Int. J. Dev. Biol. 40: 157-165 (1996)

Regulation and function of the terminal gap gene *huckebein* in the *Drosophila* blastoderm

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ABSTRACT Pattern formation in *Drosophila* involves a cascade of maternal and zygotic factors which are spatially restricted in the blastoderm embryo. Here we show that the *Drosophila* gene *huckebein* (*hkb*), a member of the gap gene class of segmentation genes, is not only required for suppression of segmentation in the terminal regions of the embryo but also to spatially restrict germ layer formation at the beginning of gastrulation. *hkb* encodes a Sp1/egr-like zinc finger protein, likely to be a transcription factor. Its absence in *hkb* mutants causes the ectodermal and mesodermal primordia to expand at the expense of endoderm anlagen, which are completely absent in null alleles of *hkb*. Conversely, ectopic expression of *hkb* inhibits the formation of the major gastrulation fold which gives rise to the mesoderm and prevents normal segmentation in the ectoderm of the trunk region.

KEY WORDS: *Drosophila embryogenesis, germ layer formation, terminal gap genes, spatial gene expression, gene regulation, zinc finger protein*

Introduction

Much of our current understanding of the biological processes involved in pattern formation is derived from an extensive analysis of segmentation in *Drosophila* (St. Johnston and Nüsslein, 1992; Hoch and Jäckle, 1993). This research was initiated by mutagenesis experiments that were designed to identify the genetic components required to establish the typical body pattern as defined by the larva's cuticle structures (Nüsslein-Volhard *et al.*, 1984; Wieschaus *et al.*, 1984). Genetics combined with molecular analysis have subsequently revealed an elaborate cascade of hierarchical and cross-regulatory interactions among transcription factors whose local activities form a pre-pattern of the larval body within a single-layered epithelium, the blastoderm (Akam, 1987; Ingham, 1988; Jäckle *et al.*, 1990; St. Johnston and Nüsslein-Volhard, 1992). The regulatory circuitry leading to the establishment of this prepattern along the anterior-posterior and dorsal-ventral axis of the embryo is initiated through spatially localized morphogenetic signals of maternal origin (Nüsslein-Volhard and Roth, 1989) which operate during the syncytial phase of *Drosophila* development. At this developmental phase, which lasts until cellularization occurs at the blastoderm, single morphogenetic signalling molecules are able to diffuse throughout the embryo to instruct groups of rapidly dividing nuclei thereby defining distinct domains of the body (Gaul and Jäckle, 1990).

Once the homogeneous sheet of cellular blastoderm is established, the three different germ layers begin to separate by two major morphogenetic movements during gastrulation. The separation of the mesoderm from the ectoderm is initiated by the formation of the ventral furrow, which arises through invagination of a row of cells along the ventral-most position between approximately, 10-90% of egg length (Simpson, 1983; Grau *et al.*, 1984; Nüsslein-Volhard *et al.*, 1984). On both sides of the ventral furrow lie the endoderm anlagen. The anterior endoderm, located on the ventral side anteriorly to the ventral furrow will invaginate to give rise to the anterior midgut. The posterior endoderm, which eventually forms the posterior midgut, derives from the posterior cap of the blastoderm embryo involving a process called amnioproctodeal invagination. After the initial invaginations had occurred at both ends of the embryo, the endodermal midgut anlagen grow towards the center where they fuse and finally surround the yolk mass. The combined anterior and posterior midgut forms a wide tube which connects the foregut, a derivative of the stomodeal invagination consisting of pharynx, esophagus and proventriculus, and the hindgut throughout the embryo (for review see Skaer, 1993). The hindgut, an ectodermal structure, derives from a circumferential band of cells which separates the posterior midgut anlage from the prospective segmented trunk region of the blastoderm (Technau and Campos-Ortega, 1985). The cells remaining at the surface of the gastrulating embryo develop mainly epidermis and neural tissue, which separates from the epidermal sheet of cells through a process called neuroblast segregation.

All these pattern forming processes depend on the morphogenetic signals generated by four different maternal patterning systems. The most conspicuous function of the anterior and posterior maternal patterning systems is to set up the segment pat-

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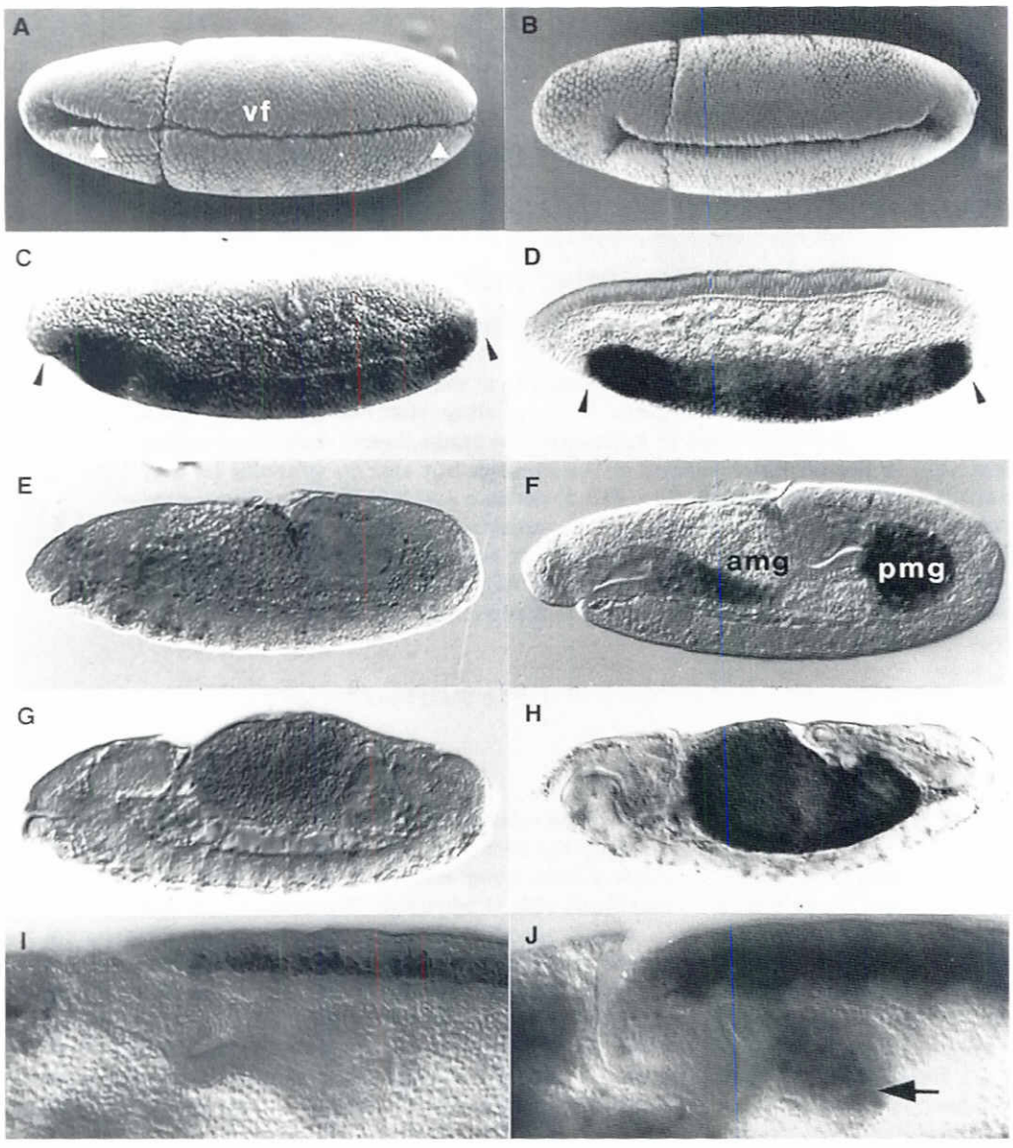

