The segmentation gene *Krüppel* of *Drosophila melanogaster* has homeotic properties

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In *Drosophila* hindgut, Malpighian tubules and posterior midgut develop from the most posterior region of the blastoderm. One of the genes that influences the differentiation of the Malpighian tubules is *Krüppel* (*Kr*), a segmentation gene of the gap class. *Kr* homozygous embryos lack thoracic and abdominal segments and, depending on the allele, develop nearly normal Malpighian tubules or do not differentiate them at all. In the wild type, injection of horseradish peroxidase (HRP) into cells of the early gastrula at various posterior positions results in labeling of hindgut (93%), Malpighian tubules (46%), and posterior midgut (20%). Malpighian tubules were labeled only in combination with hindgut. In *Kr¹* homozygous embryos that lack Malpighian tubules, the label was restricted to hindgut (84%) and posterior midgut (24%). Because we could not find significant cell death in the posterior region of *Kr¹* embryos, we counted the cell nuclei in the hindguts of wild-type and mutant embryos. The results show that the hindgut in *Kr¹* embryos contains those cells that would differentiate into Malpighian tubules in wild type. Therefore, we conclude that the *Krüppel* gene exhibits a homeotic function in addition to its role as a segmentation gene and is involved in separating hindgut and Malpighian tubule cells and in the elongation process as well.

[Key Words: *Drosophila*; *Krüppel*; segmentation; homeosis; HRP injection; Malpighian tubules]

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In *Drosophila melanogaster*, embryological observations (Poulson 1950; Hartenstein and Campos-Ortega 1985) and experimental procedures, such as destroying cells by UV-irradiation (Lohs-Schardin et al. 1979a,b), injection of horseradish peroxidase (HRP) into cells of the early gastrula stage (Technau and Campos-Ortega 1985), or analyses of gynandromorphs (for review, see Janning 1978), revealed the fate map of the blastoderm embryo. As in many other species, the fate map shows the positions of anlagen from which corresponding organs differentiate during normal development without information on developmental potentialities of anlagen cells. Clonal analysis shows that blastoderm cells are restricted in developmental potentialities (Wieschaus and Gehring 1976). Especially by using the *Minute* technique, the compartmental organization of the early embryo could be visualized (Garcia-Bellido 1975; Garcia-Bellido et al. 1976; Steiner 1976). Genes responsible for these determinative decisions were characterized, and, mainly during the past decade, the genetics of *Drosophila* embryogenesis has been worked out (for review, see Mahowald and Hardy 1985). In principle, there are

three sets of genes that organize the fate map: (1) maternal genes, which act during oogenesis to establish the global coordinate values of the egg; (2) zygotically active segmentation genes, which divide the developing embryo into metameres; and (3) homeotic genes, which are responsible for segmental and/or compartmental identities (Nüsslein-Volhard 1979; Nüsslein-Volhard et al. 1982; Gehring and Hiromi 1986; Anderson 1987). In contrast to these definitions, there are some alleles of the segmentation genes *fushi tarazu* (*ftz*) and *hunchback* (*hb*) that show homeotic phenotypes (Laughon and Scott 1984; Lehmann and Nüsslein-Volhard 1987). Molecular cloning of genes from all three classes led to descriptions of the spatiotemporal distributions of RNA and protein-gene products during embryogenesis which, in many cases, correspond to the mutant phenotypes (e.g., Hafen et al. 1984; Carroll and Scott 1985; Knipple et al. 1985).

Posterior midgut, hindgut, and the Malpighian tubules develop from the posterior region of the blastoderm (Poulson 1950; Janning 1976; Hartenstein et al. 1985). Clonal analysis of the anlage of the Malpighian tubules revealed that there are ~80 cells that undergo, on average, two to three (but up to five) mitoses before differentiation of ~480 cells of the Malpighian tubules (Janning et al. 1986).

