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# A Cascade of Transcriptional Control Leading to Axis Determination in *Drosophila*

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The early *Drosophila* embryo develops through a series of rapid syncytial nuclear divisions. The nuclei migrate to the egg membrane at the periphery to form individual cells. This formation is achieved by the membrane extending between the nuclei to generate a single-layered cellular epithelium, the blastoderm (1). These processes occur in parallel with pattern-forming events that establish a molecular prepattern of the *Drosophila* body plan as defined by the expression of segmentation genes in a series of stripes along the anterior-posterior axis of the blastoderm embryo (2).

Pattern formation along the anterior-posterior axis is initiated by three asymmetrically distributed maternal transcription factors, and their activities are mediated through zygotic transcription factors that subdivide the embryo into increasingly smaller units [for reviews, see (2–4)]. At the first zygotic level of this cascade of transcriptional events, the locally activated gap genes add a new series of overlapping short-range transcription factor gradients to the preexisting maternal long-range gradients. The gap gene-encoded transcription factors (2) act horizontally by locally repressing the activation of neighboring gap genes and vertically through interactions with distinct cis-acting modules mediating stripe expression of the pair-rule genes (2). We report that most of the established genetic interactions within the segmentation gene cascade depend on direct DNA–protein interactions. The mechanisms allowing to generate positional information for localized gene expression along the anterior-posterior axis are discussed.

## MATERNAL GRADIENTS THROUGH DIFFUSION AND TRANSLATIONAL REPRESSION

The homeodomain transcription factor Bicoid forms an anterior-to-posterior concentration gradient that emanates from maternally prelocalized mRNA in the anterior pole region of the egg (3). After translation, Bicoid diffuses to form a concentration gradient extending from the source, the site of mRNA localization, toward the posterior. Bicoid is required for the activation of zygotic segmentation genes, which establish head and thoracic segments in the embryo [reviewed in (2–4)]. Recently, a second function of Bicoid appeared with the discovery that Bicoid binds to evenly distributed maternal *caudal* mRNA via its homeodomain and represses its translation (5,6). This phenomenon, translational suppression, causes a second homeodomain protein gradient in posterior-to-anterior direction (7,8), thus complementing the gradient of Bicoid (Fig. 1a).

The third maternal transcription factor is the zinc finger protein Hunchback, which becomes asymmetrically distributed in response to *nanos* activity [reviewed in (3)]. The *nanos* mRNA is localized to the posterior pole region, and the protein emanating from this source results in a posterior-to-anterior concentration gradient, which is required for abdomen formation. Nanos (with uniformly distributed Pumilio and other not yet identified proteins) binds to the *nanos* response element located within the 3′ untranslated region of evenly distributed maternal *hunchback* mRNA and causes its translational repression in the posterior region of the embryo (3,9,10). Hunchback acts as a strong transcriptional repressor of posteriorly expressed gap genes such as *knirps* and *giant* [reviewed in (2,4)]. The generation of a Hunchback-free region in the posterior half of the embryo (by combined *nanos* and *pumilio* activities) is therefore necessary for the expression of the posterior gap genes by Caudal and Bicoid [reviewed in (4)].

## CONTROL OF FIRST ZYGOTIC GENE ACTIVITIES: MATERNAL INPUT AND CROSS TALK

Activation of the gap genes depends on Bicoid, Caudal, Hunchback, [reviewed in (3,4)] and an as yet unknown transcription factor, which is activated in the terminal regions of the embryo in response to the *torso*-dependent raf/ras signal transduction pathway [reviewed in (11)]. Expression of the gap genes are found in specific regions of the preblastoderm embryo (Fig. 1b), which fail to develop in the respective mutants [reviewed in (2)]. They include the terminal gap genes *tailless* and *huckebein,* the head segmentation genes *orthodenticle, empty spiracles,* and *buttonhead,* and the central gap genes *hunchback, Krüppel, knirps,* and *giant,* which encode transcription factors containing homeodomains, zinc fingers, or a basic leucin zipper as their DNA-binding motif [reviewed in (2)]. Gene interaction studies (2,4) have revealed an elaborate genetic network (Fig. 2) showing that (a) terminal gap genes are activated by the maternal terminal system, the *torso*-dependent ras/raf signal transduction pathway,

