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Determination of the embryonic axes of *Drosophila**

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Summary

The principles of embryonic pattern formation have been studied extensively in many systems using classical experimental approaches. In *Drosophila*, a powerful combination of genetics and transplantation experiments, as well as molecular biology, have helped to elucidate the mechanisms that operate during oogenesis and early embryogenesis to establish a set of positional cues required for axis determination in the early embryo.

In systematic searches for maternal effect mutations a small number of about 30 genes have been identified that specifically affect the process of determination of the embryonic axes. These 'coordinate' genes define four systems that determine the anteroposterior (AP) axis (three systems) and the dorsoventral (DV) axis (one system) independently. In the anteroposterior axis, the *anterior* system determines the segmented region of head and thorax, the *posterior* system determines the segmented abdominal region, and the *terminal* system is responsible for the formation of the nonsegmented termini at the anterior and posterior egg tips, the acron and telson. In contrast, pattern along the dorsoventral axis is determined by one system only. Although all four systems use different biochemical mechanisms, they share several properties. (1) The product of one gene in each system is localized in a specific region of the freshly laid egg and functions as a spatial signal. (2) In each system, this spatial information finally results in the asymmetrical distribution of one gene product that functions as a transcription factor. (3) This transcription factor is distributed in a concentration gradient that defines the spatial limits of expression of one or more zygotic target genes.

The combined action of these three anteroposterior systems as well as the dorsoventral system defines the expression of zygotic target genes in at least seven distinct regions along the anteroposterior and at least three in the dorsoventral axis. These longitudinal and transverse domains provide a coarse spatial prepattern which is then further refined by the action and interaction of zygotic pattern genes.

Key words: *Drosophila*, embryo, embryonic axis, zygotic target genes, zygotic pattern genes.

Introduction

In the life cycle of higher animals, complex forms alternate with simple ones. An individual begins its life as a zygote (a fertilized egg cell), a morphologically simple structure without recognizable similarity to the body of the adult organism. Development often proceeds through a series of juvenile forms, the structure of which corresponds to the way of life of the respective animals. Thus, in every generation, complex form arises *de novo* from a much less complex egg cell.

How complex is the egg cell really? In what manner and to what extent is the body plan of the living animal contained in the structural organization of the egg cell? How many morphogenetic components are already present in the egg cell, where are they localized, and how does their distribution relate to their function in pattern formation? These fundamental questions have occupied the minds of biologists for a long time. Approaches to an understanding must include experiments in which the informational content of the egg cell is artificially altered. In the ideal experiment, one would wish to remove single morphogenetic components from the system, one by one, without affecting any other parameter of the system. The formation of an aberrant pattern would be the consequence. These kinds of experiments are extremely difficult and, in general, it is not possible experimentally to manipulate a system as complex as the egg cell without inflicting severe, unspecific damage. Extraction, pricking, constriction, irradiation, local destruction – all these methods simultaneously affect all components in the respective region, and the required specificity can be obtained only under extremely favourable circumstances. Nevertheless, experiments of these kinds in insect embryos have shown that determinants localized at the anterior and posterior egg pole are involved in the determination of the anteroposterior axis. Destruction (anterior) or transposition (posterior) of the cytoplasm of these, but not of other regions, had most

dramatic consequences on the formation of the embryonic pattern (Kalthoff, 1983; Sander, 1975). These approaches, however, did not lead to the identification and purification of particular morphogenetic components.

For the analysis of the pattern-forming processes during embryonic development, we chose the genetical approach. The experimental basis is to eliminate the function of a gene coding for a morphogenetic component by mutation. The resulting phenotype is a specific abnormal pattern formed by the embryo. The kind of deviation from normal development reflects the function of the respective gene. The power of the genetic approach for the analysis of complex metabolic and regulatory pathways in procaryotes as well as eucaryotes has been demonstrated in numerous cases. However, the applicability of genetics is restricted to organisms that can be used in breeding experiments. Among higher organisms, *Drosophila* is best suited, although initially in chosing it as an object of genetical research, the analysis of embryonic development was not considered at all. For *Drosophila*, methods have been developed that allow the screening of a very large number of mutational hits, a number that is necessary for the isolation of a representative number of mutations affecting embryonic pattern (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard *et al.* 1984; Wieschaus and Nüsslein-Volhard, 1986). It is possible to identify most, if not all, genes, whose products are specifically involved in a particular process with appropriately large scale mutant screens. The analysis of the phenotypes that result from the lack of function of a single component allows important conclusions to be drawn about the properties of the system and the function of a particular gene. With the modern techniques of molecular biology, it is now possible to clone every gene identified by mutations in order to elucidate the structure of the gene product *via* the DNA sequence of the gene. In this essay, the analysis of the processes determining the embryonic axes of *Drosophila* using this genetic approach is described.

