lie upstream of the germline-dependent activities. For example, this signal might actually be in the form of a ventral site that stimulates the local activation of one of the serine proteases, which then acts through a protease cascade to generate the ligand. A number of gain-of-function *easter* mutations produce ventralized or lateralized embryos, and these phenotypes presumably arise because the spatial distribution of the Toll ligand has been altered (Chasan and Anderson, 1989; Jin and Anderson, 1990). It is hard to explain how *easter* mutations can alter the localization of this ligand unless wild-type easter activity is also localized. Thus, it has been suggested that the easter serine protease is normally activated only in the ventral region of the perivitelline space, while the gain-of-function alleles encode products that are released from this spatial regulation. Although there are other possibilities, this interpretation strongly favors the second model, in which the follicle cell signal controls the ventral activation of the germline products such as easter.

Delayed Induction and Limited Diffusible Ligands

Although the components of the dorsoventral and terminal systems are different, the basic features of the two pathways are quite similar. One particularly striking aspect of both is that the initial asymmetric signals seem to originate from the follicle cells that surround the developing egg. Thus, the formation of ventral and terminal pattern can be seen as an inductive process, in which the follicle cells provide an inducing signal to specific regions of the egg. Unlike classical inductive events, there is a large temporal delay between the production of the inducing signal and the response. In fact, the inducing cells have degenerated long before the fertilized egg responds to the localized signals. Several lines of evidence indicate that the egg does not receive the ventral or terminal signals until after fertilization. For example, mutations in many of the germline-dependent terminal and dorsal group genes can be rescued by cytoplasmic transplantation or RNA injection into the syncytial blastoderm embryo (e.g., Anderson and Nüsslein-Volhard, 1984; Klingler et al., 1988). Furthermore, torso, the receptor for the terminal signal, is not translated during oogenesis, demonstrating that the egg is not competent to respond until after egg deposition (Casanova and Struhl, 1989). In contrast, the contribution of the follicle cells must occur during oogenesis, as these cells die at the end of this process. Since mature eggs can be held in the female for up to 15 days before being fertilized, the localization and activity of the follicle cell signals must remain stable for long periods of time between their synthesis during oogenesis and their activation after the egg has been laid.

After fertilization, the localized follicle cell signals result in the production of ligands for the two receptors in the egg membrane. Unlike their precursors during oogenesis, these ligands appear to be freely diffusible in the perivitelline space. For example, perivitelline fluid taken from the dorsal side of *Toll*$^-$ embryos contains the same amount of polarizing activity as fluid taken from the ventral side (Stein et al., 1991). These results create an apparent paradox. It is clearly important that the receptor, Toll, is only activated on the ventral side of the embryo, yet the polarizing activity seems not to be localized. The most likely solution to this problem is that Toll limits the diffusion of its own ligand. In

wild-type embryos all of the ligand probably binds to Toll on the ventral side of the egg, but the ligand cannot be sequestered in the absence of Toll and remains free to diffuse into the dorsal perivitelline fluid. This model requires that the amount of ligand is limited, and that Toll is present in excess. In situations where the amount of Toll is reduced, the polarity of the embryo can be determined by the localization of Toll rather than the external signal. When *Toll*$^{+}$ cytoplasm or *Toll* RNA is injected into the dorsal side of *Toll*$^{-}$ embryos, the site of injection determines where ventral structures will form (Anderson et al., 1985b). Since there is no Toll protein on the ventral side of these eggs, the ligand can diffuse in the perivitelline space until it binds to the dorsal patch of Toll that is synthesized from the injected RNA. This leads to the activation of dorsal protein nuclear localization on the dorsal rather than ventral side, and results in the development of embryos with reversed polarity.

