Development 104 Supplement, 29–34 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

Regulation and putative function of the *Drosophila* gap gene *Krüppel*

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Summary

The *Drosophila* segmentation gene *Krüppel* (*Kr*) is expressed in a broad band of cells that covers about four-segment primordia in the blastoderm embryo. Examination of size and position of the *Kr* protein domain in various mutant embryos revealed that the establishment of the domain of *Kr* gene expression is under the control of the maternal effect pattern organizers which act at the poles. The lack of *Kr* activity causes a gap in the segment pattern of the embryo which is about twice the size of the *Kr* expression domain and extends posterior to it. This indicates that *Kr* activity *per se* is not directly responsible for the establishment of the pattern elements which are deleted in the mutant embryo. Examination of the molecular lesions in four *Kr* alleles indicated that each of them is a point mutant within the coding sequence of the *Kr* gene and each mutation results in a different replacement of a single amino acid within the 'finger domain' of the *Kr* protein. Thus, this region of the *Kr* protein is essential for *Kr* function. Since this portion of the *Kr* protein shares structural homology with the DNA-binding domain of several transcription factors, we propose that *Kr* acts as a transcription factor on subordinate genes that process the spatial cues provided by *Kr* activity to establish eventually the segments in the central region of the embryo.

Key words: *Drosophila*, gap gene, *Krüppel*, *Kr*, gene expression, maternal effect, pattern organizer.

Introduction

The anterior–posterior body pattern of the *Drosophila* embryo consists of a defined number and sequence of segmental units. The establishment of this metameric pattern depends on the activity of different classes of genes. The earliest acting genes are the maternal-effect genes, which can be subdivided into three groups: the anterior pattern organizer genes, the posterior pattern organizer genes and the genes required for the normal establishment of the termini (Nüsslein-Volhard *et al.* 1987). The maternal information is interpreted and refined by the zygotically active segmentation genes (reviewed by Akam, 1987) which are required to establish the number of metameric units in the embryo. According to the phenotype of the mutant embryos, the segmentation genes can be grouped into three major classes: gap genes, pair-rule genes and segment-polarity genes. Gap mutants are characterized by a deletion of a group of adjacent segments in the segment pattern and thus, the wild-type function may be to provide a coarse subdivision of the anterior–posterior axis of the embryo into contiguous regions. The pair-rule genes define the boundaries of single metameric units in a double segment periodicity, and the segment-polarity genes act within single metameres (Nüsslein-Volhard & Wieschaus, 1980).

As early as the cellular blastoderm stage, specific groups of cells are already committed to specific developmental pathways. Molecular probes for different pair-rule genes have revealed that their domains of expression at this stage roughly coincide with the primordia of the pattern elements missing in the corresponding mutant embryos (for review see Akam, 1987). In addition, such probes were used to monitor the patterns of expression of a segmentation gene in embryos mutant for other segmentation genes. Changes of the normal expression pattern are indicative of a wild-type gene function which is required for the normal expression of the gene examined. Such experiments have shown that ex-

pression of the pair-rule genes require the preceding activity of gap genes and that pair-rule gene activity is necessary to establish metameric units in the embryo, and to regulate homeotic gene activities required to specify segment identity (for a review see Akam, 1987).

In contrast to the pair-rule genes, the role of the gap genes in the process of segmentation is less apparent. Here we describe the regulation of the gap gene *Krüppel (Kr)* and its role in the establishment of the segment pattern of the *Drosophila* embryo.

Results

Kr gene activity is required for the establishment of the three thoracic and the five anterior abdominal segments in embryos as can be deduced from the pattern deletions developed by the mutant embryo (Fig. 1A,B). We have produced antibodies directed against the *Kr* protein which allow visualization of the pattern of *Kr* expression. At the blastoderm stage, when *Kr* activity is first required (Wieschaus *et al.* 1984), the *Kr* protein is expressed in a broad band in the centre of the embryo covering about 12 cells dorsally and about 16 cells ventrally (Fig. 2A; for a detailed description see Gaul *et al.* 1987). The size and position of the *Kr* domain (Fig. 2A) is constant in wild-type embryos. In order to find out how this normal pattern of *Kr* activity is established, we examined the expression of *Kr* in maternal-effect segmentation mutants.