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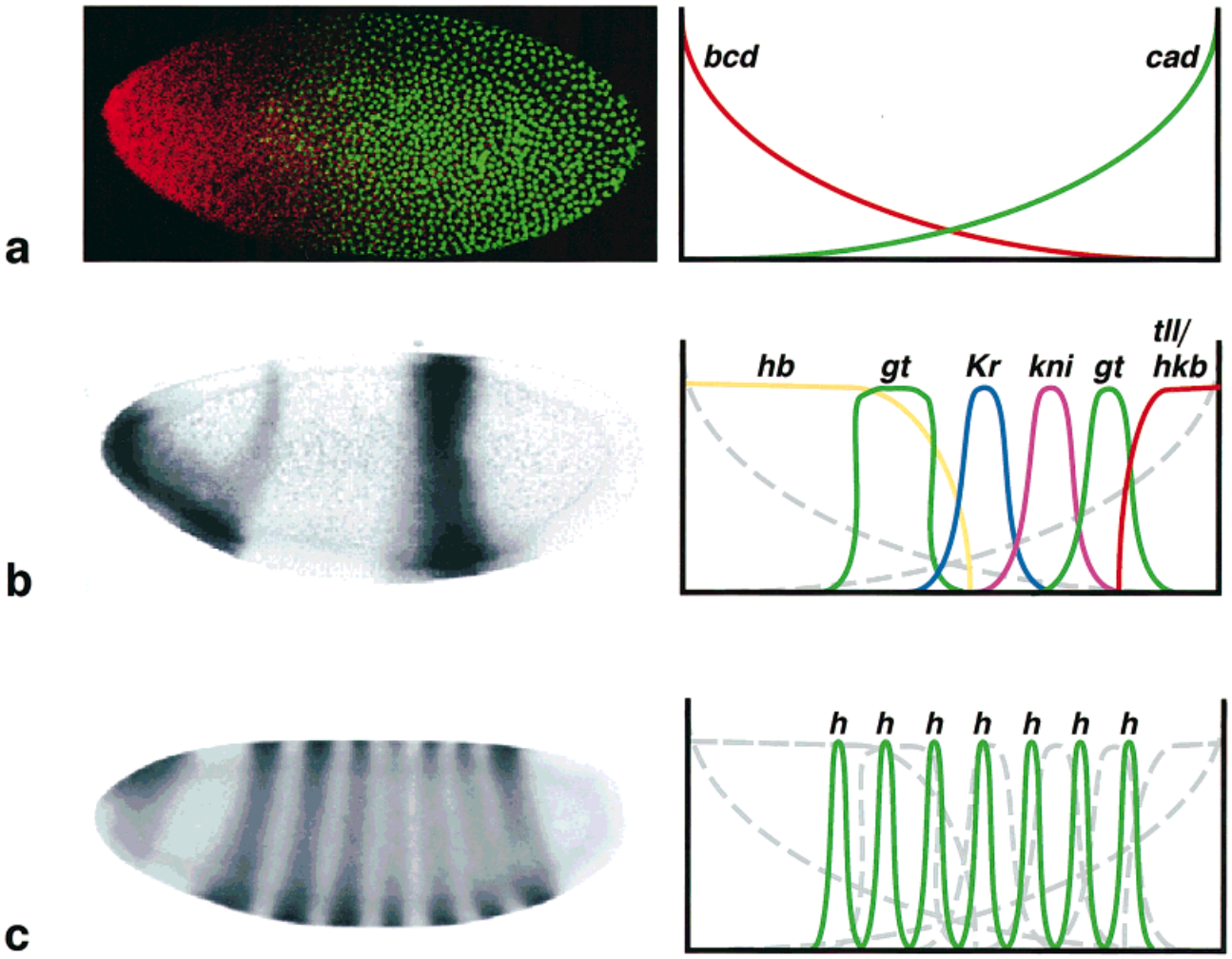


Fig. 1. Examples of maternal and zygotic *Drosophila* patterning gene expression (**left**) and a schematic illustration of the distribution of maternal and zygotic transcription factors at different levels of the segmentation gene cascade (**right**). Orientation of embryos is anterior is left and dorsal is up. **a:** Pattern of the distribution of Bicoid (red) and Caudal (green) and their distribution (right) along the anterior-to-posterior axis in the preblastoderm embryo. **b:** Example of gap gene expression (*knirps*; left) and distribution of the various gap gene-encoded transcription factors along the longitudinal axis, adding to the preexisting maternal gradients (right). **c:** Expression of a pair-rule gene (*hairy*), which adds a series of seven evenly spaced stripes along the anterior-posterior axis (right). bcd, *bicoid*; cad, *caudal*; h, *hairy*; also see legend of Figure 2.

