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Regulatory Interactions between the Segmentation Genes *fushi tarazu*, *hairy*, and *engrailed* in the Drosophila Blastoderm

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Summary

The genetic loci *fushi tarazu* (*ftz*), *hairy* (*h*), and *engrailed* (*en*) must be expressed during embryogenesis for the correct segmental organization of the Drosophila embryo to be established. Transcripts from these genes accumulate in periodic patterns at the blastoderm stage of development. We demonstrate that generation of the normal pattern of *ftz* RNA requires the h^+ function whereas formation of the *h* pattern does not require the *ftz* gene. In addition, we show that the *en* pattern is altered in both *h* and *ftz* mutant embryos. The nature of these changes and the cuticular phenotype of *h* mutant larvae suggest that both *h* and *ftz* phenotypes are a consequence of incorrect compartmentalization and that the effect of *h* is mediated via *ftz* and other genes.

Introduction

Segmentation in Drosophila occurs at the blastoderm stage of development (reviewed in Lawrence, 1981), when the embryo consists of a monolayer of nuclei in the cortical cytoplasm that surrounds the yolk-rich core of the egg (Poulson, 1950; Zalokar and Erk, 1976; Foe and Alberts, 1983). As cellularization of these nuclei concludes the blastoderm stage, the cells are assigned to a series of polyclones (Crick and Lawrence, 1975), dividing the embryo along the anteroposterior axis into an alternating series of posterior and anterior compartments (Garcia-Bellido et al., 1973, 1979). The posterior compartments are distinguished by a requirement for the *engrailed* gene (Morata and Lawrence, 1975; Kornberg, 1981). In the late blastoderm embryo, *en* transcripts can be detected by in situ hybridization in 14 discrete bands, corresponding to the 14 posterior compartments of the gnathal, thoracic, and abdominal segments (Fjose et al., 1985; Kornberg et al., 1985). Thus, accumulation of *en* transcripts in the early embryo is a criterion for formation of posterior compartments.

In contrast to *en*, transcripts from both *hairy* (*h*) (Ingham et al., 1985a) and *fushi tarazu* (*ftz*) (Hafen et al., 1984) are present in patterns having double segment periodicity in which seven bands of transcript lie along the anteroposterior axis of the blastoderm (*h* transcript also accumulates in a patch in the anterodorsal region of the embryo). Both *h* and *ftz* belong to the pair-rule class of loci whose characteristic defect is deletion of pattern elements in alternate segments (Nusslein-Volhard and Wieschaus, 1980). For both genes there is an approximate correlation between the primordia of structures deleted in mutant animals and the position of accumulation of transcripts in the blastoderm. The *ftz* phenotype has been interpreted as reflecting an autonomous requirement for the *ftz* gene function in those cells that accumulate the transcript. The role of *ftz* would accordingly be to specify particular pattern elements by its activity in specific groups of cells (Hafen et al., 1984). Here we present data that argue against a similar explanation of the *h* phenotype and suggest that *h* and *ftz* play quite different roles in the process of segmentation, although a consequence of the activity of each is to establish the appropriate pattern of *en* expression. We propose a subdivision of the pair-rule class based on the functional hierarchy revealed by these observations.

These conclusions rest on four major observations: *h* expression is independent of *ftz* expression; the *ftz* pattern is altered in *h* mutants—aspects of the novel *ftz* patterns correlate with the *h* mutant phenotype; h^- larval cuticle can exhibit pattern elements whose primordia normally lie in regions where *h* transcripts finally accumulate; and the compartmental organization of *h* and *ftz* embryos is changed so that in each case half the normal number of bands of *en* expression form.

Results

Expression of *h* Is Unaltered in *ftz* Mutants

We have analyzed the expression of *h* at the blastoderm stage in embryos lacking wild-type *ftz* activity. To identify such embryos in samples necessarily produced from heterozygous adult flies, we used deletion *Df(3R)4Scb* (Jurgens et al., 1984), known from genetic analysis to delete *ftz* and the two flanking loci *Scr* and *Antp* (see Experimental Procedures). As expected, one-fourth of these samples were found not to express *ftz* sequences during embryogenesis. Those embryos that were at the appropriate developmental stage but showed no *ftz* RNA in six alternate sections from a series of 12 were identified as homozygous for the deletion. The other six sections from the series were processed to reveal the localization of *h* RNA in the *ftz* mutant animals.

