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From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps

ROLANDO RIVERA-POMAR AND HERBERT JÄCKLE

Pattern formation along the anterior–posterior axis of the Drosophila embryo is organized by asymmetrically distributed maternal transcription factors. They initiate a cascade of spatially restricted and interacting zygotic gene activities that provide a molecular blueprint of the larval body at blastoderm stage. The key players in the pattern forming process have been identified. Recent progress has begun to reveal the mechanisms by which coherent positional information of maternal origin becomes transferred into serially repeated zygotic gene expression domains reflecting the metameric body plan of the larva.

Drosophila pattern formation along the anterior–posterior axis involves three maternal genetic pathways: the anterior, the posterior and the terminal organizer systems. Key components of the systems are *bicoid*, *nanos* and *torso*, respectively, which set in motion an elaborate cascade of zygotically expressed transcription factors (reviewed in Refs 1, 2). In effect, this gives a prepattern of the segmented larval body at the blastoderm stage by establishing a series of repetitive domains of pair-rule gene expression (reviewed in Refs 1, 2). Up to this stage, the embryo develops in a syncytium where the nuclei divide without being separated by cellular membranes. This mode of development facilitates the diffusion of morphoregulatory factors of maternal and early zygotic origin, and allows them to instruct single-layered preblastoderm nuclei according to their position in the embryo.

Anterior–posterior polarity is initiated by cell communication events between the oocyte and the surrounding epithelium of somatic follicle cells, which depend on the *gurken–torpedo* signalling pathway (Fig. 1a; reviewed in Ref. 3). This results in the microtubule-dependent localization of *bicoid* mRNA to the anterior pole of the oocyte and *oskar* mRNA to the posterior. *oskar* organizes the assembly of the pole plasm required for the co-localization of *nanos* mRNA needed for the establishment of the abdominal segments (reviewed in Ref. 3). In addition, components of the terminal organizer system generate a follicle-cell-dependent signal, which is deposited between the egg membrane and the surrounding vitelline membrane (Fig. 1b; reviewed in Ref. 4). This signal activates the *torso*-dependent RAF–RAS signalling pathway[5], which overrules the activity of the anterior and posterior maternal systems to establish the terminal regions.

Asymmetry by diffusion and translational repression

Translation of the localized *bicoid* mRNA occurs after egg deposition. This is regulated, in part, by cytoplasmic polyadenylation[6], a process disrupted in the maternal mutants *grauzone* and *cortex*[7]. BICOID diffuses from the anterior pole and thereby forms a concentration gradient extending posteriorly[1] (Fig. 2a). It is a homeodomain transcription factor required for the activation of zygotic genes that establish the head and thoracic segments[1].

NANOS, which is required for abdomen formation[1,3], forms a posterior-to-anterior concentration gradient. It acts along with uniformly distributed PUMILIO, which binds a *nanos* response element within the 3′ untranslated region (3′ UTR) of evenly distributed maternal *hunchback* mRNA. This leads to translational repression of *hunchback* mRNA in the posterior half of the embryo[8]. The zinc-finger-type transcription factor HUNCHBACK is a repressor of the posteriorly expressed gap genes *knirps* and *giant* (Ref. 2; Fig. 2b, 2c). Thus, *nanos*-dependent repression of *hunchback* serves to derepress activation of posterior segmentation genes[1,2] by an activator for which the search took almost a decade.

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BICOID and CAUDAL are redundant posterior activators

The identity of the activator of posterior segmentation genes emerged recently through the analysis of the *cis*-acting control region of the posterior gap gene *knirps* (Refs 9, 10). This control element contains a number of separate modules including a small activator element for ubiquitous gene expression and several repressor elements mediating either *hunchback*-dependent repression from anterior or *tailless*-dependent repression from posterior[9] (Figs 2, 3; see below). Furthermore, binding sites were found for the gap gene proteins KRÜPPEL and GIANT, which enhance and repress *knirps* expression, respectively[9,10].

