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Cis-acting control elements for *Krüppel* expression in the *Drosophila* embryo

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***Krüppel* (*Kr*), a gap gene of *Drosophila*, shows complex spatial patterns of expression during the different stages of embryogenesis. In order to identify *cis*-acting sequences required for normal *Kr* gene expression, we analysed the expression patterns of fusion gene constructs in transgenic embryos. In these constructs, bacterial *lacZ* expression was placed under the control of *Kr* sequences in front of a basal promoter. We identified *cis*-acting *Kr* control units which drive β-galactosidase expression in 10 known locations of *Kr* expression in early and late embryos. More than one *cis*-regulatory element drives the expression in the anterior domain at the blastoderm stage, in the nervous system, the midline precursor cells and in the amnio-serosa. In addition, two *cis*-acting elements direct the first zygotic expression of *Kr* in a striped subpattern within the central region of the blastoderm embryo. Both elements respond to alterations in the activities of maternal organizer genes known to be required for *Kr* expression in establishing the thoracic and anterior abdominal segments in the wild-type embryo.**

Key words: *cis*-regulation/*Drosophila*/*Krüppel*/maternal organizer/tissue-specific expression

Introduction

The establishment of the *Drosophila* body pattern is governed by a hierarchy of interactions between segmentation genes which are expressed in the early embryo (Nüsslein-Volhard and Wieschaus, 1980; for reviews see Akam, 1987; Ingham, 1988). The members of the gap class of zygotic segmentation genes are required for the first subdivision of the embryo into broad areas which correspond to several adjacent segment precursors on the blastoderm fate map. The first zygotic expression of the gap genes *hunchback* (*hb*), *Krüppel* (*Kr*) and *knirps* (*kni*) occurs in defined positions along the anterior–posterior axis of the blastoderm embryo (Tautz *et al.*, 1987; Knipple *et al.*, 1985; Rothe *et al.*, 1989) and requires the preceding activities of the maternal organizer gene products that are initially localized in the polar regions of the egg (Nüsslein-Volhard *et al.*, 1987). *hb*, *Kr* and *kni* encode DNA-binding zinc finger proteins (Tautz *et al.*, 1987; Rosenberg *et al.*, 1986; Nauber *et al.*, 1988) which form overlapping concentration gradients along the longitudinal axis of the blastoderm embryo (Gaul and Jäckle, 1989; Pankratz *et al.*, 1989; Stanojevic *et al.*, 1989; Pankratz *et al.*, 1990). The information generated by these gradients is required for the periodic expression of the pair-rule segmentation genes (Pankratz *et al.*, 1990).

Initial *Kr* expression can be detected by *in situ* hybridization during the syncytial blastoderm stage when the transcripts accumulate within the central region of the embryo. Shortly thereafter, at midblastoderm, *Kr* protein becomes detectable by anti-*Kr* antibodies. The *Kr* protein forms a bell shaped concentration gradient which covers the central region of the embryo (central domain; 'CD') that roughly corresponds to the segment primordia of the three thoracic and the five anterior abdominal segments. These segments fail to develop in *Kr* lack of function mutations, indicating that *Kr* activity is required for the formation of normal thoracic and anterior abdominal segments. During late blastoderm and at later stages of embryogenesis, *Kr* is also expressed in several spatially restricted domains. In view of the fact that *Kr* acts at blastoderm as a transcription factor (Pankratz *et al.*, 1989) within the regulatory cascade of segmentation genes, it may well be that *Kr* has regulatory functions at later stages of embryogenesis to establish several other developmental pathways (Gaul and Jäckle, 1987).

At late blastoderm, *Kr* expression can be observed in an anterior domain ('AD'; at ~80% of egg length, the posterior pole being 0%) and in the posterior pole region (Gaul and Jäckle, 1987). *Kr* expression in this posterior domain ('PD') corresponds in position to the combined hindgut and Malpighian tubule precursors on the blastoderm fate map. At the beginning of gastrulation, *Kr* is also expressed at the anterior pole ('AP'; Harding and Levine, 1988). During gastrulation, these cells enter the stomodeum. They represent the precursor cells of the stomatogastric nervous system (Campos-Ortega and Hartenstein, 1985). While the expression of *Kr* protein in the CD is transient and disappears during gastrulation, *Kr* protein in the PD continues to accumulate in the dorsally migrating polar plate, and remains strong in the precursors of the Malpighian tubules ('MT'), a secretory organ which originates from a ring of cells located between the hindgut and posterior midgut (Harbecke and Janning, 1989). The expression of *Kr* in the MT anlagen and its requirement for MT development are consistent with the lack of MT in *Kr* lack of function mutant embryos (Gloor, 1954) due to a transformation of MT into hindgut (Harbecke and Janning, 1989).

In the elongated germ band, *Kr* protein appears in all cells of the developing amnio-serosa ('AS') and becomes strongly expressed in cells entering the stomodeum (derivatives of the AP; for details see Gaul and Jäckle, 1987). At the same stage, *Kr* protein is observed in a subset of neuroblasts of the developing nervous system ('NS') in which the pattern of *Kr* expression undergoes several complex pattern changes