In a sample of 12 *ftz* mutant embryos, no significant changes in either the stage of the appearance or the pattern of *h* RNA could be detected. One such embryo is shown in Figure 1; it shows a perfectly normal pattern of *h* transcript despite its being ftz^-. It follows that the ftz^+ gene is not necessary for the organization of the *h* RNA into its characteristic pattern.

Expression of *ftz* Depends on h^+ Activity

Expression of *ftz* in the absence of h^+ was analyzed in embryos homozygous for either of two alleles of the *h* gene, $Df(3L)h^{i22}$ and h^{K1} (Ingham et al., 1985b). Neither

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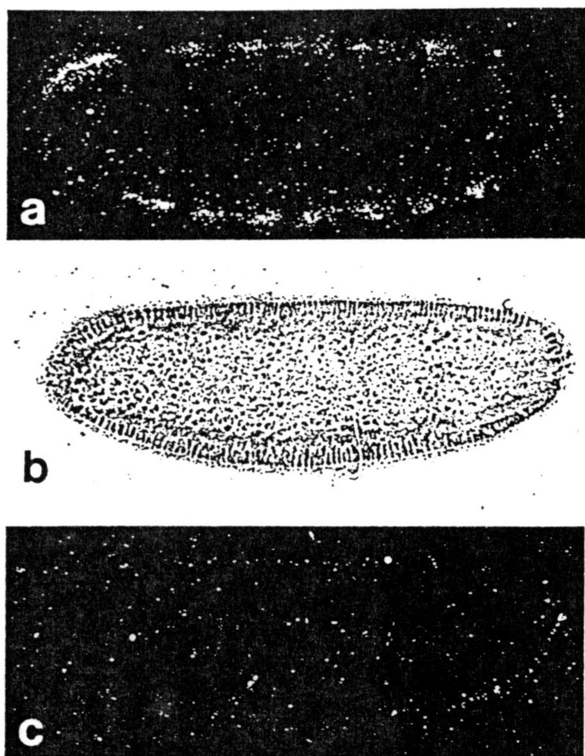


Figure 1. Expression of *hairy* in ftz^- *Embryos*

(a) Dark field photomicrograph of a stage 14 embryo homozygous for *Df(3R)4Scb* processed to reveal *h* transcript. This pattern is, within the limits of resolution of our technique, perfectly normal. Note the anterodorsal patch and the seven stripes typical of *h* (see Ingham et al., 1985a). (b) Phase contrast photomicrograph of the section shown in (a). (c) Dark field photomicrograph of an adjacent section of the same embryo processed to reveal *ftz* transcript. This *ftz* mutant produces no *ftz* RNA. In this and all subsequent figures, all sections are oriented anterior to the left, dorsal surface uppermost. Exposure: (a) and (b), 9 days; (c), 14 days.

homozygote produces a transcript detectable by an *h* cDNA probe that includes the entire putative coding sequence (C. A. Rushlow, personal communication). The *h* mutant embryos were identified in a procedure directly analogous to that used for *ftz*.

The wild-type pattern of seven stripes of *ftz* transcript evolves from a state in which *ftz* expression is almost uniform between 15% and 65% egg length. This evolution occurs during the early part of the final interphase of the blastoderm (stage 14 of Foe and Alberts, 1983), before nuclear elongation is complete (Hafen et al., 1984). Although a similar evolution is seen in h^- animals, the final seven stripes are much broader than those formed in the wild type. The resolution of the pattern is significantly delayed, so that *ftz* expression can be almost uniform when the nuclei are fully elongated, a situation that never occurs in wild-type animals. Furthermore, the rate at which these *ftz* patterns evolve varies with respect to the rate of morphological development (see Figures 3f, 3g, and legend), so that a population of h^- embryos at any given stage will exhibit a spectrum of different *ftz* patterns.

Examination of the *ftz* pattern in 39 h^- embryos at stage 14 reveals that the evolution of *ftz* expression shows a fairly regular behavior. This is reflected in the fact that states intermediate between uniform expression and the seven broad *ftz* bands can be identified and ordered into a phenotypic series. This series shows some parallels with the time course of the evolution of the *ftz* pattern in wild-type embryos, in particular a four-band state seen in both cases (compare Figure 3c with 3h; see also Weir and Kornberg, 1985).