The activator element of *knirps* was found to bind BICOID and a second homeodomain protein, CAUDAL (Ref. 10). BICOID binds to multiple sites within a 60 bp DNA fragment, while CAUDAL binds to clustered sites within an adjacent fragment. CAUDAL forms a concentration gradient with reversed polarity to BICOID (Refs 11, 12; Fig. 2a). It was noted, however, that embryos lacking both maternal and zygotic *caudal* activity develop abdominal segments, although their pattern was characterized by deletions and fusions of segments[11]. Based on this observation, *caudal* was not considered as the posterior equivalent of *bicoid* that activates posterior segmentation genes. However, the expression patterns of *knirps*, *giant* and the pair-rule gene *hairy* in the blastoderm embryo and the resulting larval phenotype show that the absence of CAUDAL affects posterior segmentation and that the effect is enhanced in embryos lacking both CAUDAL and BICOID (Fig. 3). This argues that BICOID and CAUDAL combine their activating functions to generate the segments along the entire axis. Binding-site deletion studies with the activator element of *knirps* combined with reporter gene expression in mutant embryos revealed that CAUDAL is indeed an activator of posterior *knirps* expression and that BICOID can compensate for the lack of CAUDAL in the posterior region of the blastoderm embryo[10]. This finding explains why the absence of *caudal* activity does not cause the absence of posterior segmentation: BICOID has the ability to substitute, at least partially, for the lack of *caudal* activity in the posterior region of the embryo, indicating that BICOID does not act as a determinant in the anterior region of the embryo exclusively[1].

BICOID controls CAUDAL gradient formation

CAUDAL and HUNCHBACK gradient formation have two features in common. *caudal* and *hunchback* are maternally and zygotically expressed. Their maternal mRNAs remain evenly distributed in the egg whereas the proteins form gradients[1,11–13]. Also, HUNCHBACK is evenly distributed in embryos lacking NANOS (Refs 1, 13) as CAUDAL is in embryos lacking BICOID (Ref. 14). The latter observation indicates that CAUDAL gradient formation involves *bicoid* activity. Recent evidence has shown that the BICOID homeodomain, which was known to act as a DNA-binding motif, can also bind RNA. In fact, BICOID binds via its homeodomain to regulatory sequences present in the 3′ UTR of *caudal* mRNA, blocks cap-dependent translation initiation and thereby prevents CAUDAL synthesis in response to the BICOID gradient[15,16]. This surprising result shows that