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There are several genes that influence the differentiation of the Malpighian tubules. One of these is *Krüppel* (*Kr*), a segmentation gene of the gap class, which was first described by Gloor (1950). *Kr*⁻ homozygotes, which die before hatching, lack all three thoracic and the anterior abdominal segments, whereas parts of the posterior abdominal segments, which most likely correspond to parasegment 11 (Ingham et al. 1986), are mirror image duplicated (Wieschaus et al. 1984). In addition, in *Kr*¹/*Kr*¹ embryos, Malpighian tubules do not differentiate (Gloor 1950). Hybridization of cloned *Kr* probes to tissue sections revealed a spatiotemporal pattern of gene expression consistent with the mutant phenotype of weak *Kr* alleles. Around the blastoderm stage, a *Kr* central domain of gene expression covers the anlagen of the three thoracic and the first abdominal segments. After onset of gastrulation, a posterior and an anterior *Kr* domain appear. At the end of germ-band extension, the *Kr* posterior domain is restricted to the anlage of the Malpighian tubules (Knipple et al. 1985; Preiss et al. 1985).

In this paper we investigate the fate of the blastoderm cells in *Kr*¹/*Kr*¹ embryos that would give rise to the Malpighian tubules in the wild type. We present evidence that these cells do not die in mutant embryos, and we show that the *Kr*⁺ gene determines cells of the hindgut/Malpighian tubules anlage to develop into Malpighian tubule cells.

Results

Morphology of gut structures in Kr mutations

Kr mutations are characterized by the deletion of thoracic and abdominal segments. In addition, homozygous *Kr*¹ and *Kr*⁹ embryos do not differentiate Malpighian tubules (Fig. 1). Because the segmentation phenotype is very variable in different *Kr* alleles (Preiss et al. 1985) it was important to determine whether there is a correlation between the severity of the segmentation defect and the capacity to differentiate gut structures.

Guts of homozygous lethal *Kr* embryos were dissected out at the end of embryogenesis and stained with the fluorescence dye Hoechst 33258 to investigate the morphology and the distribution of nuclei. *Kr* embryos could be distinguished from their non-*Kr* sibs by anatomical criteria. The results (Table 1) show that the ability to differentiate proper Malpighian tubules is as variable as the segmentation phenotype in different *Kr* alleles; however, there is no strict correlation between these two characteristics. *Kr*¹ and *Kr*⁹ embryos lack Malpighian tubules, whereas in *Kr*²¹⁻¹¹, also an allele with strong segmentation phenotype, the tubules seem to be developed normally. We found two types in the weak alleles. In *Kr*¹⁰⁶⁶ (Fig. 2a), Malpighian tubules are differentiated but shorter than normal, with irregular outbuddings and disturbances in the zigzag pattern of nuclei. In *Kr*⁶²⁻¹⁴ (Fig. 2b), there are only short and thickened buds instead of long tubules. The number of buds is very often more than four, and the distribution of their nuclei resembles that of the gut, i.e., no zigzag pattern is observed. A

Figure 1. Posterior region of early stage 14 embryos. Malpighian tubules develop between the hindgut and the posterior midgut in the wild type (a), whereas *Kr* homozygous embryos (b) do not differentiate Malpighian cells. Fuchsin-stained whole-mount preparations. (HG) Hindgut; (MT) Malpighian tubules; (PM) posterior midgut.

striking feature of this phenotype is the occurrence of nests of nuclei (cells) in the region of origin of the Malpighian tubules between posterior midgut and hindgut. In *KrV* and *KrX* embryos, similar phenotypes to *Kr*¹⁰⁶⁶ and *Kr*⁶²⁻¹⁴, respectively, are found, but in different proportions (see Table 1). Due to the fragile nature of the preparations and the fast fading of the Hoechst fluorescence, it was not possible to count nuclei; however, we have the impression that a more or less normal number of Malpighian tubule cells is present. It seems that the elongation process is disturbed by the mutation.

