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Gene regulation in the *Drosophila* embryo

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SUMMARY

Pattern formation in *Drosophila* depends on hierarchical interactions between the maternal and zygotic gene activities which subdivide the embryo into increasingly smaller metameric units along the anterior–posterior axis. Here we describe those genes that encode the transcription factors which control precisely the expression of subordinate transcription factors in time and space. This regulation operates through the protein–protein interactions between transcription factors bound to the cis-acting enhancers, which eventually determine the frequency of transcription initiation by polymerase II. Our data show that taking into account the multiple transcriptional activators and repressors that bind to a typical enhancer element, it is likely that the regulation of gene expression in a given cell is defined by their concentration-dependent interplay which directs target gene expression in a position-dependent fashion.

1. INTRODUCTION

Much of our current understanding of the biological processes involved in pattern formation is derived from an extensive analysis of segmentation in *Drosophila* (reviewed in Ingham 1988; St Johnston & Nüsslein-Volhard 1992; Pankratz & Jäckle 1993). This research was initiated by mutagenesis experiments which were designed to identify the genetic components required to establish the typical larval body pattern. Genetics combined with molecular analyses have subsequently defined the elaborate cascade of hierarchical and cross-regulatory interactions of transcription factors which are required to form the pre-pattern of the body within a single-layer epithelium: the blastoderm. Along the longitudinal axis, the regulatory circuitry leading to head, eleven trunk segments and the morphologically non-segmented tail is based on spatially localized positional signals which cause the asymmetric distribution of transcription factors that derive from or through maternally deposited mRNAs in the egg (reviewed in St Johnston & Nüsslein-Volhard 1992).

Segmentation along the anterior–posterior (AP) axis is controlled by three maternal key signals. Their activities establish the spatially restricted domains of zygotic segmentation gene expression either by acting as transcription factors or by controlling their distribution. At the first level, this results in the localized expression of gap gene activities which give rise to a number of overlapping protein gradients along the AP axis generated in response to the long-range transcription factor gradients of maternal origin. Combinatorial inputs from the gap genes then control the periodic patterns of pair-rule gene expression, defining the metameric blueprint of the embryo (reviewed in Pankratz & Jäckle 1993). Here we summarize the functional aspects of the various transcription factors that operate in the regulatory cascade leading to the blastoderm pre-pattern along the AP axis of the embryo. We emphasise the regulatory properties and the molecular mechanisms underlying the principle of how the molecular blueprint for the early body pattern is established and drawn out.

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2. MATERNAL AND EARLY ZYGOTIC TRANSCRIPTION FACTORS

Pattern formation along the AP axis of the *Drosophila* embryo originates from the combined action of three maternal systems: the anterior, the posterior, and the terminal organizers. The terminal system acts through a signal transduction pathway that originates from outside the egg. Of the six known maternal components of the terminal system, *torso-like* is the only one required in the somatic cells around the oocyte. It is thought to either encode or to generate a ligand molecule for the perivitelline fluid surrounding the egg which locally activates the uniformly distributed transmembrane receptor tyrosine kinase encoded by *torso*. Factors acting downstream of the *torso* receptor kinase such as the *Drosophila* homologue of *raf* or the product of *cork screw* suggest that phosphorylation might be crucial for the local activity of a not yet identified transcription factor. This factor is thought to be responsible for the local activation of two gap genes, *huckebein* and *tailless*, which are both expressed and required for setting up the non-segmented terminal regions of the embryo. Although this system has been studied in great detail, it will not be considered further here. For a recent review see Perrimon & Desplan (1994).

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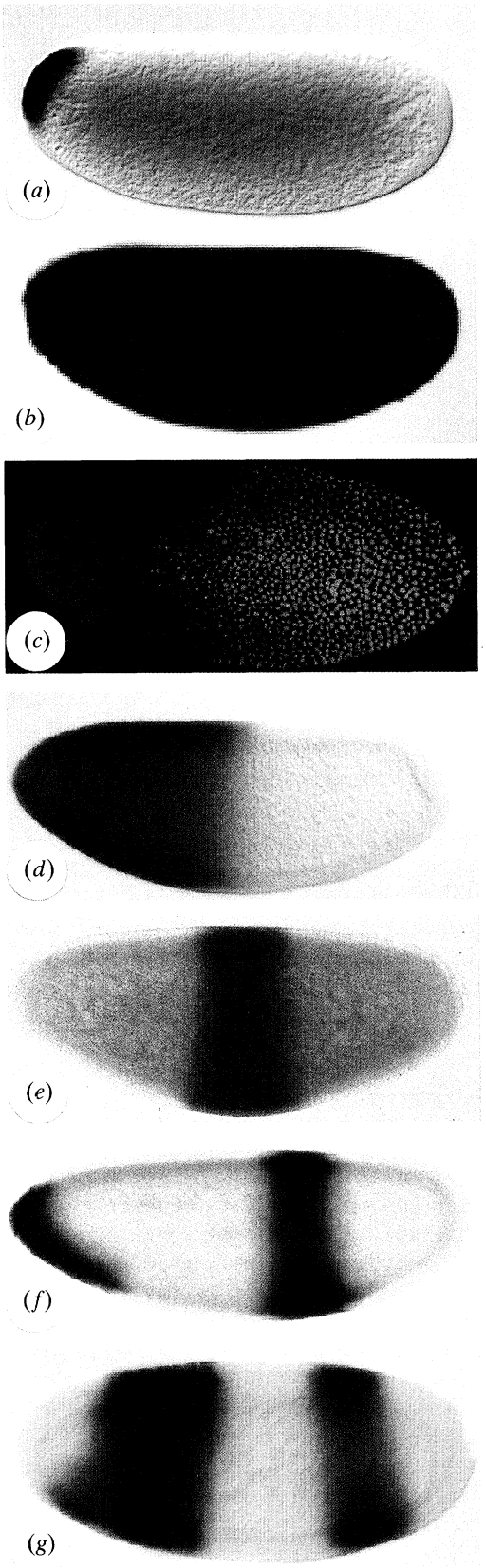

Figure 1. Localization of *bicoid* and *caudal* mRNAs, their protein gradients and the localized expression of the gap genes at blastoderm stage. (*a*) Localized bicoid mRNA in the anterior pole region of the egg and (*b*) evenly distributed caudal mRNA visualized by *in situ* hybridization. (*c*) Anterior to posterior bicoid gradient (red) and the complementary caudal gradient (green) as visualized by double staining with anti-bicoid and anti-caudal antibodies. (*d*) Expression patterns of zygotic *hunchback*; (*e*) *Krüppel*; (*f*) *knirps*; and (*g*) *giant* at precellular blastoderm stage are visualized by *in situ* hybridization. Orientation of embryos is anterior to the left and dorsal side up.