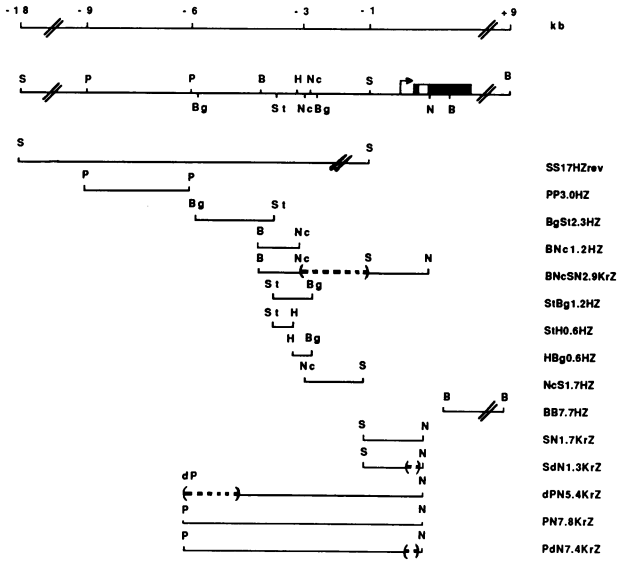

Fig. 1. Localization of *Kr* sequences used to drive *lacZ* fusion gene constructs in transformed embryos. The physical map of the *Kr* gene (below the kb marker; top line) covers ~27 kb of DNA. The arrow marks the transcription start site followed by the open reading frame (black bar) interrupted by an intron (open bar). For details see Rosenberg *et al.* (1986). Diagnostic restriction sites are indicated as B (*Bam*HI), Bg (*Bgl*II), H (*Hin*dIII), N (*Not*I), Nc (*Nco*I), P (*Pst*I), S (*Sal*I), and St (*Stu*I). Different restriction fragments shown below the physical map (thin lines; end labelled by characters designating restriction enzymes) were linked either to the heat shock basal promoter fused to the *lacZ* gene or contained the authentic *Kr* promoter region directly fused to the *lacZ* gene (see Materials and methods). The designation of the fusion gene constructs is shown on the right. Characters refer to restriction sites, numbers to the length of the DNA fragment in kb, HZ refers to constructs containing the heat shock basal promoter and KrZ to constructs containing the authentic *Kr* promoter. Note that the SS17HZrev construct contains the 17 kb *Sal*I–*Sal*I fragment in reverse orientation (rev) in front of the heat shock basal promoter. The thick, dotted lines within brackets indicate the limits of a sequence deletion within the indicated restriction fragments. The domains of expression of the different constructs are summarized in Table I; examples of the expression patterns are shown in Figures 2 and 3.

that have not been analysed in detail. In addition, *Kr* protein appears in the midline precursor cells ('MP') between the commissures and connectives of the nervous system, and in muscle ('M') precursor cells. At the end of embryogenesis, *Kr* protein is most prominent in a small cluster of cells which give rise to the Bolwig organ ('BO'), a light sensory organ (see Campos-Ortega and Hartenstein, 1985) and in a segmentally repeated pattern of cells of the NS, including the brain (Gaul and Jäckle, 1987).

As a first step towards a molecular understanding of how the complex temporal and spatial pattern of *Kr* expression is controlled, we have examined the function of *cis*-regulatory elements of the *Kr* gene by analysing the expression of reporter gene constructs in transgenic embryos. Our results demonstrate multiple and independent *cis*-acting control units within ~18 kb of *Kr* upstream DNA which drive expression in 10 different locations during embryogenesis. Two separate *cis*-acting control units are required to drive *Kr* expression in the central region of the blastoderm embryo, each representing a target for an interaction with known maternal pattern organizer genes and with the gap gene *hb*.

Table I. Domains of *Kr* sequence dependent β-gal expression of fusion gene constructs in the *Drosophila* embryo

	AP	AD	CD	PD	MT	AS	M	NS	BO	MP	ref.
SS17HZrev	+	a	+	+	+	+	(+)	+	+	+	–
PP3.OHZ	–	–	–	–	–	+	–	+	–	+	–
BgSt2.3HZ	–	–	–	–	–	b	c	d	+	–	–
BNcl.2HZ	–	–	+	–	–	(+)	–	–	–	e	–
StBgl.2HZ	–	–	–	–	–	(+)	+	–	–	e	f
StH0.6HZ	–	–	–	–	–	+	–	–	–	+	–
HBg0.6HZ	–	–	–	–	–	+	(+)	–	–	–	–
NcS1.7HZ	–	–	+	–	–	+	(+)	–	–	–	–
BB7.7HZ	–	–	–	–	–	–	–	–	–	–	–
BNcSN2.9KrZ	–	+	+	–	–	+	+	+	–	–	–
SN1.7KrZ	–	+	–	–	–	–	–	+	–	–	g
SdN1.3KrZ	–	+	–	–	–	–	–	(+)	–	–	g
dPN5.4KrZ	–	+	+	–	–	+	+	(+)	–	(+)	–
PN7.8KrZ	–	+	+	–	–	+	+	(+)	+	(+)	–
PdN7.4KrZ	–	+	+	–	–	+	+	(+)	–	(+)	–

+ refers to expression of β-gal in one of the 10 different locations (top row) of *Kr* protein expression (see text) driven by a particular *Kr* β-gal fusion gene construct (left; for details of the construct see Figure 1). – refers to no expression observed. (+) refers to expression at very low levels. Abbreviations are AP (anterior pole), AD (anterior domain), CD (central domain), PD (posterior domain). MT (Malpighian tubules), AS (amnio-serosa), M (muscle precursor cells), NS (nervous system), BO (Bolwig organ) and MP (midline precursor cells).

(a) AD expression could not be observed. We assume that this could be due to the experimentally increased distance from the promoter (see text).

(b,c,d) From five independent transformant lines all expressing β-gal in the BO, one also showed expression in the AS, one in the M and one in the NS. We assume that this might be due to different chromosomal integration sites (discussed in Weigel *et al.*, 1990b).

(e) Although the MP2-element maps onto these constructs, no expression has been observed. Thus, we assume that they contain a repressor element in addition (discussed in Weigel *et al.*, 1990b).

(f) Additional expression in the head region of the late embryo (no correspondence to the *Kr* expression pattern) was noted in several independent lines.

(g) We observed an early striped pattern throughout the gastrulating embryo in several independent lines that has no correspondence to the *Kr* expression pattern.

Results

Localization and functional analysis of cis-acting Kr elements

In order to analyse the *cis*-regulatory elements which drive the *Kr* expression at blastoderm (AD, CD and PD) and during the later stages of embryogenesis (AP, M, AS, MT, NS, BO and MP), we examined the patterns of expression of reporter gene constructs in transformed embryos. Expression of these reporter gene constructs was driven by various DNA fragments of the *Kr* gene. They were linked either to a heat shock basal promoter or to the authentic *Kr* promoter which were fused to the bacterial *lacZ* gene (for details see Materials and methods). The different fusion gene constructs were inserted downstream of the 3′ end of the *rosy* gene in derivatives of the Carnegie 20 P-element vector (Rubin and Spradling, 1982; see Materials and methods). Several independently transformed lines were analysed for each of the fusion gene constructs shown in Figure 1. Embryos were collected from these lines and whole mount preparations were stained with an anti-β-galactosidase ('β-

