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Control and function of terminal gap gene activity in the posterior pole region of the *Drosophila* embryo

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We have studied the genetic requirement for the normal expression of the terminal gap genes *huckebein (hkb)* and *tailless (tll)* and their possible function in the posterior pole region of the *Drosophila* embryo. At the early blastoderm stage, both genes are expressed in largely coextensive expression domains. Our results show that in the posterior region of the embryo both the activation and the control of the spatial limits of *tll* and *hkb* expression are critically dependent on *torso* (*tor*) activity, which is thought to be a crucial component of a cellular signal transduction pathway provided by the terminal maternal system. Furthermore, the spatial control of *hkb* and *tll* expression does not require mutual interactions among each other, nor does it require regulatory input from other gap genes which are essential for the establishment of segmentation in the trunk region of the embryo ("central gap genes"). Therefore, the terminal gap genes have unique regulatory features which are distinct from the central gap genes. In the absence of terminal gap gene activities, as in *hkb* and *tll* mutant embryos, the expression domains of the central gap genes expand posteriorly, indicating that the terminal gap gene activities prevent central gap gene expression in the posterior pole region of the wildtype embryo. This, in turn, suggests that the terminal gap gene activities prevent metamerization by repression of central gap genes, thereby distinguishing the segmented trunk from the nonsegmented tail region of the embryo.

Control of gap gene expression; *Drosophila* pattern formation; Terminal system

Introduction

The basic body pattern of the *Drosophila* embryo consists of a segmented central trunk region which is flanked by the apparently unsegmented terminal regions of the head and tail (reviewed by Nüsslein-Volhard et al., 1987). Segmentation of the trunk region requires the zygotic gap genes *hunchback* (*hb*), *Krüppel* (*Kr*), *knirps* (*kni*) and *giant* (*gt*) (reviewed in Gaul and Jäckle, 1990; Kraut and Levine, 1991). The early and local activities of these "central" gap genes at blastoderm stage define distinct areas within the embryo where they control the expression of subordinate pair-rule genes required for the further subdivision of the embryo into metameric units (reviewed by Akam, 1987; Ingham, 1988; Pankratz and Jäckle, 1990). The spatial control of the localized expression domains of the central gap genes are controlled by the maternal anterior-posterior organizer genes and by mutual interactions among the different gap genes (e.g. Jäckle et al., 1986; Gaul and Jäckle, 1987; Hülskamp et al., 1990; Kraut and Levine, 1991; Eldon and Pirrotta, 1991).

While substantial progress has been made towards an understanding of the molecular basis of segmentation in the trunk region (reviewed by Akam, 1987; Ingham, 1988), the development of the terminal regions of the embryo is still poorly understood. Recent genetic studies have shown that the establishment of the terminal pattern elements in the head and tail regions of the embryo depends on the maternal terminal system, which acts fairly independently of the anterior-posterior organizer systems that establish the anterior and posterior segmented patterns in the embryo (Nüsslein-Volhard et al., 1987). While the role of the maternal terminal organizer system in establishing anterior terminal pattern elements is unclear, genetic and molecular studies have shown that in the posterior

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region the activity of the maternal terminal system is mediated, at the zygotic level, by the two "terminal gap genes", *tailless* (*tll*) (Strecker et al., 1988) and *huckebein* (*hkb*) (Weigel et al., 1990).

The activity of the maternal anterior and posterior organizer systems, in which the gene products of *bicoid* (*bcd*) and *nanos* (*nos*) play the key roles, is germline-dependent. This means that the expression of both *bcd* and *nos* as well as the localized activities of their gene products are controlled by factors which are synthesized and active in the germline cells (Nüsslein-Volhard et al., 1987). In contrast, the activity of the maternal terminal system originates from outside the germline (Stevens et al., 1990), i.e. the signal for terminal development is thought to be transmitted from the somatic follicle cells into the egg cell. This transmission of positional information in the embryo is likely to involve a signal transduction pathway composed of at least six identified maternal components (Nüsslein-Volhard et al., 1987; Stevens et al., 1990). The key component of this morphogenetic signal transduction pathway is the gene *torso* (*tor*), which encodes a putative transmembrane receptor tyrosine kinase (Sprenger et al., 1989). Embryos which lack any one of the six known maternal components of this pathway fail to develop the terminal pattern elements which are the last abdominal segment (A8) and the apparently unsegmented tail region in the posterior region of the embryo (Nüsslein-Volhard et al., 1987; Stevens et al., 1990). An identical phenotype is observed in embryos which are mutant for the combined activities of the terminal gap genes *hkb* and *tll* (Weigel et al., 1990). This suggests that the activities of the two genes require a regulatory input of the maternal terminal system, but it leaves open the question whether the spatial control of their expression also requires a regulatory input of neighboring gap genes as has been observed with the central gap genes.