which acts through an unknown transcription factor; (b) head segmentation genes are activated by Bicoid (which is likely to involve a synergistic interaction with maternal Hunchback); (c) central gap genes are activated by either Bicoid and Hunchback (zygotic *hunchback* expression) through Bicoid and Hunchback independently (*Krüppel*) or by Bicoid and Caudal (*knirps* and *giant*); (d) the setting of the spatial limits of the central gap gene expression domains involves repression by the neighboring gap genes except for terminal gap genes, which are controlled independently of the others by the terminal raf/ras signal transduction pathway; (e) *tailless* and *huckebein* activities repress the other zygotic segmentation genes that would otherwise be activated at both ends of the embryo; (f) segmentation genes are able to control target gene expression in several different ways. For example, Hunchback synergistically interacts with Bicoid to control zygotic *hunchback* expression, acts as an independent activator of *Krüppel*, and is a strong repressor of *knirps* expression in the posterior region of the embryo.

A key conclusion of the genetic analysis was that, except for the terminal gap genes, gap gene activation rests on the two activators Bicoid and Caudal [reviewed in (4)]. The absence of Bicoid causes the absence of the head segmentation gene expression and zygotic *hunchback* expression, whereas *Krüppel, knirps,* and *giant* in the central and posterior region of the embryo are not affected. In contrast, the absence of Caudal (which, like Hunchback, is expressed both maternally and zygotically) (7,8) has no effect on *Krüppel* but causes the absence of posterior *giant* expression and reduces *knirps*