Fig. 1. Phenotypic consequences of the *huckebein* (*hkb*) mutation. **(Left column)** *Embryos missing hkb activity;* **(right column)** *wild type embryos.* **(A,B)** *Scanning electron microphotograghs showing the anterior and posterior expansion of the ventral furrow (vf) in a hkb mutant* **(A)** *and a wild type embryo* **(B)**, *the beginning of gastrulation; arrowheads in* **(A)** *mark the wild type extend of the ventral furrow.* **(C,D)** *In situ hybridization showing the expression of the zfh 1 gene (Lai et al., 1991), a marker for the early mesoderm. Corresponding to the embryos shown in* **(A and B)**, *zfh 1 expression expands into the pole regions of hkb mutant embryos* **(C)**. *Arrowheads mark the morphological extend of the ventral furrow.* **(E-J)** *The endoderm is missing in hkb mutant embryos as shown by the expression of the enhancer trap line PS11 (Bellen et al., 1989). PS11 is expressed in the early anterior (amg) and posterior (pmg) midgut anlagen* **(F)**, *the only endodermal tissues of the fly, and continues in the differentiated midgut after fusion of the anterior and posterior portions (H). In hkb mutants the midgut is missing and no PS 11 expression is detectable* **(E,G)**. *Note that the yolk, which is normally surrounded by the midgut, distributes in ectopic places* **(G)**. **(I,J)** *prospero expression (Olivier et al., 1993) labels a subset of the embryonic midgut cells in wild type* **(J**, *arrow). hkb mutants lack prospero expression* **(I)**, *also indicating the absence of the endodermal midgut. Orientation of embryos is anterior to the left, dorsal side up.*

tern in the ectoderm giving rise to the anterior-posterior cuticle pattern later seen in the larva. These systems generate the asymmetric distribution of transcriptional regulators, starting at both poles of the embryo, respectively. The regulators control the local expression of a series of zygotic transcription factors along the longitudinal axis of the blastoderm embryo (Gaul and Jäckle, 1990; St. Johnston and Nüsslein-Volhard, 1992). In contrast, the dorso-ventral system involves a signal transduction system which generates a gradient of nuclear localization of the NF-kB homologous transcription factor encoded by the gene *dorsal* (*dl*) (Roth *et al.*, 1989; Rushlow *et al.*, 1989; Steward, 1989; Gaul and Jäckle, 1990; St. Johnston and Nüsslein-Volhard, 1992). The *dl* protein acts as a morphogen that determines dorsal-ventral pattern elements in the ectoderm and it specifies the mesoderm anlage in the most ventral position of the embryo. Mesoderm as opposed to ectoderm development is determined by the *dl*-dependent action of at least two zygotic transcription factors encoded by *twist* (Nüsslein-Volhard *et al.*, 1984) and *snail* (Wieschaus *et al.*, 1984). They are thought to activate genes required for cell shape changes leading to the formation of the ventral furrow and for mesodermal differentiation (Bodmer *et al.*, 1990; Leptin and Grunewald, 1990; Leptin, 1991; Shishido *et al.*, 1993), while genes involved in ectodermal development are suppressed by *sna* activity (Leptin, 1991).

The fourth maternal patterning system, the terminal system, uses a signal transduction pathway that controls patterning in two domains at the ends of the blastoderm embryo. From these domains derive the tail and parts of the head, the ectodermal hindgut, and the endodermal posterior midgut (Schüpbach and Wieschaus, 1986; Nüsslein-Volhard *et al.*, 1987; Klingler *et al.*, 1988). The key player of the terminal system, the receptor protein-tyrosine kinase encoded by *torso* (*tor*), is uniformly distributed along the egg cell membrane (Schüpbach and Wieschaus, 1986; Nüsslein-Volhard *et al.*, 1987; Klingler *et al.*, 1988; Sprenger *et al.*, 1989). Upon activation by a ligand, dependent on the gene *torso-like* (Stevens *et al.*, 1990;

Sprenger and Nüsslein-Volhard, 1992; Savant-Bhonsale and Montell, 1993; Martin *et al.*, 1994), and present in the fluid-filled perivitelline space that surrounds the embryo, *tor* triggers a cascade of events that involve highly conserved factors known from mammalian signal transduction pathways (for review see Perrimon, 1993). They link the activated *tor* signal to an unknown nuclear transcription factor (or factors) (Brönner and Jäckle, 1991; Perrimon, 1993) which activates or derepresses a number of zygotic genes in the terminal regions of the embryo.

The best characterized target gene of the *tor*-dependent signalling pathway is *tailless* (*tll*). *tll* encodes a transcription factor which is expressed in the terminal regions of the embryo (Pignoni *et al.*, 1990). Its activity is required for the formation of ectodermal structures posterior to the seventh abdominal segment including the hindgut and the Malpighian tubules as well as for anterior epidermal structures such as foregut, distinct portions of the brain and the head skeleton (Strecker *et al.*, 1988). Recently it has been shown that *huckebein* (*hkb*) in addition to *tll* is specifically involved in mediating *tor*-dependent terminal positional information in the posterior region of the embryo (Weigel *et al.*, 1990; Brönner and Jäckle, 1991). Here we describe the functional and molecular analysis of *hkb* which plays a key role for the development and spatial assignment of the three germ layers in the blastoderm embryo.

Phenotypic consequences of missing *hkb* activity

hkb was recently identified as a terminal gap gene of *Drosophila* (Weigel *et al.*, 1990). Genetic analysis indicated that *hkb* together with *tll* is specifically involved in mediating the maternal terminal information of the *torso* signalling pathway (Weigel *et al.*, 1990; Brönner and Jäckle, 1991). In the absence of the two terminal gap gene activities, as in *hkb,tll* double