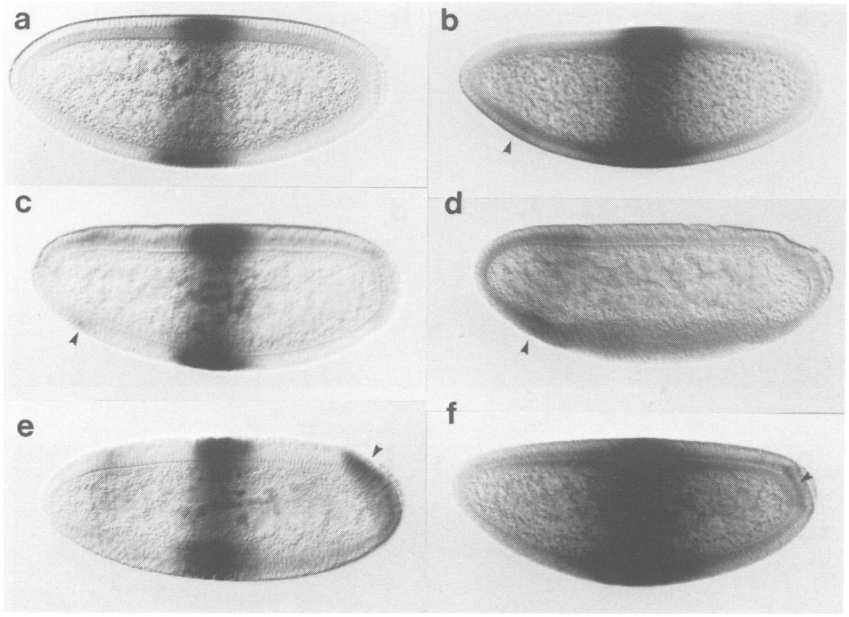

Fig. 2. *Kr* protein distribution and β-gal expression driven by *Kr cis*-acting elements at the blastoderm stage. *Kr* protein expression at early blastoderm occurs (**a**) in a single broad band forming the CD in the centre of the embryo. At mid to late blastoderm (**c**), *Kr* protein can be seen in the AD (arrowhead). At late blastoderm/beginning of gastrulation (**e**), *Kr* protein appears in the PD (arrowhead). *LacZ* fusion genes driven by different *Kr* upstream sequences present in the constructs dPN5.4KrZ (**b**), SdN1.3KrZ (**d**) and SS17HZrev (**f**) show β-gal expression in the corresponding domains of the embryos (b, d, f). Note that the size of these domains varies depending on the staining conditions. In addition, β-gal is cytoplasmic, while the *Kr* protein has a nuclear location. We refer to 'identical patterns' if staining conditions could be obtained which showed *Kr* protein and β-gal expression in congruent domains. For *Kr* transcript patterns see Figure 5a–d. Orientation of embryos is with the anterior to the left and dorsal upwards.

gal') antibody. The domains of expression of the different fusion gene constructs (Figure 1) are summarized in Table I. The *Kr* protein in wild-type embryos and examples of β-gal expression in the various locations of *Kr* protein expression are shown in Figures 2 and 3.

The SS17HZrev construct contains 17 bp of *Kr* upstream DNA starting ~0.9 kb upstream of the *Kr* transcription start site fused to the *hsp/lacZ* reporter gene. Even in reverse orientation, this 17 kb *SalI–SalI* fragment directs β-gal expression in the AP, CD, PD, MT, AS, M, BO, NS and MP, but not in the AD (see Table I). The fusion gene construct SN1.7KrZ, which contains sequences extending from 0.9 kb upstream to 0.8 kb downstream of the *Kr* transcription start site, is expressed in the AD at blastoderm, and later in the NS. The BB7.7HZ construct, which contains ~8 kb of *Kr* downstream sequences (from about +1.3 kb to +9 kb downstream of the *Kr* transcription start site), lacks reproducible expression in wild-type embryos (Table I). Taken together, these results indicate that the sequence information required for embryonic *Kr* expression is contained within *Kr* DNA extending from the *Kr* intron to less than 18 kb upstream of the *Kr* transcription start site.

The 18 kb *Kr* upstream sequence was analysed in further detail to search for individual *cis*-acting control units of the *Kr* gene. The results summarized in Figure 4 and Table I show that the *Kr* upstream region present in the SS17HZrev and SN1.7KrZ constructs contain a complex array of multiple and independent *cis*-acting control elements which direct *Kr* expression in 10 different locations. More than one element drives the initial expression of *Kr* in the central domain and in the anterior domain of the blastoderm embryo, in the NS, the MP and the AS at later stages of embryogenesis.

Elements for the initial Kr expression at blastoderm stage

Between precellular blastoderm and early gastrulation, expression of *Kr* protein can be observed in four distinct locations. Initially it is expressed in the CD, later on in the AD and PD, and finally in the anterior pole region of the late blastoderm embryo (see above). In the CD, high resolution *Kr* antibody staining revealed a graded *Kr* protein distribution which extends from 60% to 33% egg length (Gaul and Jäckle, 1989). The domain of maximum *Kr* protein concentration (~40–50%; see Figure 2a, c, e) corresponds in position to the *Kr* transcript expression domain which broadens towards the ventral side, forming a band of uniform *Kr* transcript accumulation around the blastoderm embryo (Knipple *et al.*, 1985).

Two β-gal constructs (NcS1.7HZ and BNc1.2HZ, Figure 1), containing different portions of the *Kr* upstream sequence in front of the heat shock basal promoter, are expressed at the correct stage in the central domain of the blastoderm embryo. However, β-gal expression driven by the 'CD1-element' (Figures 4 and 5e,f,g; present in BNc1.2HZ) or the 'CD2-element (Figures 4 and 6i; present in NcS1.7HZ) occurs within a region smaller than the *Kr* protein domain. Furthermore, each of the two subdomains shows a striped pattern (Figure 5e,f,g) rather than a contiguous band of expression as seen with the *Kr* protein. With the CD1-element, stripes are observed at the early blastoderm, forming a dynamic pattern which is maintained