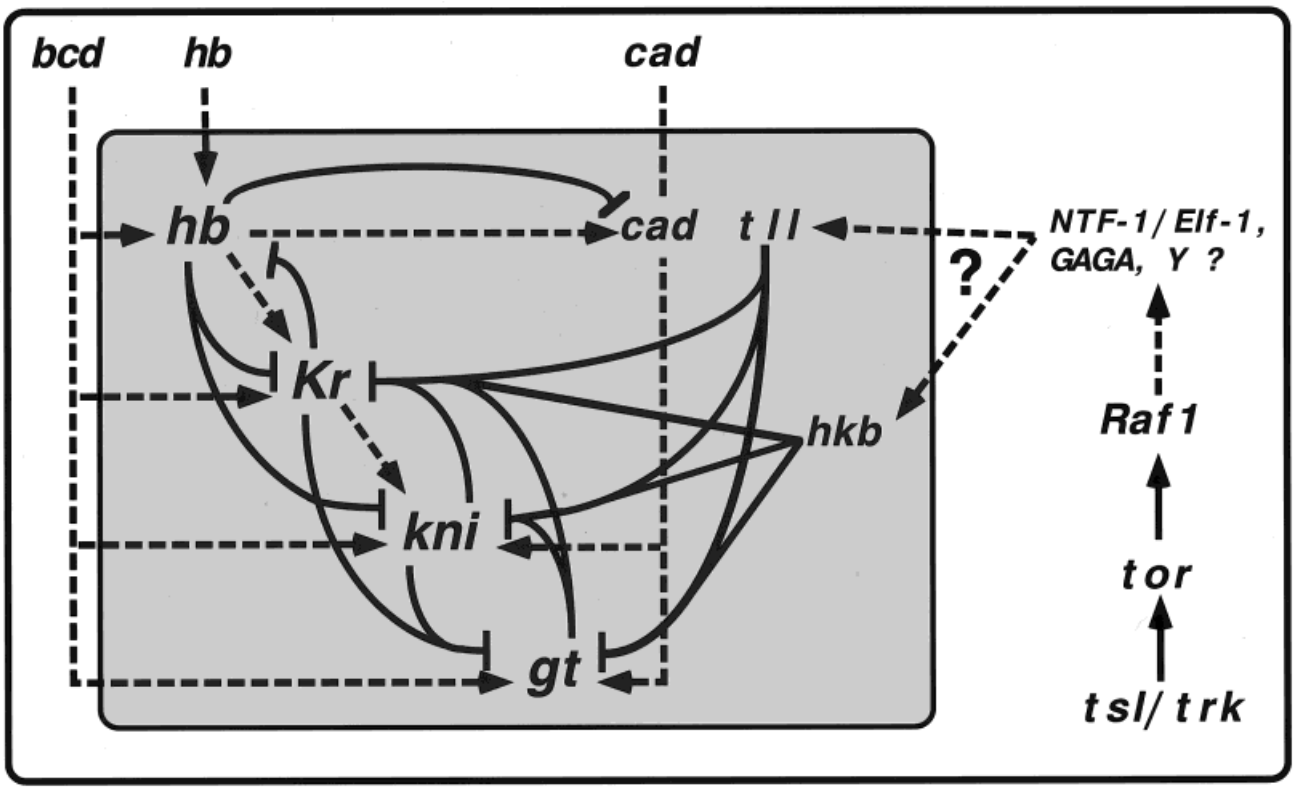


Fig. 2. Genetic interactions required for the proper spatial expression of the gap genes *hunchback* (hb), *Krüppel* (Kr), *knirps* (kni), and *giant* (gt), which are required for segmentation of the trunk region. They are under the control of maternal factors encoded by bicoid (bcd), hb, and caudal (cad). Activation of these "central" gap genes is antagonized by repression by the terminal gap genes *tailless* (tll) and *huckebein* (hkb), which are activated and spatially controlled through the activity of the maternal *torso* (tor)-dependent signal transduction pathway. For a description of this pathway, which is not part of this review, see (11).

expression (Fig. 3a–f). The *knirps* is still expressed in embryos lacking *caudal* activity (Fig. 3d) because Bicoid can partly substitute for the lack of Caudal activity by activating *knirps* (12). When both Bicoid and Caudal activities are absent from the embryo (Fig. 3g,h), the anteriorly and posteriorly acting gap genes fail to be expressed, and consequently, the expression of pair-rule genes, as visualized by *hairy* expression (Fig 1c) [reviewed in (2)], does not occur. The resulting embryo shows no sign of segmentation (Fig. 3i).

In summary, genetic analysis revealed all the factors that are necessary for the activation and for the spatial control of the first zygotic genes, which are required to generate segments in the trunk region of the embryo. Bicoid and maternal Hunchback act in the anterior and central regions of the embryo, and the posterior activation of gap gene rests on the redundant activities of Bicoid and Caudal. All of the Bicoid- and Caudal-dependent genes are suppressed by terminal gap gene activities.

## GENERATING ADJACENT GAP GENE DOMAINS

Molecular dissection of the cis-acting sequences, which are both necessary and sufficient to conduct reporter gene expression within the *hunchback*, and *Krüppel* and *knirps* expression domains of transgenic embryos provided mechanistic models of how the genetically defined trans-acting factors control position-dependent gap gene expression in the preblastoderm embryo (2,12).

Bicoid binds to the cis-acting region required for zygotic *hunchback* expression (13,14). This binding is sufficient for the activation of the gene but fails to spatially regulate the expression domain in Hunchback-depleted embryos (15). Cell-free transcription reactions have been described (16,17) that recapitulate transcriptional synergism directed by Bicoid and maternal Hunchback. Two specific coactivator subunits of the basal transcription factor TFIID serve as targets to mediate transcriptional activation by synergistic Bicoid and Hunchback activities. The TATA-binding protein and three coactivator subunits (TAF$_{II}$250, TAF$_{II}$110, and TAF$_{II}$I60) mediated transcriptional synergism in response to Bicoid and Hunchback, whereas complexes lacking either TAFII110 or TAFII60 resulted in nonsynergistic activation (16,17). This in vitro finding provides a model of how the concerted action of the two factors establishes the spatial domain of zygotic *hunchback* expression: spatial information is generated by a synergistic interplay between the two factors and the basal transcription machinery.