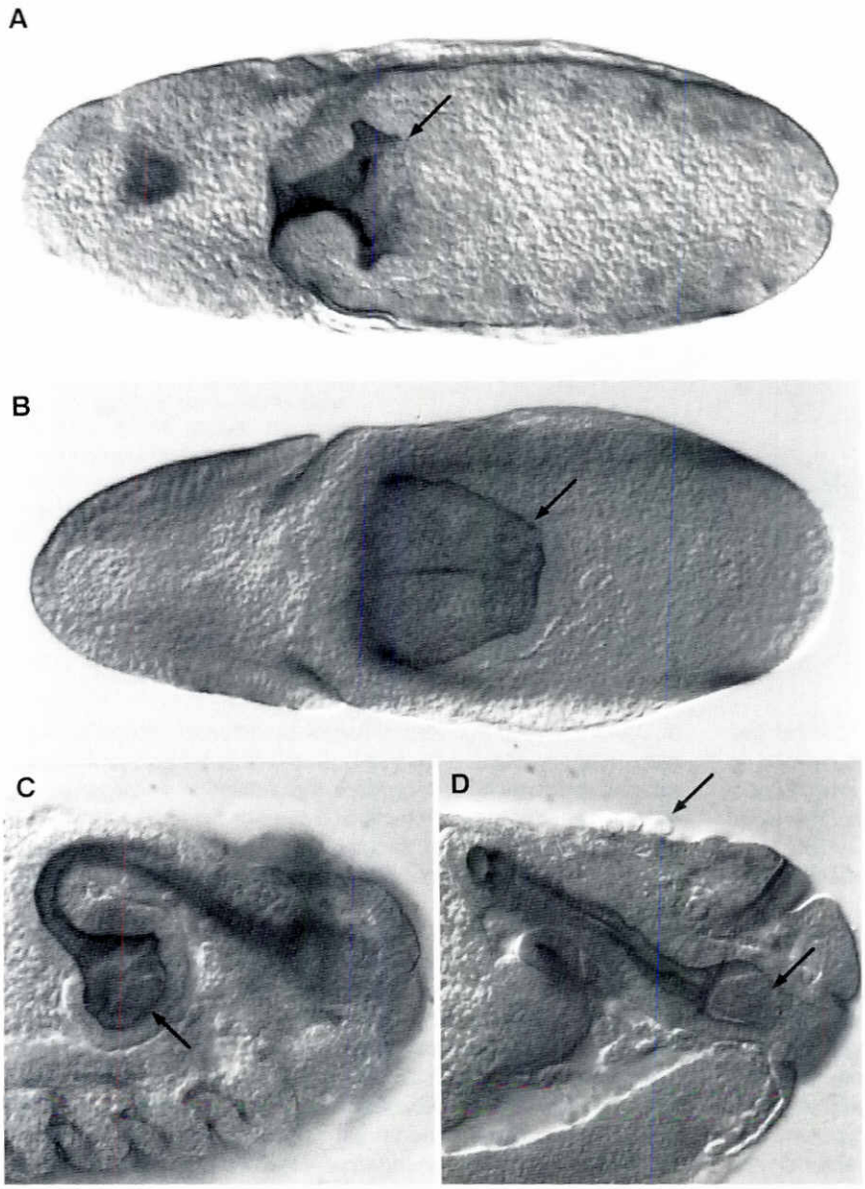

Fig. 2. Anti-*crumbs* (*crb*) antibody stainings in wild type and *hkb* mutant embryos. *crb expression is a marker for ectodermal epithelial cells (Tepass et al., 1990) found in the hindgut but not in the endodermal midgut. In wild type embryos, crb expression forms a steep gradient in the hindgut/midgut transition zone (***A***, arrow), where the hindgut is connected to the midgut. In hkb mutant embryos no crb expression gradient can be seen and crb expressing cells form a pocket closing the hindgut (***B***, arrow). As a consequence, the pole cells which normally would penetrate the midgut on their way towards the embryonic gonads are trapped in the closed tube (***C***, arrow). At later stages of development, however, the pole cells migrate in that they move in reverse orientation than normal to leave the hindgut via the anus (lower arrow) and eventually attach to the outer dorsal surface of the embryo (***D***, upper arrow).* **(A,B)** *Dorsal view, anterior is left.* **(C,D)** *Posterior pole region (anterior is left, dorsal up) of late embryos.*

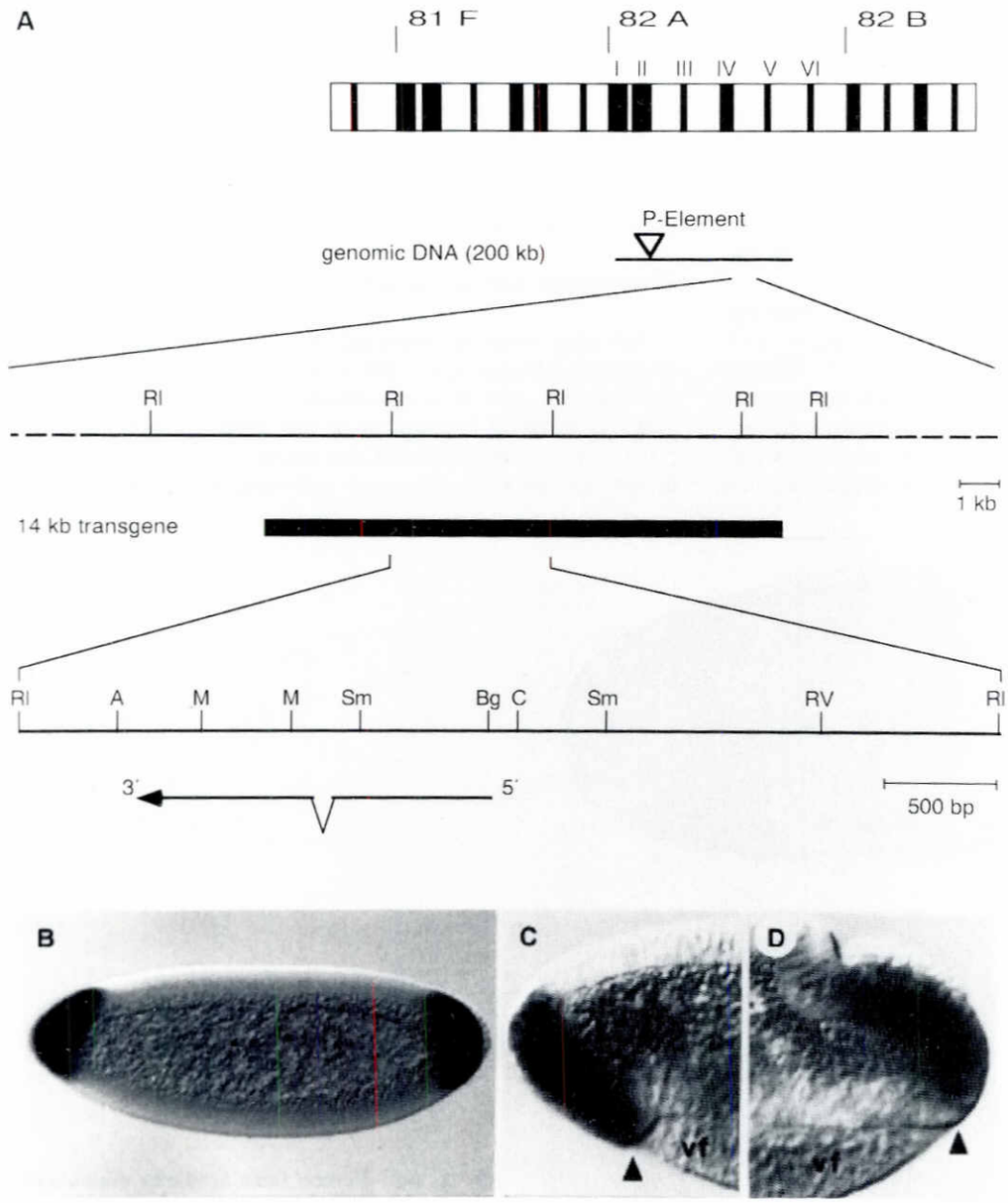

Fig. 3. Cloning and wild type expression of *hkb*. **(A)** *The hkb gene is located in the chromosomal region 82 A (top). Starting from the P-element insertion A321 (Bellen et al., 1989) approx. 200 kb of genomic DNA have been cloned in a chromosomal walk. In this walk a 1.8 kb transcript (bottom) was detected, showing expression in the terminal regions of the embryo (see* **B-D***). A 14 kb transgene containing only this transcript was able to rescue hkb^1/hkb^2 mutant embryos to adulthood. (A, AccI; Bg, BglII; C, ClaI; M, MluI; RI, EcoRI; RV, EcoRV; Sm, SmaI).* **(B)** *In situ hybridization showing hkb expression in the terminal regions of the blastoderm embryo. The anterior* **(C)** *and posterior* **(D)** *domains of hkb expression border the extend of the ventral furrow (vf, arrowheads) in the gastrulating embryo are shown. For a detailed description see Brönner et al. (1994).*

mutant embryos, the expression domains of the "central gap genes" expand posteriorly and the trunk anlagen, as defined by the stripe expression of pair-rule genes, are shifted into the posterior pole region of the embryo (Weigel *et al.,* 1990). The same expansion occurs in embryos lacking maternal *tor* activity, suggesting that *hkb* and *tll* transmit *tor* activity at the posterior end of the blastoderm embryo.