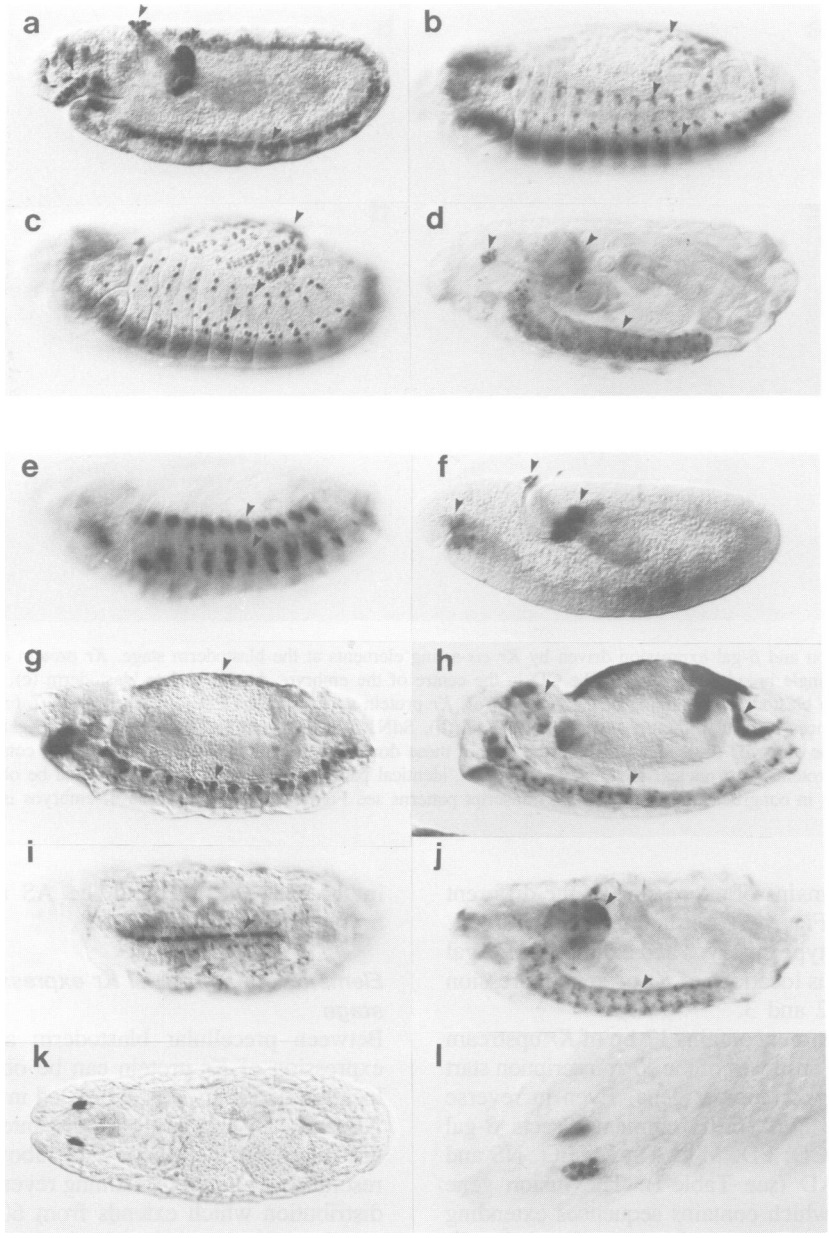

Fig. 3. *Kr* protein and β-gal expression driven by *Kr cis*-acting elements after gastrulation. *Kr* protein during extended germband stage (**a**) showing expression in the stromodeum, amnio-serosa, Malpighian tubule precursor cells and in the central nervous system (arrowheads from left to right). After (**b**) and during (**c**) germband shortening, *Kr* protein can be seen in the amnio-serosa (top arrowhead), muscle precursor cells (lower arrowheads) and in the nervous system including parts of the brain (out of focus; for details see Gaul and Jäckle, 1987). After cuticle formation (**d**), *Kr* protein can be observed in the Bolwig organ, parts of the brain and in a subset of cells in the ventral nervous system (arrowheads left to right). β-gal expression under the control of *Kr* upstream sequences (e–l) can be observed in the *Kr* protein expression domains. (**e**) β-gal expression in muscle precursor cells (arrowheads) under the control of construct StBg1.2HZ. (**f**) β-gal expression in the stromodeum, amnio-serosa and the Malpighian tubule precursor cells of the SS17HZrev construct (compare expression with the embryo shown in (a); arrowheads from left to right. (**g**) Construct PP3.OHZ shows β-gal expression in the amnio-serosa and in a subset of cells in the nervous system (arrowheads from top to bottom). (**h**) SS17HZrev showing β-gal expression in parts of the brain, the Malpighian tubules and in the ventral nervous system (arrowheads from top to bottom). (**i**) Construct StHO.6HZ drives β-gal expression in midline cells (arrowhead). (**j**) β-gal expression in the brain and a subset of cells in the ventral nervous system (arrowheads from top to bottom) derived from construct SN1.7KrZ. β-gal expression of construct BgSt2.3HZ in the Bolwig organ at the late stage of embryogenesis (**k**) and in the hatching larva (**l**). The embryos shown in (e–l) represent examples of β-gal expression patterns of only some of the constructs listed in Figure 1. A summary of the expression patterns of all constructs made is given in Table I. Note that several of these constructs drive β-gal expression in more than one *Kr* domain and that in several domains, β-gal expression is under the control of more than one *Kr* upstream element (see text and Figure 4). Orientation of embryos is with anterior to the left and dorsal upwards.

until gastrulation. With the CD2-element, stripes are observed at midblastoderm stage, i.e. later than with the CD1-element, and the level of expression is lower than that observed with CD1.

As shown in Figure 5a–d, high resolution *in situ* hybridization on whole mount embryos (Tautz and Pfeifle, 1989) reveals a single broad band of high levels of *Kr* expression in the central region of the syncytial blastoderm embryo under all staining conditions applied. However, at the late blastoderm stage when the accumulation of *Kr*