*Krüppel* expression (posteriorly adjacent to the zygotic *hunchback* expression domain) may be activated

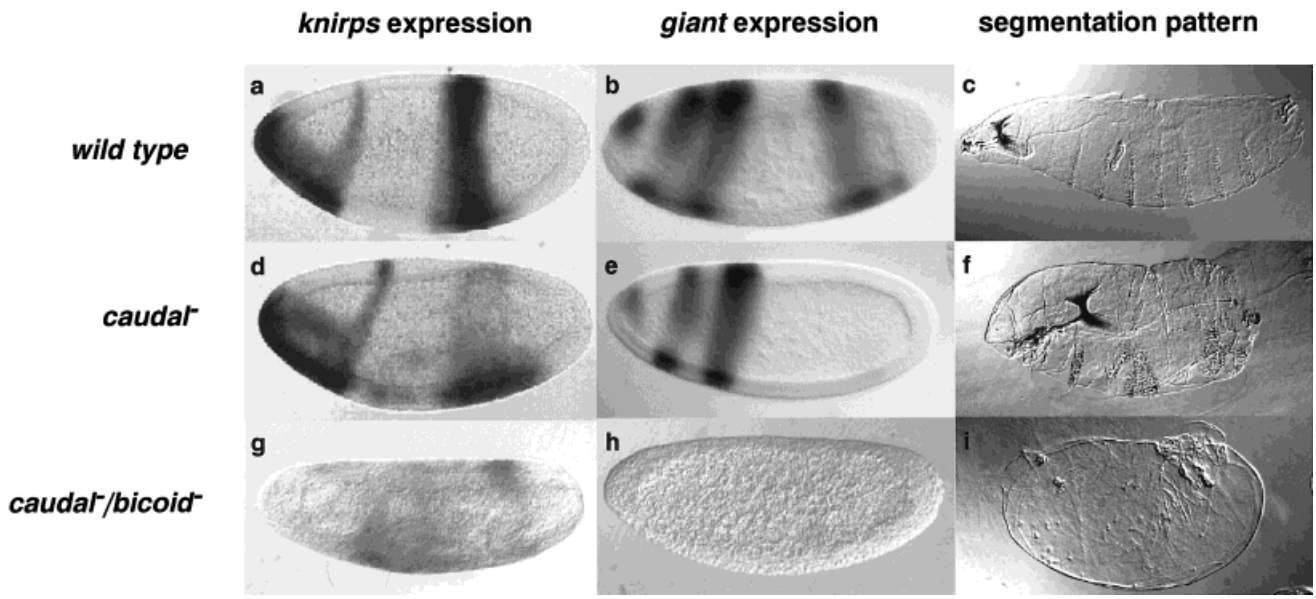


Fig. 3. Expression of the gap genes *knirps* and *giant* in wild-type (**a,b**), *caudal*-deficient embryos (**d,e**), and in embryos lacking *caudal* and *bicoid* activities at the same time (**g,h**). Cuticle preparations showing the wild-type segmentation pattern (**c**), the *caudal* lack-of-function phenotype (**f**), and the lack of segmentation in embryos lacking both *caudal* and *bicoid* activities (**i**). Note the formation of abdominal segments in *caudal*-deficient embryos (f) due to the activation of posteriorly acting genes through Bicoid.

in a similar manner, and activation of *Krüppel* in the region occupied by zygotic Hunchback may be suppressed. However, Bicoid and Hunchback can act as independent activators of *Krüppel* expression, and gap genes expressed adjacent to the *Krüppel* domain counteract the activation through repression (18). The *Krüppel* cis-acting element contains multiple overlapping binding sites for the activators Hunchback and Bicoid and the repressors Knirps, Giant, and Tailless. In vitro studies combined with cell culture experiments have shown that the binding of activators and repressors are mutually exclusive and that high repressor concentrations prevent activators from binding (18). These findings would explain why the activation of *Krüppel* occurs only in the central region of the embryo, where the concentrations of the multiple repressors are too low to compete for the binding of the activators. In fact, when the repressors Knirps, Giant, or Tailless were expressed ubiquitously (under the control of an inducible heat-shock promoter), their activities were found to either reduce or abolish *Krüppel* expression (18) (unpublished results). Thus, the current data suggest that *Krüppel* is activated broadly and that its spatial restriction is brought about by a redundant repressor system composed of the activities of neighboring gap genes.

The *knirps* expression, which is posteriorly adjacent to the *Krüppel* domain, is mediated by a modular array of nonoverlapping elements, where activators and repressors can bind in parallel (12). Activators are Bicoid and Caudal, which bind to separate activator modules; repressors include Tailless, Giant and Hunchback, which bind to separate repressor modules (12). Thus, the repressors cannot function by preventing the binding of the activators but rather through a phenomenon termed "quenching" (19) by interfering with activator function through protein–protein interactions with the activators or the basal transcription machinery [reviewed in (20)].