To ask whether the localized expression of the terminal gap genes depends on other factors than that provided by the terminal system, and to determine their possible function with respect to and in comparison with the central gap genes, we have examined the expression patterns of *tll* and *hkb* in wildtype and in different mutant embryos. We have focussed our attention on the posterior region of the embryo, since both by genetic and morphological criteria pattern formation in the head region is much more complex than in the tail region (Jürgens and Weigel, 1988; Weigel et al., 1990). Here we show that the activation and the spatial limits of *hkb* and *tll* expression in the posterior region of the embryo are controlled exclusively by positional information provided by maternal gene activity, and are independent of the activity of the known central gap genes. In contrast, the activities of *tll* and *hkb* prevent the expression of central gap genes in the posterior pole region of the embryo. These findings suggest that central and terminal gap genes differ in the way in which their activities are spatially regulated in the embryo, and that the terminal gap gene activities repress the activation of central gap genes and thereby also the proper expression of the central gap gene-dependent segmentation genes which establish metameric patterns in the embryo.

Results and Discussion

Expression patterns and control of terminal gap genes

Initial *tll* gene expression occurs in two domains in the opposite ends of the blastoderm embryo (Fig. 1). In the anterior region of the embryo, *tll* transcripts can be observed in a dorso-lateral stripe; in the posterior region they form a cap (Pignoni et al., 1990). *hkb* is expressed in two domains of the blastoderm embryo as well (Fig. 1a). While the anterior *hkb* expression domain forms a cap which is in a position anteriorly adjacent to the *tll* anterior dorso-lateral stripe, the posterior *hkb* and *tll* expression domains are largely coextensive (compare Fig. 1a, b). *tll* expression covers the region between 0–15% egg length (posterior pole is 0%), and *hkb* is expressed in a slightly smaller domain between 0–12% (Fig. 1 a, b). In this paper we focus our attention on *tll* and *hkb* expression in the posterior region of the embryo.

Based on recent genetic analysis, it has been proposed that *hkb* and *tll* represent the zygotic mediators of the maternal terminal system in the posterior region of the embryo (Weigel et al., 1990). This finding left the possibilities that the maternal terminal system might be required to activate and/or to spatially restrict the limits of posterior *tll* and *hkb* expression, or that it may activate their gene products which then become localized through the activity of other factors such as the central gap gene products. To distinguish between these possibilities, we examined the expression patterns of *tll* and *hkb* in embryos in which the activity of the maternal terminal system, the maternal posterior organizer system, and/or the activity of central gap genes had been altered by mutations.

tll and *hkb* fail to be expressed in the posterior region of embryos lacking the terminally localized activity of *tor* (Fig. 1c, d), a key component of the maternal terminal system (Nüsslein-Volhard et al., 1987). This result suggests that the activity of the terminal system is required for the activation of the two genes in the posterior region of the embryo. We then asked whether the preceding activity of the terminal system is also sufficient for *tll* and *hkb* expression. For this, we made use of the *tor* gain of function mutation tor^{4021}, which causes ectopic activity of *tor*

throughout the embryo (Klingler et al., 1988). In such embryos, high levels of ectopic *hkb* and *tll* expression are observed (Fig. 1e, f). These findings demonstrate that the activity of the maternal terminal system is both required and sufficient for the transcriptional activation of *hkb* and *tll*, but they leave open the question whether transacting factors other than those provided by the terminal system are necessary for the control of the spatial limits of the *tll* and *hkb* expression domains in wildtype embryos.