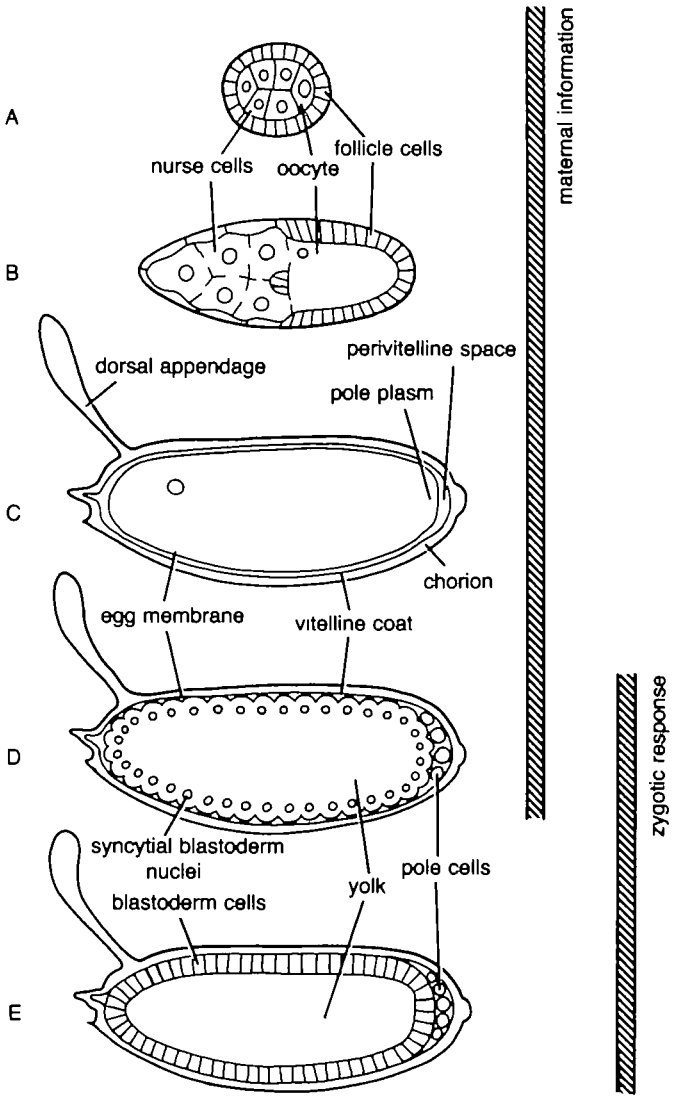

Fig. 1. Scheme of oogenesis and early embryogenesis in *Drosophila*. (A) Follicle at an early stage of oogenesis. The germline-derived nurse cell–oocyte complex is surrounded by a sheet of mesodermally derived follicle cells. (B) Follicle at stage 10. The growing oocyte is located posteriorly to the nurse cells. The follicle cells surrounding the oocyte are thickened. (C) Freshly laid egg. The egg cell is surrounded by two coverings, the vitelline coat and the chorion with its dorsal appendages. Between egg membrane and vitelline coat a space is indicated, the perivitelline space. In the living embryo, this space is only visible at the anterior and posterior tip of the egg, however. (D) Syncytial blastoderm stage embryo (2 h after egg deposition). The cleavage nuclei have migrated to the periphery of the egg cell. The pole cells have formed at the posterior pole. In this stage, transcription of the zygotic genome begins. (E) Cellular blastoderm. First cellular stage of the embryo. Monolayer of 6000 uniform cells.

The identification of coordinate genes

The informational content of the egg cell is built during oogenesis. All the substances present in the freshly laid egg are synthesized and deposited in it during oogenesis. Most of these substances serve metabolic functions of the rapidly developing embryo, while only a few components participate in the formation of the embryonic pattern. Several cell types are involved in building the egg. Follicle cells of somatic origin surround a complex of 15 nurse cells and the posteriorly located oocyte. This complex originates from a single germ cell by mitosis and the 16 sister cells are interconnected by cytoplasmic bridges (Fig. 1). The nurse cells are synthetically active during oogenesis and produce RNA species as well as proteins that are transported into the oocyte. A further supply of metabolic substances occurs *via* uptake from the hemolymph through the follicle cells. The follicle cells produce the egg coverings, the vitelline coat and chorion. Genetically, all substances in the mature egg are under the control of the maternal genome. Genes that fulfil specific functions during oogenesis and early embryogenesis and that are transcribed during

oogenesis are called, in short, 'maternal genes'. In contrast, those genes that are expressed only after fertilization in the embryo are called 'zygotic genes'. For mutants in maternal genes, the embryonic phenotype is displayed in eggs from genetically mutant females, whereas, in the case of zygotic genes, the genotype of the embryo itself determines its phenotype (Fig. 1).

Large-scale mutagenesis experiments for the isolation of maternal effect mutants have been carried out in several laboratories (Gans *et al.* 1975; Mohler, 1977; Perrimon *et al.* 1986; Anderson and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986*a*; Nüsslein-Volhard *et al.* 1987; Schüpbach and Wieschaus, 1989). The analysis of phenotypes, genetic complementation tests and mapping experiments resulted in the identification of a small number of genes that have specific functions in embryonic pattern formation, in the establishment of the spatial coordinates of the developing embryo ('coordinate genes': Sander, 1975; Nüsslein-Volhard, 1979). Mutations in coordinate genes have certain properties. Homozygous mutant flies are viable if they are derived from heterozygous females. Homozygous mutant females produce eggs of normal egg shell morphology, the embryos developing in these eggs, however, die (independent of their own genotype). The lethal phenotype is not caused by a defect in general functions such as nuclear division, cell formation, cell division and differentiation, but concerns the arrangement and formation of body regions. The spatial organization of the embryo is disturbed.

In addition to the strictly maternal coordinate genes, genes with an additional function during the life cycle of the fly are also involved in the establishment of the spatial organisation of the egg. As the lack of function alleles are lethal, their contribution during oogenesis can only be deduced from the existence of viable alleles that only affect the maternal function or the analysis of germ line chimeras (Perrimon *et al.* 1984; Lehmann and Nüsslein-Volhard, 1987).

About 30 coordinate genes have been identified so far (Fig. 3). A number of arguments (e.g. frequency of alleles obtained in the various mutagenesis experiments) suggest that these 30 genes represent more than 70 % of all coordinate genes. It is not yet possible to estimate the total number of the genes with both maternal and zygotic contribution.

The four systems of axis determination

Embryos derived from mutants in a coordinate gene generally lack particular body regions while others may be enlarged or duplicated. The number of different phenotypes is smaller than the number of genes, which means that groups of genes show similar or identical phenotypes. This finding suggests that the genes within such a group participate in one pattern-forming system. Each system specifies those regions of the body that are absent in the mutant phenotype of the group. Four pattern-forming systems can be defined by such groups of genes, three of which determine the anteroposterior (AP) axis and one the DV axis. The pattern along the dorsoventral (DV) axis thus is determined independently of that of the AP axis (Nüsslein-Volhard *et al.* 1987; Nüsslein-Volhard and Roth, 1989).

Within the AP axis, individual regions are determined largely independently. In this manner, the *anterior* system (A) is responsible for the segmented region of head and thorax, the *posterior* system (P) determines the segmented abdomen, and a third system, the *terminal* system (T), determines the nonsegmented acron and telson. In Fig. 2, the phenotypes of the three AP systems are illustrated (Fig. 2B,C,D). The large degree of independence of the systems is indicated by the general additivity of phenotypes; if the function of two of the AP systems is eliminated simultaneously by constructing double mutant embryos, a partial pattern is still formed that reflects the function of the third system (Fig. 2F,G,H). Superposition of the partial patterns of the three possible double mutant combinations results in a fairly complete larval pattern. Only if all three systems are eliminated, does the embryo no longer develop a pattern (Fig. 2E). This means that the three AP systems are necessary and sufficient for the specification of the entire AP axis. There is one exception to the independence that concerns the specification of the terminal nonsegmented regions. The acron depends on both the terminal and anterior systems. If the anterior system is eliminated, the terminal system specifies telson also in the anterior egg region.

Whereas each AP system has one principal lack-of-function phenotype (see Fig. 2), the DV system has two: most of the genes display a dorsalisation as the lack-of-function phenotype (Fig. 7B), but null mutations in one gene, in contrast, produce partial ventralisation (Fig. 7C). Before describing the individual systems, their essential common features will be briefly summarized. The components and their relationships are displayed schematically in Fig. 3.

Each system starts with the localization of a spatial signal within the egg (Figs 4, 6). In two cases, the signal is represented by an RNA that is localized at the anterior or posterior egg pole respectively. In the two remaining systems, the spatial stimulus emanates from the follicle cells, which produce a spatially restricted signal that is released into the perivitelline space surrounding the egg cell. In each system, the local signal, acting through different mechanisms, finally causes the asymmetrical distribution of a maternal gene product that functions as a transcription factor. This factor is often distributed in the form of a gradient that controls the threshold of expression of one or more zygotic genes along the AP or DV axis (Fig. 6). A superposition of the patterns of expression of the zygotic target genes results in a sequence of at least seven unique domains along the AP axis and at least three along the DV axis (Fig. 4).