In *trans*-heterozygotes, the phenotype of Malpighian tubule differentiation corresponds to that of the weaker allele: in *Kr*²¹⁻¹¹/*Kr*⁶²⁻¹⁴ and *Kr*²¹⁻¹¹/*Kr*¹, tubules are similar to those in *Kr*²¹⁻¹¹ homozygotes, and in *Kr*¹/*Kr*⁶²⁻¹⁴, the tubules consist of (even shorter) buds as in *Kr*⁶²⁻¹⁴ embryos. In all alleles investigated, posterior midgut and hindgut are not visibly affected by the mutation, with the exception of *Kr*¹ and *Kr*⁹, which differentiate a larger hindgut than the wild type.

Injection of HRP into cells of the early gastrula

Because it was shown by Technau and Campos-Ortega (1985) that the anlagen cells of the Malpighian tubules

Table 1. Differentiation of Malpighian tubules in *Kr* mutations

Allele	Segmentation phenotype	Morphology of MT	Reference ^a
<i>Kr¹</i>	strong	no MT	2, 6
<i>Kr⁹</i>	strong	no MT	4
<i>Kr¹⁰⁶⁶</i>	weak	all MT shorter than normal (MP longer than MA) with irregular buds; zigzag pattern of nuclei disturbed; ureter $\pm$ normal	3, 5
<i>Kr^V</i>	weak	phenotype variable: in most embryos, short thickened buds, often more than four; in $\sim$ 30% of embryos, MP tubules are elongated; distribution of nuclei similar as in the gut (no zigzag pattern); nests of nuclei in PM/HG region; nearly no ureter	4, 5
<i>Kr^X</i>	weak	phenotype variable: in most embryos, all MT shorter than normal (MP longer than MA) with irregular buds; sometimes short thickened buds; ureter $\pm$ normal	4, 5
<i>Kr²¹⁻¹¹</i>	strong	normal, sometimes buds at ureter	1, 5
<i>Kr⁶²⁻¹⁴</i>	weak	short thickened buds, often more than four; distribution of nuclei similar as in the gut (no zigzag pattern); nests of nuclei in PM/HG region; nearly no ureter	1, 5

(HG) Hindgut; (MA) anterior MT; (MP) posterior MT; (MT) Malpighian tubules; (PM) posterior midgut.

^a 1. Allele from P-mutagenesis by E. Wieschaus (H. Jäckle, pers. comm.); 2. Gloor (1950); 3. Nüsslein-Volhard et al. (1984); 4. Preiss et al. (1985); 5. this paper; 6. Wieschaus et al. (1984).

are located at the posterior end of the early gastrula, we injected HRP in cells at various posterior positions [20% egg length (EL) at the dorsal midline (100% VD), 15% EL/100% VD (ventral–dorsal), and 10% EL/50% VD]. In contrast to other injection sites where labeling of small cell groups is possible (Fig. 3a), injections of HRP into cells at these sites result in a spreading of the injected enzyme over many cells (Fig. 3b).

In wild-type embryos, staining for HRP after completion of germ-band shortening (stage 13 of Campos-Ortega and Hartenstein 1985) shows distribution of the label in the hindgut, the Malpighian tubules, and less frequently in the posterior midgut. The data in Table 2 show that in 93% of all successful injections, marked cells were found in the hindgut, in 46% of the preparations, they were found in Malpighian tubules, and in 20%, posterior midgut cells were labeled. Due to the spreading of HRP at the time of injection, we have observed a very frequent overlapping of the label (Table 2). However, it is worth noting that Malpighian tubules are labeled only in combination with hindgut (Fig. 4a). The results are very similar over the relatively large area of all three injection sites. Differences may be region dependent or due to small numbers.