The *ftz* patterns in h^- may be described as arising by fusions of the seven broad stripes so that larger domains of *ftz* expression are formed. There is a definite order to these fusions; for example, the fourth and fifth bands (counting from the anterior) are the most frequently fused and are always fused if any of the other bands are. This pattern of fusions may vary between sections of the same embryo, suggesting a slight irregularity of the pattern. Embryos were classified based on the section showing the best resolved pattern. Using a plus symbol to denote a fusion and a slash to denote separation of the bands, the phenotypic series may be described as follows: 1+2+3+4+5+6+7 (unresolved expression), thirteen cases; 1+2+3/4+5/6+7, four cases; 1/2+3/4+5/6+7, eight cases; 1/2/3/4+5/6/7, four cases; and 1/2/3/4/5/6/7, ten cases. An example of each of these classes is shown in Figures 3a-3e respectively. The stability of the *ftz* pattern clearly varies along the anteroposterior axis, with the region between the fourth and fifth bands being the least stable and the regions between the third and fourth and between the fifth and sixth being the most stable. We also note a difference in the stability of the pattern on the dorsal and ventral sides of the embryo, the pattern generally being better resolved dorsally than ventrally (data not shown).

Absence of h^+ and the Final Differentiated Pattern

To determine the consequence of complete absence of *h* coding sequences during embryogenesis we have analyzed the larval cuticular patterns of animals homozygous for *Df(3L)h*^{i22}. Although this allele is a deletion of the sequences encoded in the embryonic *h* transcript (Ish-Horowicz et al., 1985), the phenotype of the homozygotes is variable. Since animals heterozygous for *Df(3L)h*^{i22} also show some pattern defects and so might be confused with the homozygotes, cuticle was prepared from the progeny of adults of the genotype *Df(3L)h*^{i22} *Ubx*^{1}*/TM1*. Homozygous animals were then identified by the homeotic transformation of the ventral denticle band of the first abdominal segment (A1) to a thoracic character (Lewis, 1978).

In larvae exhibiting the regular pair-rule phenotype, only half the normal number of ventral denticle bands form (compare the *Df(3L)h*^{i22} homozygote in Figure 2b with the wild-type cuticle in Figure 2a). This phenotype may be described as a deletion of pattern elements from approximately the middle of one segment to a homologous position in the succeeding segment, so that segments A1 and A2, A3 and A4, and so on fuse together. These mutant animals are invariably smaller than wild types.