The anterior and the posterior systems are responsible for setting up the metameric segment pattern in the trunk region of the embryo. Both systems act through gradients of morphogenetic proteins derived from mRNA molecules localized to the anterior and the posterior poles of the egg. *bicoid* mRNA is localized at the anterior tip (Berleth *et al.* 1988). Upon translation, the homeodomain-containing transcription factor bicoid diffuses from its site of translation and thereby forms a concentration gradient with a high point in the anterior tip region of the embryo (Driever & Nüsslein-Volhard 1988) (figure 1*a*, *c*). Conversely, *nanos* mRNA is localized at the posterior pole. Nanos consequently forms a concentration gradient with reverse orientation (Wang & Lehmann 1991). *nanos* activity, possibly in conjunction with the uniformly distributed protein encoded by *pumilio*, prevents the translation of maternally derived *hunchback* mRNA which is evenly distributed throughout the egg (Tautz *et al.* 1987). As a consequence of *nanos* activity in the posterior region, hunchback protein (a zinc finger-type transcription factor) forms a concentration gradient that roughly parallels that of bicoid (Tautz *et al.* 1987; Tautz 1988). Taken together, the anterior system provides the asymmetric distribution of the transcription factor bicoid, whereas the posterior system provides the asymmetric distribution of a post-transcriptional repressor which causes hunchback to form a second anterior-to-posterior protein concentration gradient.

The mutagenesis screen had not revealed a gene which may act as a transcriptional activator for zygotic genes in the posterior region of the embryo. However, Mlodzik *et al.* (1985) identified a maternal homeodomain transcription factor encoded by the gene *caudal*. In a manner analogous to *hunchback*, its mRNA is equally distributed throughout the egg (figure 1*b*). *caudal* mRNA slowly decays in the anterior region and the protein forms a gradient in opposite direction to bicoid (figure 1*c*) (Macdonald & Struhl 1986; Mlodzik & Gehring 1987*a*, *b*). This suggests that translation of *caudal* mRNA is suppressed by post-transcriptional regulation in the anterior region of the embryo (R. Rivera Pomar, D. Niessing & U. Schmidt-Ott, unpublished data). As shown below, the caudal transcription factor and the anterior determining transcription factor bicoid cooperate to form a partly redundant activator system in the posterior region of the embryo.

What are the targets of the maternal transcription factors? Mutations of zygotic segmentation genes cause defects in contiguous sets of segments (gap genes), in alternating segments (pair-rule genes) or in each segment (segment polarity genes), implying that the AP axis of the embryo becomes successively subdivided into progressively smaller units (reviewed in Hoch & Jäckle 1993; Pankratz & Jäckle 1993). As the immediate targets of bicoid, hunchback and caudal, the gap genes *hunchback* (i.e. the zygotic expression of the gene; figure 1*d*), *Krüppel* (figure 1*e*), *knirps* (figure

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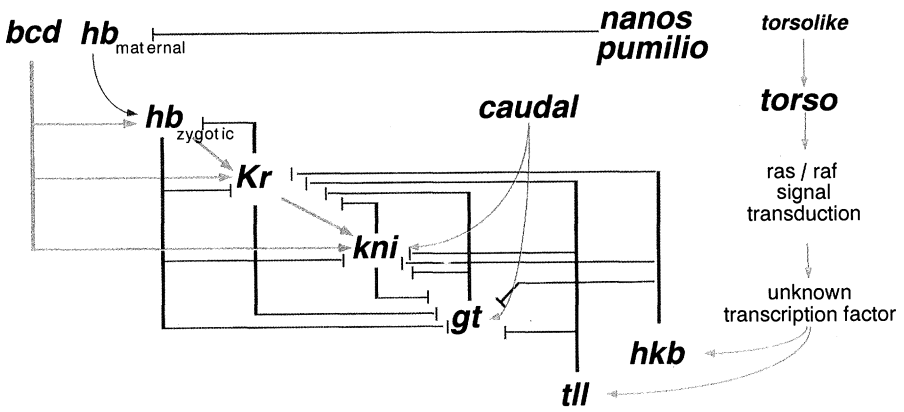

Figure 2. Genetic circuitry establishing the localized expression domains of the gap genes at blastoderm stage. Red lines represent negative interactions, green arrows represent activating interactions. Blue arrow indicates that maternal hunchback (hb) activity is not required for activation of zygotic hb expression but likely to interact synergistically with the activator bicoid (bcd). Note that the maternal terminal pathway (torso-like down to the unknown transcription factor) leads to the activation of huckebein (hkb) and tailless (tll) (reviewed in Perrimon & Desplan 1994) which are strong repressors of the gap genes *Krüppel* (*Kr*), *knirps* (*kni*) and *giant* (*gt*) and thereby delimit the region of the embryo where segmentation occurs (reviewed in Pankratz & Jäckle 1993). For details see text.

1*f*) and *giant* (figure 1*g*) – which are required for trunk formation – are expressed in a series of contiguous domains shortly before or during blastoderm formation. They encode DNA-binding proteins which diffuse from the site of mRNA localization to form a series of short-range concentration gradients (reviewed in Pankratz & Jäckle 1993). Figure 2 shows the genetic interactions necessary to control the spatial expression domains of the gap genes. Once expressed, the maternal- and gap-gene encoded transcription factors combine their activities to control the repetitive stripe patterns of subordinated pair-rule gene expression within the prospective trunk region of the embryo (reviewed in Hoch & Jäckle 1993; Pankratz & Jäckle 1993).

3. MATERNAL FACTORS AND CROSSTALK

The zygotic enhancer of *hunchback* (Schröder *et al.* 1988) mediates gene expression to make the total amount of embryonic hunchback (the combined maternal and zygotic complements) required for anterior development. Zygotic *hunchback* expression cannot be activated in the absence of *bicoid* activity (reviewed in St Johnston & Nüsslein-Volhard 1992). The discovery of bicoid binding sites of different affinities within the corresponding enhancer element suggested the possibility that a single transcription factor gradient can provide positional information through different threshold values: transcriptional activation mediated by low-affinity binding sites would mediate gene expression above a high bicoid concentration threshold value, whereas high-affinity binding sites would allow for gene expression more posteriorly at lower bicoid concentrations (Driever & Nüsslein-Volhard 1988, 1989; Driever *et al.* 1989; Struhl *et al.* 1989). It has been shown, however, that bicoid does not provide the normal spatial limit of the zygotic *hunchback* expression domain in the absence of maternal *hunchback* activity but rather causes activation at the peak level of the bicoid gradient exclusively (Simpson-Brose *et al.* 1994). This result suggests that the spatial limit of the zygotic *hunchback* expression domain (figure 1*d*) is set in response to a synergistic interaction between the activator bicoid and hunchback which by itself is insufficient to activate via the zygotic hunchback promoter (Simpson-Brose *et al.* 1994).