The phenotypic consequences of absent *hkb* activity can be detected as early as the beginning of gastrulation. Both by morphological criteria (Fig. 1A,B) and mesoderm specific marker gene expression (Fig. 1C,D), *hkb* mutant embryos show an abnormal expansion of the ventral furrow towards the pole regions in *hkb* mutant embryos (Fig. 1A,C). This indicates that *hkb* activity is required to spatially restrict the limits of the ventral furrow to the central portion of the gastrulating wild type embryo. Reuter and Leptin have shown that *hkb* sets the anterior and posterior limits of the ventral furrow by different modes of regulation (Reuter and Leptin, 1994). In the anterior part of the blastoderm, *hkb* activity antagonizes the activation of target genes by the mesoderm-determining activities of *twi* and *sna*, while in the posterior pole region, *hkb* activity suppresses the expression of *sna* in the endodermal primordium which is adjacent to the mesoderm primordium.

In *hkb* mutants, the formation of the "polar plate", which carries the combined anlagen of the posterior endoderm and the ectodermal hindgut, as well as the subsequent process termed amnioproctodeal invagination (Campos-Ortega and Hartenstein, 1985) are indistinguishable from wild type embryos by morphological criteria. In order to determine whether the amnioproctodeal invagination of *hkb* mutant embryos carries both the ectodermal hindgut and endodermal midgut anlagen, we analyzed the expression of the endodermal markers PS11 (Bellen *et al.,*

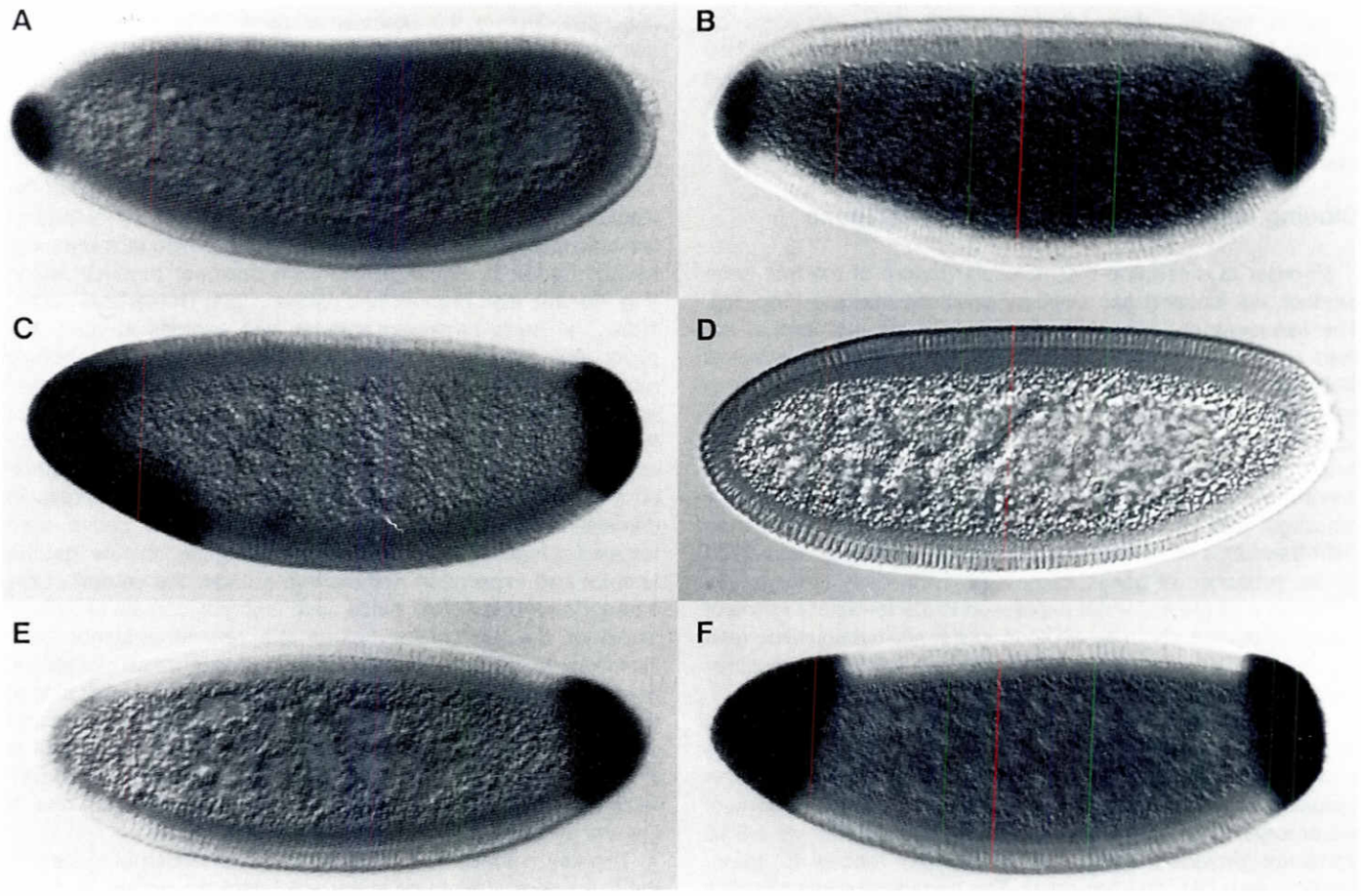

Fig. 4. *In situ* hybridization showing *hkb* expression at blastoderm stage in embryos of females lacking the activity of maternal pattern organizer systems. (A) *In embryos lacking maternal torso (tor) activity, posterior hkb expression is absent and only a small spot of anterior expression remains.* **(B)** *Without bicoid (bcd) activity the anterior expression domain is reduced.* **(C)** *In embryos of females containing four copies of the bcd gene, the anterior expression domain is enlarged. Note the different enlargement on the ventral as compared to the dorsal side.* **(D)** *In embryos lacking both bcd and tor activity hkb expression is absent.* **(E)** *In embryos from dorsal (dl) mutant females the anterior expression domain disappears with the beginning of the cellular blastoderm stage. Note that hkb expression was normal at the preceding syncycial blastoderm (not shown).* **(F)** *In embryos from females carrying the dominant Toll10B allele (note that dl is active in the nuclei both along the dorsal and ventral side of the embryo). Anterior hkb expression extends posteriorly only at the dorsal side. Orientation of blastoderm embryos is anterior left and dorsal up.*

1989) and *prospero* (Olivier *et al.*, 1993). PS11 expression labels the expanding endoderm anlagen (Fig. 1E,F) and it continues to label the midgut throughout embryonic development (Fig. 1G,H); *prospero* labels a subset of cells of the embryonic midgut endoderm (Fig. 1I,J) which express different neuronal marker genes (Olivier *et al.*, 1993). *hkb* mutant embryos fail to express PS11 (Fig. 1E,G) and *prospero* expression is absent in the region where the endoderm anlagen normally develop (Fig. 1I,J). Morphological inspection of differentiated *hkb* embryos indicates that the endodermal midgut is missing; consequently, the yolk of *hkb* mutant embryos distributes into ectopic positions of the embryo since it is not surrounded by the midgut. The remaining ectodermal hindgut of *hkb* mutant embryos forms a closed tube. This is shown by *crumbs* (*crb*) gene expression (Fig. 2) which serves as a cellular marker for epithelial cells of ectodermal origin (Tepass *et al.*, 1990) and thereby allows to distinguish between hindgut (ectodermal origin) and midgut (endodermal origin) development. In the wild-type embryo, *crb* expression forms a steep gradient at the inner end of the hindgut and ends abruptly at the ectodermal hindgut/endodermal midgut transition zone (Fig. 2A). In *hkb* mutant embryos, *crb* expression is seen in both the hindgut and in cells closing the hindgut tube (Fig. 2B,C).