Kr *expression requires the maternal gene pattern organizer genes*

The maternal-effect segmentation genes can be grouped into those affecting segmentation of the abdominal region ('posterior pattern organizer genes'), the acron and telson regions ('terminal organizer genes'), and the head and thorax region ('anterior pattern organizer genes') (Nüsslein-Volhard *et al.* 1987).

Embryos that lack posterior pattern organizer gene activities such as *staufen* (*stau*), *vasa* (*vas*), *oskar* (*osk*) and *pumillio* (*pum*) have the abdominal segments deleted; occasionally some abdominal dentical bands can be seen. *stau* mutations cause a defective abdominal segment pattern and, in addition, head defects (for details on the mutant phenotypes see Nüsslein-Volhard *et al.* 1987). The *Kr* domain is clearly changed in embryos mutant for these genes. In *vas*, *osk* and *pum*, the *Kr* protein domain is expanded only towards posterior. An example of such posterior expansion in an *osk* embryo is shown in Fig. 2B. The most prominent changes were found in *stau* embryos

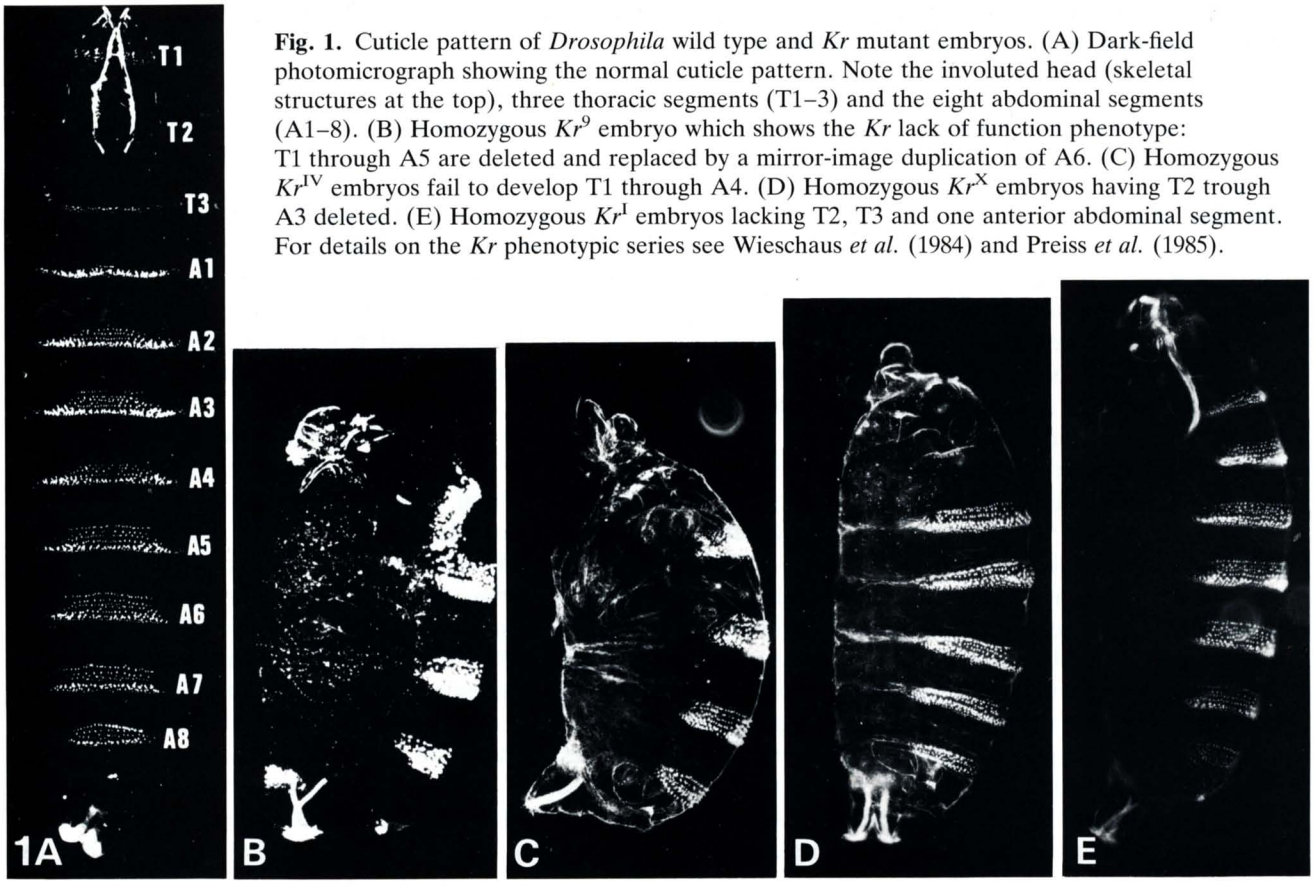

Fig. 1. Cuticle pattern of *Drosophila* wild type and *Kr* mutant embryos. (A) Dark-field photomicrograph showing the normal cuticle pattern. Note the involuted head (skeletal structures at the top), three thoracic segments (T1–3) and the eight abdominal segments (A1–8). (B) Homozygous Kr^{9} embryo which shows the *Kr* lack of function