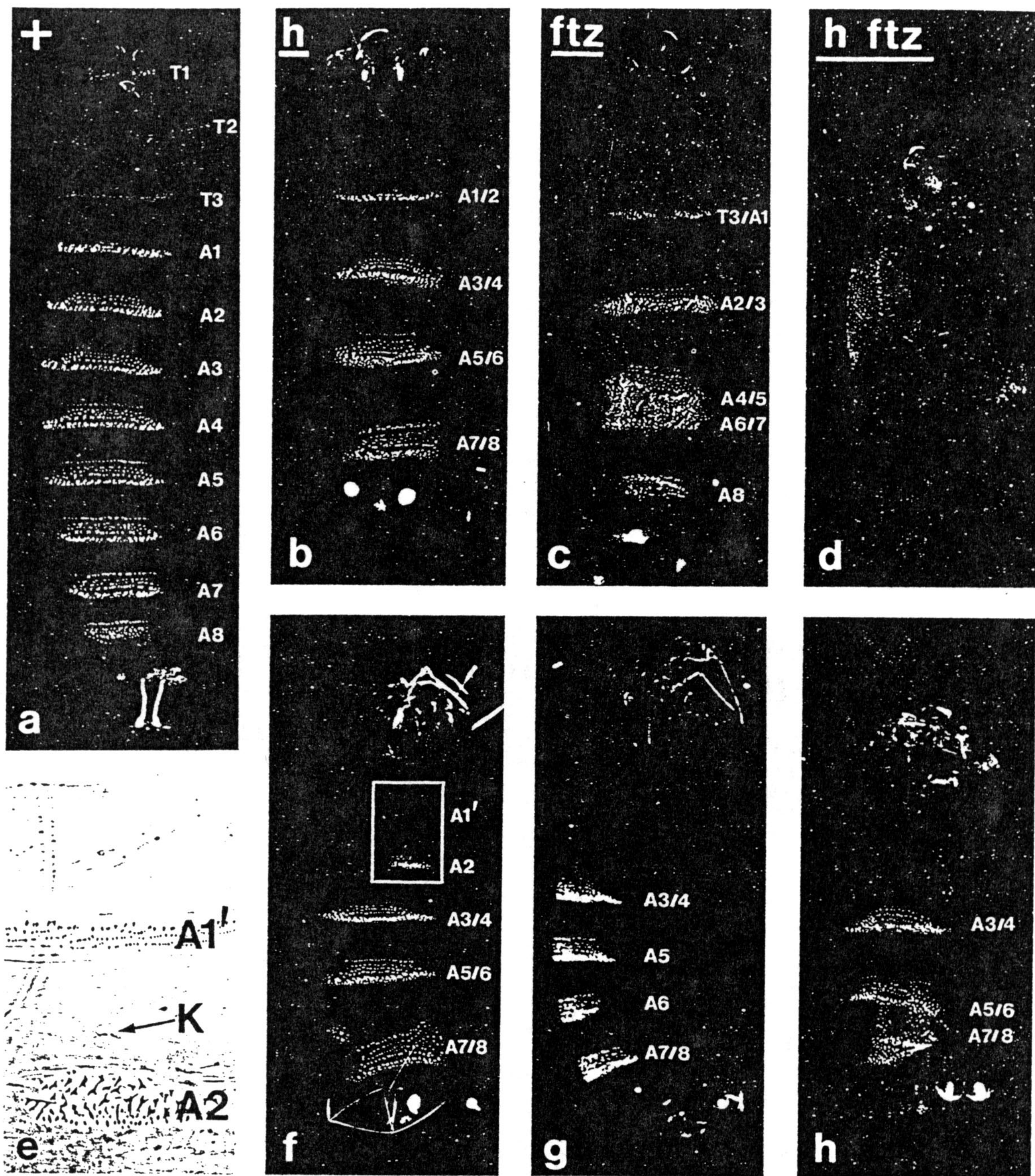


Figure 2. Cuticular *hairy* and *ftz* Phenotypes

Dark field photomicrographs of the ventral patterns of pharate first instar larvae. (a) The wild type has three thoracic (T1, T2, T3) and eight abdominal (A1–A8) ventral denticle belts. (b) Homozygous *Df(3L)h*^{i22} cuticle showing the regular pair-rule phenotype; (c) homozygous *Df(3R)4Scb* cuticle showing fusion of the compound A4–A5 and A6–A7 segments, typical of these animals. Note that because *Df(3R)4Scb* is also *Antp*$^-$ and *Scr*$^-$, it is possible to identify the homozygotes independently of their *ftz* phenotype on the basis of the homeotic transformation of the thoracic segments (see Wakimoto and Kaufman, 1981); (d) cuticle of animal homozygous for both *Df(3L)h*^{i22} and *Df(3R)4Scb*. A single aperiodic mass of denticles is secreted. The same phenotype is observed in *h*^{5H07} *ftz*^{9H34} double homozygotes and can therefore be attributed uniquely to the loss of *h*$^+$ and *ftz*$^+$ and not to loss of any of the other loci absent from the two deletions. (e–h) Homozygous *Df(3L)h*^{i22} *Ubx*^{1} cuticle, in each case identified by the transformation of the A1 denticle belt to a thoracic character (A1′), see (e). In (f) part of the A2 denticle band is retained; the detail (boxed region) is shown in (e): note the presence of a two-haired Keilin's organ, a derivative of the transformed anterior A1. (g) Shows another example in which a complete A6 denticle band is present. (h) Typical extreme *h*$^-$ phenotype, displaying fusion of compound segments.