The anterior system – a morphogenetic gradient

The anterior system is the simplest of the four systems;

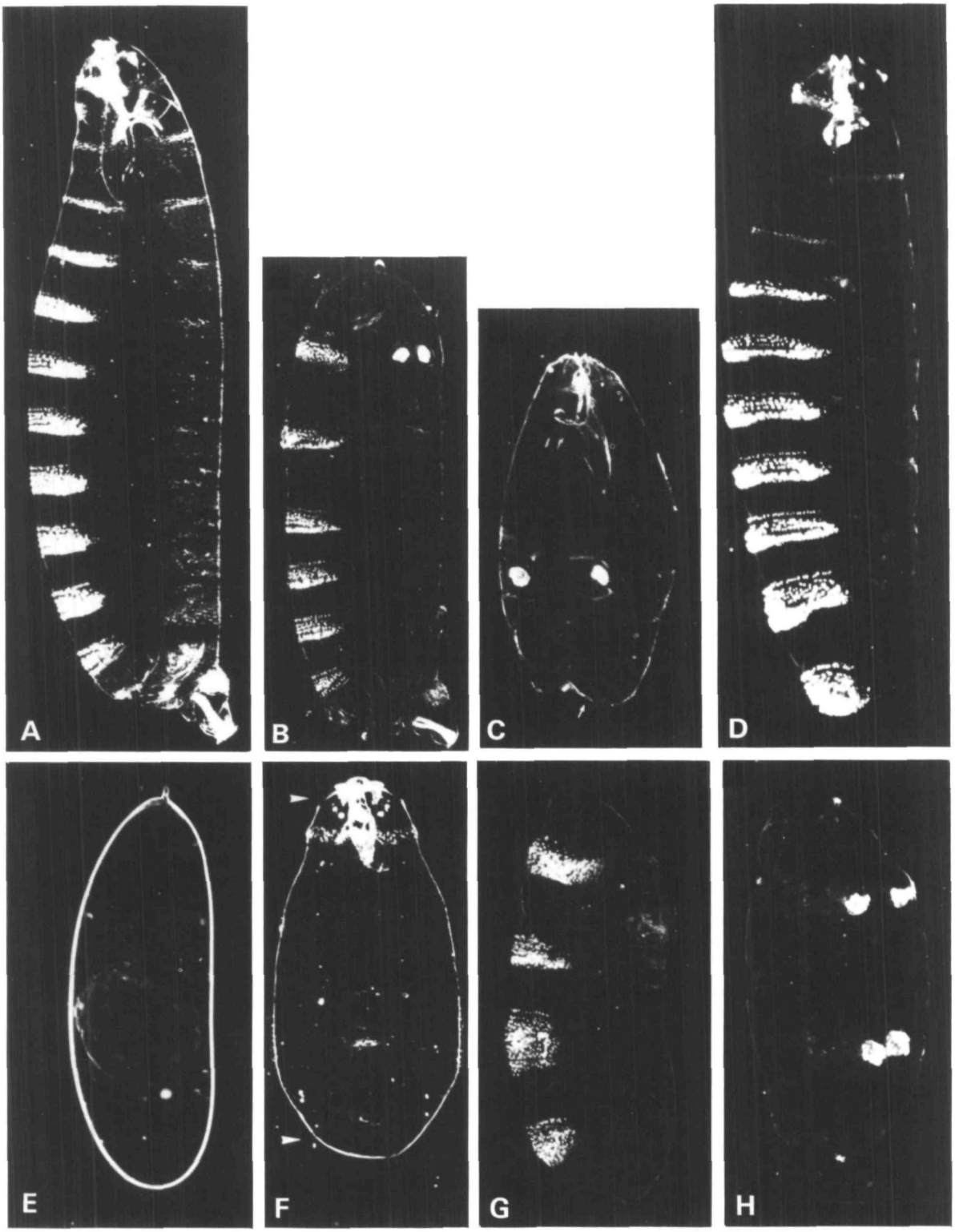

Fig. 2. Phenotype of mutant embryos of the 3 AP systems. Cuticle preparations of differentiated embryos. (A) Normal larva. (B) Phenotype of the A-system (*bicoid*$^-$ embryo). (C) Phenotype of the P-System (*oskar*$^-$ embryo). (D) Phenotype of the T-system (*torsolike*$^-$ embryo). (E) Phenotype of A$^-$, P$^-$ and T$^-$ (triple mutant *bcd osk tsl*). This embryo is still in the vitelline coat. (F) Phenotype of P$^-$ T$^-$ (double mutant *osk tsl*). (G) Phenotype of A$^-$ T$^-$ (double mutant *bcd tsl*). (H) Phenotype of A$^-$ P$^-$ (double mutant *bcd osk*).

further, it is for the time being the best understood. Only one gene, *bicoid* (*bcd*), is indispensable for the determination of all anterior structures, while mutants of the other genes of the system have only partial effects on anterior pattern (Frohnhöfer and Nüsslein-Volhard, 1986, 1987; Schüpbach and Wieschaus, 1986*a*). The products of the *bcd* gene provide both the localized signal and the transcription factor. The *bcd* mRNA is synthesized during oogenesis in the nurse cells and deposited at the anterior egg pole (Frigerio *et al.* 1986; Berleth *et al.* 1988; St. Johnston *et al.* 1989) where it functions as the source of a *bcd* protein gradient (Fig. 5). The gradient probably is formed by diffusion away from the local source and dispersed decay (Driever and Nüsslein-Volhard, 1988*a*). The *bcd* protein contains a homeobox (Frigerio *et al.* 1986; Berleth *et al.* 1988) and functions as an activator of transcription of zygotic target genes (Driever and Nüsslein-Volhard, 1989; Driever *et al.* 1989*b*; Struhl *et al.* 1989). One of these target genes is the gap gene *hunchback* (*hb*). *hb* is transcribed at uniform levels above a particular *bcd* protein concentration (Struhl *et al.* 1989). The threshold concentration that is required for *hb* transcription is reached at about 50 % egg length (Fig. 5). It is determined by the affinity of the promoter for the *bcd* protein. In experiments involving artificial promoter constructs, it has been demonstrated that lowering the affinity of the promoter leads to an anterior shift of the boundary of the expression domain. Assuming the existence of other target genes whose promoters differ in their affinity for the *bcd* protein, the smooth *bcd* gradient thus leads to a subdivision of the egg into several clearly defined domains (Driever *et al.* 1989*a*) (Fig. 6). Candidates for such target genes are *btd, ems* and *otd* (Finkelstein and Perrimon, 1990; Dalton *et al.* 1989; Cohen and Jürgens, 1990).