In *Kr¹* homozygous embryos, we found very similar results, except that Malpighian tubules were missing (Table 2; Fig. 4b). The label was restricted to hindgut (84%) and posterior midgut (24%). It is important to know that in this series, we have never found label in organs other than those mentioned. So the question arises: What is the fate of anlage cells in *Kr* embryos that develop Malpighian tubules in the wild type? It was determined previously by Jäckle et al. (1985) that cells in the region of the *Kr* central domain die. Therefore, we stained serial sections of embryos at the stage of completed germ-band shortening with methylene blue. As is shown in Figure 5, accumulations of dead cells could be seen between the mesodermal and neural tissue on the ventral side. Cell death was not observed in the hindgut or posterior midgut of *Kr* embryos that could account for the absence of the Malpighian tubules.

Figure 2. Gut structures in homozygous lethal *Kr* embryos dissected out at the end of embryogenesis. (a) Malpighian tubules in *Kr¹⁰⁶⁶* with some outbuddings in the ureter regions. (b) Short Malpighian buds in *Kr⁶²⁻¹⁴*. Fluorescence in Hoechst 33258-stained preparations. (HG) Hindgut; (IC) imaginal hindgut cells; (PM) posterior midgut.

Figure 3. Injection of HRP into cells of the early gastrula stage. Enzyme staining immediately after the injection procedure shows that labeling of only a few cells is possible (a). At 20% EL, 100% VD enzyme activity spreads over many cells (b). Whole-mount preparations; Nomarski optics.

Table 2. Distribution of labeled cells in wild-type or *Kr*¹ heterozygous and in *Kr*¹ homozygous embryos after injection of HRP into cells of beginning gastrulae

Injection site	Labeled cells in	Genotype			
		<i>Kr</i> ¹ /+ or +/+ (n)	(%)	<i>Kr</i> ¹ / <i>Kr</i> ¹ (n)	(%)
20% EL/100% VD	HG	42	45	28	78
	HG/MT	36	39	—	—
	HG/PM	0	0	5	14
	HG/PM/MT	10	11	—	—
	PM	5	5	3	8
	Total	93	100	36	100
15% EL/100% VD	HG	23	45	10	67
	HG/MT	14	27	—	—
	HG/PM	1	2	0	0
	HG/PM/MT	7	14	—	—
	PM	6	12	5	33
	Total	51	100	15	100
10% EL/50% VD	HG	12	55	6	86
	HG/MT	6	27	—	—
	HG/PM	0	0	0	0
	HG/PM/MT	4	18	—	—
	PM	0	0	1	14
	Total	22	100	7	100

See footnote to Table 1 for abbreviations.

Size of the proctodeum (hindgut) in normal and *Kr* mutant embryos

Comparison of the sizes of hindguts in wild-type versus *Kr*¹ homozygous embryos in histological sections and whole mounts (Fig. 4) led to the assumption that cells normally taking part in the formation of Malpighian tubules have lost this property in *Kr*¹ mutant embryos but are still there. To prove this hypothesis, we counted the cell nuclei of the embryonic hindgut at completed germ-band shortening in normally developed (*Kr*¹/+ and wild-type) and in *Kr* homozygous embryos.

The results (Table 3) show that the proctodeum of normal embryos comprises 820 cells, whereas there are 1174 cells in the hindgut in *Kr* mutant embryos. As a control, we counted the hindgut cell number in wild-type first-instar larvae and found that the mean number was 871 cells (Table 3). The larval cell number is larger by 6.2%, compared to the embryonic cell number. This might be due to experimental procedures as, for example, different staining methods. It is also possible that the imaginal hindgut cells, which lie as a ring around the proximal part of the larval hindgut, have already begun

Figure 4. Injection of HRP into cells of the early gastrula at 20% EL, 100% VD. Cells of the hindgut and Malpighian tubules are labeled in stage 13 embryos in the wild-type (a). The enzyme label is restricted to the hindgut in homozygous *Kr* embryos (b), which seems to be larger than that in the wild type. Whole-mount preparations; Nomarski optics. (HG) Hindgut; (MT) Malpighian tubules.