Germ line precursor cells, referred to as "pole cells", normally migrate through the endodermal midgut portion of the gut into the gonadal mesoderm (Campos-Ortega and Hartenstein, 1985). In *hkb* mutant embryos, they are initially trapped within the hindgut at the site where the tube is closed (Fig. 2C). Then, however, they migrate in opposite direction than normal and eventually leave the hindgut through the anus. Consequently, the pole cells of *hkb* mutants end up outside the embryo and can be found attached to the dorsal surface of the embryo (Fig. 2D). This suggests that the ability to migrate is an intrinsic property of the pole cells and that they may normally require guidance by midgut-specific cell surface antigens to follow their stereotype pathway into the developing gonads.

Taken together, these results indicate that *hkb* plays an essential role in specifying endodermal cell fate and to spatially restrict ventral furrow formation at both ends of the wild-type blastoderm embryo. Since the ventral furrow carries the anlagen of the mesoderm, *hkb* is functionally required for the normal formation of the three germ layers in *Drosophila*.

Cloning and characterization of the *hkb* gene

In order to determine the molecular nature of the *hkb* gene product, we isolated *hkb* DNA by positional cloning (Fig. 3A). The *hkb* locus of *Drosophila* is located on the right arm of the third chromosome. Cytogenetic analysis indicated that *hkb* is included in the deletion Df(3R)A321RI which was obtained by γ-ray induced excision of the P-element, a retroviral-like transposable element that had been inserted into the 82A region (Bellen *et al.*, 1989). Genomic sequences flanking the P-element insertion site were cloned to initiate a chromosomal walk which covers about 200 kb of DNA of the 82A region, and overlapping DNA fragments encompassing the cloned region were examined for the presence of genes expressed in the early embryo. We identified a single transcript expressed in the terminal regions of the blastoderm embryo by whole mount *in situ* hybridization (see Fig. 3B,D). The expression pattern of this transcript coincides with the regions known to require *hkb* activity.

In order to identify the corresponding transcription unit as the one encoded by *hkb*, we performed genetic rescue experiments with *hkb* mutants using transgenes containing DNA fragments including the transcription unit expressed in the pole regions of the embryo. A 14 kb transgene which codes for a single 1.8 kb transcript provided significant phenotypic rescue to trans-heterozygous hkb^{1}/hkb^{2} embryos. The transgene-containing *hkb* mutant embryos developed a normal embryonic midgut, a normally sized ventral furrow and the head involution was normal although labral derivatives were often abnormal (Brönner *et al.*, 1994). This indicates that the 1.8 kb transcript contained within the transgene DNA carries *hkb* function. It encodes a Sp1/egr-related protein with three zinc finger domains (Kadonaga *et al.*, 1987; Joseph *et al.*, 1988; Suggs *et al.*, 1990), a glutamine-rich region corresponding to an activation domain (Courey and Tjian, 1988) and an alanine-rich region similar to repressor domains of *Drosophila* transcription factors (Han and Manley, 1993). Based on these similarities, the *hkb* protein can be tentatively grouped as a DNA-binding transcriptional regulator.

Spatiotemporal patterns of *hkb* expression and their control

The spatial and temporal patterns of *hkb* expression were determined using the *hkb* cDNA as a probe for *in situ* hybridization on embryos at different stages of development. *hkb* transcripts were first detected during syncytial blastoderm stage, forming two symmetrical polar caps in the terminal regions of the embryo (Fig. 3B-D). The posterior cap extends from 0-12% of egg length (0% is posterior), the anterior from 90-100% of egg length. With the beginning of gastrulation, the anterior cap expands on the ventral side to a position where the ventral furrow starts to form (Fig. 3C). Similarly, the posterior expression domain of *hkb* delimits the posterior extent of the ventral furrow (Fig. 3D). During the process of germ band extension, *hkb* expression is found in a variety of tissues including the developing central nervous system (CNS). The aspects of *hkb* expression after blastoderm formation are beyond the scope of the present report where we will focus exclusively on the early function of *hkb* expression in the terminal domains at blastoderm.

The activation of *hkb* expression in the posterior region of the blastoderm embryo is exclusively dependent on the activity of the maternal terminal system, i.e. embryos which lack maternal *tor* activity fail to express *hkb* in the posterior terminal region (Fig. 4A; see also Brönner and Jäckle, 1991; Reuter and Leptin, 1994). In such embryos, anterior *hkb* expression does still occur, but the extent of the expression domain is strongly reduced dorsally; only a small spot remains in a anterior-ventral position (Fig. 4A). Similarly, embryos which lack the activity of the anterior morphogen *bicoid* (*bcd*) show a reduced anterior expression domain, whereas the posterior domain is not affected (Fig. 4B). The observation that the anterior *hkb* expression domain expands posteriorly in embryos which derive from females containing four copies of the *bcd* gene indicate that the anterior *hkb* expression domain forms under the control of the anterior morphogen *bcd*, since such embryos contain increased levels of the *bcd* protein along the anterior-posterior axis. However, the posterior expansion of the anterior *hkb* expression domain is asymmetrical and much stronger at the ventral side of the embryo (Fig. 4C; see also below). In *bcd tor* double mutant embryos, *hkb* expression is absent (Fig. 4D). This indicates that in the anterior pole region, the activities of both maternal patterning systems combine to activate *hkb* although each one of the two systems is sufficient for *hkb* activation *per se*.

The key component of the dorsoventral maternal system is the gene *dorsal* (*dl*). In embryos which lack the activity of *dl*, the initial *hkb* anterior expression during syncytial blastoderm stage appears to be normal. However, during cellular blastoderm stage when the anterior *hkb* expression domain in wild type embryos shifts ventro-posteriorly, anterior *hkb* expression is shut down and no transcripts can be detected anymore in the absence of *dl* activity, while the posterior domain of expression remains unaffected (Fig. 4E). Conversely, when *dl* activity is evenly distributed in the nuclei along the dorsoventral axis as found in embryos laid by females containing the dominant $Toll^{10B}$ allele (Roth *et al.*, 1989), the anterior *hkb* expression domain is enlarged symmetrically showing ventrally and dorsally the same posterior extension (Fig. 4F). These results demonstrate that the anterior and posterior terminal expression domains of *hkb* are controlled differently: while the posterior domain of expression is exclusively dependent on the terminal maternal system which involves the *tor*-dependent signalling pathway, the anterior domain of expression requires regulatory inputs from three maternal organizer systems, i.e. the terminal, the anterior and the dorsoventral systems. Both *bcd* and *tor* activity are required for the normal activation of *hkb* in the anterior region, a process initially independent of *dl* activity. During blastoderm, however, when the anterior *hkb* expression domain expands posteriorly in wild type embryos, *dl* protein is required both for maintenance of *hkb* anterior expression and for the posterior expansion along the ventral side of the embryo.