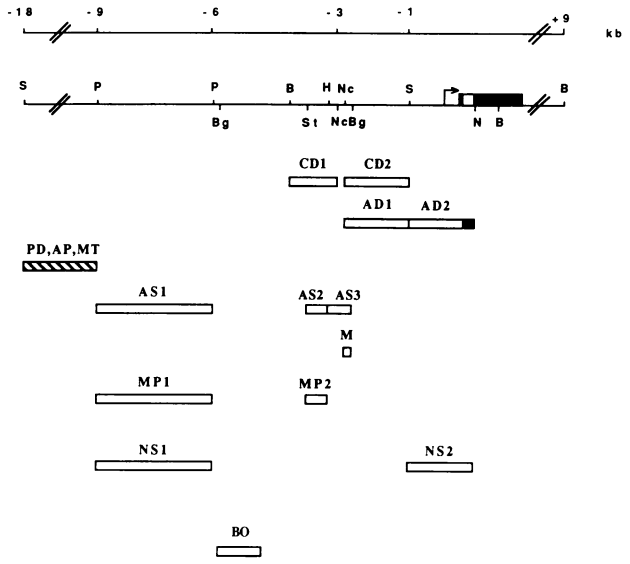

Fig. 4. Localization of *cis*-acting control elements within the *Kr* gene. The uppermost line serves as a distance marker (in kb) for the physical map of the *Kr* gene shown below (for details see legend to Figure 1). The bars shown below the physical map indicate the location of *cis*-acting control elements derived from the analysis of the expression patterns (see summary in Table I) of the constructs listed in Figure 1. The sizes of the bars indicate the length of the DNA fragments which contain a certain element. Elements are labelled (top of the bar) using the abbrevations for the 10 different domains (see text and Table I). Numbers in combinations with elements were used when more than one element has been identified which drives the expression independently in the same domain. Open bars correspond to individual elements which were separated and act in front of the heterologous heat shock basal promoter. Note that the M expression was obtained with both StBg1.2HZ and NcS1.7HZ (Figure 1; Table I). This observation places the M element into the overlap region of the *Kr* upstream fragments (size of the bar). Similarly, BO expression is found with construct BgSt2.3HZ, but not with construct dPN5.4KrZ (Figure 1; Table I) placing the BO elements in the DNA fragment indicated by the bar. Expression in PD, AP and MP was only observed with the SS17HZrev construct (Figure 1; Table I). Therefore, the corresponding elements were not individualized as the others, but must lie in the region labelled by the hatched bar. The black square within AD2 indicates that the *Kr* intron sequences were deleted from the construct and thus, they cannot contain the AD2-element.

transcripts in the central domain has decreased significantly, the former contiguous band of *Kr* transcripts is split into four stripes. Two strong stripes occur in the centre of the *Kr* expression domain, and are flanked asymmetrically by two weak stripes at either side (Figure 5a–d). The four stripes correspond in position and relative staining intensity to the stripes expressed under the control of the CD1- and CD2-elements. With both CD-elements, expression is always observed in the position of stripes 2 and 3. In addition, low level expression can only be observed in stripes 4 and 1 if the embryos are overstained.

The *cis*-acting sequences of both CD1 and CD2 were combined in a single construct which was driven by the authentic *Kr* promoter (dPN5.4KrZ; Figures 1 and 2b). In addition, we made β-gal fusion gene constructs containing the CD1-element linked to the *Kr* basal promoter (BNcSN2.9KrZ) instead of the *hsp* promoter (BNc1.2HZ; see Figures 1 and 5h). In each case, and under all staining conditions applied, only a single and contiguous domain of β-gal expression can be detected. The size of this domain corresponds in size to the *Kr* protein domain (Figure 2a, c, e).

Elements for Kr expression at late blastoderm stage

As shown in Figure 5a–d, whole mount *in situ* hybridization with a *Kr* cDNA probe reveals a pair of narrow stripes in the anterior region of the late blastoderm embryo which had been previously observed as a single band using conventional *in situ* hybridization techniques (Knipple *et al.*, 1985). As in the case of the earlier central domain (see above), we have identified two separate *cis*-acting elements (AD1, AD2; Figures 4 and 5d) which drive expression of β-gal in the AD. The AD1-element is present in the NcS1.7HZ construct that contains the CD2-element (see above). The AD2-element is located within the *SalI–NotI* fragment (region −1 to +0.7) from which the intron sequence has been deleted (Figures 1 and 4). At this point, we do not know if the two AD-elements each gives rise to a single stripe of anterior *Kr* expression.

The two AD-elements map within less than 2 kb upstream of the *Kr* transcription unit (Figure 4). When one of them, AD1, is shifted to a more upstream position, such as in the case of the SS17HZrev construct, no β-gal expression can be observed. Thus, either reversal of the sequence or its increased distance relative to the promoter prevents expression in the anterior domain. Therefore, the AD1-element may not have a typical 'enhancer' function, in contrast to CD1 and/or CD2 which act over a long distance and in reversed orientation as in the SS17HZrev construct (Table I).

The *cis*-acting sequences required for AP expression are far upstream (region −9 to −17; Figure 4). They map to the same *SalI–PstI* fragment which contains the sequences that direct expression in the *Kr* PD. Taken together, the *cis*-acting control units that drive β-gal expression in the *Kr* blastoderm domains are scattered throughout the 18 kb of *Kr* DNA. The elements for *Kr* expression in the pole regions (AP, PD) map far upstream, while the elements for expression in the AD map close to the *Kr* transcription start site (Figures 1 and 4). The CD-elements (CD1, CD2) are in close vicinity, ~3–4 kb upstream of the *Kr* transcribed region.

Elements for late Kr expression

Expression of *Kr* after the blastoderm stage is under the control of at least 10 different regulatory units that drive the expression in the Malpighian tubule anlagen, amnio-serosa cells, muscle precursor cells, the developing nervous system, midline precursor cells and the Bolwig organ (see Figure 3a–d for the *Kr* protein patterns in wild-type embryos and compare with the β-gal expression patterns shown in Figure 3e–l). In extended germband stage embryos, strong β-gal expression can be observed in AS cells and in MT precursor cells (Figure 3f, g, h). For AS expression, three separate *cis*-acting control units were identified (Figure 4; Table I). Two elements are located within adjacent DNA fragments ~3 kb upstream of the *Kr* transcription start site, while the third AS-element maps to the *PstI–PstI* fragment covering the −6 to −9 *Kr* upstream position (Figures 1 and 4). The element(s) required for MT expression lies within the furthest upstream 8 kb *SalI–PstI* fragment which contains the AP- and PD-control region.