Krüppel is expressed in a region posteriorly adjacent to the zygotic *hunchback* expression domain (figure 1*e*). One could imagine that *Krüppel* expression is also activated by a synergistic interaction of hunchback and bicoid as described above, and that an anteriorly acting repressor prevents *Krüppel* gene activation in the region taken by zygotic hunchback. However, the expression pattern of *Krüppel* in various mutant embryos indicates that the setting of the spatial limits of the *Krüppel* expression domain is more complex: both bicoid and hunchback act as independent activators of *Krüppel* expression and each one of the gap genes expressed outside the *Krüppel* expression domain contributes, to various extents, to suppression of the *Krüppel* gene activation. This model is supported by experiments in which gap genes are ubiquitously expressed throughout the blastoderm embryo as achieved by transgenes under the control of a heat shock-inducible promoter fused to the transcribed region of *knirps*, *giant* or *tailless* (Hoch *et al.* 1992). *knirps* expression causes a reduction of the level of *Krüppel* expression which is less dramatic than the effect caused by ubiquitous *giant* expression. In the extreme case, the heat shock-induced expression of *tailless*, the *Krüppel* gene is not activated at all. The available data therefore suggest that in the absence of the repressors, both hunchback or bicoid would lead to a broad activation of *Krüppel* within the trunk region of the embryo. The spatial restriction of the expression domain is brought about by repression in response to the activities of the other gap genes which combine their functions to act as a redundant repressor system (Hoch & Jäckle 1993). This also alludes to the possibility that the transcription factor hunchback may play a dual role: in case of

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zygotic *hunchback* expression, the maternal hunchback is required to synergize so that the cis-acting element recognizes a given bicoid threshold level above which a gene can be activated. In the case of *Krüppel*, hunchback acts as an independent activator in addition to bicoid.

The *Krüppel* enhancer element which is necessary and sufficient to provide reporter gene expression in the authentic *Krüppel* expression domain contains multiple binding sites for hunchback, bicoid, giant and tailless and a single binding site for knirps (Hoch *et al.* 1992). Each one of the bicoid binding sites as well as most of the hunchback binding sites either overlap or are in close proximity to binding sites for those gap gene-encoded transcription factors which act as repressors. *In vitro* binding studies combined with tissue culture experiments have shown that binding of the activators and the repressors is mutually exclusive and that high concentrations of repressors prevent the activators from functioning. The results obtained are consistent with a model which proposes that within the *Krüppel* enhancer element, activators and repressors compete for binding and that thus activation occurs in a region of the embryo where the activators can bind (Hoch *et al.* 1992; Hoch & Jäckle 1993). As outlined above, therefore, *Krüppel* is only activated in the central region of the embryo (which contains sufficient amounts of the activators bicoid and hunchback) in the absence of repressor concentrations that prevent the activation. We should add that the repressors not only act by competing the binding of activators to the enhancer but also through 'quenching' (Levine & Manley 1989) i.e. they extinguish the function of the bound activators within the enhancer.

knirps expression in a region posteriorly adjacent to the *Krüppel* domain (figure 1*f*) is mediated by a cis-acting element composed of an activator module, a hunchback-dependent repressor module, a module upon which *Krüppel* exerts coactivator function and a second, tailless-dependent repressor module (Pankratz *et al.* 1992). The available evidence (based on studies involving mutant embryos (figure 1*g*), and reporter gene studies on transgene-containing wild-type embryos) suggested that the *knirps* expression domain involves ubiquitous activation and hunchback- and tailless-dependent repression in the anterior and posterior regions, respectively (Pankratz *et al.* 1992). The arrangement of the repressor binding sites next to the activator module (Pankratz *et al.* 1992; Rivera-Pomar *et al.* 1995) implies that both repressors act upon quenching, thereby preventing the ubiquitous activator from functioning in regions where *knirps* is not expressed. Thus hunchback not only has the ability to synergize with bicoid and to activate *Krüppel* gene expression, but it can also function as a repressor.

Identification of an activator for *knirps* and *giant* (which is expressed in the region posteriorly adjacent to *knirps*) did not come through genetics. The posterior maternal system, initially expected to act in a similar manner as the anterior system, does not function at the level of transcription. Instead it prevents translation of the hunchback mRNA in the posterior half of the embryo so that the posterior gap genes *knirps* and *giant* can be activated (Hülskamp *et al.* 1989; Irish *et al.* 1989; Struhl 1989).

In the search for factor(s) that activate posterior *knirps* gene expression, we examined deletion constructs of the 2 kb *knirps* cis-acting region for their ability to conduct reporter gene expression in transgenic embryos. Gene activation in the *knirps* domain depends on two separate activator modules, the combination of which mediates gene expression throughout the embryo (Rivera-Pomar *et al.* 1995). One of them, a 64 base pair element (b.p.-element), contains six *bicoid* binding sites and mediates bicoid-dependent gene expression in an anterior-to-posterior gradient. The second, 223 b.p. cis-acting element contains caudal binding sites. It is 5′ adjacent to the 64 b.p.-element and mediates caudal-dependent gene activation. In the presence of hunchback, the combination of the two cis-acting elements mediates gene expression in the posterior half of the blastoderm-stage embryo in a manner indicating that the hunchback binding sites provide repression in the anterior half. If hunchback binding sites are removed from the combined 64 b.p./223 b.p. element, gene expression occurs throughout the embryo. The results suggest that *knirps* activation occurs in response to the two homeodomain-containing transcriptional activators, bicoid and caudal, by combining their graded activities to potentially activate gene expression throughout the embryo. hunchback acts as a repressor in the anterior region; tailless (which acts upon six *in vitro* binding sites) suppresses the activation in the posterior pole region of the embryo (Pankratz *et al.* 1992; Rivera-Pomar *et al.* 1995).

In embryos lacking bicoid activity, maternal *caudal* is present throughout the embryo. Such embryos develop a normal posterior abdominal-segmentation pattern. When caudal is absent, the fourth and eighth abdominal segments are missing and the other abdominal segments are variably affected (Macdonald & Struhl 1986). These phenotypes are consistent with the argument that in the absence of either bicoid or caudal, posterior segmentation genes are not expressed at biologically relevant levels. They also suggest a partial complementation of the lack of caudal function by bicoid and vice versa. Consistent with this proposal, the absence of both bicoid and caudal at the same time causes the absence of anterior and posterior segments in the trunk region of the embryo (R. Rivera-Pomar, unpublished result). Thus it is likely that the activation of segmentation genes in the posterior region of the embryo is dependent on the two complementing and, in part, redundant activators bicoid and caudal, instead of a ubiquitous transcription factor. The organization of binding sites for activators and repressors in distinct cis-acting modules suggests that the repressors can only act over short distances within the *knirps* enhancer and that they possibly act through quenching. The transacting factors required to activate and spatially control the posterior *giant* expression domain (figure 1*g*) are summarized in figure 2. Based on the available evidence (Rivera-Pomar *et al.* 1995) *giant* is also activated by *caudal* (and most likely also by *bicoid*) and the spatial control is dependent on

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repression by the neighbouring gap genes i.e. *knirps* and *giant* may be the only two gap genes which are controlled in an analogous fashion.