A similar phenomenon has also been observed in the terminal system. The injection of *torso* RNA into the middle of *torso*$^{-}$ recipients leads to the suppression of segmentation, and sometimes causes the development of telson structures in the middle of the embryo (F. Sprenger and C. N.-V., unpublished data). The induction of this phenotype depends upon the presence of ligand and the absence of endogenous torso with a wild-type extracellular domain. These results indicate that when there is no torso at the poles to bind the ligand, this activity can diffuse to the middle of the egg and activate any wild-type torso protein that is expressed there. For an inductive process to be spatially controlled, either the inducer or the competence to respond must be localized. In the dorsoventral and terminal pathways, it is normally the inducing activity that is localized, but in the exceptional cases described above this situation is reversed and it is the distribution of responsiveness that determines the final pattern.

Morphogen Gradients

Both bicoid and dorsal proteins form gradients in the nuclei of the syncytial blastoderm embryo, and the shapes of these gradients correspond well with the resulting patterns. However, to prove that either molecule functions as a morphogen, a third criterion must be satisfied. A morphogen has been defined as a "factor which can evoke more than one positive response from the responding tissue" (Slack, 1991). Thus, the presence of the morphogen alone must be sufficient to generate at least two different responses or cell states with different threshold concentrations. In general, it is hard to be certain that all of the observed cell states are determined directly by the absolute concentration of the morphogen, and that they do not arise as a result of secondary interactions. For example, even though retinoic acid behaves as a morphogen when applied to the chick limb bud, one cannot rule out that it acts as a local inducer of one cell state and that the pattern is actually formed by a series of cell–cell interactions or by a gradient of a second molecule (Brockes, 1991; Noji et al., 1991; Wanek et al., 1991). One way to exclude the possibility that different cell states are being generated by subsequent cell–cell interactions or secondary gradients is to expose single cells to various concentrations of the factor in question (Green and Smith, 1990; Simeone et al., 1990). Since the maternal signals in Drosophila act before cellularization has occurred, this approach cannot be used for bicoid and dorsal. Instead, it is possible to generate a uniform concentration of either protein across the whole axis. Since all the nuclei will therefore be exposed to the same concentration of morphogen, they should all respond in the same way, thereby eliminating any effects of secondary interactions between different regions. In the dorsoventral system, a number of different mutant combinations give rise to apolar phenotypes in which all nuclei contain the same concentration of dorsal protein (Roth et al., 1989). At a molecular level, two different thresholds can be distinguished for the response to the dorsal protein concentration, leading to the specification of three cell states (Figure 8). When no dorsal is present in the nuclei, *zen* and *dpp* are expressed everywhere. Above the first threshold, *zen* and *dpp* are repressed, but *twist* and *snail* are not activated; above the second threshold, *zen* and *dpp* remain repressed, and *twist* and *snail* are turned on. Using various combinations of dorsal group and *cactus* alleles, it is possible to generate two additional apolar lateralized phenotypes (Roth et al., 1991). This suggests that the dorsal nuclear concentration gradient actually defines five different positional values. At present, the three apolar lateralized phenotypes cannot be distinguished by their patterns of zygotic gene expression, and it is possible that there is another zygotic target of dorsal regulation that has not yet been identified.

To create a uniform distribution of bicoid protein, it is necessary to prevent the localization of both *bicoid* and *nanos* RNAs, since localized nanos activity will inhibit the translation of bicoid in the posterior of the embryo. This can be done by using *exuperantia staufen* double mutant embryos and varying the number of maternal copies of *bicoid*. In this way, it has been possible to show that bicoid also acts as a morphogen, with at least two threshold concentrations (W. Driever and C. N.-V., unpublished data).