The control of head segmentation gene expression and the patterns of *giant* expression have not yet been studied beyond genetic analysis. The collection of players and their impact on gene expression domains make it likely, however, that the regulation employs mechanisms similar to those described for gap genes. Also, activation of the terminal gap genes *huckebein* and *tailless* is not yet fully understood. Studies on *tailless* regulation suggest that the raf/ras-dependent activation involves a derepression mechanism through the transcription factors GAGA and NTF-1/Elf-1 (21).

## MECHANISMS

The available evidence suggests that the spatial control of gap gene expression domains mainly involves mutual repression [reviewed in (2,12)]. One mechanism of repression involves the competitive binding of repressors and activators to overlapping sites within the cis-acting element (Fig. 4a). Different affinities of corresponding binding sites within the enhancer sense local combinations and concentrations of the relevant factors and thereby determine the spatial limits of the expression domain (20,22). Such a mechanism is intuitively easy to understand, but it could not account for the sharp on/off border of gene expression, as seen in the embryo [reviewed in (20)], which argues for cooperative interactions between the factors that bind. Quenching, as an additional mode of repression, implies that the factors that are bound to the cis-acting region are able to interact with each other before or while they communicate with the basal transcription machinery (Fig. 4b). Such interactions have been observed in tissue culture and in vitro by showing that Krüppel, Hunchback, and Knirps are able to associate when bound to DNA [re-