At the same time that muscle precursor cells become detectable in the embryo by anti-*Kr* antibody staining (Gaul

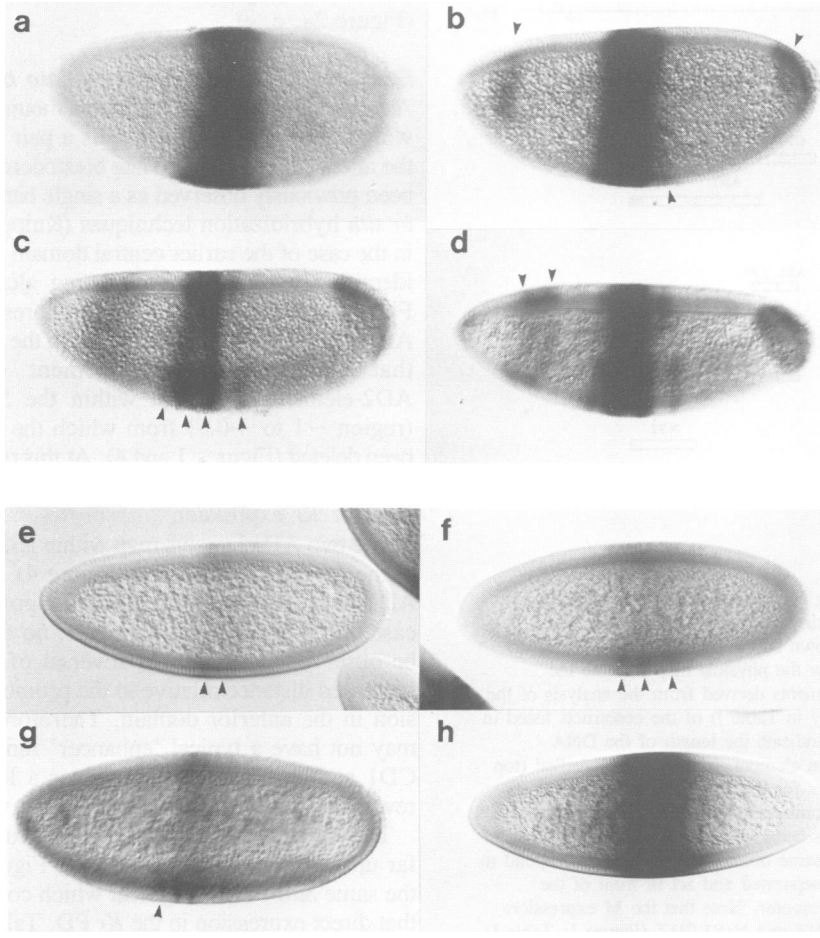

Fig. 5. Early *Kr* transcript pattern and β-gal expression driven by the CD1-element at blastoderm stage. Early *Kr* gene expression (**a**–**d**) revealed by whole mount *in situ* hybridization. (**a**) Shows a contiguous band of transcripts at early blastoderm stage which splits into a total of four stripes prior to gastrulation (arrowheads in b,c). (**b**) Late blastoderm embryo showing a single stripe in the AD, first signs of stripes in the CD and the PD (arrowheads from left to right). (**c, d**) Embryos at the beginning of gastrulation showing, in different focal planes, two stripes in the AD, four stripes of different intensities in the CD and the PD. Note that slight overstaining of the embryo in (d) masks the two strong stripes in the CD clearly separated in (c). The CD1-element shows a striped pattern of β-gal expression in front of the heat shock basal promoter (**e**–**g**). The striped pattern forms dynamically, i.e. stripes 2 and 3 appear first (arrowheads left to right in e). Under optimal staining conditions, stripe 4 (arrowhead to the right in f) and stripe 1 (arrowhead in g) can be observed. We note that β-gal expression of the CD1-element is dramatically increased by replacing the heat shock promoter with a DNA fragment covering the authentic *Kr* promoter as shown in (**h**) with the BNcSN2.9KrZ construct (see Figure 1). Staining conditions could not be adjusted to reveal the striped pattern shown in (e–g). Orientation of embryos is with anterior to the left and dorsal upwards.

and Jäckle, 1987), β-gal expression under the control of *Kr* upstream sequences can be observed in these cells (Figure 3e; 'M-element'; see Table I and Figure 4). In contrast to the *Kr* staining pattern which is only transient during early muscle development (Gaul and Jäckle, 1987), β-gal activity persists in muscle cells of the late embryo (possibly due to differential regulation of β-gal and the *Kr* protein).

At least two different *cis*-acting control units drive *Kr* expression in the developing nervous system and in the midline precursor cells (Figure 3i, j; see Table I and Figure 4). The expression patterns under the control of the two NS-elements (region −0.9 to +0.8; −6 to −9) have not been analysed in detail. It appears likely, however, that each NS-element drives expression in different as well as overlapping subsets of NS cells. We note that the complexity of the NS expression pattern is further increased when the *Kr* intron is present in the *lacZ* constructs (Figure 1; Table I). Thus, we suspect that regulatory sequences for NS expression also lie within the *Kr* intron. The elements for MP expression lead to segmentally repeated patterns which are distinctly different from each other, i.e. they occur in a different subset of midline precursor cells (Figure 3i).

Late expression of *Kr* is found in the Bolwig organ (Gaul and Jäckle, 1987). The sequences required for expression in this organ (Figure 3k, l) are located within the *BglII*–*StuI* fragment which maps ~5 kb upstream of the *Kr* transcription start site (Figure 4). A detailed molecular and functional analysis of *Kr* expression in this organ as well as in the nervous system is beyond the scope of the present study and will be presented elsewhere.

Control of the CD-elements in the early embryo

Kr expression in the central domain (CD) of the blastoderm embryo is required for the normal formation of thoracic and anterior abdominal segments (Wieschaus *et al.*, 1984; see