In a sample of 152 such *Df(3L)h*^{i22}*Ubx*^{1} pharate larvae, 40% (61) showed this regular pair-rule fusion. A further 48% (73) showed phenotypes in which the deletions span more than a segment width, resulting in the fusion of the compound segments (Figure 2h). Weaker phenotypes were also seen. These involve the formation of denticles corresponding to A2 (3/152), A6 (14/152), or both (1/152). In the former case, the A2 denticles are separated from

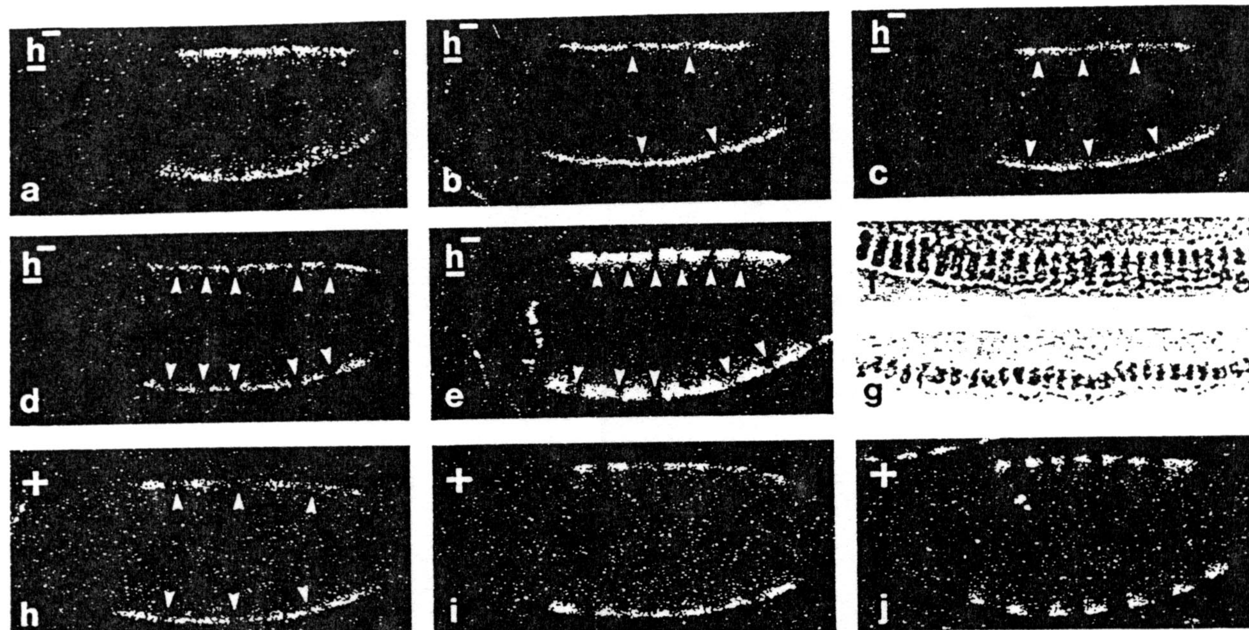


Figure 3. Expression of *ftz* in h^- Embryos

Dark field photomicrographs of medial sagittal sections of *hairy* mutant (h^-) and wild-type (+) embryos hybridized with *ftz* probe.

(a–e) Show examples of the various patterns of *ftz* expression observed in h^- embryos and correspond to classes a–e described in the text. Each of these embryos is homozygous for the deficiency *Df(3L)h*^{i22} and was identified as such by examination of adjacent sections hybridized with *h* probe (data not shown, see text).

(f and g) Show the nuclear morphology at the anteroventral region of sections illustrating the stage of embryos (a) and (c) respectively. Note that the *ftz* pattern is less well resolved in the later embryo; at the equivalent stage of wild-type development the pattern is clearly resolved (Hafen et al., 1984; see also j).

(h–j) Show the evolution of the *ftz* pattern in wild-type embryos through stage 14. Note the four-segment periodicity at the earliest phase of the evolution (h).

the Keilin's organ of the transformed A1 by naked cuticle that represents posterior A1, and from the denticles of A3 by another patch of naked cuticle, which corresponds to either or both of the anterior and posterior compartments of A2 (Figures 2e and 2f). The deletion frame in these cases encompasses considerably less than a full segmental unit.

This variability of phenotype contrasts with the effect of absence of *ftz*; comparable weaker phenotypes were not seen in a sample of 80 *Df(3R)4Scb* cuticle preparations. The most common pattern is one in which the compound A4/A5 and A6/A7 segments are themselves fused (Figure 2c).