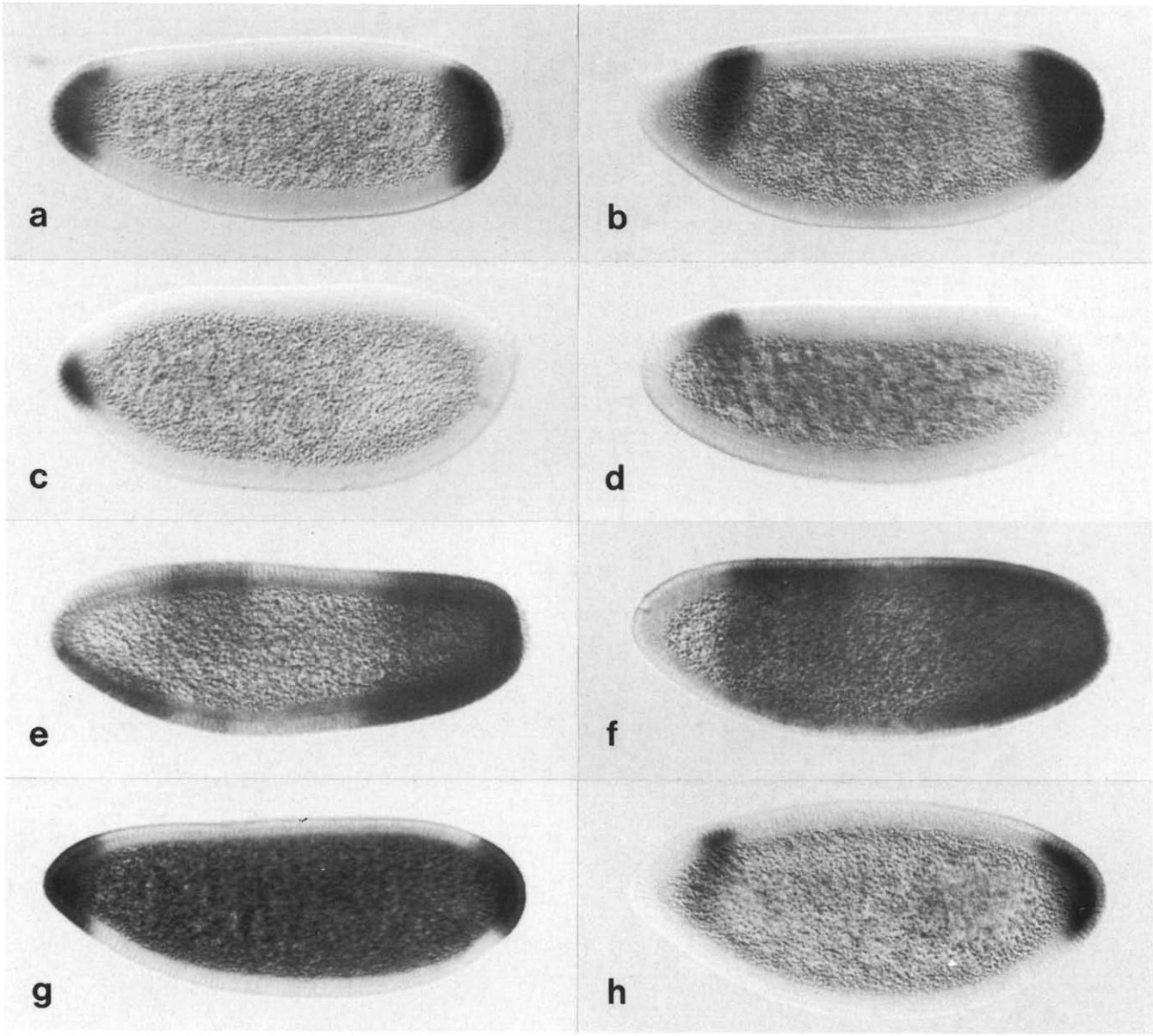

Fig. 1. Patterns of *hkb* and *tll* expression in wildtype and mutant blastoderm embryos. (a, c, e, g): in situ localisation of *hkb* in wildtype embryos (a), in tor^{PM} embryos (c), in tor^{4021} embryos (e), and in osk^{166} Kr^{1} double mutants embryos (g). (b, d, f, h): in situ localisation of *tll* transcripts in wildtype embryos (b), in tor^{PM} embryos (d), in tor^{4021} embryos (f), and in osk^{166} Kr^{1} double mutants embryos (h). Note the absence of terminal gap gene expression in the posterior region in the embryos containing the *tor* lack of function mutation, tor^{PM}, and ectopic expression of *hkb* and *tll* in embryos in which *tor* activity is ectopically expressed due to the tor^{4021} mutation (Klingler et al., 1988). The patterns of *tll* and *hkb* expression in the posterior region of *osk* embryos (not shown) are indistinguishable from the patterns in *osk Kr* double mutants (g, h). Due to the *osk* mutation, embryos lack the activity of two central gap genes, *kni* and *gt*, while the activity of a third one, *Kr*, expands posteriorly. This could mean that the reduction of the terminal gap gene expression domains in *osk* embryos might be due to the posteriorly expanded *Kr* activity. However, since the same reduction as in *osk* embryos could be observed in the *osk Kr* double mutant embryos (which lack the activity of *Kr*, *kni* and *gt* at the same time), the expanded *Kr* activity in *osk* embryos cannot be responsible for the reduction observed (see text). The *osk Kr* double mutant embryos were identified by a double staining involving a *Kr* cDNA probe (Rosenberg et al., 1986). Orientation of embryos is anterior left and dorsal side up. The anterior *hkb* and *tll* expression are not completely absent in tor^{PM} mutant embryos. This indicates that another factor than a *tor*-dependent one must be involved in the activation of the two terminal gap genes in the anterior region of the embryo.