The results obtained so far suggest that hunchback can either synergize bicoid activity, as in the case of zygotic *hunchback* expression, or suppress bicoid-dependent activation, as in the case of *knirps*. Within the *Krüppel* cis-acting control region, hunchback can also act as an activator and functions in parallel to bicoid. This puzzling regulatory feature does not seem to be hunchback-specific, as Krüppel may also act both ways. It can repress through quenching (thereby interferring with the bicoid- and hunchback-dependent activation required to form a distinct pair-rule gene expression domain i.e. *even-skipped* stripe 2; see Stanojevic *et al*. 1989, 1991; Small *et al*. 1992). It is also required for the coactivation of *knirps* and for the activation of the gene *knirps-related* in the same spatial domain (Rothe *et al*. 1994). In addition, *Krüppel* functions to repress *giant* (reviewed in Hoch & Jäckle 1993). These observations leave the possibility that each one of the zinc finger-encoding gap genes, which are both expressed and required in multiple tissues and organs of the embryo at later stages, may regulate within different enhancers in a context-dependent manner (Harbecke & Janning 1989; Hoch *et al*. 1990; Gaul & Weigel 1991; Schmucker *et al*. 1992; S. Romani *et al*., unpublished data).

4. REGULATORY POTENTIAL OF KRÜPPEL

To assess the regulatory potential of Krüppel we made use of the finding that Krüppel acts from a single binding site of the sequence -AAAAGGGTTAA- in front of a heterologous promoter in cotransfected *Drosophila* Schneider cells (Sauer & Jäckle 1991, 1993). At low concentrations, Krüppel causes activation whereas at high concentrations Krüppel causes repression below the basal level of reporter gene expression. At low concentrations, Krüppel acts as a monomer. When the concentration of Krüppel increases it is able to form homodimers involving its C-terminal region while still bound to the single binding site (Sauer & Jäckle 1991, 1993).

The discovery that both target gene activation and repression are mediated through a single *in vitro* Krüppel binding site suggested that the opposite regulatory effects of Krüppel might be generated by different functional interactions with components of the basal transcription machinery, which includes the initiation factors TFIIA, B and E–I as well as the TATA-binding protein (TBP) and TBP-associated effectors (TAFs) contained in the multisubunit TFIID (reviewed in Roeder 1991; Gill & Tjian 1992; Tjian & Maniatis 1994). Co-immunoprecipitation experiments with Krüppel and recombinant initiation factors indicate that Krüppel can associate with recombinant TFIIB and the β-subunit of TFIIE, provided that Krüppel is bound to DNA (Sauer *et al*. 1995). Mutant *Krüppel*, which lacks the C-terminal homodimerization domain, interacts with TFIIB but not with TFIIEβ. Conversely, mutant *Krüppel* which lacks the N-terminal region (including an activation domain) or the Krüppel dimer co-precipitate with TFIIEβ but not with TFIIB (Sauer *et al*. 1995). These data suggest that monomeric Krüppel can act as a transcriptional activator by interactions with TFIIB, while the Krüppel homodimer interacts with TFIIEβ and thereby causes repression of transcription.

To demonstrate the functional significance of the different interactions of Krüppel with the basal transcription machinery, we assayed transcriptional regulation by Krüppel *in vitro*. In a manner similar to that which had been observed in tissue culture cells, Krüppel can activate or repress *in vitro* transcription in a concentration-dependent manner i.e. monomeric Krüppel activates transcription whereas the cross-linked Krüppel homodimer represses transcription. Activation by monomeric Krüppel was inhibited in the presence of antibodies directed against TFIIB whereas repression was inhibited by antibodies directed against TFIIEβ. Neither of the antibodies interfered with basal transcription, presumably because the antibodies recognize only those domains of TFIIB and TFIIEβ required for functions which are not apparent from the *in vitro* transcription assay. In both cases, the addition of recombinant factors restored the corresponding activities of Krüppel (Sauer *et al*. 1995). These results suggest that the association of Krüppel dimers and TFIIEβ causes transcriptional repression whereas the association of Krüppel monomers with TFIIB causes activation. Within this context, it is interesting to note that control experiments in which the repressor even-skipped exerts repression in the presence of anti-TFIIEβ antibodies that otherwise prevent Krüppel-dependent transcriptional repression (Sauer *et al*. 1995). Thus the mechanisms of repression by Krüppel and even-skipped are different. This finding is consistent with the subsequent finding that even-skipped prevents the binding of TFIID to the promoter (Austin & Biggin 1995; Um *et al*. 1995)

5. KRÜPPEL AND OTHER GAP PROTEINS

The discovery that Krüppel is capable of protein–protein interactions prompted us to examine Krüppel-dependent reporter gene regulation in the presence of other gap gene proteins such as hunchback and knirps. For this we used cotransfected *Drosophila* Schneider cells serving as a biological test tube to examine whether hunchback or knirps may interfere with Krüppel-dependent transcriptional regulation when coexpressed in the cell nucleus. Neither hunchback nor knirps affect the basal level of reporter gene expression mediated by the single Krüppel *in vitro* binding site described above. However, they interfere with Krüppel-dependent transcriptional regulation specifically. Krüppel-dependent activation changed to repression when increasing concentrations of knirps are added, whereas knirps had no effect on Krüppel-dependent repression. Except for when DNA containing hunchback *in vitro* binding sites was added together with the reporter gene-containing plasmid, hunchback had no observed effect on Krüppel-dependent regulation. In this case, Krüppel-dependent activation changes to repression and Krüppel-depen-