The localization of the *bcd* RNA at the anterior egg pole during oogenesis is dependent on the activity of at least three coordinate genes of the anterior system. The elimination of the function of *exuperantia (exu), swallow (swa)* or *staufen (stau)* results in a spread of the mRNA towards more posterior regions and a corresponding change in the embryonic fate map (Frohnhöfer and Nüsslein-Volhard, 1987; Berleth *et al.* 1988; Driever and Nüsslein-Volhard, 1988*b*; St. Johnston *et al.* 1989). The analysis of the distribution of *bcd* mRNA during oogenesis and early stages of embryogenesis by *in situ* hybridization has revealed several steps in localization. The RNA is already localized in the nurse cells to apical regions. Upon entering the oocyte, it is bound to the cortex at the anterior of the oocyte. In the freshly laid egg, the RNA occupies a more central position at the anterior dorsal tip of the egg (Fig. 5). The *exu* product is already required at an early stage for the localization in the nurse cells, while the *sww* product appears to be involved in attaching the *bcd* mRNA (perhaps bound to the *exu* protein) to the cortex of the oocyte. In *stau* embryos, the *bcd* mRNA is distributed in a shallow anterior gradient in the egg, while all the early processes occur normally (St Johnston *et al.* 1989).

The 3′ nontranslated end of the *bcd* mRNA contains nucleotide sequences that are required for the anterior localization (MacDonald and Struhl, 1988). These sequences may include sites for specific interaction with the proteins involved in RNA localization. *bcd* RNA, when transplanted into the egg cell, is not transported to the anterior pole; rather it remains localized, as other RNA molecules, at the site of transplantation and may function as the source of the protein gradient at the respective location. In such experiments, a dramatic reorganization of the entire spatial pattern of the embryo can be induced. The *bcd* RNA thus has properties of an organizer determining polarity and pattern with long range influence (Frohnhöfer and Nüsslein-Volhard, 1986; Driever *et al.* 1990).

The posterior system – double negative control

In several ways, the posterior system is similar to the anterior system. Like the anterior cytoplasm, the posterior pole plasm, when transplanted, displays long range effects on pattern formation, and the posterior group of genes is responsible for the formation and activity of this localized source (Lehmann and Nüsslein-Volhard, 1986, 1987; Sander and Lehmann, 1988; Lehmann and Frohnhöfer, 1989; Lehmann and Nüsslein-Volhard, 1991). A closer analysis of the components of the posterior system with embryological, genetic and molecular approaches has revealed, however, that it functions in a strikingly different way from the anterior system (Hülskamp *et al.* 1989; Irish *et al.* 1989; Struhl, 1989; Lehmann and Frohnhöfer, 1989). A central component is the product of the gene *nanos*, (*nos*) that was identified as a localized activity that can induce abdomen formation in mutant embryos of the posterior system (Lehmann and Nüsslein-Volhard, 1991). Recent results indicate that it is the *nos* mRNA that is localized at the posterior pole (Wang and Lehmann, 1991). There is evidence that the *nos* dependent activity spreads anteriorly to about the middle of the egg. For this spread, the function of the gene *pumilio* is required (Lehmann and Nüsslein-Volhard, 1987). Cytoplasmic transplantation experiments in which posterior pole plasm was injected into embryos of various mutant genotypes at various positions indicated that, in the case of *nos*, the activity does not determine polarity and pattern in an autonomous and concentration-dependent manner, as was observed with *bcd*; while the *nos* activity determines abdominal structures, their segmental quality within the abdomen, as well as their polarity, seems to depend on the influence of zygotic target genes expressed in the adjacent regions (Lehmann and Frohnhöfer, 1989). In other words, *nos* is required for abdomen formation, but patterning within the abdomen is determined by the interaction of the products of zygotic target genes of the gap class that depend on all three maternal systems.

The *nos* function is required for the transcriptional activation of the zygotic gap gene *knirps*. Surprisingly, *nos* is not a transcription factor. Its function is indirect, and it acts by elimination of a transcriptional repressor of *knirps*. This repressor is normally present throughout the embryo and is removed from the posterior egg half

by the *nos* function (Fig. 6). If this repressor is absent from the beginning, *nos* function is no longer required for the specification of the abdomen. The repressor of *knirps* has been identified as the maternal product of the gap gene *hunchback* which is homogeneously distributed in the freshly laid egg (Hülskamp *et al.* 1989; Irish *et al.* 1989; Struhl, 1989). As already mentioned, the *hunchback* gene has a later function as a zygotic target gene of *bicoid*. The rationale for this dual function and for the rather complex double negative control is not obvious. Perhaps it can be understood only in the context of the origin of the pattern-forming systems during evolution.

Most of the posterior group genes (Fig. 3) are required for the localization of the *nos* product in the posterior pole plasm of the egg cell. In mutants of five of the seven posterior group genes, the pole plasm is not formed and thus the localization of *nos* mRNA and in addition the formation of the pole cells, the germ line precursors, is blocked (Schüpbach and Wieschaus, 1986*a*,*b*; Lehmann and Nüsslein-Volhard, 1991). For one of these genes, *vasa*, it is shown that the protein product but not the mRNA is localized in the pole plasm (Lasko and Ashburner, 1988; Hay *et al.* 1988). Preliminary evidence suggests that also the products of other posterior group genes, in the form of mRNA or protein, are localized in the pole plasm (Ephrussi *et al.* 1991; St Johnston *et al.* 1991). How these products become localized to the posterior pole after being synthesized in the anterior nurse cells, is still obscure.

The terminal system – local activation of a receptor

While, in the anterior and posterior system, the origins of polarity reside within the germ line, in both the terminal and the dorsoventral system inductive influences coming from the follicle cells play a leading role. In the case of the terminal system, five maternal coordinate genes have been identified (Fig. 3) that share a common phenotype: deletion of the anterior-most and posterior-most regions of the embryo, acron and telson (Schüpbach and Wieschaus, 1986*a*; Nüsslein-Volhard *et al.* 1987; Klingler *et al.* 1988). In germ line chimeras and, more recently, in clones induced by mitotic recombination in the follicle cells, it has been shown that one of these genes, *torsolike*, is active in a subpopulation of follicle cells located at the anterior and posterior tips of the oocyte (Stevens *et al.* 1990). These experiments suggest that these cells produce a signal (perhaps the *torsolike* product) that can activate the egg cell at the anterior and posterior egg pole (Fig. 6). The receptor of this local signal is the protein product of the gene *torso* (Sprenger *et al.* 1989).

Among the genes of the terminal system, *torso* is exceptional as dominant alleles exist, which produce a phenotype that is complementary to the lack-of-function phenotype. In embryos from such mutant females, the segmented middle region is defective while the termini are enlarged (Klingler *et al.* 1988). Genetic experiments indicated that, in these dominant mutants, active *torso* product is also present in the middle region of the egg, which is normally segmented. *torso* activity inhibits segmentation in this middle region by activating the target genes of the terminal system in the wrong position (Klingler *et al.* 1988; Strecker *et al.* 1989). The molecular analysis of the *torso* gene has helped to understand this striking behavior. It encodes a membrane-bound receptor tyrosine kinase (Sprenger *et al.* 1989), which is incorporated in the egg cell membrane (oolemma) of the early embryo (Casanova and Struhl, 1990). The present working hypothesis is that *torso*, in order to fulfill its function, has to be activated by a ligand. This activation normally only occurs at the ends where the ligand is present. Dominant alleles code for a ligand-independent, constitutively active product (Sprenger *et al.* in preparation). The ligand itself may be the product of the gene *torsolike* (Stevens *et al.* 1990).