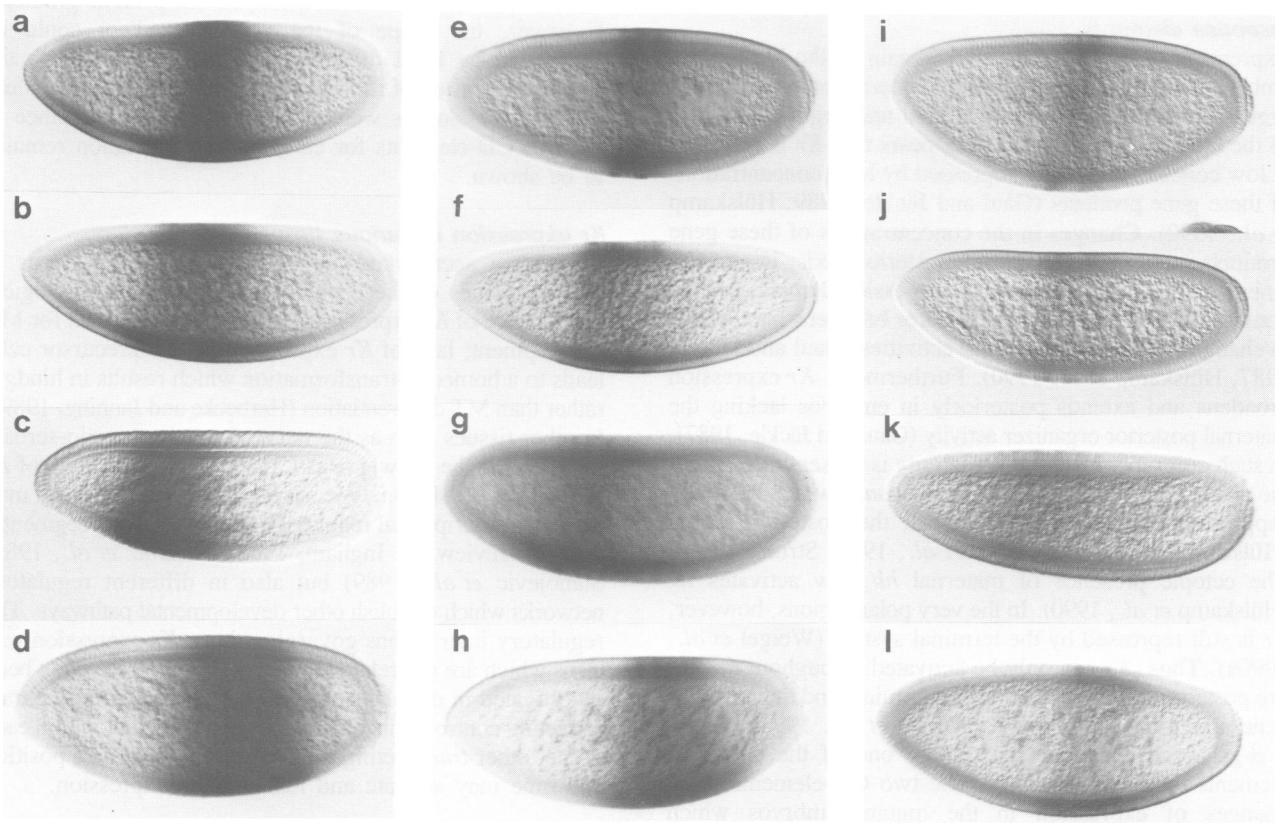

Fig. 6. CD1- and CD2-dependent β-gal expression in maternal effect and gap mutant embryos. β-gal under the control of both CD1- and CD2-elements (**a**–**d**; construct dPN5.4KrZ, Figure 1) or the CD1-element (**e**–**h**; construct BNc1.2HZ, Figure 1) or the CD2-element (**i**–**l**; construct NcS1.7HZ, Figure 1) in wild-type embryos (**a, e, f**) and embryos which are mutant for *hb* (**b, f, j**), *bcd* (c, g, k) or *nos* (d, h, l). Note the similar changes in the expression patterns as has been observed for the *Kr* protein pattern (Gaul and Jäckle, 1987). In the case of CD2-embryos (**i**–**l**), in which the CD is expressed at the lowest level (just above the detection limit), we measured the CD domain of β-gal staining in several embryos and in different optical sections under the microscope: for details, see the text. Orientation of embryos is dorsal upwards and anterior to the left.

also Introduction). The control of *Kr* expression in this domain is primarily under the control of maternal organizer genes (Gaul and Jäckle, 1987, 1989). We therefore asked whether the two constructs which drive β-gal expression in the CD contain the target sequences for regulation by the maternal organizer activities. To investigate this, we examined the β-gal expression patterns of the two fusion gene constructs (BNc1.2HZ, NcS1.7HZ; Figure 1) containing either the CD1- or the CD2-element (Figure 4) in front of the heat shock basal promoter, and the dPN5.4KrZ (Figure 1) containing both CD-elements in front of the authentic *Kr* promoter in embryos which lacked the activity of maternal organizer genes (Figure 6).

In embryos which lack the activity of the posterior organizer gene *nos* (Figure 6d, h, i), the expression domain of β-gal is extended posteriorly; similar results have been observed for the *Kr* protein (Gaul and Jackle, 1987). Conversely, when embryos lack the activity of the anterior organizer gene *bcd* (Figure 6c, g, h), the β-gal expression domain is shifted anteriorly. Furthermore, in the absence of zygotic *hb* activity (Figure 6b, f, j), the β-gal expression domains extend towards the anterior. Thus, each of the two CD-elements must contain the *cis*-regulatory sequences that act as targets for the direct or indirect interaction with *bcd*, *nos* and *hb*, and which are required for the normal expression of *Kr* in the central domain. Note that the expression of the CD2 construct is always weaker than that observed with CD1.

Discussion

We have identified *cis*-regulatory elements within ~18 kb of *Kr* DNA sequences (Figure 4; Table I) which are sufficient to generate the qualitative aspects of the complex spatial patterns of *Kr* gene expression. The late expression of *Kr* in amnio-serosa and in particular in the nervous system is under the control of several *cis*-acting elements which may act in concert to generate the normal levels of *Kr* protein within a given tissue. This aspect of the *Kr* expression pattern, which may be of important biological significance, cannot be addressed here because of extraneous parameters such as the differential stability of *Kr* and β-gal RNAs or proteins. We note that the level of β-gal differs significantly when the *cis*-acting control units were used in conjunction with the authentic *Kr* basal promoter instead of the heat shock basal promoter. This observation leaves open the question of whether the *Kr* basal promoter is intrinsically stronger than the *hsp* promoter or whether the higher level of expression requires a specific interaction of the *Kr cis*-regulatory elements with its own promoter. Alternatively, the stronger expression could also be due to *Kr* sequences of the 3′ untranslated region which is present in the constructs containing the authentic *Kr* basal promoter.