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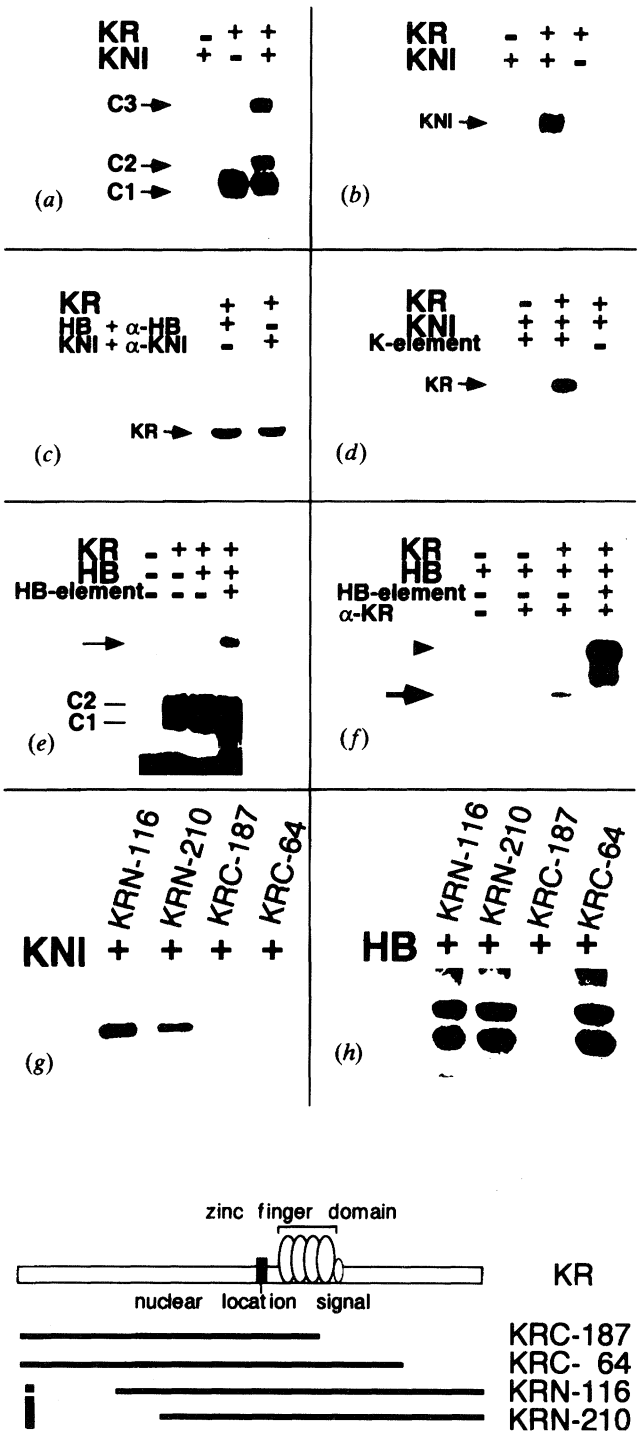

Figure 3. Association of Krüppel (KR) with knirps (KNI) and hunchback (HB) *in vitro*. (*a*) Gel mobility shift assays; (*b–d*) and co-immunoprecipitation experiments involving *in vitro* translated KR, KNI and HB. (*a*) Gel mobility shift assays were performed with the ^{32}P-labelled K-element (a single KR binding site), *in vitro* translated KR and KNI. C_1, DNA-bound KR monomer; C_2, DNA-bound KR homodimer (Sauer & Jäckle 1993); C_3, DNA-bound KNI/KR heterodimer. (*b*) Co-immunoprecipitation experiments using anti-KR antibodies, *in vitro* translated KR and ^{35}S-methionine-labelled KNI (arrow). The K-element was included in the reactions. (*c*) ^{35}S-labelled KR (arrow) was co-precipitated with unlabelled HB or KNI using anti-HB or anti-KNI antibodies. Note that KR only co-precipitates with HB when both the K-element and DNA containing HB *in vitro* binding sites were present in the reaction mixture. (*d*) Co-immunoprecipitation experiments using anti-KNI antibodies, *in vitro* translated KNI and ^{35}S-methionine-labelled KR (arrow). The K-element was present (+) or absent (−) in the reaction mixture. Co-precipitates were separated by SDS-PAGE, and labelled proteins were visualized by fluorography. (*e*) Gel mobility shift assays included the ^{32}P-labelled K-element, *in vitro* translated KR and *in vitro* translated HB in the absence (−) or presence (+) of HB binding sites. C_1 and C_2, DNA-bound KR monomer and KR homodimer, respectively. Arrow marks an additional complex obtained in the presence of HB and the HB-element. (*f*) Co-immunoprecipitation experiments using anti-KR antibodies, ^{35}S-labelled HB or ^{35}S-labelled KR (arrow) and unlabelled KR. ^{35}S-labelled HB (arrowhead) served as a marker for HB (see last lane). Immunoprecipitates were separated by SDS-PAGE and

dent repression becomes stronger with increasing concentrations of hunchback (Sauer & Jäckle 1995). Thus both knirps and hunchback can interfere with Krüppel in a specific manner.

To see whether Krüppel can interact with knirps and hunchback directly, we performed immuno-co-precipitation experiments and gel mobility assays. The results summarized in figure 3 show that DNA-bound Krüppel is able to bind to knirps and to hunchback, provided that the latter is itself bound to DNA. The regions required for each interaction are different. Deletion analysis of Krüppel indicates that knirps binds to the C-terminal region of Krüppel, whereas hunchback binds to a more central region which includes part of the Krüppel zinc finger domain. These results and the effects observed in the cotransfection studies on *Drosophila* Schneider cells consistently argue the possibility of direct and functional protein–protein interactions between Krüppel and hunchback as well as between Krüppel and knirps. As the regulatory effects of knirps and hunchback are provided in the absence of functional binding sites for these proteins within the control region of the target gene, these results also suggest that knirps and hunchback act upon binding to DNA-bound Krüppel which serves as a tether (Sauer & Jäckle 1995).

6. CONCLUSIONS

The results described establish that the *Drosophila* segmentation gene hierarchy acts to subdivide the embryo. The maternal systems act by providing localized components at the pole regions which either result directly in the asymmetric distribution of a transcription factor through localized mRNA (bicoid) or function to generate asymmetric transcription factor gradients (hunchback, caudal) from evenly distributed mRNA. The maternal transcription factors activate another set of spatially more restricted zygotic transcription factors as their immediate targets, the gap genes. Their localized activity results in a series of overlapping transcription factor gradients which add to the pre-existing long-range maternal factors. This scenario of factors along the AP axis provides locally distinct transcription factor concentrations that can be received and functionally integrated by the cis-acting enhancer elements which provide sharp on/off boundaries of gene expression (figure 1). The latter require gap gene activities which either antagonize or enhance the activating functions provided by the maternal transcription factors. This crosstalk between the gap genes leads to a highly dynamic pattern of gap gene expression: the initially diffuse borders at the syncytial blastoderm stage sharpen up in response to increasing gap gene expression until the cellular blastoderm is reached i.e. nuclei become isolated from each other by ingrown membranes.