Previous studies have shown that the localized expression of the central gap gene domains depends not only on maternal genes but also on mutual interactions among the gap genes (Jäckle et al., 1986; Gaul and Jäckle, 1987; Hülskamp et al., 1990; Kraut and Levine, 1991; Eldon and Pirrotta, 1991). We therefore examined the *hkb* and *tll* expression patterns in embryos which lack central gap gene activities. In the absence of *hb*, *Kr*, *kni* or *gt* activities, the spatial domains of the *hkb* and *tll* expression are indistinguishable from wildtype embryos. Furthermore, *tll* expression is normal in *hkb* mutant embryos, as is *hkb* expression in *tll* mutant embryos (data not shown). These results indicate that the spatial domains of *hkb* and *tll* expression do not require interactions at the level of the gap genes.

In order to see whether the spatial limits of the posterior expression domains of the terminal gap genes are then controlled by the activity of the maternal posterior organizer system, we examined *hkb* and *tll* expression in embryos which lack the activity of *oskar* (*osk*), one of the key components of the maternal posterior organizer system (Nüsslein-Volhard et al., 1987). In embryos which derived from homozygous *osk* mutant females, the size of the *tll* and *hkb* expression domains is slightly reduced when compared to wildtype (compare Fig. 1 a,b and Fig. 1g,h). We have no straight explanation for this slight reduction of the terminal gap gene expression domains in embryos lacking posterior organizer activity, but for reasons outlined in the legend to Fig. 1, this reduction is unlikely to be caused by the altered zygotic gap gene activities in response to the *osk* mutation. However, the zygotic gap gene function has a maternal complement known to be the mediator of the posterior organizer system (Hülskamp et al., 1989; Irish et al., 1989; Struhl, 1989). In *osk* mutant embryos, maternal *hb* activity is not removed from the posterior portion of the embryo (Tautz, 1988), and this activity may then be responsible for the small reduction of the domains of terminal gap gene expression. Taken together these results indicate that the spatial patterns of terminal gap gene expression are only slightly affected by the absence of the maternal