Figure 5. Semithin sections of stage 13 embryos stained with methylene blue. In comparison to the wild type (a), dead cells can be seen in the ventral region of homozygous *Kr* embryos but not in the hindgut (HG) (b).

their mitotic activity at the time of dissection. This could explain the larger standard deviation (20 cells) in relation to that of normal embryos (9 cells).

In *Kr²¹⁻¹¹* embryos, the differentiation of the Malpighian tubules seems relatively normal (Table 1). To prove whether the hindgut also contains a normal number of cells, we counted the nuclei in *Kr²¹⁻¹¹* homozygous embryos. We found that the hindgut in this *Kr* allele has a wild-type size of 842 cells (Table 3).

Table 3. Hindgut cell number of *Kr¹* and *Kr²¹⁻¹¹* homozygous, and of *Kr¹* heterozygous or wild-type embryos

	<i>Kr¹/Kr¹</i>	<i>Kr¹/+ or +/+</i>	<i>Kr²¹⁻¹¹/Kr²¹⁻¹¹</i>	First larva ^a
Cell numbers	1183	831	858	843
	1195	807	825	888
	1138	813	847	861
	1183	830	838	892
	1191	827	841	873
	1173	819	852	
	1154	815	835	
Mean	1174 ± 21	820 ± 9	842 ± 11	871 ± 20

^a Cell number of first-instar larval hindgut.

Distribution of caudal protein in gut structures of *Kr* mutations

Recently, it was found that gut structures are part of the spatiotemporal pattern of expression of the *caudal* (*cad*) gene. Levine et al. (1985) localized their S67 transcripts in the Malpighian tubules and regions of the posterior midgut and hindgut. Mlodzik and Gehring (1987) and Mlodzik et al. (1987) found that at the end of embryogenesis, the *cad⁺* transcript and *cad* protein are located in cells of the posterior midgut, the Malpighian tubules, and the hindgut. In contrast to these findings, Macdonald and Struhl (1986) showed that the pattern of *cad* protein distribution does not include the hindgut. We used this anti-*cad* antibody (a gift from P. Macdonald and G. Struhl) for whole-mount staining of wild-type and various *Kr* embryos with peroxidase-linked secondary antibody. Our results show that in *Kr¹* and *Kr²* embryos, *cad* protein is located in the proximal part of the posterior midgut, but not in any part of the hindgut (Fig. 6). In all other *Kr* alleles investigated, additional staining is found in differentiated parts of the Malpighian tubules (Fig. 6; for description, see Table 1).

Discussion

Development of the Malpighian tubules

The Malpighian tubules of *Drosophila melanogaster* consist of an anterior and a posterior pair of tubules that function as excretory organs throughout all stages of development. They are not histolyzed during metamorphosis but remain functional in the adult. The identity of larval and imaginal cells of the tubules was shown by cell counts (Wissen 1983) and by morphological observations by Meise (1987), who was able to show that the anatomical differences in larval and imaginal anterior tubules (the so-called initial segments) are due to changes in cell shape during metamorphosis but not to cell loss.

An extensive clonal analysis (Janning et al. 1986) showed that the 480 cells of the differentiated Malpighian tubules arise from a blastodermal anlage of ~80 cells that divide, on average, two to three times. Analysis of sizes and frequencies of clones induced by mitotic recombination after variation of the X-ray dose led to a model in which a decreasing number of anlagen cells go through up to five mitoses before differentiation. By statistical analysis of overlapping clones in different tubules and pairs, it could be shown that the anlage of the Malpighian tubules is not compartmentalized but represents a common cell pool for further development.

The *Kr* gene has a homeotic function

The results from HRP injection experiments (Table 2) and cell counts (Table 3) led to the suggestion that cells giving rise to Malpighian tubules in the wild type take part in the differentiation of hindgut in *Kr* embryos. Death of prospective Malpighian cells in *Kr* embryos cannot be excluded but was not observed (Jäckle et al.