The Localization of the Maternal Signals

Although the way that the four maternal signals generate polarity in the embryo is quite well understood, much less is known about how this asymmetry initially arises during oogenesis, when the maternal signals themselves are localized. In the case of the terminal and dorsoventral systems, this seems to be a question of how different follicle cell populations are defined, and most probably involves cell–cell interactions. The determination of follicle cell states along the dorsoventral axis also seems to require signaling between the oocyte and the follicle cells. The soma-dependent *torpedo* mutations in the Drosophila epidermal growth factor receptor homolog cause all of the follicle cells to adopt a ventral fate, leading to the production of ventralized egg coverings and embryos (Schüpbach, 1987; Price et al., 1989; Schejter and Shilo, 1989). Two other genes involved in this signaling, *K10* and *gurken*, are required in the germline, and *K10* RNA accumulates specifically around the oocyte nucleus, which lies on the dorsal side of the cell (Wieschaus et al., 1978; Haenlin

et al., 1987; Schüpbach, 1987). These observations have led to a model in which the oocyte nucleus produces a signal that diffuses to the nearby follicle cells and binds to the Drosophila epidermal growth factor receptor homolog, thereby inducing the cells to adopt a dorsal fate.

The anterior and posterior determinants must be localized by quite a different mechanism, since these are both maternal RNAs that reside in the cytoplasm at opposite ends of the oocyte. The localization of *bicoid* RNA is mediated through the 3′ untranslated region of the transcript and occurs in several steps, in which the RNA first localizes to the apical regions of the nurse cells before being transported into the oocyte and binding to the cortex at the anterior pole (Macdonald and Struhl, 1988; St Johnston et al., 1989). The initial phase of this process is disrupted by *exuperantia* mutations, while *swallow* mutations cause the RNA to fall off the cortex in the middle of oogenesis (Berleth et al., 1988; Stephenson et al., 1988). Before the egg is laid, *bicoid* RNA is released from the cortex into the anterior cytoplasm, where the product of the *staufen* gene seems to be required to hold the RNA in position (St Johnston et al., 1989). staufen protein is concentrated in the anterior cytoplasm in the same region as *bicoid* RNA, suggesting that staufen may bind to the RNA to prevent its diffusion (St Johnston et al., 1991). While almost nothing is known about the mechanisms that direct the localization of *bicoid* RNA, this process does require microtubules, and this raises the possibility that the RNA is transported along the microtubule network (Pokrywka and Stephenson, 1991).

Localization to the posterior pole seems to be an even more complicated process. One reason for this is that it is not just *nanos* RNA that is localized, but all the components of the pole plasm as well. The first identified molecules to reach the posterior pole are staufen protein and *oskar* RNA (Ephrussi et al., 1991; Kim-Ha et al., 1991; St Johnston et al., 1991). These are followed by vasa protein, cyclin B RNA, and toward the end of oogenesis, *nanos* RNA and pole cell–determining activity (Illmensee et al., 1976; Hay et al., 1988; Whitfield et al., 1989; Lasko and Ashburner, 1990; Wang and Lehmann, 1991). The pole plasm forms in a stepwise manner, in which the components that localize early are required for the subsequent localization of those that arrive later in oogenesis (Whitfield et al., 1989; Hay et al., 1990; Lasko and Ashburner, 1990). Although the majority of these molecules may accumulate in the pole plasm by binding to other components that have already been localized, this model cannot account for the localization of the first molecules to reach the posterior pole, most probably staufen protein and *oskar* RNA. Several lines of evidence suggest that staufen protein associates with *oskar* RNA at the anterior of the oocyte and the two are then transported around the cortex to the posterior pole as a complex, in a process that requires the activities of two other posterior group genes, *cappuccino* and *spire* (Manseau and Schüpbach, 1989; Ephrussi et al., 1991; Kim-Ha et al., 1991; St Johnston et al., 1991). Thus, staufen protein seems to associate with both *oskar* and *bicoid* RNAs, mediating the transport of the former to the posterior end of the egg and anchoring the latter at the anterior end.

Although many of the genes that are involved in localization in the oocyte have now been identified, the cell biology of these processes is not well understood, nor is it known how the anterior and posterior ends of the egg are first defined as the sites for localization. The polarity of the oocyte probably depends upon the geometry of the nurse cell–oocyte complex, which is initially established near the beginning of oogenesis when the oocyte migrates to the posterior of the follicle. However, recent results suggest that the follicle cells are also involved in defining the two ends of the oocyte, since the reduction of the activities of the neurogenic genes *Notch* and *Delta* in these cells frequently results in the localization of *bicoid* RNA to the posterior as well as the anterior pole (Ruohola et al., 1991).