phenotype: T1 through A5 are deleted and replaced by a mirror-image duplication of A6. (C) Homozygous Kr^{IV} embryos fail to develop T1 through A4. (D) Homozygous Kr^{X} embryos having T2 trough A3 deleted. (E) Homozygous Kr^{I} embryos lacking T2, T3 and one anterior abdominal segment. For details on the *Kr* phenotypic series see Wieschaus *et al.* (1984) and Preiss *et al.* (1985).

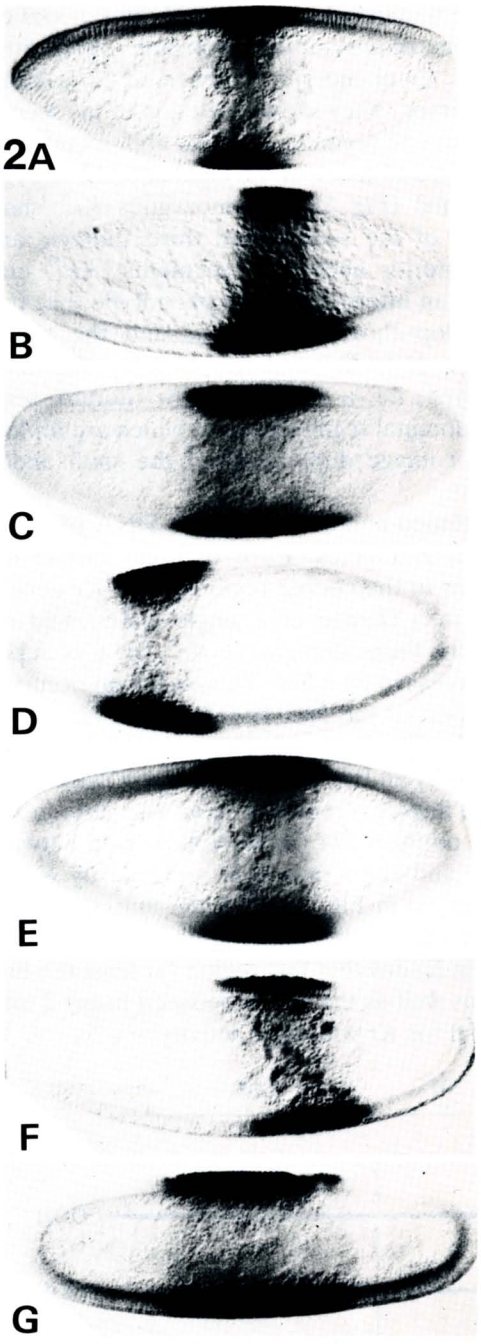

Fig. 2. Size and position of the *Kr* protein domain in wild-type and maternal effect mutant embryos. Optical sections of embryos stained with the *Kr* antibodies using the procedures described in Gaul *et al.* (1987). The *Kr* domain appears as a dark band of *Kr* antibody staining in the middle of the embryos. Sagittal focus on whole-mount embryos; orientation is anterior left and dorsal up. (A) *Kr* wild-type domain. *Kr* domain in embryos developing the (B) *osk*, (C) *stau*, (D) *bcd* (weak allele), (E) *exu*, (F) *swa*, and (G) *osk/bcd* phenotype. The genotype of maternal effect mutant females producing the embryos which were stained with the *Kr* antibodies as well as the staining procedures, are described in Gaul & Jäckle (1987).

where both the anterior and posterior borders of the *Kr* protein domain are shifted from the centre (Fig. 2C). This results in an enlarged *Kr* protein domain (twice the size of the normal) which correlates with the head and abdominal pattern defects observed in the *stau* mutants.