Figure 6. *cad* protein distribution in wild-type and in *Kr* embryos at stage 14–15. Immunohistochemical staining with anti-*cad* antibody is restricted to the anterior part of the posterior midgut, the Malpighian tubules, and to a cap at the posterior pole. (a) Wild type, (b) *Kr*¹, (c) *Kr*⁹, (d) *Kr*¹⁰⁶⁶, (e) *KrX*, (f) *Kr*⁶²⁻¹⁴. Whole-mount preparations, posterior end of embryos to the right; Nomarski optics. (HG) Hindgut; (MT) Malpighian tubules; (PM) posterior midgut.

1985; R. Lehmann, pers. comm.; this paper). In the wild type there are 820 cells in the hindgut and 484 cells in the Malpighian tubules (Janning et al. 1986). The sum of ~1300 cells for both organs is higher than the number of 1174 cells found in the hindgut of homozygous *Kr* embryos. This difference would be even larger if we take into account the cells of both ureters.

A possible explanation for these contradictory data may be found in the different proliferation activity/pattern of proctodeal and Malpighian tubule cells. In their embryological analysis, Hartenstein and Campos-Ortega (1985) have found that there are 300 blastoderm cells that develop into hindgut and Malpighian tubules. Prospective hindgut cells go through two mitotic cycles, with the exception of the distal hindgut portions, which undergo a third mitosis; whereas Malpighian tubule cells divide approximately three times (Hartenstein and Campos-Ortega 1985; Janning et al. 1986). The following conclusions can be drawn from these results (Table 4). In

the *Kr* embryo, 300 anlage cells divide two times and differentiate 1200 hindgut cells. In the wild type, these 300 cells are separated by gene activity into Malpighian tubules and hindgut cells. Because no ureter formation could be observed in *Kr*¹ homozygous embryos, both ureters are probably part of the Malpighian tubules and develop from the same anlage. Therefore, the postulated number of 80 anlage cells for the Malpighian tubules proper (Janning et al. 1986) might be too small for Malpighian tubules including the ureters. We estimate a number of 80 plus 20 cells for the whole Malpighian anlage. Then, different mitotic activity leads to 800 hindgut cells, 480 cells in the Malpighian tubules, and an estimated number of ~120 cells in both ureters (Table 4).

From these data, we conclude that the *Kr* gene exhibits a homeotic function besides its role as a segmentation gene of the gap class. Homeosis does not need transformation of segments or parts thereof, per se, as

Table 4. Proliferation of anlagen cells of hindgut and Malpighian tubules in *Kr* and wild-type embryos

<i>Kr</i>			
HG + MT (incl. UR): 300 $\xrightarrow{\text{I}}$ 600 $\xrightarrow{\text{II}}$ 1200 HG cells			
Wild type			
HG:	200 $\xrightarrow{\text{I}}$ 400 $\xrightarrow{\text{II}}$	800 HG cells	
MT:	80 $\xrightarrow{\text{I}}$ 160 $\xrightarrow{\text{II-V}}$	480 MT cells	
UR:	20 $\xrightarrow{\text{I}}$ 40 $\xrightarrow{\text{II-V}}$	120 UR cells	

(HG) Hindgut; (MT) Malpighian tubules, including ureters (UR). Mitotic divisions are given in roman numbers. In the wild type, divisions II–V are according to the proliferation model in Janning et al. (1986).

was shown by Hafen et al. (1987) for the *sevenless* (*sev*) gene of *Drosophila*. In the wild type, the *Kr*⁺ gene product is somehow involved in the process of specifying the identities of hindgut and Malpighian tubule cells. In *Kr* mutant embryos where this gene product is missing, all anlagen cells remain in the hindgut state. (Note that the homeosis of *Kr* comprises the participation of Malpighian cells in the differentiation, as well as in the proliferation pattern of hindgut cells.)