In order to determine whether the activities of the maternal pattern organizers are mediated by known responder genes

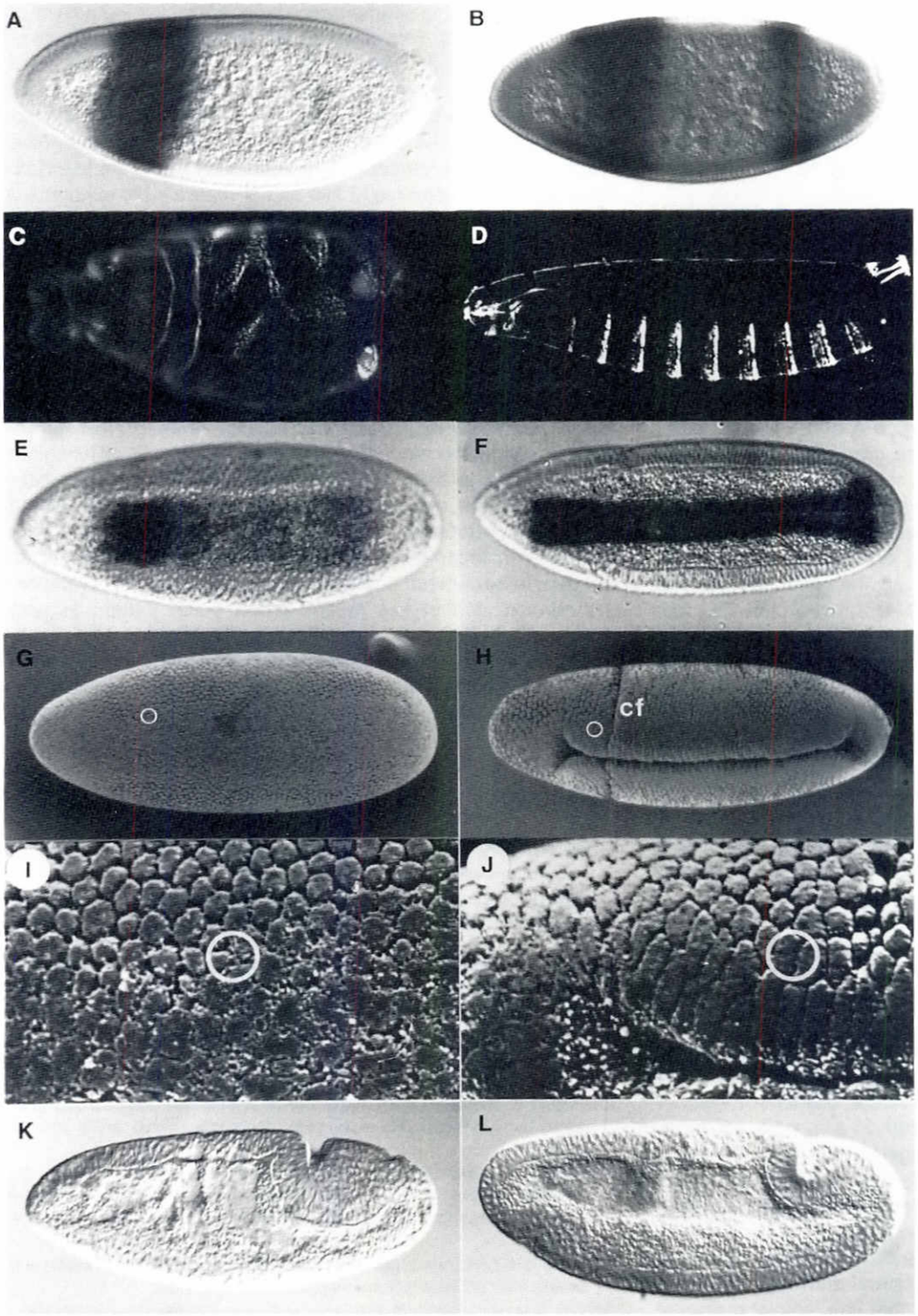

Fig. 5. Effects of ectopic *hkb* expression. *Ectopic expression was achieved by heat treatment of syncytial blastoderm embryos containing a hkb transgene under control of a heatshock inducible promoter. Left column: heat treated embryos; right column: control embryos. Orientation of embryos and larvae is anterior left and dorsal up.* **(A,B)** *In situ hybridization showing expression of the gap gene giant (gt), an integral part the segmentation cascade. Note that abdominal gt expression is repressed by ectopic hkb activity* **(A)**. *Orientation of embryos and larvae is anterior left and dorsal up.* **(C,D)** *Cuticle preparations showing the severe disruption of segmentation by ectopic hkb expression* **(C)** *as compared to wild type* **(D)**. **(E-L)** *Invagination of the ventral furrow is inhibited by ectopic hkb expression.* **(E,F)** *In situ hybridization showing zfh 1 expression (ventral view; anterior left). Heat shocked embryos do not form the slit* **(E,G)** *which is visible in wild type embryos after ventral furrow invagination* **(F,H)**. *zfh 1 expression is not absent upon ectopic hkb expression though a reduction in staining intensity can be seen (compare* **E** *and* **F**). **(G,H)** *Scanning electron micrographs showing that the ventral furrow invagination is absent after ectopic hkb expression (***G***; compare to wild type embryo at the corresponding stage in* **H**). *Circles mark the enlarged areas shown in* **(I,J)**; *cf; cephalic furrow.* **(I,J)** *Enlargements of the ectodermal/mesodermal boundary. Note that ventral cells flatten in a manner similar to mesodermal cells, although the invagination does not occur after ectopic hkb expression while the morphology of ectodermal cells seem unaffected (compare* **I** *with the corresponding wild type embryo shown in* **J**). **(K,L)** *Formation of the amnioproctodeal plate is normal after ectopic hkb expression* **(K)**, *although it seems broadened compared to the wild type situation* **(L)**. *Orientation of gastrulating embryos is anterior left, dorsal up.*

such as *twi*, *sna*, *zerknüllt* (*zen*) and *decapentaplegic* (*dpp*) in the case of *dl* (Nüsslein-Volhard *et al.*, 1984; Wakimoto *et al.*, 1984; Wieschaus *et al.*, 1984; Irish and Gelbart, 1987) or *orthodenticles*, *empty spiracles*, *buttonhead* and *hunchback* in the case of *bcd* (Jürgens *et al.*, 1984; Wieschaus *et al.*, 1984; Lehmann and Nüsslein-Volhard, 1987; Cohen and Jürgens, 1990), we analyzed the *hkb* expression pattern in embryos which lack each of these gene activities. They all showed a normal expression pattern of *hkb* both in the anterior and posterior domains (data not shown). Since *tll* and *hkb* are coexpressed at posterior pole of the embryo, we tested if their expression domains depend on each others' activities. We found, however, that *hkb* is expressed normally in the absence of *tll* activity and *vice versa*. Taken together, these results suggest that *hkb* is like-

ly to be a direct target of the transcription factors encoded by *dl* and *bcd* in the anterior region and of the unidentified transcription factor that is acting in response to *tor* activity at both ends of the embryo.

Ectopic expression of *hkb* suppresses ventral furrow formation and central gap gene activities

The early expression pattern of *hkb* is consistent with the mutant phenotype suggesting that *hkb* activity is required for endoderm development and to spatially restrict the other two germ layers in the blastoderm embryo. To further test this proposal, we generated a transgene that allows for the ectopic expression of *hkb* under heat shock control. Ectopic *hkb* expression at blastoderm stage affects both ectoderm and mesoderm. In the ectoderm, ectopic *hkb* activity represses the activity of the central gap gene *giant* (compare Fig. 5 A and B), an integral component of the segmentation gene cascade (Mohler *et al.,* 1989; Eldon and Pirrotta, 1991).