Mutations of the anterior pattern organizer genes cause anterior pattern defects. In addition, mutations in the different genes of this class cause specific defects in other regions of the embryo: *bicoid* (*bcd*) embryos have defects in the anterior part of the segment pattern, while *exuperantia* (*exu*) and *swallow* (*swa*) embryos show defects in the posterior abdomen (for details on the mutants and different alleles see Nüsslein-Volhard *et al.* 1987). The *Kr* protein domain in *bcd* mutants is dependent on the strength of the allele analysed. In weak alleles, the *Kr* domain is both enlarged and shifted anteriorly (Fig. 2D). In strong alleles that represent the amorphic phenotype, the *Kr* domain is shifted into a similar position, but it is not markedly enlarged (Fig. 2D). In both *exu* and *swa* embryos, the *Kr* domain expands anteriorly and posteriorly with the centre of the enlarged domain shifted (Fig. 2E,F). The abdominal defects in these mutants can be correlated with the posterior shift which is more pronounced in *swa* embryos having a stronger abdominal defect than *exu* embryos. Thus, the mutations of each of the anterior pattern organizer genes cause a mutant- and allele-specific shift and/or enlargement of the *Kr* protein domain.

Mutations of the terminal pattern organizer genes *torso* (*tor*) and *trunk* (*trk*) cause defects in both acron and telson (for details see review by Nüsslein-Volhard *et al.* 1987). In such embryos, the *Kr* protein domain is unchanged.

Double-mutant *bcd* and *osk* embryos lack two of the gene products that are required to establish the anterior and posterior segment pattern. These embryos fail to develop any segmented pattern and consist of just two telsons arranged in mirror-image symmetry (Nüsslein-Volhard *et al.* 1987). The *Kr* domain of *bdc/osk* embryos is dramatically enlarged. It covers most of the region of the blastoderm-stage embryo that will give rise to the segmented part of the embryo (Lohs-Schardin *et al.* 1979). The lack of segmentation in these embryos indicates that *Kr* activity *per se* is insufficient to establish segmentation.

Biochemical properties of the Kr *protein product*

Sequencing of wild-type *Kr* DNA (Rosenberg *et al.* 1986) revealed that the putative *Kr* protein contains several 'zinc fingers', a motif for DNA-binding proteins that emerged from the analysis of TF IIIA (Miller *et al.* 1985; Brown & Argos, 1986), a transcription factor of *Xenopus*. The finger structure is

Discussion

Regulation of Kr *protein expression*

The *Kr* protein pattern is significantly changed in maternal-effect mutants. This finding is consistent with the proposed dependence of *Kr* activity on maternal positional information already present in the egg (Meinhardt, 1986 and this volume).

The maternal-effect genes so far identified can be grouped into anterior pattern organizer genes, posterior pattern organizer genes and terminal pattern organizer genes (Nüsslein-Volhard *et al.* 1987; Lehmann, this volume). Thus, none of the maternal-effect mutants cause a phenotype that resembles the *Kr* phenotype, i.e. no maternal-effect mutation causes the deletion of the middle region of the segment pattern exclusively. This observation suggests that *Kr* gene expression is not under the control of a prelocalized transcription activator, the lack of which should cause a *Kr*-like phenotype. Thus, *Kr* expression is more likely to be activated by one (or several) transcription factor(s) which is (are) present throughout the blastoderm embryo. *Kr* expression is always activated in maternal-effect mutants; the absence of each of the maternal-effect gene products examined causes a shift and/or an expansion of the *Kr* protein domain towards the pole regions of the blastoderm-stage embryo. These findings suggest that the normal pattern of *Kr* expression is generated by its repression in the anterior and posterior regions of the embryo by maternal organizers such as *bcd* and *osk*. Since *Kr* has no maternal counterpart, the organization of the central segment pattern elements of the embryo may depend exclusively on zygotic activity provided by the gap gene *Kr*. The expression pattern of *Kr* in *bcd/osk* double-mutant embryos demonstrates that *Kr* activity *per se* is insufficient to establish segments in the embryo. Thus, the establishment of a normal segmentation pattern in the middle region of the embryo requires additional gene activities. Candidates that are likely to provide these additional gene activities are those gap genes that act in the domains adjacent to *Kr*.