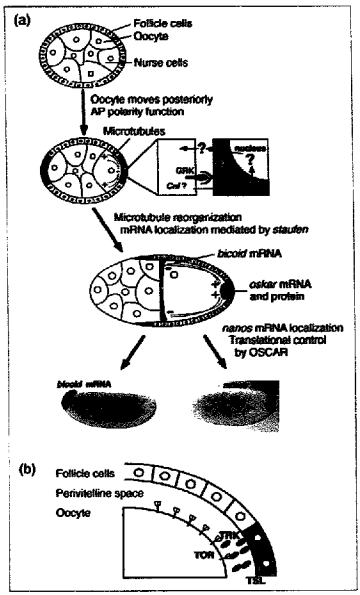

FIGURE 1. Generation of anterior–posterior (AP) polarity and non-segmented regions of the embryo involves two signal transduction pathways. In all figures, anterior is to the left and dorsal to the top. (a) The *Drosophila* egg develops in an egg chamber consisting of an oocyte and 15 siblings, the nurse cells[43]. It gains its polarity by intercellular communication events involving the surrounding epithelium of follicle cells. After an initial move of the oocyte, a signal involving the *gurken–torpedo* signalling pathway, involving cornichon (*cni*), reaches the adjacent posterior follicle cells (reviewed in Ref. 5). *gurken* (*grk*) encodes a transforming growth factor α-like molecule produced in the oocyte, while *torpedo* (*top*) encodes an epidermal growth-factor-receptor-like molecule present in the follicle cells. Due to the position of the oocyte, the *gurken* signal is limited to the posterior-most epithelial cells, which respond by a signal that causes re-orientation of the microtubule cytoskeleton (red) in the oocyte, which allows for *bicoid* mRNA localization at the anterior end and the transport of *oskar* mRNA to the posterior pole. This process also involves the activity of *staufen*, which codes for an RNA-binding microtubule-associated protein that mediates the transport of the mRNAs in a microtubule-dependent manner (reviewed in Refs 3, 44). (b) The 'terminal maternal system', required to establish the terminal pattern elements in the embryo, involves a signal transduction cascade active between the follicle cells and the oocyte. The genes *torso-like* (*tsl*) and *trunk* (*trk*), which are active in the follicle cells[45–47], generate an extraembryonic signal, a putative ligand molecule likely to be the *trunk* gene product, which is stored as a signal in the perivitelline space[47]. After egg deposition, it locally activates the TOR5O (TOR) tyrosine receptor kinase at both ends of the embryo[46], which, in turn, activates a cascade of serine/threonine kinases of the RAF–RAS signal transduction pathway (reviewed in Refs 5, 48).

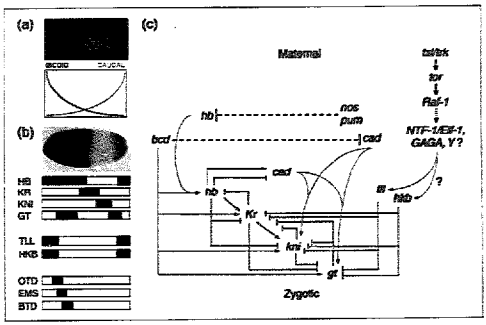

FIGURE 2. Maternal and first zygotic transcription factors, their expression domains and genetic interactions in the pre-blastoderm embryo. (a) BICOID (red) and CAUDAL (green) form opposing concentration gradients along the anterior–posterior axis. (b) Blastoderm expression domain of zygotic *hunchback* (*hb*), and schematic representation of the gap gene expression domains. Note the zygotic *hb* expression in the anterior half, the central domains of *Krüppel* (*Kr*), *knirps* (*kni*) and *giant* (*gt*), the terminal gap genes *tailless* (*tll*) and *huckebein* (*hkb*) at both ends of the embryo, and the domains of the gap-like head genes *orthodenticle* (*otd*), *empty spiracles* (*ems*) and *buttonhead* (*btd*). (For details on the expression domains and the molecular nature of the gap gene proteins, see Ref. 2.) (c) Genetic circuitry establishing the localized expression domains of the gap genes. Red lines represent negative interactions, green arrows represent activating interactions. Note that the maternal terminal pathway, which involves *torso-like* (*tsl*)[35], *trunk* (*trk*)[47] and *torso* (*tor*)[46] activities, the RAS–RAF transduction pathway[5,48], the transcription factors NTF-1/Elf-1 (Ref. 31), GAGA (Ref. 31) and possibly an unknown transcription factor Y (Ref. 1) causes the activation of *tll* and *hkb*. The *tll* and *hkb* activities provide repression of the central gap genes and, thereby, delimit the region of the embryo where segmentation occurs. Note that this pathway acts on both ends of the embryo (only posterior shown). For details of the interactions, see text. Abbreviations other than in (b) are *bicoid* (*bcd*), *caudal* (*cad*), *nanos* (*nos*) and *pumilio* (*pum*).

BICOID functions at different regulatory levels and, thereby, combines the two separate functions provided in the posterior region by NANOS (translational control) and CAUDAL (transcriptional control). Also, the anterior and posterior systems, previously thought to act independently, are linked through the BICOID-dependent spatial control of CAUDAL (Refs 15, 16), which generates a second, complementing, homeodomain protein gradient (Fig. 2a).