The posterior domain of *gt* expression extends posteriorly in *tll* mutant embryos. While no such expansion was seen in *hkb* mutant embryos alone, *gt* expansion extends to the tip of the posterior pole of the embryos which lack the combined *hkb* and *tll* activities (Brönner and Jäckle, 1991). This indicates that *hkb* activity together with *tll* activity is responsible for *gt* repression in the posterior pole region. This finding and the absence of the posterior *gt* expression domain in embryos ectopically expressing *hkb* is therefore consistent with the argument that *hkb* activity is able to repress the activation of central gap genes in the posterior region of the embryo in a manner analogous to *tll* activity (Steingrimsson *et al.,* 1991). This function of *hkb* is independent of *tll,* based on the following two observations. First, *tll* expression is not affected in *hkb* mutants and vice versa and secondly, *tll* expression is not significantly altered upon ectopic expression of *hkb* (data not shown).

The interference of ectopic *hkb* expression with the activity of the segmentation gene cascade is also seen by the severe disruption of the segment pattern of the larvae (Fig. 5C,D). Furthermore ectopic *hkb* activity prevents ventral furrow formation along the entire length of the embryo (Fig. 5E-H). This is the opposite of the *hkb* lack-of-function phenotype indicating that *hkb* activity restricts the spatial limits of the ventral furrow in the wild type embryo. However, the mid-ventral blastoderm cells of the *hkb* gain-of-function embryos flatten in a manner similar to mesodermal cells, but they fail to invaginate (Fig. 5I,J). This suggests that the mid ventral region of the embryo begins its normal differentiation pattern, but can not invaginate because *hkb* activity interferes with their proper genetic program. In contrast, no gross morphological defects could be observed with respect to the formation of the combined endodermal and ectodermal gut anlagen of these embryos, except that the amnioproctodeal invagination appears slightly enlarged (Fig. 5K,L).

Conclusions and perspectives

Both the lack-of-function and the gain-of-function conditions show that *hkb* is a morphoregulatory component in the blastoderm which acts in or upon each of the three germ layers of the *Drosophila* embryo. In the ectoderm, *hkb* functions as a terminal gap gene preventing segmentation by suppressing the activity of central gap genes. This effect of *hkb* is similar to the effects of ectopic *tll* activity but independent of it. Thus, the activities of both terminal gap genes combine to a repressor system which provides a stringent border for central gap gene action in the terminal regions of the blastoderm embryo and there interferes negatively with the process of segmentation in the ectoderm. In other words, the activities of *hkb* and *tll* separate the segmented trunk from the non-segmented tail region of the embryo.

Posterior expression of *hkb* is exclusively dependent on the *torso*-dependent terminal signal transduction pathway, while its anterior expression is controlled, in addition to *torso* activity, by the combined activities of the anterior and dorsoventral maternal pattern-forming systems. Once activated, terminal *hkb* activity is essential for endoderm development and it overrules within its domains of activity both ectoderm and mesoderm development. When ectopically expressed, *hkb* inhibits ventral furrow formation throughout the embryo, while the amnioproctodeal invagination continues to occur normally, except that it might be broadened. Thus, the two major morphogenetic movements during *Drosophila* gastrulation, which separate mesoderm and endoderm from ectoderm, are under separate genetic control. Given the generality of these ontogenetic processes and the evolutionary conservation of key factors which guide pattern forming processes in animal development (e.g. Beddington and Smith, 1993; Krumlauf, 1993; Morata, 1993), the findings presented here may provide valuable leads into the factors and mechanisms which cause the separation and differentiation of germ layers.

References

AKAM, M. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development 101:* 1-22.

BEDDINGTON, R.S.P. and SMITH, J.C. (1993). The control of vertebrate gastrulation: inducing signals and responding genes. *Curr. Opin. Genet. Dev. 3:* 655-661.

BELLEN, H.J., O'KANE, C.J., WILSON, C., GROSSNIKLAUS, U., PEARSON, R.K. and GEHRING, W.J. (1989). P-element-mediated enhancer detection: a versatile method to study development in *Drosophila. Genes Dev 3:* 1301-13.

BODMER, R., JAN, L.Y. and JAN, Y.N. (1990). A new homeobox-containing gene, msh-2, is transiently expressed early during mesoderm formation of Drosophila. *Development 110:* 661-669.

BRÖNNER, G. and JÄCKLE, H. (1991). Control and function of terminal gap gene activity in the posterior pole region of the *Drosophila* embryo. *Mech. Dev. 35:* 205-211.

BRÖNNER, G., CHU-LAGRAFF, Q., DOE, C.Q., COHEN, B., WEIGEL, D., TAUBERT, H. and JÄCKLE, H. (1994). Sp1/egr-like zinc-finger protein required for endoderm specification and germ-layer formation in *Drosophila. Nature 369:* 664-668.

CAMPOS-ORTEGA, J.A. and HARTENSTEIN, V. (1985). *The Embryonic Development of Drosophila melanogaster.* Berlin, Springer.

COHEN, S.M. and JÜRGENS, G. (1990). Mediation of *Drosophila* head development by gap-like segmentation genes. *Nature 346:* 482-485.

COUREY, A.J. and TJIAN, R. (1988). Analysis of Sp1 *in vivo* reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. *Cell 55:* 887-98.

ELDON, E.D. and PIRROTTA, V. (1991). Interactions of the *Drosophila* gap gene giant with maternal and zygotic pattern-forming genes. *Development 111:* 367-78.

GAUL, U.V JÄCKLE, H. (1990). Role of gap genes in early *Drosophila* development. *Adv. Genet. 27:* 239-272.

GRAU, Y., CARTERET, G. and SIMPSON, P. (1984). Mutation and chromosomal rearrangements affecting the expression of snail, a gene involved in embryonic patterning in Drosophila melanogaster. *Genetics 108:* 347-360.

HAN, K. and MANLEY, J.L. (1993). Transcriptional repression by the *Drosophila* even-skipped protein: definition of a minimal repression domain. *Genes Dev. 7:* 491-503.

HOCH, M. and JÄCKLE, H. (1993). Transcriptional regulation and spatial patterning in *Drosophila. Curr. Biol. 3:* 566-573.

INGHAM, P.W. (1988). The molecular genetic of embryonic pattern formation in *Drosophila. Nature 335:* 25-34.

IRISH, V.F. and GELBART, W.M. (1987). The *decapentaplegic* gene is required for dorsal-ventral patterning of the *Drosophila* embryo. *Genes Dev. 1:* 868-879.

JÄCKLE, H., PANKRATZ, M.J., BRÖNNER, G., BUSCH, M., ROTHE, M., GERWIN, N., HALBRITTER, H-P., NAUBER, U., HOCH, M., PEHL, M., HARTMANN, C., SAUER, F. and FORSCHBACH, V. (1990). Etablierung metamerer Einheiten in *Drosophila*-Embryo. *Verh. Dtsch. Zool. Ges. 83:* 197-209.

JOSEPH, L.J., LE, B.M., JAMIESON, G.J., ACHARYA, S., SHOWS, T.B., ROWLEY, J.D. and SUKHATME, V.P. (1988). Molecular cloning, sequencing, and mapping of EGR2, a human early growth response gene encoding a protein with "zinc-binding finger" structure. *Proc. Natl. Acad. Sci. USA. 85:* 7164-8.