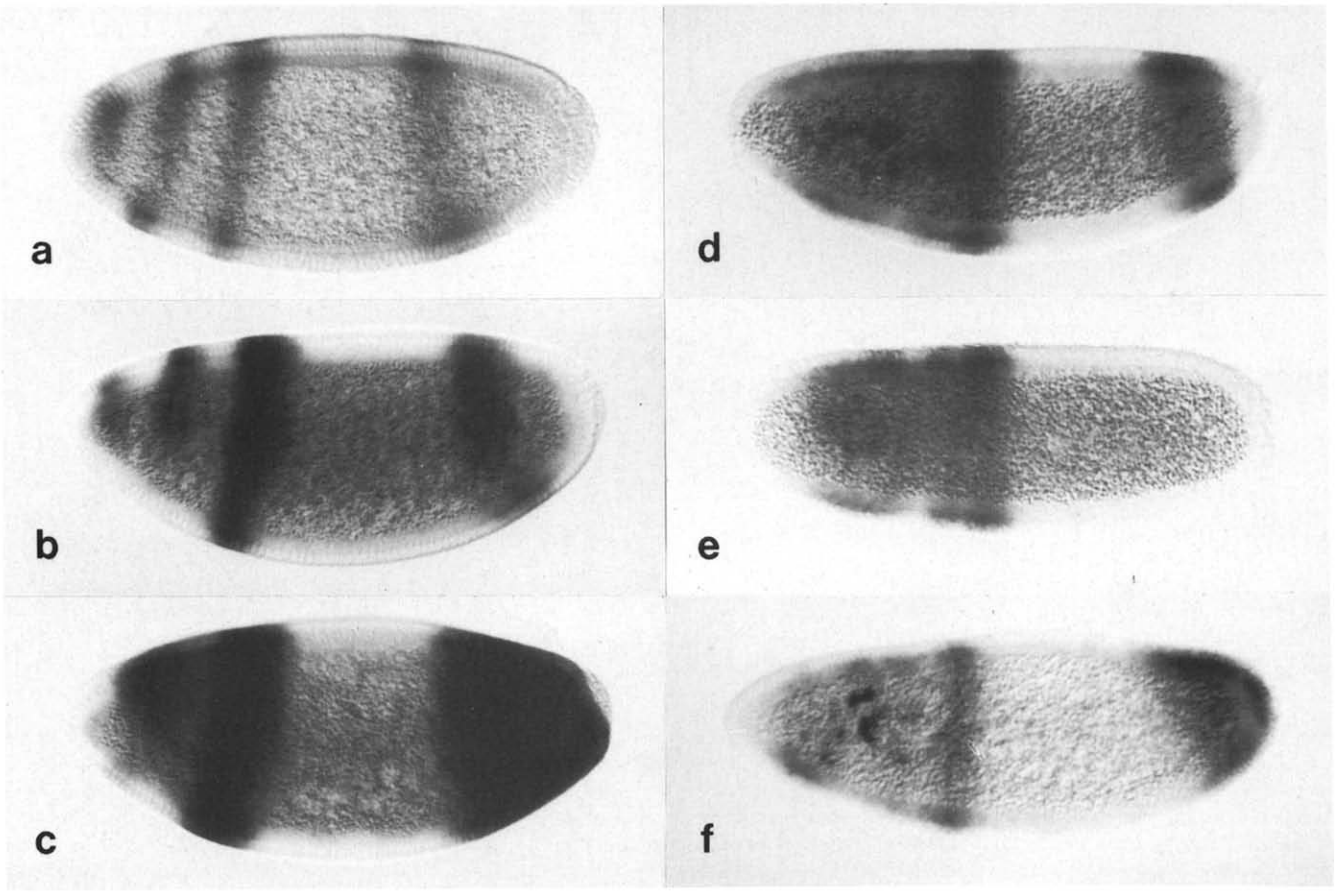

Fig. 2. In situ localisation of *gt* (a–c) and *hb* (d–f) expression in wildtype embryos (a, d) and in mutant embryos such as tll^{g} (b, e), tor^{PM} (c) and *hkb* (f). Note that *gt* is expressed more posteriorly in *tll* mutant embryos (b) and that *gt* expression expands to the posterior tip in *tor* mutants (c). This expansion is also seen in *hkb tll* double mutants (not shown). *gt* and *kni* expression is not changed in *hkb* mutant embryos (not shown). Alterations in *gt* expression, such as the loss of the anterior most stripe, have also been observed in *tor* mutants. A more detailed analysis of the anterior terminal system is beyond the scope of our present study. The posterior domain of *hb* expression (d) is absent from *tll* embryos (e) and does not retract from the posterior pole of *hkb* embryos. Orientation of embryos is anterior left and dorsal up.

posterior organizer activity, and not at all influenced by the central gap genes acting in the regions adjacent to the terminal gap gene expression domain. Therefore, our results reflect a fundamental difference in the regulation of the central and terminal gap genes in the sense that the local expression of the latter depends primarily on the maternal components of the terminal system and not at all on interactions with the other known zygotic genes.

Another important conclusion of our results is that the known components of the terminal system do not include the transacting factor(s) responsible for the transcriptional activation of the terminal gap genes in the posterior region of the embryo. Genetic experiments have led to the conclusion that out of the six known components of the maternal terminal system, only one of them, *lethal(l)polehole* (*l(l)ph*), is acting downstream of *tor* (Ambrosio et al., 1989). However, this downstream most known component of the terminal system represents the *Drosophila raf* oncogene homolog, a putative protein kinase (Nishida et al., 1988; Ambrosio et al., 1989), which is unlikely to account for the direct transcriptional activation of *hkb* and *tll*. Therefore one must postulate that at least one unknown component of the terminal signal transduction pathway is likely to be a transcription factor which is locally active in the posterior terminal region of the wildtype embryo. The active form of this transcription factor may derive from phosphorylation by the *raf* homolog, which could result in a short-range activity gradient with a maximum level in the posterior pole region. By analogy to the known long range morphogen gradient provided by *bicoid* in the anterior region of the embryo (Driever and Nüsslein-Volhard, 1988,1989; Struhl et al., 1989), both *hkb* and *tll* might be activated above different concentration values of the active form of this transcription factor. *tll* expression, which is found localized from the posterior pole to a more anterior position than *hkb*, would then be activated at lower concentration values and therefore in a more anterior position than *hkb*, and both the *hkb* and *tll* expression domains would extend to the posterior tip as observed in the wildtype embryo (Fig. 1a, b).