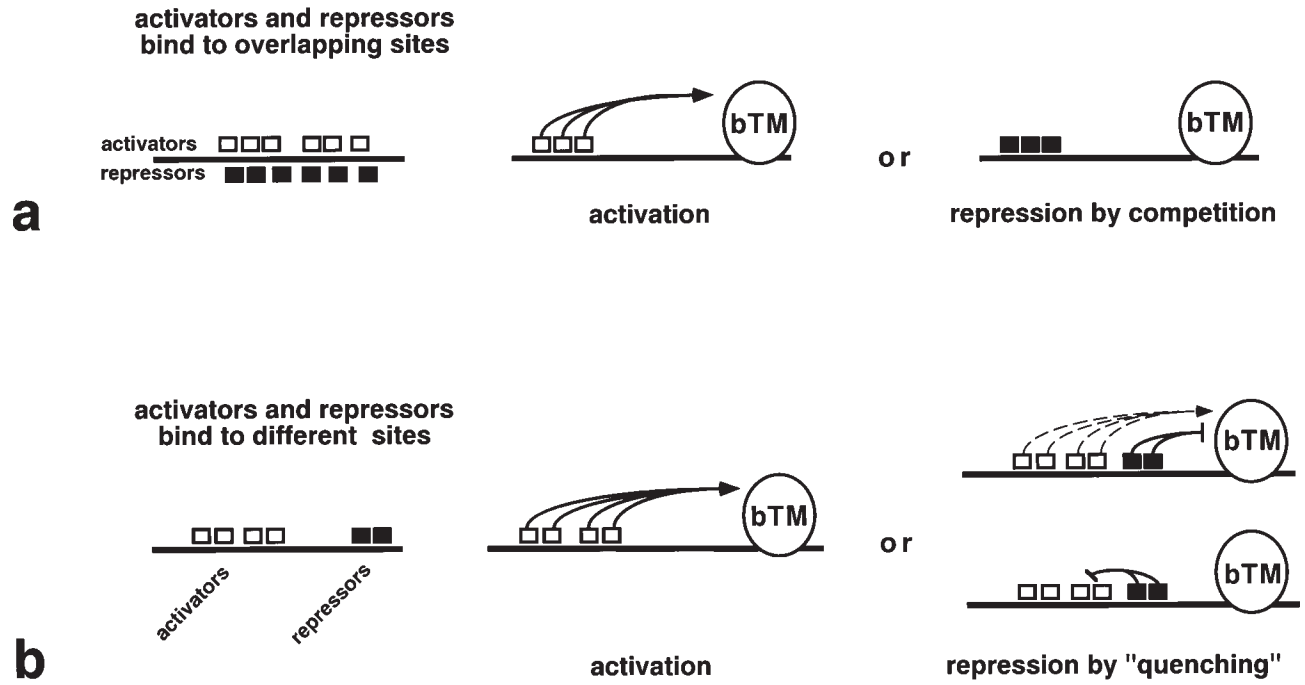


Fig. 4. Mechanisms of spatially regulated gene expression in the blastoderm embryo. **a:** Schematic representation of a cis-acting element (black bar) containing overlapping binding sites for activators (open boxes) and repressors (filled boxes). In the absence of repressors, the activators are able to bind and to interact with the basal transcription machinery (bTM) causing activation. When the concentration of repressors is high enough, they compete for the binding of the repressors and thereby prevent activation. **b:** The cis-acting element contains distinct binding sites for activators (open boxes) and nearby sites for repressors (filled boxes). Activation occurs in the absence of repressors, and repression occurs through quenching. Quenching may occur through an interaction of the repressors with the basal transcription machinery or through interactions with the activators, which prevent them from interacting with the basal transcription machinery. For details, see text and (20).

viewed in (23)]. Also, Krüppel causes both activation and repression in a concentration-dependent manner when acting from a single site in front of a heterologous promoter in tissue culture. The opposite actions of Krüppel are provided through its function as a monomer or as a dimer, which are capable of different interactions with components of the basal transcription machinery involving TFIIB for activation and TFIIE for repression [reviewed in (23)]. These results indicate that, although the functional binding sites and players of most enhancers are known, the mechanism of how spatial control is brought about in the embryo is still illusive.

## GRADIENTS TURN INTO STRIPES

Gap gene expression results in the accumulation of mRNA in adjacent domains along the anterior-posterior axis of the embryo (Fig. 1b). Because at this stage the embryo develops still as a syncytium, the transcription factors encoded by the localized mRNAs are able to diffuse and to form a series of short-range concentration gradients. How does the distribution of the different gradients regulate the expression of pair-rule genes, each in a distinct pattern of seven repetitive stripes along the anterior-posterior axis?

The 10 known pair-rule genes act at two different levels [reviewed in (2)]. Accordingly, they were grouped as primary and secondary pair-rule genes. The cis-acting control elements of the primary pair-rule genes *even-skipped* and *hairy* [reviewed in (2,4)] are composed of a modular array of distinct stripe elements. Each stripe element contains a specific set of binding sites for the multiple activators and repressors that emerged from genetic analysis: maternal transcription factors appear to be activators and the gap gene-encoded transcription factors act mainly as repressors [reviewed in (2,4,20)].

Expression of *even-skipped* in a position anterior to *Krüppel* ("stripe 2") is activated in response to Bicoid and Hunchback and is suppressed by the framing activities of Giant and Krüppel [(22), reviewed in (2,20)]. As seen with the cis-acting elements for the gap genes, overlapping binding sites for activators and repressors were found, and much of the repressor action is provided through quenching. Conversely, expression of the two posterior-most stripes of *hairy* (stripes 6 and 7) are activated in response to Knirps and Caudal in the case of hairy stripe 6 or in response to Caudal, Bicoid, and Krüppel in the case of hairy stripe 7 (T. Häder and A. La Rosée, unpublished results). Repression and thus stripe formation is brought about by gap gene products such as Krüppel, Hunchback, Knirps, and Tailless [reviewed in (4) (A. La Rosée, unpublished results). This scenario suggests that the control of the primary pair-rule genes involves the combined activities of maternal and gap genes that employ the mechanisms established for gap gene regulation, eventually adding a new series of transcription factors, in series of seven evenly spaced stripes, to the preexisting ones (Fig. 1c). Stripe expres-

sion also involves cross-regulatory interactions among the pair-rule genes that, like the gap genes, encode transcription factors [reviewed in (2)]. Thus, the spatial control of pair-rule gene expression is not exclusively dependent on preexisting transcription factors but also on the cross talk between the pair-rule genes.

The results reported here establish that the body pattern of the *Drosophila* embryo is generated through a three-tiered cascade of transcription factors that add on top of each other (Fig. 1). It is easy to foresee how cells are programmed in a position-dependent manner because the nuclei within different positions of the blastoderm embryo receive different local concentrations and combinations of the transcription factors. Future efforts will undoubtedly focus more deeply on how the scenario of transcription factors work, how the factors interact, and how they eventually control the cellular factors that cause cells to differentiate and to behave according to their position in the embryo.

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