In common with eachother, the three cis-acting elements of zygotic *hunchback*, *Krüppel* and *knirps* need either two factors to mediate the spatial domain of expression; or two activators to guarantee the required level of expression. hunchback serves a different function within each of the three enhancers, and the functions (synergistic interactions with bicoid, repressor and activator) do not necessarily correlate with the hunchback concentration along the AP axis. This suggests that the different cis-acting control regions may integrate hunchback activity in a context-dependent manner. In other words: whether hunchback acts synergistically with bicoid as seen in the zygotic *hunchback* enhancer, or whether it functions as an activator (in parallel to bicoid) as seen in the *Krüppel* enhancer or finally, whether it provides a repressor that overcomes activation (by bicoid and caudal) may depend on the arrangement of the binding sites within the enhancer and the other factors that bind in parallel. Our results show that the gap gene-encoded transcription factors are able to undergo direct protein–protein interactions and interactions with components of the general transcription machinery. It is likely that such interactions impose structural constraints which result in the exposure of distinct protein domains. In fact, we noted a repressor domain and a transactivation domain in Krüppel in addition to a previously identified repressor domain (Licht *et al.* 1989) which function in different cellular contexts. Furthermore, hunchback is able to bind to Krüppel only when hunchback target DNA is present. This suggests that a DNA-dependent change in conformation is necessary for the interaction of hunchback with Krüppel. A change in Krüppel conformation may also explain why the monomer is able to interact functionally with TFIIB (involving the Krüppel activation domain) and not with TFIIEβ; whereas the Krüppel dimer interacts functionally with TFIIEβ (involving both repressor domains) (Sauer *et al.* 1995) but not with TFIIB.

The interactions of DNA-bound Krüppel with hunchback and knirps and the effects of these transcription factors on Krüppel-dependent trans-

labelled proteins were visualized by fluorography. Note two forms of HB (last lane) recognized by anti-HB antibodies. The form with higher molecular mass is always predominant (arrowhead). We do not know whether the lower molecular mass band represents a specific degradation product or a second translation initiation site within the *hb* mRNA. Note also that in the absence of HB *in vitro* binding sites, KR and HB fail to associate. (*g*, *h*) Localization of the regions of KR necessary for the association with KNI and HB (see also *i*). Fluorographs show co-immunoprecipitation experiments using *in vitro* translated ^{35}S-methionine-labelled KNI (*g*) or HB (*h*) and the truncated versions of KR. Note that KNI and HB require different regions of KR to associate *in vitro*. For the appearance of two forms of *in vitro* translated ^{35}S-labelled HB see (*f*). (*i*) Structure of Kr and deletions used in (*g* and *h*). KRC-187 and KRC-64 are C-terminal deletions having lost the C-terminal repressor and dimerization domains. Note that the HB and KNI require different KR regions for the interaction. KRN-116 and KRN-210 are N-terminal deletions which do not affect the interactions with HB and KNI (see *g*, *h*). For details see Sauer & Jäckle (1995).

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criptional regulation in cotransfected tissue culture cells suggest that transcription factors can interfere with the activity of other transcription factors even though they are not bound to the promoter or enhancer directly. This phenomenon suggests the possibility that transcription factors such as Krüppel may not only act by quenching or through a direct interaction with the basal transcription machinery but also serve as a tether to allow transcription factors to join the enhancer region without binding to its DNA directly (Sauer & Jäckle 1995). Such an arrangement would provide an opportunity for different or additional interactions with other factors assembled in the enhancer region and/or with components of the basal transcription machinery such as the TAFs.

REFERENCES

Austin, R. J. & Biggin, M. D. 1995 A domain of the *even-skipped* protein represses transcription by preventing TFIID binding to a promotor: repression by cooperative blocking. *Molec. Cell. Biol.* **15**, 4683–4693.

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. & Nüsslein-Volhard, C. 1988 The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7**, 1749–1756.

Driever, W. & Nüsslein-Volhard, C. 1988 The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95–104.

Driever, W. & Nüsslein-Volhard, C. 1989 The *bicoid* protein is a positive regulator of *hunchback* transcription in the early *Drosophila* embryo. *Nature, Lond.* **337**, 138–143.

Driever, W., Thoma, G. & Nüsslein-Volhard, C. 1989 Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the *bicoid* morphogen. *Nature, Lond.* **340**, 363–367.

Gaul, U. & Weigel, D. 1991 Regulation of *Kr* expression in the anlage of the Malpighian tubules in the *Drosophila* embryo. *Mech. Dev.* **33**, 57–68.

Gill, G. & Tjian, R. 1992 Eukaryotic coactivators associated with the TATA box binding protein. *Curr. Opin. Gen. Dev.* **3**, 234–241.

Harbecke, R. & Janning, W. 1989 The segmentation gene *Krüppel* of *Drosophila melanogaster* has homeotic properties. *Genes Dev.* **3**, 114–122.

Hoch, M., Schröder, C., Seifert, E. & Jäckle, H. 1990 *Cis*-acting control elements for *Krüppel* expression in the *Drosophila* embryo. *EMBO J.* **9**, 2587–2595.

Hoch, M., Gerwin, N., Taubert, H. & Jäckle, H. 1992 Competition for overlapping sites in the regulatory region of the *Drosophila* gene *Krüppel*. *Science, Wash.* **256**, 94–97.

Hoch, M. & Jäckle, H. 1993 Transcriptional regulation and spatial patterning in *Drosophila*. *Curr. Opin. Gen. Dev.* **3**, 566–573.

Hülskamp, M., Schröder, C., Pfeifle, C., Häckle, H. & Tautz, D. 1989 Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene. *Nature, Lond.* **338**, 629–632.

Ingham, P. W. 1988 The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature, Lond.* 335, 25–34.

Irish, V. F., Lehmann, R. & Akam, M. 1989 The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. *Nature, Lond.* **338**, 646–648.

Levine, M. & Manley, J. L. 1989 Transcriptional repression of eukaryotic promotors. *Cell* **59**, 405–408.

Licht, J. D., Grossel, M. J., Figge, J. & Hansen, U. 1990 *Krüppel* is a transcriptional repressor. *Nature, Lond.* **346**, 76–79.

Macdonald, P. M. & Struhl, G. 1986 A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature, Lond.* **324**, 537–545.

Mlodzik, M., Fjose, A. & Gehring, W. J. 1985 Isolation of *caudal*, a Drosophila homeobox-containing gene with maternal expression, whose transcripts form a concentration gradient at the preblastoderm stage. *EMBO J.* **4**, 2961–2969.

Mlodzik, W. & Gehring, W. J. 1987*a* Expression of the *caudal* gene in the germ line of *Drosophila*. Formation of an RNA and protein gradient during early embryogenesis. *Cell* **48**, 465–478.