First subdivisions by gap gene activities

The first zygotically expressed segmentation genes are the gap genes. Their activities are found in specific regions of the preblastoderm that fail to develop in the respective mutants (reviewed in Refs 1, 2). This class of genes includes the terminal gap genes *tailless* and *huckebein*, the gap-like head genes *orthodenticle*, *empty spiracles* and *buttonhead*, and the central gap genes *hunchback*, *Krüppel*, *knirps* and *giant* (Fig. 2b). Gene expression studies in mutant embryos revealed an elaborate genetic network (Fig. 2c), which established that: (1) terminal gap genes are activated by the maternal terminal system; (2) gap-like head genes are activated by *bicoid*; (3) central gap genes are activated either by a synergistic interaction of *bicoid* and *hunchback* (in the case of zygotic *hunchback*) or by *bicoid* and *hunchback* independently (in the case of *Krüppel*) or by *bicoid* and *caudal* (in the case of *knirps* and *giant*); and (4) the setting of the spatial limits of the central gap gene expression domains involves repression activities by adjacent gap gene expression domains (reviewed in Ref. 2). Only the terminal gap gene activities are controlled independently of the other gap gene activities. However, *tailless* and *huckebein* repress the activity of other zygotic segmentation genes that otherwise would be activated at both ends of the embryo[2,3]. Also, activated *torso* is thought to interfere directly with BICOID and prevent its function in the anterior-most position[17]. Finally, the genetic interactions suggest that gap genes control target gene expression in several different ways. For example, *hunchback* helps *bicoid* to control spatially zygotic *hunchback* expression, and it acts as an activator of *Krüppel* and as a repressor of *knirps* (Ref. 2; Fig. 2c).

Generating adjacent gap gene domains

Molecular dissection of zygotic *hunchback* activation provides a mechanistic model of how the BICOID gradient controls position-dependent target gene expression[18,19]. High-affinity BICOID binding sites within an enhancer cause gene expression at low BICOID concentrations, while low-affinity binding sites cause gene expression at correspondingly higher concentrations within the gradient[18]. This observation implies that binding sites of the highest affinity within a promoter/enhancer define the posterior-most position to which gene activation extends in the BICOID gradient (reviewed in Ref. 1). This would elegantly explain how BICOID defines different posterior limits of gene expression, but more-recent results reach a different conclusion.

BICOID is necessary and sufficient for the activation of zygotic *hunchback* expression. However, it lacks the ability to regulate spatially the expression domain in HUNCHBACK-depleted embryos[20], suggesting that the spatial control by BICOID requires a synergistic interaction with maternal HUNCHBACK in wild-type embryos (Fig. 4a). Cell-free transcription reactions were described that recapitulate transcriptional synergism directed by BICOID and HUNCHBACK (Refs 21, 22). Two specific coactivator subunits (TAF_H110 and TAF_H60) of the basal transcription factor IID (TFIID; reviewed in Ref. 25) served as targets to mediate transcriptional activation by BICOID and HUNCHBACK activities. Quadruple complexes containing the TATA binding protein (TBP) and three coactivator subunits (TAF_H250, TAF_H110 and TAF_H60)

mediated transcriptional synergism in response to BICOID and HUNCHBACK, while complexes lacking $TAF_{II}110$ or $TAF_{II}60$ resulted in non-synergistic activation[21,22]. This finding provides a model of how the concerted action of BICOID and HUNCHBACK with different coactivators establishes the pattern of zygotic *hunchback* expression: BICOID is necessary and sufficient for the activation but does not provide the spatial information for the limits of the expression domain directly. Instead, spatial information is generated by a synergistic interaction between BICOID and maternal HUNCHBACK, causing the efficient recruitment of the $TBP–TAF_{II}$ complexes to the promoter (Fig. 4a).