Correlation between the Kr *expression domain and phenotype*

The *Kr* protein domain embraces the primordia of approximately four segmental units at the blastoderm stage. However, the absence of the *Kr* gene product in this domain causes a deletion of eight segments from the larval body. The deleted elements include those segments where precursors express the *Kr* protein, as well as derivatives of more posterior segment primorida. Thus, in contrast to the pair-rule genes, there is no direct correspondence between the *Kr* expression domain and the segmental deletions of the strong, amorphic *Kr* phenotype. If the formation of the segments that are deleted in the *Kr* amorphic mutants depended directly on *Kr* activity, one might consider that the formation of the more posterior segments affected in strong *Kr* alleles requires the spreading of *Kr* gene expression towards posterior observed during early gastrulation (Knipple *et al.* 1985). However, the *ftz* expression pattern (a molecular marker for segment primordia) is altered in this region as early as the blastoderm stage in *Kr* mutant embryos (Ingham *et al.* 1986; Howard, this volume). Thus, the spreading of *Kr* gene expression during gastrulation cannot be responsible for the large gap developed by the mutants. These data not only confirm that *Kr* activity is required for the establishment of the *ftz* pattern, but they also restrict the critical period for determining the gap region to the blastoderm. The mechanism that provides the asymmetrical long-range effect of *Kr* activity towards posterior is not yet understood (see below).

Molecular features of the Kr *protein product*

The *Kr* gene codes for a protein that can potentially fold into a structure that is conserved in a series of eukaryotic proteins, several of which are known transcription activators. This structure, the zinc finger, has been shown to possess DNA-binding activity (for review see Evans & Hollenberg, 1988).

Our molecular analysis of four *Kr* alelles showed that single amino acid exchanges within the *Kr* finger domain can result in different *Kr* phenotypes. This indicates that the *Kr* finger domain is essential for *Kr* function. By analogy with other finger proteins, this domain should be responsible for specific contacts made by the *Kr* protein with the target DNA. Such a role for the protein is in line with its nuclear location (Gaul *et al.* 1987) as well as its general DNA-binding properties (Ollo & Maniatis, 1987). These observations, in conjunction with the finding that single amino acid exchanges can impair Kr^+ activity, strongly support the argument that *Kr* codes for a DNA-binding protein possibly involving the activation of other genes.

Prospects

Both the genetic and molecular studies have failed so far to yield a mechanistic understanding of how the segmentation gene *Kr* establishes segmentation during *Drosophila* embryogenesis. The data accumulated suggest that the establishment of the *Kr* domain at the blastoderm stage involves repression by the anterior and posterior pattern organizer gene products. *Kr* activity then acts as the "central pattern organizer" within the region of *Kr* gene expression,

as well as in the region posteriorly adjacent to it. This asymmetric long-range effect of *Kr* activity is possibly best understood in terms of *Kr* providing spatial cues for the expression of the subordinate pair-rule genes. The observation that the other gap genes, *hunchback* and *knirps*, share the molecular features with *Kr* (Tautz *et al.* 1987; Nauber *et al.* unpublished data) suggests that each of the different gap gene products acts as a transcription factor for subordinate genes including pair-rule genes. The lack of *Kr* activity causes an abnormal patterning of pair-rule gene activities along the longitudinal axis of the embryo (Carrol & Scott, 1986; Ingham *et al.* 1986; Frasch & Levine, 1987; Howard, this volume; Ingham & Gergen, this volume). This, in turn, may result in the gap phenotype observed. The question remains how the pattern of pair-rule gene is instructed by the *Kr* activity or, in other terms, what are the decisive cues provided by the gap genes to generate the pair-rule pattern. Initially, the pair-rule genes are activated within the region of segment primordia. The initial transcription pattern is apparently uniform, but complex patterns rapidly evolve to give rise eventually to the evenly spaced stripes showing a double-segment periodicity (see Ingham & Gergen, this volume; Howard, this volume). The local order in this evolving pattern may be initiated by the gap gene products and/or their interaction at the borders of expression. A detailed examination of the domain of *Kr* gene expression, and of the other gap genes, and their correlation with the pattern of evolving pair-rule expression should help in identifying candidates among the pair-rule genes that are likely to respond to *Kr* activity and to spread the gap posterior to the domain of *Kr* gene expression.

We would like to thank Drs M. J. Pankratz and G. Rödel for critical comments on the manuscript. U. Gaul is a fellow of the Studienstiftung des deutschen Volkes. The work was supported by a DFG grant (Leibniz-Programm).

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