Conclusions

The results described in this review show that we now have a fairly clear picture of the elegant way that the four maternal signals generate asymmetry in the Drosophila embryo (Figure 9). However, our understanding of the process by which positional information is transmitted from one generation to the next is still incomplete, as so little is known about the origin of asymmetry in the oocyte. While the general organization of each pathway is probably correct, there are still many steps that are poorly characterized. It is also likely that several genes that play a part in these pathways have not yet been identified, and we have already mentioned a couple of examples where the existence of an additional gene has been proposed. Although a few maternal-specific genes may have been missed in the screens for maternal-effect mutations, a potentially much larger class of unidentified genes are those that are required in the zygote as well as in the mother, since mutations in these genes cannot be tested for maternal-effect phenotypes if the mutants die before adulthood. One such gene is *l(1)pole hole*, the Drosophila *raf* homolog. This gene is required at several developmental stages, and its maternal role was only discovered by making germline clones (Perrimon et al., 1985; Ambrosio et al., 1989). *pumilio*, *cactus*, and *torpedo* also mutate to zygotic lethality, but some alleles of these genes are viable, allowing their identification in maternal screens. Since it is possible to make sense of each maternal system without invoking more than a few additional components, it is unlikely that there are a large number of unknown zygotic lethal genes that play a specific role in these processes.

Although there are still many unsolved questions, the early development of Drosophila is probably better understood than that of any other organism. In this context it is worth considering how relevant the information gained in Drosophila is to other developmental systems. It is clear that many of the later developmental processes in flies have counterparts in vertebrates (see the reviews by Ingham and Martinez Arias, 1992; McGinnis and Krumlauf, 1992, this issue), but the early events that we have described are probably less general. Drosophila is unusual in that both major body axes are already defined in the