Our findings were confirmed by Redemann et al. (1988), who found that an amino acid exchange eliminates *Kr*⁺ function in *Kr*⁹ resulting in the lack of Malpighian tubules. Nevertheless, the defect protein is detected by the *Kr* antibody in the hindgut. The *cad* protein, which is also located in the Malpighian tubules (Levine et al. 1985; Macdonald and Struhl 1986; Mlodzik and Gehring 1987; Mlodzik et al. 1987), could have been a second candidate to find cells that do not differentiate into tubule cells. In whole mounts of *Kr*⁹ embryos stained with anti-*cad* antibody, we did not find staining of hindgut cells (Fig. 6).

Morphological observations of differentiation patterns of Malpighian tubules in different *Kr* alleles (Table 1) revealed that the *Kr* gene is also most likely involved in the process of elongation of the tubules. The elongation of the posterior tubules is variable especially in the phenotypes of *KrV* and *KrX*. Also, in *Kr*⁶²⁻¹⁴ embryos, which differentiate only buds instead of long tubules, we found many nuclei (cells) at the origin of the Malpighian tubules (see Figs. 2 and 6).

Both pairs of Malpighian tubules arise at the junction of the endodermal posterior midgut and the ectodermal hindgut. There is some question about endodermal versus ectodermal origin of the Malpighian tubules (for review, see Poulson 1950). Embryological data (Campos-Ortega and Hartenstein 1985) suggest an ectodermal origin of the Malpighian tubules. Lawrence and Johnston (1986) analyzed nuclear transplantation mosaics and also found 'that the Malpighian tubules are more closely related to the hindgut than the midgut.' Here, we present data providing strong evidence that hindgut and

Malpighian tubules have the same, i.e., ectodermal, origin.

Organization of the embryonic postabdomen

The *Drosophila* larva, as well as the adult fly, are clearly segmented into three thoracic and eight abdominal segments, whereas the segmentation of head and tail regions is less well understood because of possible fusions of segments during evolution. The imaginal terminalia that originate from a single genital imaginal disc presumably represent the embryonic segments A8 (female genitalia), A9 (male genitalia), and A10 (analia) of lower insects (Nöthiger et al. 1977; Schüpbach et al. 1978; Dübendorfer and Nöthiger 1982; Janning et al. 1983). The cuticular derivatives of the larval tail region following segment A8 were analyzed in detail by Jürgens (1987). He found by UV laser fate mapping that tail structures develop from the embryonic segments A8–A11. In the blastoderm fate map, the anlagen of the proctodeum (hindgut), the Malpighian tubules, and the posterior midgut are localized in the region between 15% and 0% EL (Hartenstein et al. 1985). This anterior–posterior sequence is also verified by embryological observation of cell movements during gastrulation (Campos-Ortega and Hartenstein 1985).

The data presented in this work, as well as the results of our clonal analysis (Janning et al. 1986), strongly suggest that the anlagen cells of the Malpighian tubules behave as a functional unit. The anlage is not divided into compartments, and the failure of *Kr* gene function transforms these cells to hindgut differentiation. This transformation in the recessive loss of function phenotype of *Kr*¹ homozygous embryos is exhibited in anterior direction of the fate map, as it is found in recessive mutants of the Bithorax complex (for review, see Gehring and Hiromi 1986). This view is further supported by results of in situ hybridizations of *engrailed* (*en*) gene products. *en* is active in all posterior compartments of the embryo and includes activity in the hindgut and posterior midgut (Fjose et al. 1985; Ingham et al. 1985; Kornberg et al. 1985). We speculate that if hindgut has posterior quality, the Malpighian tubules cells should be an anterior group of cells and should not exhibit *en* activity. This is confirmed by Fjose et al. (1985), who found *en* activity in the hindgut (in Fig. 8 of their work, the designation posterior midgut most probably has to be changed into hindgut). Ingham et al. (1985) report on *en* activity in 'portions of the Malpighian tubules.' By re-examination of their preparations, the investigators found that there seems to be doubt about the correct identification of Malpighian tubules and that *en* activity is restricted to hindgut in this region of the embryo (P.A. Lawrence, pers. comm.). These results could be confirmed in embryos of a stock in which the bacterial *lacZ* gene is under control of *en* (generously supplied by T. Kornberg). We found that staining in the posterior gut region was restricted to a dorsal stripe in the hindgut, which would then be the only region with posterior quality. Therefore, it remains unclear whether or not

hindgut, Malpighian tubules, and posterior midgut are organized segmentally.