It is conceivable that *Krüppel* expression is activated in a similar manner and that a repressor prevents activation in the region occupied by zygotic *hunchback*. However, regulation of *Krüppel* expression is more complex: both BICOID and HUNCHBACK act as independent activators and the gap genes expressed adjacent to the *Krüppel* domain restrict activation by repression[24] (Fig. 2b, 2c). In fact, when *knirps*, *giant* or *tailless* expression was ubiquitously induced, their activities were found either to reduce or to abolish *Krüppel* expression[24]. The current data suggest that HUNCHBACK and BICOID activate *Krüppel* broadly, and refined spatial restriction is brought about by redundant repression by the other gap gene activities which antagonize the activation[24] (Fig. 4b).

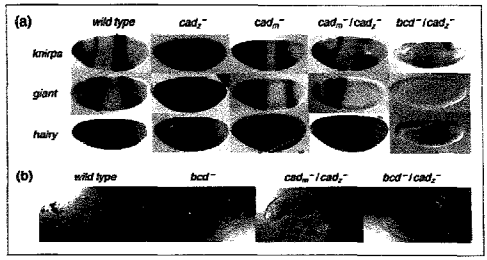

FIGURE 3. Gap and pair-rule gene expression patterns in wild type and *caudal* mutant embryos and phenotypes of the larvae. (a) Blastoderm expression of *knirps*, *giant* and the pair-rule gene *hairy* in wild type, in embryos lacking zygotic (cad_z^-) or maternal (cad_m^-) *caudal* activity, in embryos lacking maternal and zygotic *caudal* activities (cad_m^-/cad_z^-) and in embryos lacking *bicoid* and zygotic *caudal* activities (bcd^-/cad_z^-). Note the low level of *knirps* expression, the absence of *giant* expression and the low level of ubiquitous pair-rule gene *hairy* expression (except for the ends) in bcd^-/cad_z^- embryos, probably due to maternal *caudal* activity (*knirps* expression is absent in $bcd^-/cad_m^-/cad_z^-$ embryos). The anterior expression domain seen in cad_z^- embryos corresponds to β-galactosidase, a genetic marker used to distinguish them from embryos that lack zygotic *caudal* gene expression in addition (for details, see Ref. 10). (b) Cuticle preparations of a wild-type larva showing the normal segment pattern and patterns of various mutants [abbreviations as in (a)]. A low level of ubiquitous pair-rule gene expression [see (a)] is not sufficient to generate any segments in the larva.

The enhancer that is necessary and sufficient for *Krüppel* expression contains multiple overlapping binding sites for repressors and activators[25]. *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 functioning[25]. This would explain why activation of *Krüppel* occurs in the central region of the embryo where repressor concentrations are too low to compete for the binding of the activators (Fig. 4b). However, *knirps* expression, which is posteriorly adjacent to the *Krüppel* domain, is mediated by a modular array of non-overlapping elements where activators and repressors can bind in parallel (Fig. 4c). Furthermore, repressors in the *Krüppel* control region do not only compete for activator binding, but extinguish activation over short distances by a phenomenon termed 'quenching'[26]: repressors interfere with activators through protein–protein interactions and thereby prevent transcription (reviewed in Ref. 27).

The mechanisms establishing the expression domains of the gap-like head genes and the patterns of *giant* expression (Fig. 2c) are not yet studied beyond genetic analysis[28–30]. The collection of players and their impact on gene expression domains make it likely that activation and spatial control of these genes employ similar mechanisms as seen with *hunchback*, *Krüppel* or *knirps*. Activation of the terminal gap genes *huckebein* and *tailless* is also not yet fully understood. Studies on *tailless* regulation, however, suggest that *torso*-dependent activation depends on derepression involving the transcription factors GAGA and NTF-1/Elf-1 (Ref. 31; Fig. 2b).