Activation of the *torso* gene product at the egg poles results in a signal transduction chain that leads to a positive control of transcription of the zygotic target genes *huckebein* and *tailless* (Klingler *et al.* 1988; Weigel *et al.* 1990). The gene encoding the transcription factor for the terminal system (gene Y) has not yet been identified. We predict that the product of gene Y determines the domains of expression of *huckebein* and *tailless* in a concentration-dependent manner, defining two thresholds in a similar fashion to *bicoid* (Fig. 6).

The dorsoventral system – control of the nuclear morphogen concentration

Of all the four systems of axis determination, the DV system is the most complex. Polarity in this system, as in the terminal system, results from local induction by the follicle cells. Position along the DV axis, like in the anterior system, is determined by a concentration gradient of a morphogen that functions as a transcription factor. However, the formation of this gradient involves an entirely new mechanism: the spatially controlled, graded uptake of the morphogen into the nuclei of the syncytial blastoderm stage embryo.

Eleven of the twelve genes of the DV system, the *dorsal* group, display a complete dorsalization as the lack-of-function phenotype: only pattern elements that normally derive from the dorsal-most egg region are formed, with ventral and lateral elements lacking (Nüsslein-Volhard, 1979) (Fig. 7B). In weak alleles, partially dorsalized embryos are formed which lack only the ventral-most elements. For some of the genes (*easter*, *Toll*), alleles have been isolated that have a lateralized or partially ventralized phenotype (Anderson and Nüsslein-Volhard, 1984; Anderson *et al.* 1985*a*,*b*; Anderson and Nüsslein-Volhard, 1986). Finally, the gene *cactus* shows partial ventralization as a lack-of-function phenotype; pattern elements normally derived from the dorsal and dorsolateral region are absent in mutant embryos, while ventral and ventrolateral elements are formed along the entire DV axis (Schüpbach and Wieschaus, 1989; Roth *et al.* 1989) (Fig. 7C). Most double mutants of *cactus* and loss of function alleles of genes of the *dorsal* group display an apolar, lateralized pattern (Fig. 7D); only the double

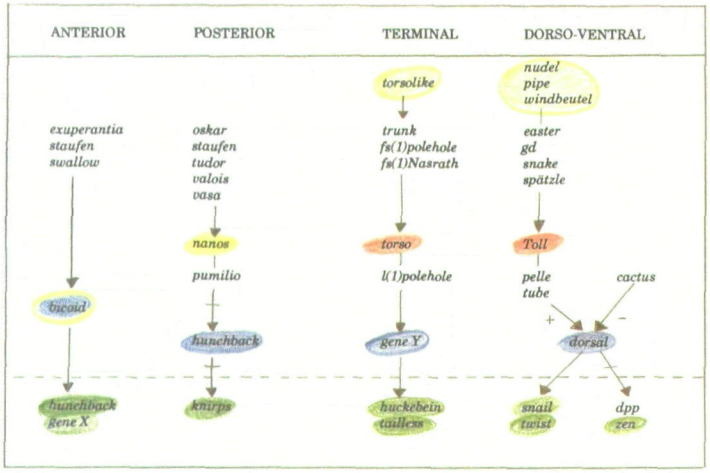

Fig. 3. The genes of the 4 systems of axis determination in *Drosphila*. Above the dotted line: maternal genes, below the dotted line: zygotic target genes of the maternal systems. Yellow: genes encoding the localized signal. In the case of the terminal and dorsoventral system, these have not yet been identified, candidates are the somadependent genes *tsl*, *ndl*, *pipe* and *wind*. Red: genes encoding a membrane-bound receptor. Blue: genes encoding the maternal transcription factor that is asymmetrically distributed. In the anterior system, the transcription factor is the product of the signal, thus both are encoded by the same gene, *bicoid*. Green: putative zygotic target genes of the maternal systems that encode transcription factors.

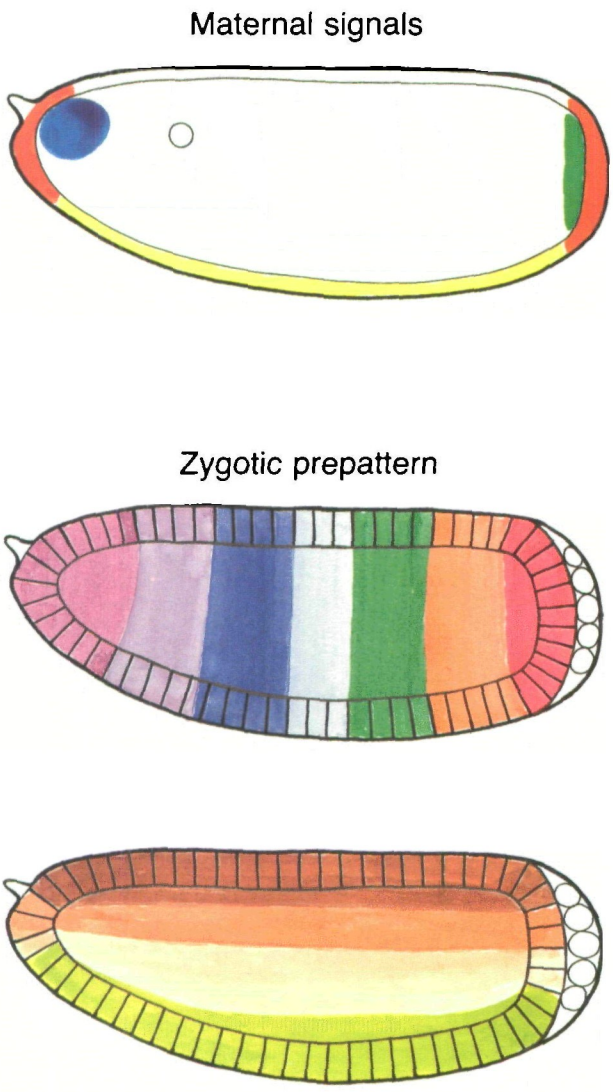

Fig. 4. Model for the development of complexity of the pattern during early embryogenesis. The maternally provided prepattern consists of 4 localized components and specifies a zygotic prepattern of at least 7 unique domains along the AP and 4 along the DV axis.