Our data demonstrate a requirement for the *h* gene in the organization of *ftz* expression. If this were its sole function, absence of h^+ should be of no consequence in ftz^- embryos, i.e. *ftz* would be epistatic to *h*. To test this the cuticular pattern differentiated by embryos mutant for both genes was examined. A typical example is shown in Figure 2d. This pattern differs significantly from that of *ftz* alone; in particular, the single patch of denticles formed shows no evidence of a periodic pattern (see also Nusslein-Volhard et al., 1985).

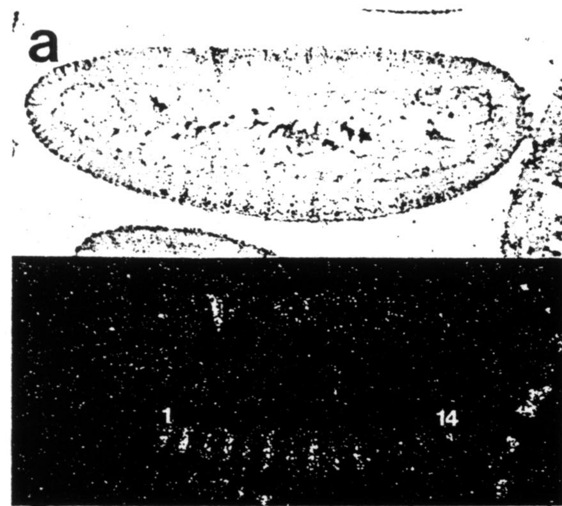
Compartmentalization in *h* and *ftz* Mutants

Expression of *en* is first detectable in situ at the conclusion of the blastoderm stage, when a pattern of 14 bands of transcript forms. These alternate in intensity, revealing a pair-rule modulation of early *en* expression (Weir and Kornberg, 1985; Di Nardo et al., 1985; see also Figures 4a,

Figure 4. Onset of *en* Expression in Wild-Type and h^- Embryos

The pairs of bright and dark field images show a temporal sequence representing about 30 min of development at 25°C. The sections are sagittal or parasagittal, with wild-type embryos on the left and h^- (h^{k1}) on the right. All sections were treated identically. Exposure 33 days.