Material and methods

Injection of HRP into cells of the early gastrula

For injection experiments we used the original *Kr* allele (*Kr¹*; Gloor 1950). *Kr¹* was marked with *cn* and *bw* and balanced over *CyO* (for detailed description of the mutants, see Lindsley and Grell 1968). To exclude a possible influence of *Cy* heterozygosity on embryonic development, *Kr¹/CyO* females were mated to males of a wild-type stock (*Sevelen*). The *Kr¹*-bearing offspring was crossed inter se. Eggs were collected from this cross and prepared for experiments.

Eggs were collected at intervals of 30 min on apple juice–agar plates (Nüsslein-Volhard et al. 1984), with a piece of filter paper moistened with one drop of 45% acetic acid. When eggs had developed to the blastoderm stage (~3 hr at 25°C), they were prepared for HRP injection as described by Technau and Campos-Ortega (1985). Embryos were injected at the beginning of gastrulation with a 10% solution of HRP in 0.2 M KCl. The injection sites were 20% EL (0% EL is at the posterior pole) on the dorsal midline (100% VD), 15% EL/100% VD, and 10% EL/50% VD. Injected embryos were allowed to develop to the completion of germ-band shortening. This stage corresponds to stage 13 of Campos-Ortega and Hartenstein (1985). After fixation of embryos (Technau and Campos-Ortega 1985), labeled cells were stained for HRP with a 10% solution of 3,3'-diaminobenzidine in 0.1 M phosphate buffer and 10 μ l 1% hydrogen peroxide. After a reaction time of 15 min, the embryos were washed in 0.1 M phosphate buffer at pH 7.2, dehydrated through an ethanol series, and transferred through propylene oxide to Epon.

Staining and counting embryonic cell nuclei

Eggs were collected on apple juice–agar dishes in 1-hr intervals and allowed to develop for another 10–11 hr at 25°C. At this time, the embryos had completed germ-band shortening. Embryos were dechorionated chemically with a saturated solution of calcium hypochlorite and prefixed in a mixture of 95% ethanol, 50% acetic acid, formalin, and *n*-heptane for 5–10 min (Zalokar and Erk 1977). The vitelline membrane was removed by adding some drops of methanol in *n*-heptane (Mitchison and Sedat 1983). Fixing and fuchsin staining of embryos was done as described by Zalokar and Erk (1977). After staining, embryos were washed in 5% acetic acid and 95% ethanol, dehydrated in 100% ethanol, and embedded through propylene oxide in Epon as whole mounts.

Cell nuclei of the hindgut of *Kr¹* and *Kr²¹⁻¹¹* homozygous and of normal embryos were counted at 600 $\times$ magnification.

Staining and counting larval cell nuclei

Preparation of first-instar larvae was done as described by Janing (1976). Cell nuclei were stained for 5–7 min in acetocarmine and washed for 1 min in 45% acetic acid. Nuclei of the larval hindgut were counted at 400 $\times$ magnification.

Staining dead embryonic cells

Histological sections were prepared as described by Zalokar and Erk (1977), fixed with 2.5% buffered glutaraldehyde, and embedded in Epon. Semithin sections (2 μ m) were stained with 0.05% methylene blue in a 0.01 M borate buffer solution (pH 9.22) for 5 min at 60°C.

Whole mounts of embryos

Staining of embryos with anti-*cad* antibody was as described by Macdonald and Struhl (1986), using a peroxidase-linked secondary antibody (Vectastain Kit).

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