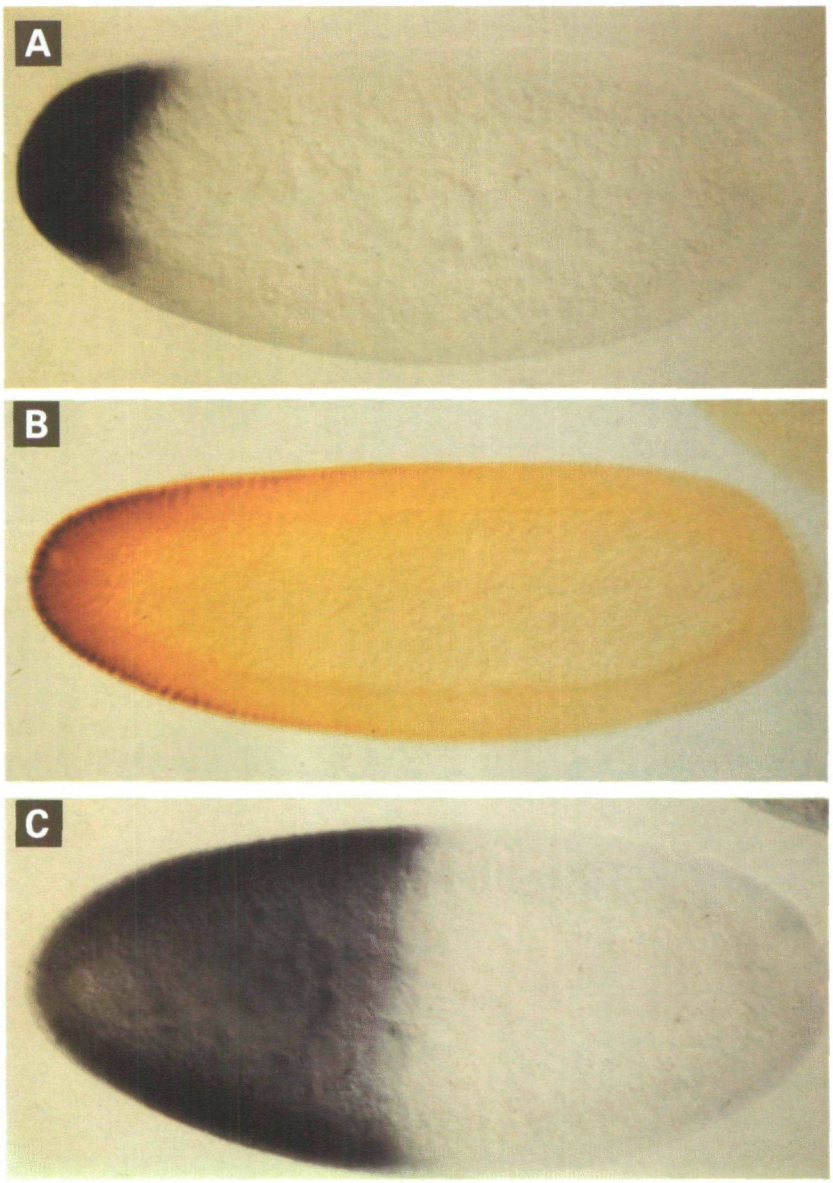

Fig. 5. The distribution of signal, transcription factor and response RNA in the case of the anterior system. (A) *bcd* RNA in the syncytial blastoderm stage embryo. *In situ* hybridization. (B) *bcd* protein distribution in a syncytial blastoderm stage embryo. *bicoid* protein antibody staining. (C) The expression pattern of *hunchback* in the early embryo. *In situ* hybridization.

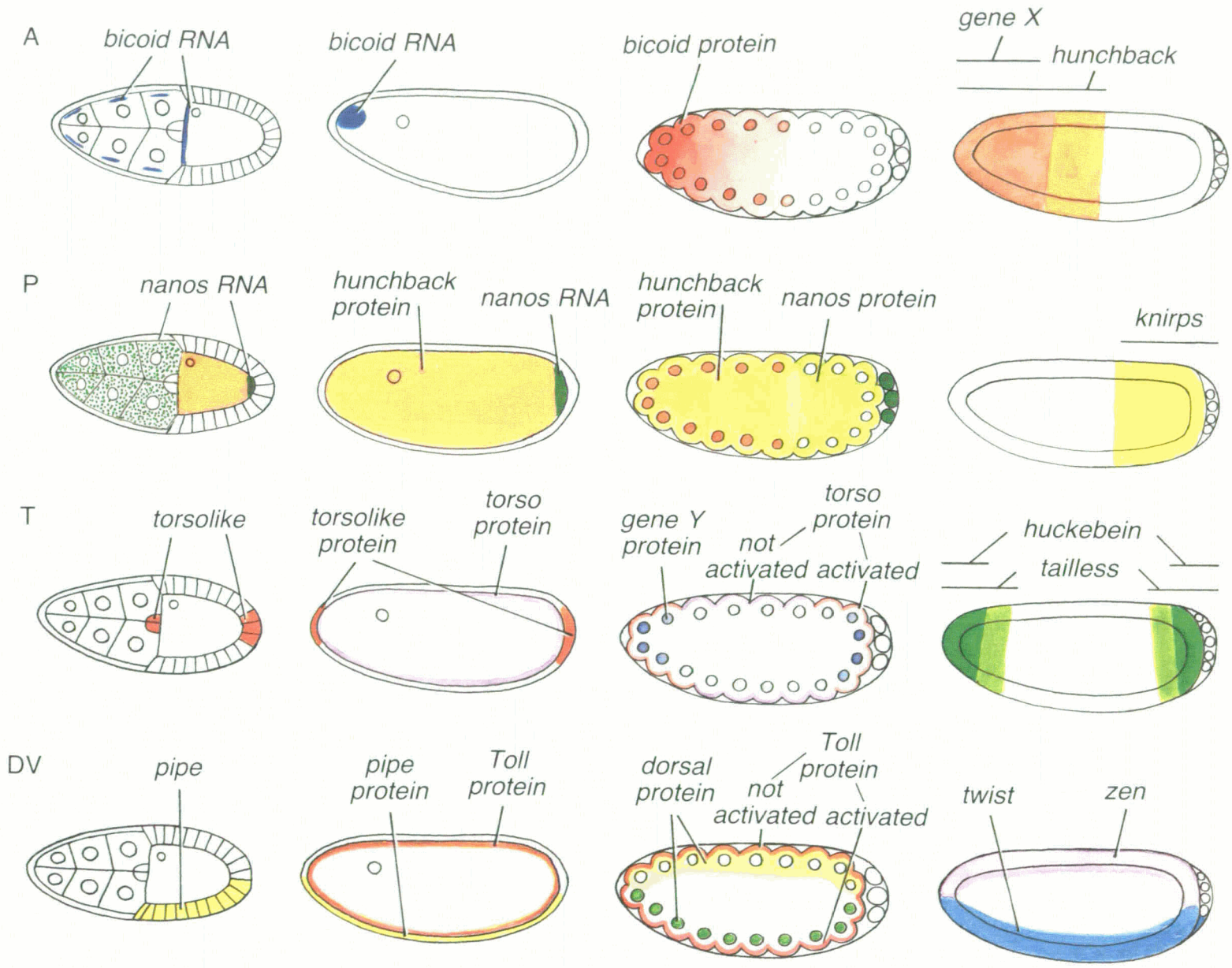

Fig. 6. Model for the function of the 4 systems of axis determination (AP axis systems: A, anterior; P, posterior; T, terminal; DV, DV axis system).