(a) Shows that in the wild-type stage 14 embryo, 14 bands of alternating intensity appear in an anteroposterior progression (1–14). Each band spans approximately one nucleus, shown in (c). In contrast, (b) shows that in h^- embryos, only seven bands of *en* expression (1–7) appear at blastoderm. Each of these is significantly broader than in wild-type, spanning two to three nuclei, shown in (d). Note that in both wild type and h^-, expression of *en* is more advanced ventrally than dorsally. The most prominent band at this stage in wild type is the second, located just posterior to the site of the cephalic fold invagination. This corresponds to the most anterior band (1) of expression in h^- embryos. This is more apparent in the early gastrula (e and f), where the cephalic fold is clearly visible (arrowed). The modulation of intensity is still visible during this stage in the wild type (e). In early h^- gastrulae (f) evidence of intervening weak bands can sometimes be seen (arrowheads). As germ band elongation proceeds the modulation ceases in the wild type (g). In h^- embryos (h) *en* continues to be expressed in seven coherent, broad bands. The large patch of signal located mediodorsally in this section is due to the compression of the dorsal surface that occurs during the process of germ band elongation.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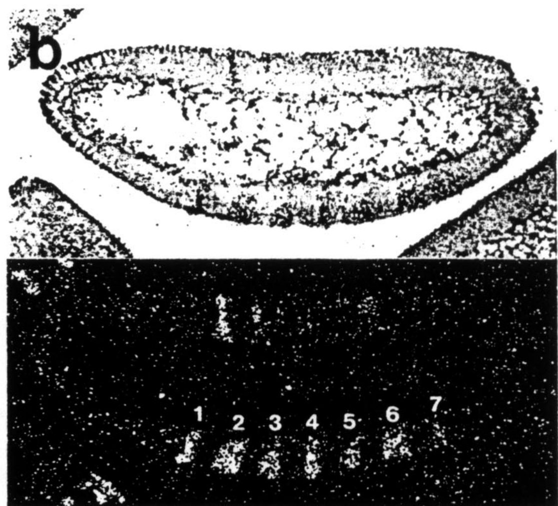