Central gap gene repression by terminal gap genes

Whereas the expression domains of the terminal gap genes are not affected by the central gap genes, the

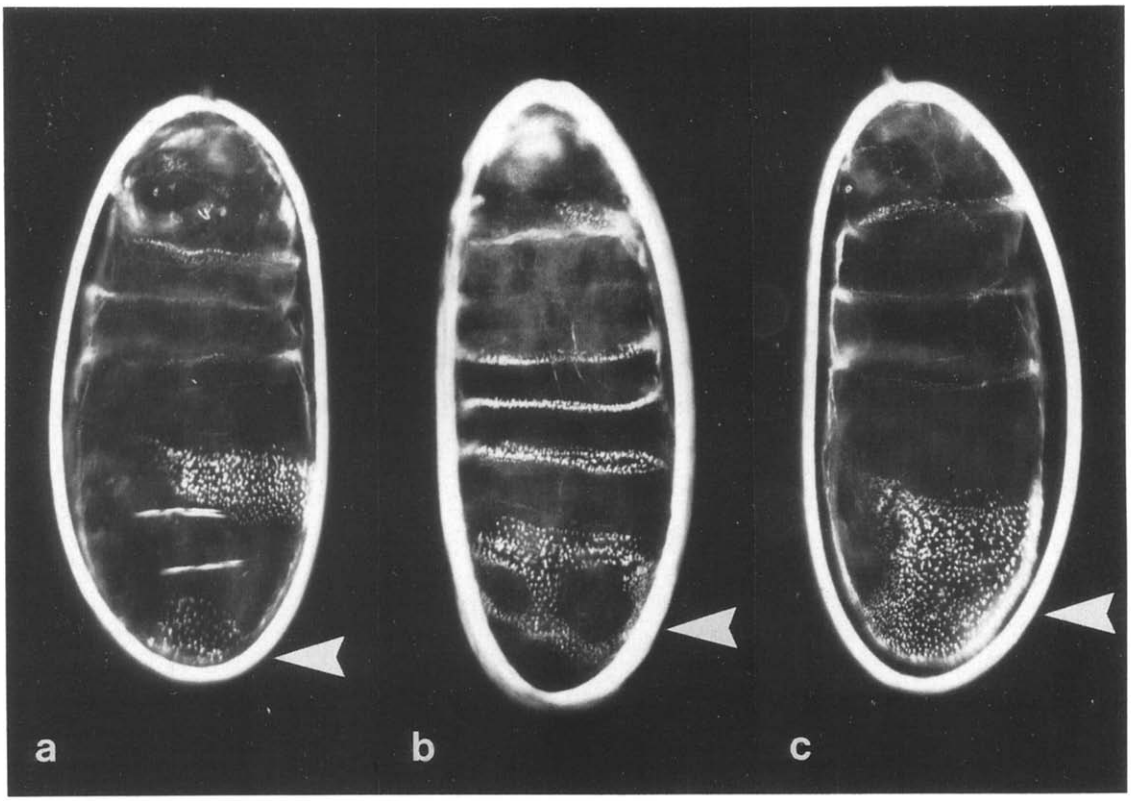

Fig. 3. Larval phenotype of different mutant combinations involving abdominal and terminal gap genes. (a) The terminal regions of *kni tll* double mutant embryos develop a single large denticle band (arrow). Note that this denticle band must be different from segment A8, which is absent from *tll* mutant embryos. (b) *gt tll* double mutant embryos develop a large denticle band resembling a segment fusion (arrow). (c) Triple mutant *kni gt tll* embryos develop a lawn of denticles which extend from the terminal into the abdominal region of the embryo (arrow). Similar phenotypes have been observed for *kni torsolike* (*tsl*) (Lehmann, 1985) and *gt Nasrat* (fs(1)*N*) (Petscheck et al., 1987). In the *tsl* and fs(1)*N* mutations, where a maternal terminal component is missing, both *hkb* and *tll* are absent. This suggests that *hkb* has no specific influence on the described phenotypes. Due to the absence of reliable phenotypic markers, the segment identity of the denticle belts cannot be determined.