Taken together, the available evidence suggests that the gap gene expression domains are mainly controlled by mutual repression. One mechanism defining the region of gene expression involves competitive binding of repressors and activators to overlapping sites within the enhancer. 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. Although such a mechanism is intuitively easy to understand, it would not explain the sharp on/off borders of gene expression, which argue for cooperative interactions between the factors that bind. Quenching as an additional mode of repression implies that enhancer-bound factors are able to interact before or while they communicate with the basal transcription machinery. Such interactions have been observed in cell culture and *in vitro* by showing the binding of KRÜPPEL to HUNCHBACK, and of KRÜPPEL to KNIRPS (reviewed in Ref. 32). The results suggested that KNIRPS and HUNCHBACK can interact with KRÜPPEL, which serves as their DNA-bound tether. Furthermore, the phenomenon that HUNCHBACK acts as a synergistic partner of BICOID, as an activator and as a repressor, has an interesting parallel in the finding that KRÜPPEL can also act two ways, at least in cell culture. In this system the KRÜPPEL monomer is able to cause transcriptional

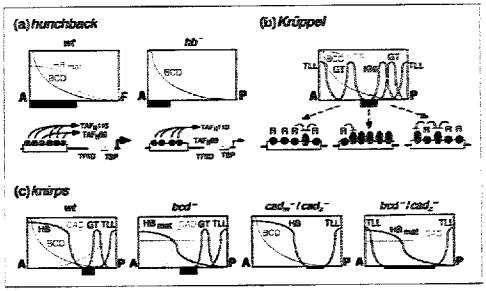

FIGURE 4. Schematic representation of *hunchback*, *Krüppel* and *knirps* expression in response to maternal activators and gap-gene-dependent repression. (a) *hunchback* expression (blue bar) is regulated in response to the graded distribution of BICOID and HUNCHBACK in the egg. The synergism between the two factors required to establish the spatial limit is due to their different contacts with different components of the basal transcription machinery (details in the text). Note that in the absence of maternal *hunchback* activity, BICOID is able to activate zygotic *hunchback* expression (diagram on the right) leading to simple activation at high concentrations of BICOID through limited contacts (TAF_II110) with the basal transcription machinery. (b) *Krüppel* activation is mediated through BICOID and HUNCHBACK independently and spatial restriction (blue bar) is brought about by repression through the adjacently expressed gap genes (see distribution in the diagram on top). In the anterior region (left side), filling of the enhancer involves competition between repressors (R) and activators (A). The central region of the embryo contains low levels of repressors (top diagram) so that activators can bind to the enhancer and, thereby, cause *Krüppel* activation. In the posterior region (right side), activation does not occur due to either a low concentration of the activators or high concentrations of the multiple repressors. (c) The mechanisms involved in *knirps* expression are still elusive. However, the expression patterns of *knirps* in wild-type embryos in response to the activator and repressor distributions suggest that CAUDAL and BICOID cause ubiquitous activation and that the spatial restriction is brought about by repression (see text). Note the expanded *knirps* expression domain in embryos lacking *bicoid* activity (*bcd*⁻) and weak expression in embryos lacking both maternal and zygotic *caudal* (*cad*⁻). In the absence of *bicoid* and zygotic *caudal* activities (*bcd*⁻/*cad*⁻), *knirps* is ubiquitously expressed (in response to maternal *caudal* activity) except at the ends. Abbreviations: BCD, BICOID; GT, GIANT; HB, maternal and zygotic HUNCHBACK; HB_{mat}, maternal HUNCHBACK; TLL, TAILLESS; wt, wild type.

activation when acting from a single binding site in front of a heterologous promotor, while the KRÜPPEL dimer functions as a repressor. The two opposite actions of KRÜPPEL are provided through different interactions with components of the basal transcription machinery involving TFIIB for activation and TFIIE for repression[32]. These results point out that although the functional binding sites and players for most enhancers are known, the mechanism of how spatial control is brought about in the embryo is still elusive.