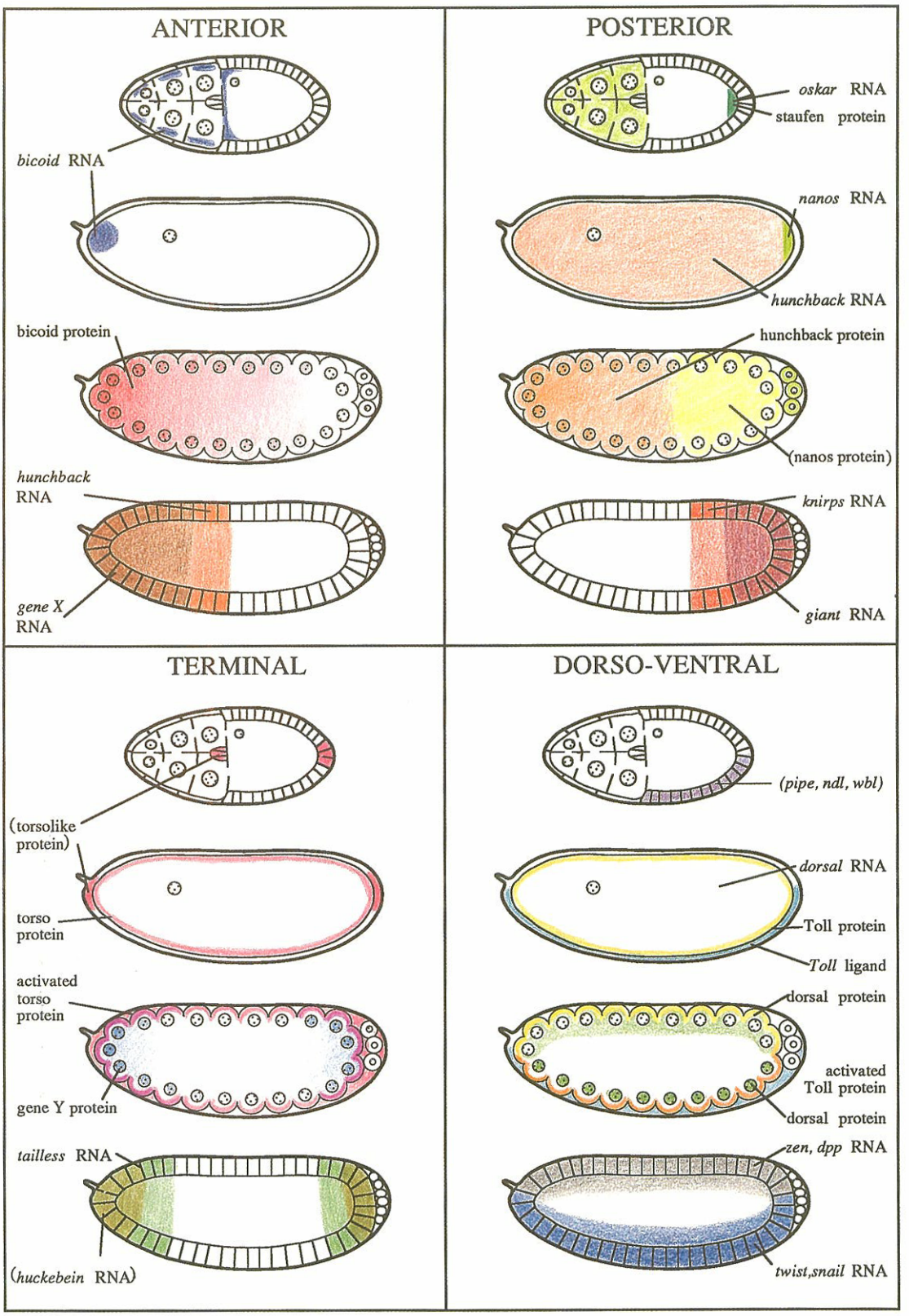

Figure 9. A Summary of the Major Steps in Each of the Four Maternal Pathways

The distributions of gene products shown in parentheses are hypothetical, as are gene X in the anterior system and gene Y in the terminal system.

unfertilized egg, and the first steps of pattern formation occur in a syncytium. However, many of the individual steps in these maternal pathways do resemble processes that occur during the development of other organisms and are therefore of more general relevance, such as RNA localization, signal transduction, induction, and the regulation of nuclear localization.

Drosophila axis formation also provides an excellent model for studying several important pattern-forming mechanisms. For instance, bicoid and dorsal provide the best-characterized examples of morphogen gradients. The gradient of bicoid protein forms by diffusion in a syncytium from a localized RNA source. This is unlikely to be a common mechanism for setting up morphogen gradients, since in most other developmental systems pattern formation takes place after cells have formed, and cell membranes will limit the movement of intracellular factors. The dorsoventral system may provide a more useful paradigm. In this case, the first gradient probably forms outside the egg in the extracellular space, and this leads via a signal transduction pathway to a graded intracellular response, the formation of the dorsal nuclear gradient.

One of the most surprising aspects of axis formation in Drosophila is that although all four maternal systems result in the region-specific activation of zygotic pattern genes, each achieves this by a unique pathway. While two of the initial asymmetric signals are localized outside the egg, the other two are found in the egg cytoplasm as localized RNAs. Furthermore, the spatial control of transcription arises in a number of different ways. The bicoid protein gradient regulates zygotic gene expression directly, nanos seems to function as a repressor of a repressor, and dorsoventral polarity is generated by the control of nuclear localization. It is known that localized maternal determinants play a role in the development of many organisms, but it is only in Drosophila that such molecules have been identified and characterized. The variety of mechanisms found in this one system suggests that maternal determinants may turn out to constitute a diverse collection of molecules that can regulate embryonic development in very different ways.

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