expression domains of the central gap genes are influenced considerably by the terminal genes. In *tll* mutant embryos, the posterior band of *gt* expression (Eldon and Pirrotta, 1991) is broader and extends posteriorly as compared to wildtype (Fig. 2a, b). In *tor* mutant embryos, as well as in *hkb tll* double mutant embryos, *gt* extends to the posterior tip (Fig. 2c). Similarly, the domain of *kni* expression expands posteriorly in *tll* mutants (Pankratz et al., 1989) or in *hkb tll* double mutant embryos (not shown). *Kr* expression is also repressed by the *hkb* and *tll* activities (Weigel et al., 1990). These results argue that the activity of the terminal gap genes delimits the region of the wildtype embryo which would become segmentally subdivided by the central gap gene dependent activities of the pair rule genes (reviewed by Ingham, 1990). The repression of the segmentation genes by the terminal genes is therefore a prerequisite for the specialized development of the head and tail regions. As both *tll* (Pignoni et al., 1990) and *hkb* (G.B., unpublished result) encode putative DNA binding proteins, they may promote terminal development by directly activating region specific homeotic genes like *forkhead* (*fkh*) (Jürgens and Weigel, 1988), which itself is likely to be a transcription factor (Weigel and Jäckle, 1990). We note, however, that the terminal system contributes to the posterior most metameric unit (Nüsslein-Volhard et al., 1987) through the positive control of a central gap gene, *hb*, which has two effects in the embryo (Casanova, 1990). First, it is expressed and required in the anterior half of the embryo. Secondly, it is required for the normal formation of the last abdominal segments A7 and A8 (Ingham, 1988; Lehmann, 1985), and it is expressed as a small stripe in the corresponding posterior position of the wildtype embryo (Tautz et al., 1987). The expression of this posterior stripe is controlled by both *tll* and *hkb* activities (see Fig. 2d–f). *tll* activates posterior *hb* expression in a contiguous domain in the posterior pole region. The stripe of *hb* expression in position of the last abdominal segments is then formed by the more posterior activity of *hkb* which acts as a direct or indirect repressor. This control of posterior *hb* expression is consistent with the fact that A8 fails to develop properly in some *hb* mutants (Lehmann, 1985) and in *tll* mutants as well (Strecker et al., 1988).

Since the elimination of the terminal gap genes results in a concomitant expansion of the central segmentation genes into the terminal region, we examined how embryos develop in the absence of both the terminal and the central gap genes. The phenotype of these mutants does not result from an additive combination of the phenotypes of the single mutations. For example, in *kni* mutant embryos A1 through A7 is fused to a single large denticle field (Lehmann, 1985), and in *tll* mutants the region posterior to A7 is deleted. In the corresponding *kni tll* double mutant embryos, one might expect the A1/A7 denticle field, and no segment posterior to it. However, *kni tll* double mutant embryos develop a supernumerary denticle field posterior to the enlarged A1/A7 segment (Fig. 3a). *gt* mutations affect the abdominal segments A5 through A8 (Petscheck et al., 1987). In *gt tll* double mutant embryos abdominal segments A1 to A3 are present, but they are followed by a single large denticle field which is larger than observed in *kni tll* double mutant embryos (Fig. 3b). Finally, in *kni gt tll* triple mutant embryos, in which all abdominal segments but A1 should be absent, the entire posterior region of the embryo is covered with denticles (Fig. 3c). These findings suggest that certain aspects of segmental patterning can occur even without the information from the known central and terminal gap genes. It may be that the denticle fields represent oversized segment portions for which novel combinations of the remaining gap gene activities, unknown single gap genes, or even gap gene independent gene activities might be responsible.

Materials and Methods

Drosophila strains, egg collection and cuticle preparations

Drosophila wildtype and mutant strains were raised under standard conditions (Wieschaus and Nüsslein-Volhard, 1986). Mutant strains referred to in the text and figure legends are described in Lehmann (1985), Wieschaus et al. (1984), Klingler et al. (1988), Nüsslein-Volhard et al. (1987). For the collection of embryos, flies were kept on apple juice agar plates. After aging to the desired developmental stage, embryos were either collected for in situ hybridization (see below) or allowed to develop until hatching. Cuticle preparations of mutant embryos were described earlier (Wieschaus and Nüsslein-Volhard, 1986).