JÜRGENS, G., WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. and KLUDING, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* II. Zygotic loci on the third chromosome. *Roux Arch. Dev. Biol. 193:* 283-295.

KADONAGA, J.T., CARNER, K.R., MASIARZ, F.R. and TJIAN, R. (1987). Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. *Cell 51:* 1079-90.

KLINGLER, M., ERDÉLI, M., SZABAD, J. and NÜSSLEIN-VOLHARD, C. (1988). Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. *Nature 335:* 275-277.

KRUMLAUF, R. (1993). Mouse *Hox* gene functions. *Curr. Opin. Genet. Dev. 3:* 621-625.

LAI, Z.C., FORTINI, M.E. and RUBIN, G.M. (1991). The embryonic expression patterns of zfh-1 and zfh-2, two *Drosophila* genes encoding novel zinc-finger homeodomain proteins. *Mech. Dev 34:* 123-34.

LEHMANN, R. and NÜSSLEIN-VOLHARD, C. (1987). hunchback, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Dev. Biol. 119:* 402-17.

LEPTIN, M. (1991). twist and snail as positive and negative regulators during Drosophila mesoderm development. *Genes Dev. 5:* 1568-1576.

LEPTIN, M. and GRUNEWALD, B. (1990). Cell shape changes during gastrulation in Drosophila. *Development 110:* 73-84.

MARTIN, J.H., RAIBAUD, A. and OLLO, R. (1994). Terminal pattern elements in Drosophila embryo induced by the Torso-like protein. *Nature 367:* 741-745.

MOHLER, J., ELDON, E.D. and PIRROTTA, V. (1989). A novel spatial transcription pattern associated with the segmentation gene, giant, of *Drosophila. EMBO J. 8:* 1539-48.

MORATA, G. (1993). Homeotic genes of *Drosophila. Curr. Opin. Genet. Dev. 3:* 606-614.

NÜSSLEIN-VOLHARD, C. and ROTH, S. (1989). Axis determination in insect embryos. *Ciba Found. Symp. 144:* 37-55.

NÜSSLEIN-VOLHARD, C., FROHNHOFER, H.G. and LEHMANN, R. (1987). Determination of anteroposterior polarity in *Drosophila. Science 238:* 1675-81.

NÜSSLEIN-VOLHARD, C., WIESCHAUS, E. and KLUDING, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I: Zygotic loci on the second chromosome. *Roux Arch. Dev. Biol. 193:* 267-282.

OLIVIER, J.P., RAABE, T., HENKEMEYER, M., DICKSON, B., MBAMALU, G., MARGOLIS, B., SCHLESSINGER, J., HAFEN, E. and PAWSON, T. (1993). A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of *ras* guanine nucleotide exchange, *Sos. Cell 73:* 179-191.

PERRIMON, N. (1993). The torso receptor protein-tyrosine kinase signaling pathway: an endless story. *Cell* 74: 219-222.

PIGNONI, F., BALDARELLI, R.M., STEINGRIMSSON, E., DIAZ, R.J., PATAPOUTIAN, A., MERRIAM, J.R. and LENGYEL, J.A. (1990). The *Drosophila* gene tailless is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell 62:* 151-63.

REUTER, R. and LEPTIN, M. (1994). Interacting functions of *snail, twist* and *huckebein* during the early development of germ layers in *Drosophila. Development 120:* 1137-1150.

ROTH, S., STEIN, D.V NÜSSLEIN-VOLHARD, C. (1989). A gradient of nuclear localization of the dorsal protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell 59:* 1189-202.

RUSHLOW, C.A., HAN, K., MANLEY, J.L. and LEVINE, M. (1989). The graded distribution of the dorsal morphogen is initiated by selective nuclear transport in *Drosophila. Cell 59:* 1165-77.

SAVANT-BHONSALE, S. and MONTELL, D.J. (1993). Torso-like encodes the localized determinant of Drosophila terminal pattern formation. *Genes Dev. 7:* 2548-2555.

SCHÜPBACH, T. and WIESCHAUS, E. (1986). Maternal effect mutations altering the anterior-posterior pattern of the *Drosophila* embryo. *Roux Arch. Dev. Biol. 195:* 302-317.

SHISHIDO, E., HIGASHIJIMA, S., EMORI, Y. and SAIGO, K. (1993). Two FGF-receptor homologues of *Drosophila:* one is expressed in mesodermal primordium in early embryos. *Development 117:* 751-761.

SIMPSON, P. (1983). Maternal-zygotic gene interactions during formation of the dorsoventral pattern in *Drosophila* embryos. *Genetics 105:* 615-632.

SKAER, H. (1993). *Development of the Alimentary Canal in Drosophila melanogaster. The Development of Drosophila.* CSH Laboratory Press, New York.

SPRENGER, F. and NÜSSLEIN-VOLHARD, C. (1992). Torso receptor activity is regulated by a diffusible ligand produced at the extracellular terminal regions of the *Drosophila* egg. *Cell 71:* 987-1001.

SPRENGER, F., STEVENS, L.M. and NÜSSLEIN-VOLHARD, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature 338:* 478-483.

St. JOHNSTON, D. and NÜSSLEIN-VOLHARD, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell 68:* 201-19.

STEINGRIMSSON, E., PIGNONI, F., LIAW, G.J. and LENGYEL, J.A. (1991). Dual role of the *Drosophila* pattern gene tailless in embryonic termini. *Science 254:* 418-421.

STEVENS, L.M., FROHNHÖFER, H-G., KLINGLER, M. and NÜSSLEIN-VOLHARD, C. (1990). Localized requirement for torsolike expression in follicle cells for the development of terminal anlagen of the *Drosophila* embryo. *Nature 346:* 660-663.

STEWARD, R. (1989). Relocalization of the dorsal protein from the cytoplasm to the nucleus correlates with its function. *Cell 59:* 1179-88.

STRECKER, T.R., MERRIAM, J.R. and LENGYEL, J.A. (1988). Graded requirement for the zygotic terminal gene, tailless, in the brain and tail region of the *Drosophila* embryo. *Development 102:* 721-34.

SUGGS, S.V., KATZOWITZ, J.L., TSAI-MORRIS, C. and SUKHATME, V.P. (1990). cDNA sequence of the human cellular early growth response gene Egr-1. *Nucleic Acids Res. 18:* 4283.

TECHNAU, G.M. and CAMPOS-ORTEGA, J.A. (1985). Fate mapping in the wild type *Drosophila melanogaster.* II. Injections of horseradish peroxidase in cells of the early gastrula stage. *Roux Arch. Dev. Biol. 194:* 196-212.

TEPASS, U., THERES, C. and KNUST, E. (1990). crumbs encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. *Cell 61:* 787-799.

WAKIMOTO, B.T., TURNER, F.R. and KAUFMANN, T.C. (1984). Defects in embryogenesis in mutants associated with the Antennapedia gene complex of *Drosophila melanogaster. Dev. Biol. 102:* 147-172.

WEIGEL, D. and JÄCKLE, H. (1990). The fork head domain: a novel DNA binding motif of eukaryotic transcription factors? *Cell 63:* 455-6.

WEIGEL, D., JÜRGENS, G., KLINGLER, M. and JÄCKLE, H. (1990). Two gap genes mediate maternal terminal pattern formation in *Drosophila. Science 248:* 495-498.

WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. and JÜRGENS, G. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster.* III. Zygotic loci on the X-Chromosome and fourth chromosome. *Roux Arch. Dev. Biol. 193:* 296-307.