Mlodzik, W. & Gehring, W. J. 1987*b* Hierarchy of the genetic interactions that specify the anteroposterior segmentation pattern of the *Drosophila* embryo as monitored by caudal protein expression. *Development* **101**, 421–430.

Pankratz, M. J., Busch, M., Hoch, M., Seifert, E. & Jäckle, H. 1992 Spatial control of the gap gene knirps in the *Drosophila* embryo by posterior morphogen system. *Science* **255**, 986–989.

Pankratz, M. J. & Jäckle, H. 1993 Blastoderm segmentation. In *The development of* Drosophila melanogaster (ed. M. Bate & A. Martinez Arias), vol. 1, pp. 467–516. New York: Cold Spring Harbor Laboratory Press.

Perrimon, N. & Desplan, C. 1994 Signal transduction in the early *Drosophila* embryo: when genetics meets biochemistry. *Trends Biochem. Sci.* **19**, 509–513.

Rivera-Pomar, R., Lu, X., Perrimon, N., Taubert, H. & Jäckle, H. 1995 Activation of posterior gap gene expression in the *Drosophila* blastoderm. *Nature, Lond.* **376**, 253–256.

Roeder, R. G. 1991 The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. *Trends Biochem. Sci.* **16**, 402–407.

Rothe, M., Wimmer, E. A., Pankratz, M. J., Gonzalez-Gaitan, M. & Jäckle, H. 1994 Identical transacting factor requirement for *knirps* and *knirps-related* gene expression in the anterior but not in the posterior region of the *Drosophila* embryo. *Mech. Dev.* **46**, 169–181.

Sauer, F. & Jäckle, H. 1991 Concentration-dependent transcriptional activation or repression by KRÜPPEL from a single binding site. *Nature, Lond.* **353**, 563–565.

Sauer, F. & Jäckle, H. 1993 Dimerization and the control of transcription by *Krüppel*. *Nature, Lond.* **364**, 454–457.

Sauer, F. & Jäckle, H. 1995 Heterodimeric *Drosophila* gap gene protein complexes acting as transcriptional repressors. *EMBO J.* **14**, 4773–4780.

Sauer, F., Fondell, J. D., Ohkuma, Y., Roeder, R. G. & Jäckle, H. 1995 Transcriptional control by *Krüppel* through interactions with TFIIB and TFIIEb. *Nature, Lond.* **375**, 162–164

Schmucker, D., Taubert, H. & Jäckle, H. 1992 Formation of the *Drosophila* larval photoreceptor organ and its neuronal differentiation require continous *Krüppel* gene activity. *Neuron* **5**, 1025–1039.

Schröder, C., Tautz, D., Seifert, E. & Jäckle, H. 1988 Different regulation of the two transcripts from the *Drosophila* gap segmentation gene *hunchback*. *EMBO J.* **7**, 2881–2887.

Simpson-Brose, M., Treisman, J. & Desplan, C. 1994 Synergy between the bicoid and hunchback morphogens is required for anterior patterning in *Drosophila*. *Cell* **78**, 855–865.

Small, S., Kraut, R., Hoey, T., Warrior, R. & Levine, M.

1991 Transcriptional regulation of a pair-rule stripe in *Drosophila*. *Genes Dev*. **5**, 827–839.

Stanojevic, D., Hoey, T. & Levine, M. 1989 Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*. *Nature*, *Lond*. **341**, 331–335.

Stanojevic, D., Small, S. & Levine, M. 1991 Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. *Science*, *Wash*. **254**, 1385–1387.

St Johnston, D. & Nüsslein-Volhard, C. 1992 The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201–220.

Struhl, G. 1989 Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. *Nature*, *Lond*. **338**, 741–744.

Struhl, G., Struhl, K. & Macdonald, P. M. 1989 The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell* **57**, 1259–1273.

Tautz, D., Lehmann, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K. & Jäckle, H. 1987 Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature*, *Lond*. **327**, 383–389.

Tautz, D. 1988 Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. *Nature*, *Lond*. **332**, 281–284.

Tjian, R. & Maniatis, T. 1994 Transcriptional activation: a complex puzzle with few easy pieces. *Cell* **77**, 5–9.

Um, M., Li, C. & Manley, J. L. 1995 The transcriptional repressor even-skipped interacts directly with TATA-binding protein. *Molec. Cell. Biol*. **15**, 5007–5016.

Wang, C. & Lehmann, R. 1991 *nanos* is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637–647.