Gradients turn into stripes

How does the distribution of maternal activators and gap-gene-encoded transcription factors regulate the expression of pair-rule genes in a pattern of seven repetitive stripes? The ten pair-rule genes that encode transcription factors act at two different levels. Accordingly, they were grouped as primary and secondary pair-rule genes[33] (reviewed in Refs 2, 34). The *cis*-acting control of the primary pair-rule genes *even-skipped* and *hairy* depends on a modular array of distinct 'stripe elements'. This phenomenon emerged from altered expression patterns of the pair-rule gene *hairy* in various *hairy* mutant embryos[35]. Subsequent molecular analysis revealed that each stripe element contains a specific set of activator and repressor binding sites[36–38]; maternal transcription factors appear to be activators, the gap-gene-encoded transcription factors act mainly as repressors (reviewed in Refs 2, 34).

Expression of *even-skipped* in stripe two (second stripe from the anterior; anterior to the *Krüppel* expression domain) is activated in response to BICOID and HUNCHBACK, while repression is provided by GIANT and KRÜPPEL (Refs 36, 39). As seen with the *Krüppel* enhancer region, overlapping binding sites for activators and repressors were found, and much of the repressor action is provided through quenching[39]. Conversely, *hairy* stripe six expression (second stripe from the posterior; posterior to the *Krüppel* expression domain) is activated in response to *knirps* (Refs 37, 40) and also by *caudal* (T. Häder and R. Rivera-Pomar, unpublished; Fig. 3). Repression is brought about by *Krüppel*, *hunchback* (the posterior expression domain) and *tailless* (Refs 37, 40). This scenario suggests that the control of the primary pair-rule genes involves the combined activities of maternal and gap genes employing the mechanisms established for gap gene regulation. In addition, stripe expression involves cross-regulatory interactions among the pair-rule genes (reviewed in Refs 2, 34). Thus, the spatial control of pair-rule gene expression is not exclusively dependent on pre-existing transcription factors but also on other pair-rule genes. The initial question of how repetitive stripes are generated has been partially answered by the finding that the composition and concentration of transcription factors is sensed and mediated by separate *cis*-acting stripe elements. The more interesting part of the question – how these elements were generated during evolution and coordinated to result in a metameric pattern – is still open.

Conclusions and perspectives

Pre-embryonic determination of anterior–posterior polarity results in the asymmetric distribution of three maternal transcription factors. Their activities and distributions are linked: BICOID controls the formation of the CAUDAL gradient by translational suppression; it also synergistically interacts with NANOS-controlled maternal HUNCHBACK to define the spatial limit of zygotic HUNCHBACK expression. These findings were

as unexpected as the result that the anterior determinant BICOID substitutes CAUDAL-dependent gene activation in the posterior region of the embryo, including *knirps* and *giant*. Furthermore, the posterior stripes of the secondary pair-rule gene *fushi tarazu* were shown to be CAUDAL dependent[11,51]. Thus, the three maternal transcription factor gradients might not only initiate the segmentation gene cascade but also represent a general activator system that, with the exception of the *torso*-controlled terminal regions, is likely to act at each level of the segmentation gene cascade and possibly also on homeotic genes. The latter proposal is consistent with the finding that the disruption of one of the murine *caudal* homologues affects axial skeletal identities by altering mesodermal expression of *hox* genes in the mouse embryo[42].

Ultimately, activation or repression is achieved through contacts established between the enhancer-bound factors and components of the basal transcription machinery. Such contacts have been unravelled *in vitro*, but it is not yet established whether they are also relevant for the embryo, which of the contacts of enhancer-bound factors will be decisive in driving or preventing transcription and how they do it. Mutually exclusive binding of factors might limit the number of actual players present on the enhancer, but most aspects of the repression involve quenching. This type of repression is likely to involve protein–protein interactions that prevent, for example, contacts of activators with the basal transcription machinery. Thus, although the transcription factors and the arrangement and affinities of their binding sites within the relevant enhancer elements are identified, the mechanism leading to restricted gene expression is still not transparent enough to reveal how the molecular blueprint of the embryonic body pattern is drawn. Future efforts will undoubtedly focus more deeply on how the factors work, how they interact and how they control transcription as well as translation mechanistically. Given the advantage of *Drosophila* for genetic and molecular studies, those basic questions relevant to cell determination and differentiation in general might soon be answered.

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