CD1 and CD2 contain maternal and gap gene response elements

Expression of *Kr* in the central domain of the blastoderm embryo is under the control of the maternal anterior pattern organizer gene *bcd* and the maternal and zygotic activities of the anterior gap gene *hb*. It appears that *Kr* is activated at low concentrations and repressed by high concentrations of these gene products (Gaul and Jäckle, 1989; Hülskamp *et al.*, 1990). Changes in the concentrations of these gene products along the anterior–posterior axis in mutant embryos results in an altered *Kr* expression domain, i.e. the domain of *Kr* expression shifts and/or broadens in response to changes in the *bcd* and/or *hb* activities (Gaul and Jäckle, 1987; Hülskamp *et al.* 1990). Furthermore, *Kr* expression broadens and extends posteriorly in embryos lacking the maternal posterior organizer activity (Gaul and Jäckle, 1987). In such embryos, maternal *hb* activity is present throughout the entire embryo due to the lack of *nanos* which normally suppresses maternal *hb* activity in the posterior region (Hülskamp *et al.*, 1989; Irish *et al.*, 1989; Struhl, 1989). The ectopic presence of maternal *hb* now activates *Kr* (Hülskamp *et al.*, 1990). In the very polar regions, however, *Kr* is still repressed by the terminal system (Weigel *et al.*, 1990a). Thus, *Kr* can only be activated throughout the entire posterior region when both the terminal and the posterior gene activities are removed (Weigel *et al.*, 1990a).

β-gal gene constructs containing one of the two CD-elements or a combination of the two CD-elements show changes of expression in the mutant embryos which correspond to those observed with the endogenous *Kr* protein. These results indicate that each of the two *cis*-acting elements functions as a separate unit in response to *bcd* and *hb* activity. Furthermore, since CD1- and CD2-dependent expression is expanded in *nos* mutant embryos but does not extend to the posterior pole (see above), each of the two elements should contain the sequences which respond to the activity of the terminal gene system.

Kr expression in the central domain

The β-gal expression driven by each of the two CD-elements is low, and it results in striped patterns. The initial expression of β-gal from each of the two CD-elements might be an artefact due to the use of the heterologous heat shock promoter or due to the separation of the two elements that normally act together in front of the *Kr* promoter. However, the superimposed striped pattern which derives from the two separate CD-constructs corresponds in position and relative intensity to the stripes of the endogenous *Kr* transcripts seen at the late blastoderm stage. At early blastoderm, *Kr* is strongly expressed in a broad, contiguous domain. This pattern of expression was observed with CD-constructs containing the authentic *Kr* promoter independent of whether only one or both CD-elements were driving the expression. These results suggest that a high level of expression masks the subpattern of *Kr* expression and that the *Kr* gene might be transcribed in a subpattern of stripes from early on.

The β-gal protein that derives from constructs containing the combined CD1/CD2-elements or the CD1-element in combination with the authentic *Kr* promoter forms a bell shaped gradient as has been observed for the *Kr* protein domain (Gaul and Jäckle, 1987, 1989). This observation suggests that passive diffusion from a local source might be sufficient to generate a bell shaped *Kr* protein gradient. However, the shape of the protein gradient could be modulated by local differences of transcripts due to the striped expression of *Kr*. The relevance of the striped pattern of *Kr* expression as well as the biological significance of the two CD-elements for early pattern formation remains to be shown.

Kr expression in various tissues

Kr protein accumulates in defined temporal patterns in various tissues of the developing embryo. The biological significance of *Kr* expression has been demonstrated for MT development; lack of *Kr* expression in MT precursor cells leads to a homeotic transformation which results in hindgut rather than MT differentiation (Harbecke and Janning, 1989). In other tissues such as the nervous system, amnio-serosa, muscles and the Bolwig organ, the biological function of *Kr* remains to be shown. We suspect, however, that *Kr* may act as a transcriptional regulator not only for body segmentation (for review see Ingham, 1988; Pankratz *et al.*, 1989; Stanojevic *et al.*, 1989) but also in different regulatory networks which establish other developmental pathways. The regulatory interactions governing those *Kr* expression patterns which are not related to metamerization have not been investigated in detail. In view of the different and separate *cis*-acting control units of *Kr*, it seems likely that within each tissue, other *trans*-acting factors present at a given position and time may activate and maintain *Kr* expression.

Materials and methods

Construction of Kr fusion genes and germ-line transformation

Various fragments of the *Kr* gene of Oregon R origin (Figure 1; for an overview see Rosenberg *et al.*, 1986) were cloned into the polylinker of the HZ50PL P-element vector (Hiromi and Gehring, 1987) using the restriction sites indicated in Figure 1. The original direction of the *Kr* transcribed region was maintained in all constructs except in SS17HZrev. The P-element constructs containing the authentic *Kr* promoter were generated by fusing a 5′ *Kr* DNA framgent (*SalI*–*NotI*; −0.9 to +0.9; see Rosenberg *et al.*, 1986) in frame to the bacterial *lacZ* coding sequence (from the vector pMc1871) linked to a 700 bp *Sau*3AI *Kr* fragment (which contains the 3′ untranslated region; see Rosenberg *et al.*, 1986). This DNA construct was inserted into Carnegie 20 vector DNA, and various upstream fragments (see Figure 1) were inserted in front of the *Kr SalI* site to give rise to the different KrZ constructs listed in Table I and Figure 1. The different P-element constructs (see Figure 1) were injected into ry^{506} embryos (300 μg/ml construct DNA, 50 μg/ml of helper plasmid DNA) as described by Mullins *et al.*, 1989. Expression of β-gal was monitored by antibody staining (see below) in the progeny of transformed flies at all stages of embryogenesis. For each construct, at least three independent lines which showed the same β-gal expression pattern were established.

Drosophila strains and mutant embryos

The P-element constructs were maintained in a ry^{506} homozygous background. In addition, the P-element constructs were crossed into several mutant lines such as bcd^{E1}, hb^{7M}, and nos^{L7} (Nüsslein-Volhard *et al.*, 1987; Lehmann, 1985). Embryos mutant for a given maternal gene were identified by the genotype of the mother and by scoring the phenotype of sibling embryos all showing a *bcd* or *nos* mutant phenotype. *hb* mutants were identified by double staining with *hb* antibodies (Tautz and Pfeifle, 1989).

Antibody staining of embryos

Anti-*Kr* antibodies have been described by Gaul *et al.* (1897); anti-β-galactosidase antibodies were purchased from Cappel. Antibody staining of whole mount embryos was carried out as described (Macdonald and Struhl, 1986) using the Vectastain ABC Elite-horseradish peroxidase system and the modifications described in Weigel *et al.* (1990b).

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