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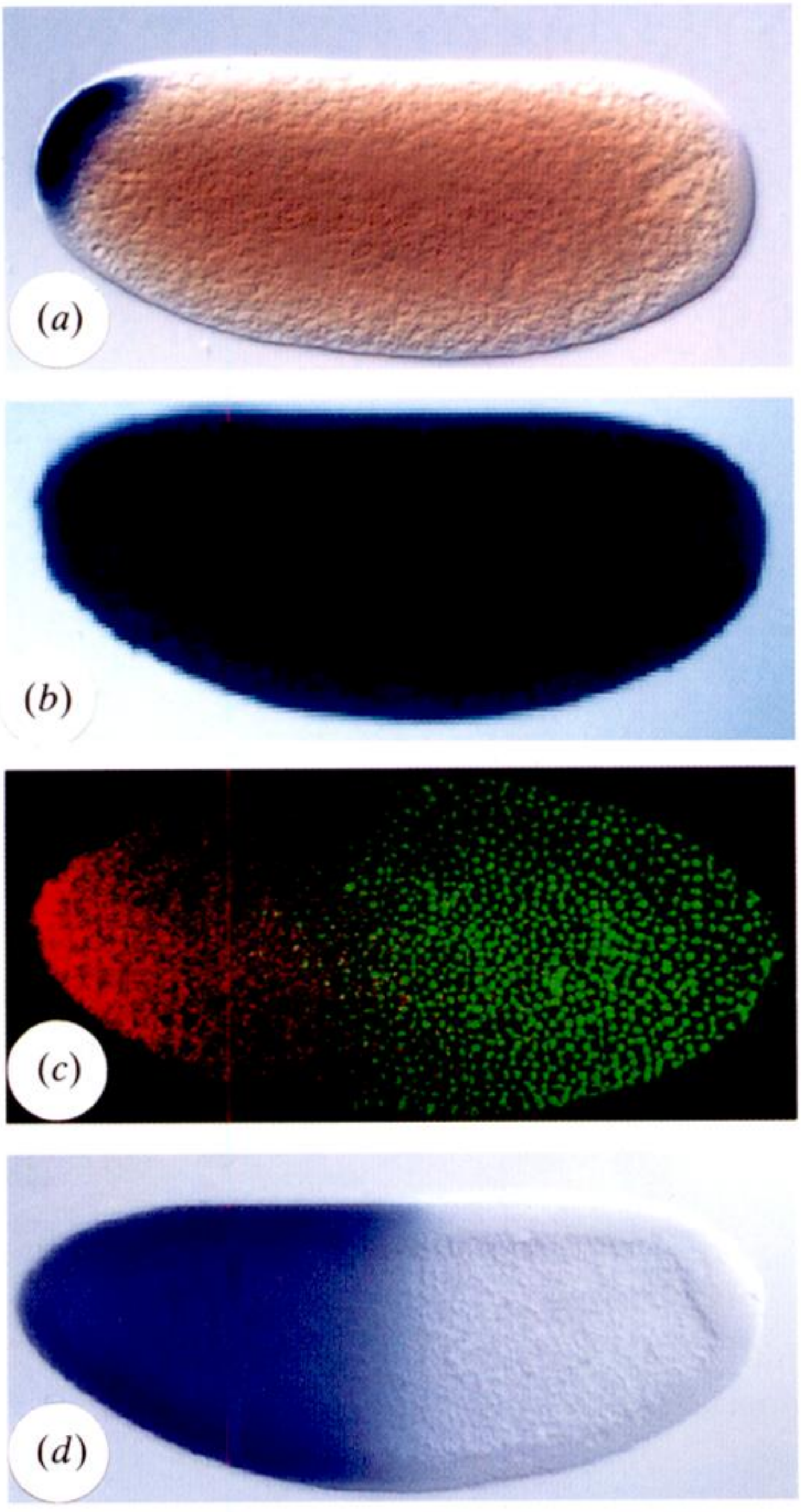
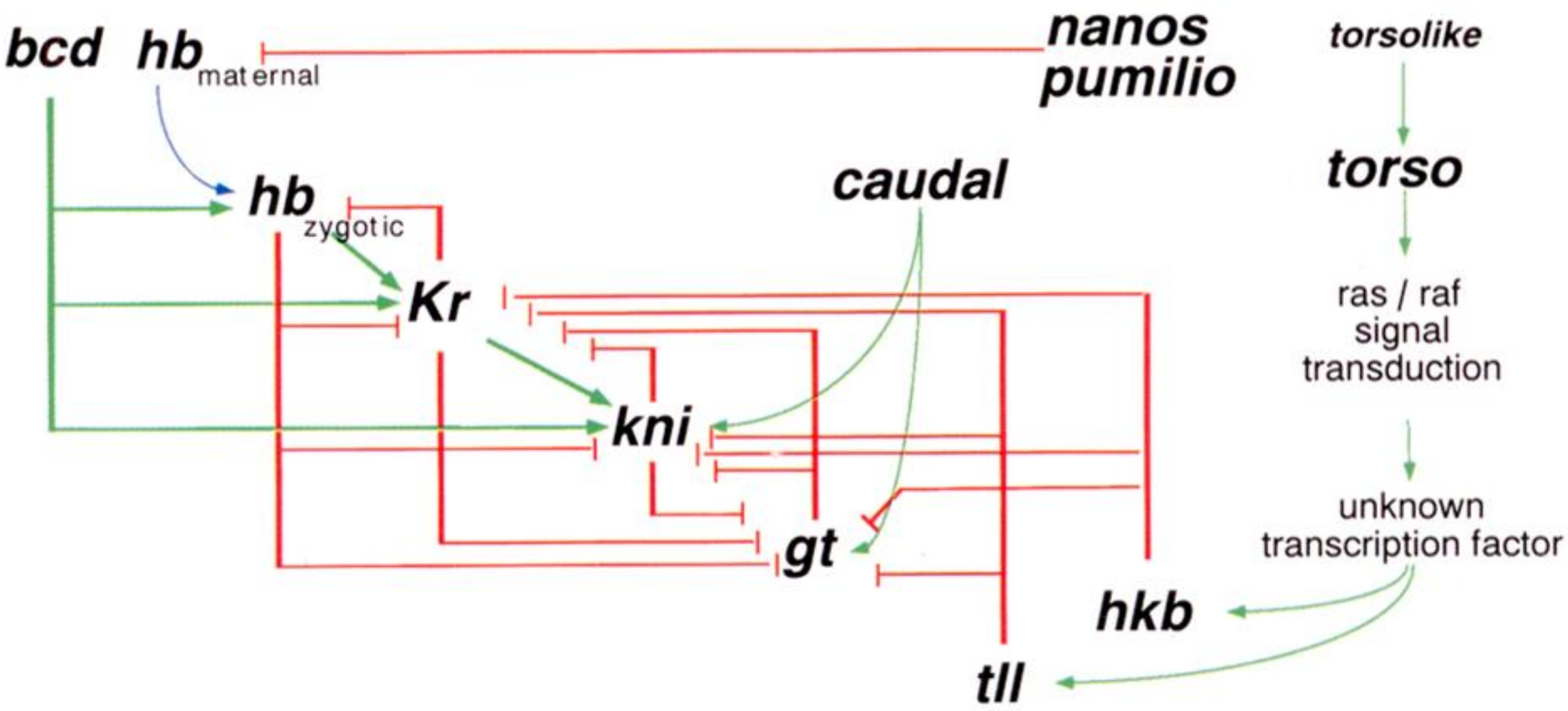


gure 1. Localization of *bicoid* and *caudal* mRNAs, their otein gradients and the localized expression of the gap nes at blastoderm stage. (*a*) Localized bicoid mRNA in the terior pole region of the egg and (*b*) evenly distributed udal mRNA visualized by *in situ* hybridization. (*c*) Anterior posterior bicoid gradient (red) and the complementary udal gradient (green) as visualized by double staining with ti-bicoid and anti-caudal antibodies. (*d*) Expression tterns of zygotic *hunchback*; (*e*) *Krüppel*; (*f*) *knirps*; and (*g*) *nt* at precellular blastoderm stage are visualized by *in situ* bridization. Orientation of embryos is anterior to the left d dorsal side up.



gure 2. Genetic circuitry establishing the localized expression domains of the gap genes at blastoderm stage. Red es represent negative interactions, green arrows represent activating interactions. Blue arrow indicates that aternal hunchback (hb) activity is not required for activation of zygotic hb expression but likely to interact nergistically with the activator bicoid (bcd). Note that the maternal terminal pathway (torso-like down to the known transcription factor) leads to the activation of huckebein (hkb) and tailless (tll) (reviewed in Perrimon & esplan 1994) which are strong repressors of the gap genes *Krüppel* (*Kr*), *knirps* (*kni*) and *giant* (*gt*) and thereby delimit e region of the embryo where segmentation occurs (reviewed in Pankratz & Jäckle 1993). For details see text.