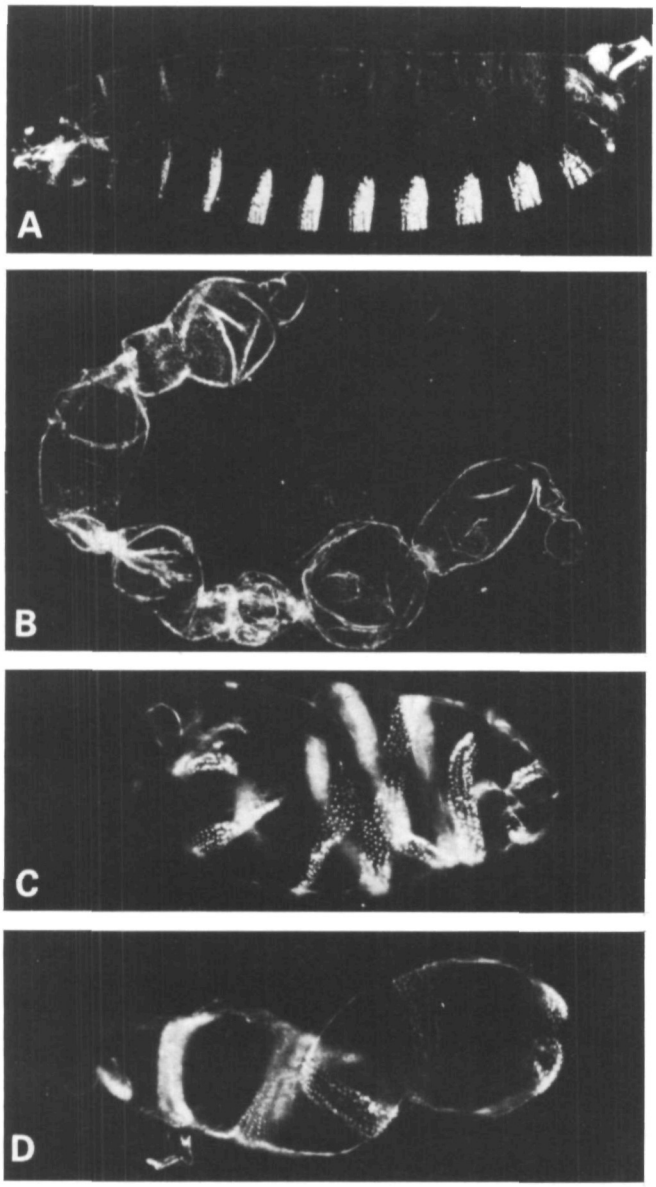

Fig. 7. The phenotypes of the genes of the DV system. (A) Normal larva, anterior left, ventral bottom. (B) Dorsalized embryo, only dorsal cuticle is differentiated (*dorsal*$^-$ embryo). (C) Partially ventralized embryo. Ventral denticle bands surround the entire embryo while dorsal cuticle is lacking (*cactus* embryo). (D) Lateralized embryo. Ventrolateral denticle bands surround the embryo while ventral and dorsal cuticle is lacking. During gastrulation, the embryos in B and D are without polarity while the partially ventralized embryo displays a polar gastrulation pattern with mesodermal invagination at the ventral side only.

mutant *cactus dorsal* has a dorsalized phenotype (Roth *et al.* 1989; Roth *et al.* 1991).

The continuous spectrum of common phenotypes that is observed in mutants of the DV system is best described with a gradient model in which the local concentration of a morphogen determines position along the DV axis (Nüsslein-Volhard, 1979). For several reasons, the gene *dorsal* is the best candidate for the gene coding for this morphogen (Santamaria and Nüsslein-Volhard, 1983; Anderson *et al.* 1985*b*). Recent molecular findings support this hypothesis (Roth *et al.* 1989). The mRNA of *dorsal* (Steward *et al.* 1985) as well as its protein product are homogeneously distributed in the freshly laid egg (Roth *et al.* 1989; Rushlow *et al.* 1989; Steward, 1989). As soon as the nuclei of the syncytial embryo reach the periphery of the egg, however, a gradient of nuclear concentration of *dorsal* protein is observed. In the nuclei of the ventral side of the embryo, the *dorsal* protein is enriched, while it remains in the cytoplasm at the dorsal side (Roth *et al.* 1989; Rushlow *et al.* 1989; Steward, 1989) (Fig. 8A). This gradient therefore is not based, as is the case for *bicoid*, on a net asymmetry of distribution of the protein, but in contrast, the total *dorsal* protein concentration is uniform along the DV axis. This fact is most apparent during the mitoses of the cleaving embryo, during which the *dorsal* protein is released from the nuclei (Roth *et al.* 1989). The *dorsal* gene codes for a protein with homology to the oncogene *rel* (Steward, 1987), and the transcription factor NFκB (Kieran *et al.* 1990; Ghosh *et al.* 1990). It presumably functions as a transcription factor. The domains of expression of the zygotic genes *twist* and *zen*, which are normally located in longitudinal stripes along the ventral (*twist*, Thisse *et al.* 1988) and dorsal (*zen*, Rushlow *et al.* 1987) midline, are dependent on the local nuclear concentration of the *dorsal* protein (Roth *et al.* 1989; Rushlow *et al.* 1989; Steward, 1989). It appears that *dorsal* can influence the transcription of target genes in both a positive (*twist*) and a negative (*zen*, Doyle *et al.* 1989) fashion.

The nuclear uptake of the *dorsal* protein is controlled by the genes of the *dorsal* group and by *cactus*. In mutant embryos of all these genes, the *dorsal* protein is present in normal amounts (Roth *et al.* 1989). In dorsalized mutants of the *dorsal* group, it is not taken up by the nuclei but remains in the cytoplasm even at ventral positions, while in *cactus* it is taken up by the nuclei also at the dorsal side (Fig. 8B,C). In lateralized embryos, it is equally distributed between cytoplasm and nuclei (Roth *et al.* 1989). Therefore, *cactus* functions as an inhibitor of the nuclear uptake of the *dorsal* protein, and the genes of the *dorsal* group, in contrast, provide a positive stimulus for the nuclear uptake on the ventral side of the egg.