In situ hybridization on embryos

DNA probes for *tll* transcripts were obtained from genomic DNA (encompassing sequence region 307–1193, Pignoni et al., 1990). The *hkb* gene had been cloned by chromosomal walking. Identification of *hkb* DNA is based on molecular analysis of chromosomal rearrangements affecting a single transcription unit (G. Brönner, unpublished result). Hybridization of the dioxygenin labelled DNA probes of *tll*, *hkb*, *gt* (Mohler et al., 1989), *Kr* (Rosenberg et al., 1986) and *hb* (Tautz et al., 1987), the subsequent processing of the whole mount embryos as well as the analysis of the expression patterns was carried out as described in the detailed protocol of Tautz and Pfeifle (1989).

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References

Akam, M. (1987) Development, 101, 1–22.
Ambrosio, L., Mahowald, A.P. and Perrimon, N. (1989) Nature, 342, 288–291.
Casanova, J. (1990) Development, 110, 621–628.
Driever, W. and Nüsslein-Volhard, C. (1988) Cell, 54, 83–93.
Driever, W. and Nüsslein-Volhard, C. (1989) Nature, 340, 363–367.
Eldon, E. and Pirrotta, V. (1991) Development, 111, 367–378.
Gaul, U. and Jäckle, H. (1987) Cell, 51, 549–555.
Gaul, U. and Jäckle, H. (1990) Adv. in Genetics, 27, 239–272.
Hülskamp, M, Schröder C. Pfeifle, C., Jäckle, H. and Tautz, D. (1989) Nature, 338, 629–632.
Hülskamp, M., Pfeifle, C. and Tautz, D. (1990) Nature, 346, 577–580.
Ingham, P.W. (1988) Nature, 335, 25–34.
Ingham, P.W. (1990) Nature, 346, 412–413.
Irish, V. F., Lehmann, R. and Akam, M. (1989) Nature 338, 646–648.
Jäckle, H. et al. (1986) Nature, 324, 668–670.
Jürgens, D. and Weigel, D. (1988) Wilhelm Roux Arch. Dev. Biol., 197, 345–354.
Klingler, M., Erdély, M., Szabad, J. and Nüsslein-Volhard, C. (1988) Nature, 335, 275–277.
Kraut, R. and Levine, M. (1991) Development, 111, 611–621.
Lehmann, R. (1985) Thesis, University of Tübingen.
Mohler, J., Eldon, E.D. and Pirrotta, V. (1989) Eur. Mol. Biol. Organ. J., 8, 1539–1548.
Nishida, Y. et al. (1988) Eur. Mol. Biol. Organ. J., 7, 775–781.
Nüsslein-Volhard, C., Frohnhöfer, H.G. and Lehmann, R. (1987) Science, 238, 1675–1681.
Pankratz, M.X., Hoch, M., Seifert, E. and Jäckle, H. (1989) Nature, 341, 337–340.
Pankratz, M.X. and Jäckle, H. (1990) Trends Genet. 6, 287–292.
Petscheck, J.P., Perrimon, N. and Mahowald, A.P. (1987) Dev. Biol., 119, 175–189.
Pignoni, F. et al. (1990) Cell, 62, 151–163.
Rosenberg, U. et al. (1986) Nature, 319, 336–339.
Sprenger, F., Stevens, L.M. and Nüsslein-Volhard, C. (1989) Nature, 338, 478–483.
Stevens, L., Frohnhöfer, H-G., Klingler, M. and Nüsslein-Volhard, C. (1990) Nature, 346, 660–663.
Strecker, T.R., Merriam, J.R. and Lengyel, J.A. (1988) Development, 102, 721–734.
Struhl, G. (1989) Nature, 338, 741–744.
Struhl, G., Struhl, K. and Macdonald, P. (1989) Cell, 57, 413–422.
Tautz, D. et al (1987) Nature, 327, 383–389.
Tautz, D. and Pfeifle, C. (1989) Chromosoma, 98, 81–85.
Weigel, D., Jürgens, G., Klingler, M. and Jäckle, H. (1990) Science, 248, 495–498.
Weigel, D. and Jäckle, H. (1990) Cell, 63, 455–456.
Wieschaus, E., Nüsslein-Volhard, C. and Jürgens, G. (1984a) Wilhelm Roux Arch. Dev. Biol., 193, 296–307.
Wieschaus, E., Nüsslein-Volhard, C. and Kluding, H. (1984b) Dev. Biol., 104, 172–186.
Wieschaus, E. and Nüsslein-Volhard, C. (1986) *Drosophila*: a practical approach. IRL Press Oxford, U.K.