In providing the ventral stimulus, among the *dorsal* group genes the *Toll* gene plays a central role (Anderson *et al.* 1985*a*,*b*). *Toll*, like *torso*, has gain-of-function alleles with a phenotype complementary to the dorsalized lack-of-function phenotype. *Toll* encodes a membrane protein that is evenly distributed in the egg membrane (Hashimoto *et al.* 1988), and therefore may, like *torso*, function as a receptor. Recent experiments (Stein *et al.* 1991) have shown that the expression of three of the *dorsal* group genes (*nudel, pipe, windbeutel)* is required in the follicle cells. By analogy to *torsolike*, they may be involved in providing a ligand for the *Toll* receptor. Two other *dorsal* group genes, *snake* and *easter*, have been shown to encode secreted

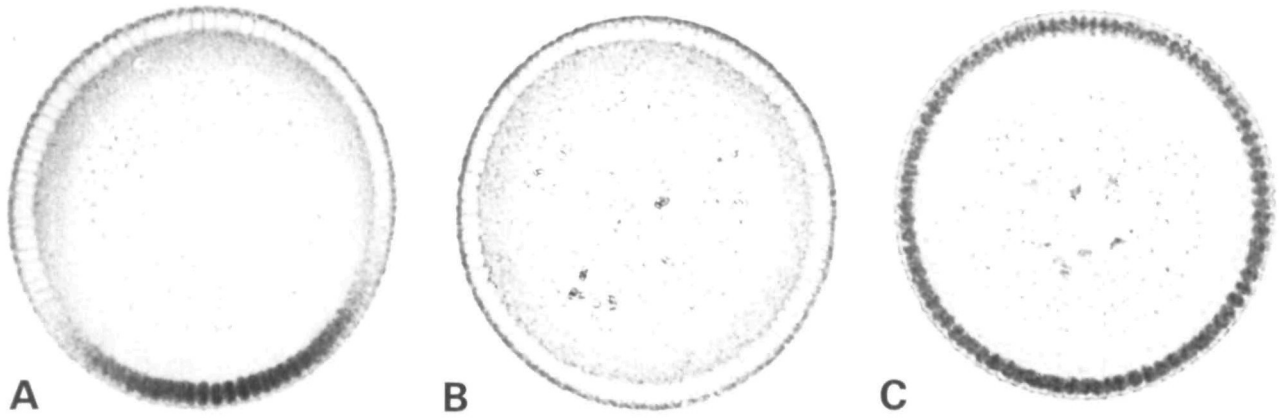

Fig. 8. Distribution of the *dorsal* protein in normal and mutant embryos in the blastoderm stage. Staining with a polyclonal *dorsal* protein antibody. 10 μm araldite sections. (A) Normal embryo. Gradient of nuclear *dorsal* protein concentration with maximum in ventral midline (bottom). (B) Dorsalized embryo (*pelle*$^{-}$). *dorsal* protein is exclusively in the cytoplasm. (C) Ventralized embryo (Tl^{10b} embryo). *dorsal* protein is almost exclusively in the nuclei all around the embryo.

serine proteases (DeLotto and Spierer, 1986; Chasan and Anderson, 1989). Analogies to the terminal system (Sprenger *et al.* 1989, and in preparation; Stevens *et al.* 1989) as well as recent transplantation experiments (Stein *et al.* 1991) suggest the following model (Fig. 8). A ligand for the *Toll* receptor is produced by specialized follicle cells facing the ventral side of the oocyte. The ligand is localized in the perivitelline space, perhaps attached to the vitelline coat. During early stages of embryogenesis, it is released by the products of *easter* and *snake* and bound by the *Toll* receptor, thereby activating it. The activation of *Toll* results, in a manner not yet understood, in the nuclear uptake of the *dorsal* protein (Fig. 6).

Synopsis

The origin of the embryonic prepattern

Starting from the four localized signals, the four maternal systems of axis determination culminate in the asymmetric distribution of four components, the gene products of the genes *bicoid*, *nanos*, gene Y and *dorsal*. With the exception of *nanos*, these are morphogens in the classical meaning of the word: they are distributed in gradients and determine positions along the axes in a concentration-dependent manner. The maternal morphogens presumably act as transcription factors that control the spatial domains of transcription of zygotic pattern genes (Fig. 6). The target genes of the morphogens of the AP systems are the gap genes (Nüsslein-Volhard and Wieschaus, 1980). Those of the DV systems are a number of zygotic genes that are expressed in longitudinal domains along the DV axis. Target genes of different affinity to the morphogen respond with different threshold concentrations, such that the graded distribution of a morphogen can define a sequence of several regions (Driever *et al.* 1989*a*,*b*). Considering the function of each individual system separately, the *bicoid* gradient determines at least two zones, *nanos* one (as mentioned earlier, in this case not in a direct manner but through a double negative control involving the transcription factor *hunchback*), and the morphogen of the terminal system specifying two symmetrically duplicated regions (Fig. 6). The interaction of the three systems finally results in the formation of a pattern of at least seven domains along the AP axis. In the case of the anterior terminal region, these interactions are combinatorial, here gene Y, together with a high *bcd* concentration, defines acron, while in the absence of *bcd* it defines telson. Further, interactions between the products of the target genes play an important role. The description of these interactions is beyond the scope of this article. They are important for the exact definition of the boundaries that initially are defined in a rather course manner by the maternal transcription factors. In the case of the DV axis, the *dorsal* gradient alone appears to define at least three, most likely four domains along the DV axis. The *dorsal* protein functions in this process both as an activator (target: *twi*) and a repressor (*zen*). The molecular prepattern of the embryo in the blastoderm stage, that is determined by the four systems of axis determination, is thus already much more complex than the distribution of the four signals present in the freshly laid egg (Fig. 4).

The origin of polarity

The spatial arrangement of the signals in the egg has its basis in the architecture of the follicle as it arises in the ovarium. In two of the systems, the signal is represented by localized mRNA. Synthesis as well as anchoring of this RNA occurs within the germ line-derived oocyte–nurse cell complex. In contrast, the two other systems, the terminal and the dorsoventral, show striking similarities with inductive systems. For the origin of polarity, a close contact between two different cell types, follicle cells and oocyte, is imperative. The signal is presumably created in a spatially restricted region

within the follicle cell sheet and is received by the activation of receptor molecules in the egg membrane. A special feature of the maternal systems, in this context, is the temporal delay between the production and the function of the signal. The signal is active in the early embryo, at a time when the follicle cells have long disappeared and have left only inviable egg coverings, the chorion and the vitelline coat, behind. Despite this peculiarity, there are several parallels in other systems of cell biology that help to explain the molecular details of these signal transduction mechanisms.

The intrinsic polarizing processes that are reflected in the localization of the signals originate during the development of the follicle and its polarity. These processes precede, both temporally and functionally, the processes in which the maternal coordinate genes are involved. The polarization of the nurse cell–oocyte complex, in particular the singling out of one of the 16 daughter cells as the oocyte, determines the orientation of the gradients of the AP axis. It is to be expected that an elaborate process, comparable to the pattern-forming process of axis determination, is responsible for pattern formation within the follicle cell epithelium. Genes whose phenotypes affect the pattern of the chorion (derived from the follicle cells) as well as that of the embryo are involved in this earlier process (Wieschaus *et al.* 1978; Schüpbach, 1987; Manseau and Schüpbach, 1989). For a complete understanding of axis determination, the elucidation of these early processes is of prime importance.

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