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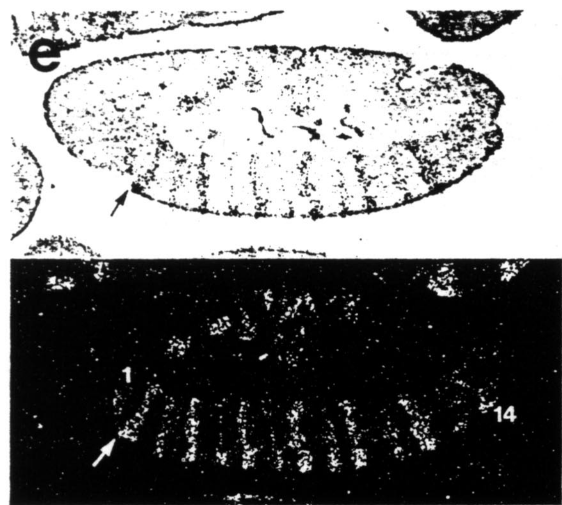
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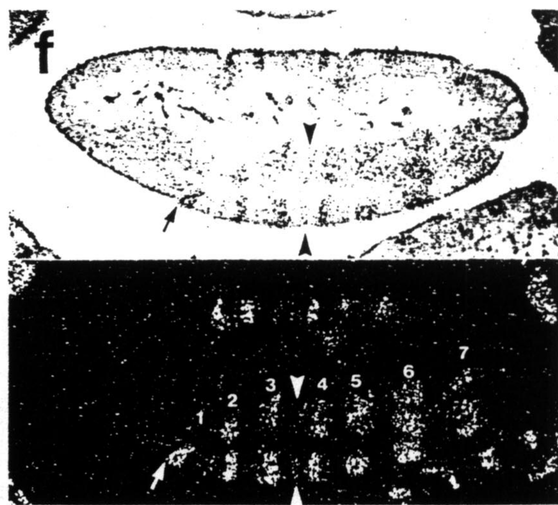
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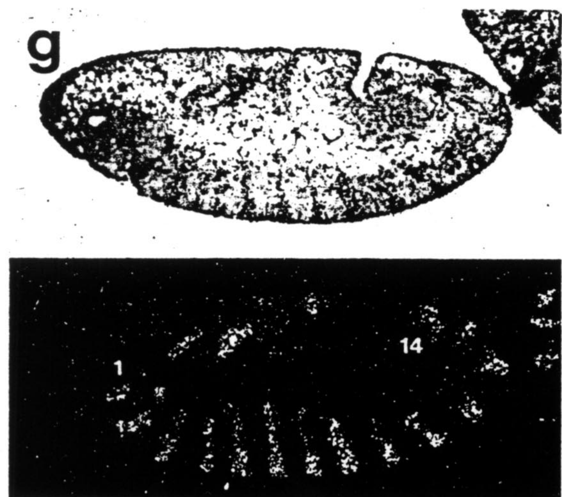
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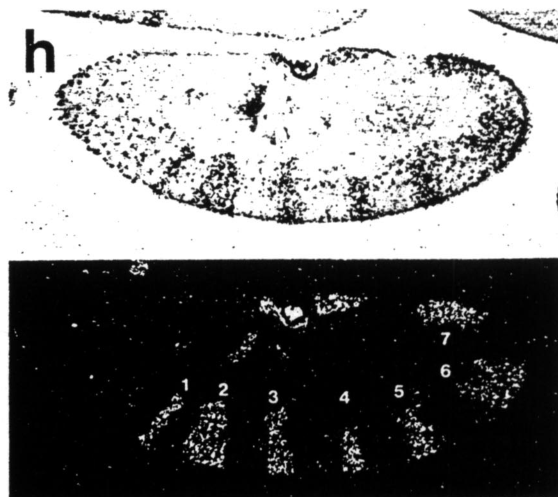
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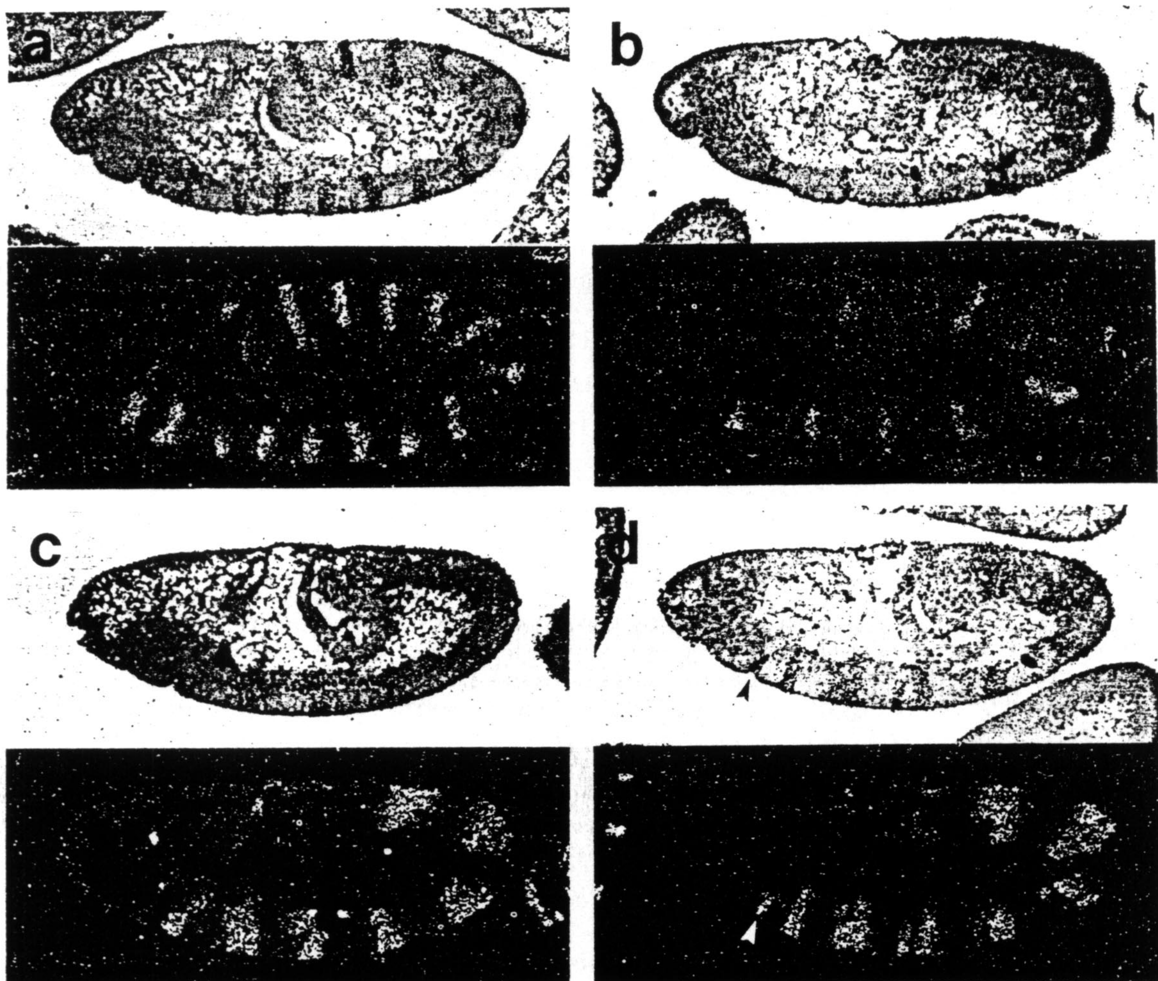

Figure 5. Expression of *en* in Wild-Type and Mutant Germ Bands

Bright and dark field images of embryos that have just completed germ band extension (after approximately 4.5 hr of development at 25°C). Note that in h^- embryos (c and d) extension proceeds less far than in wild type (a) (Ingham et al., 1985a).

(a) A wild-type embryo showing the characteristic pattern of 14 bands.

(b) At the equivalent stage, ftz^- (*Df(3R)4Scb*) embryos have only six or seven narrow bands of *en* expression. From their positions these appear to correspond to the wild-type bands 1, 3, 5, 7, 9, 11, and 13. The ftz^- phenotype can thus best be described as the suppression of even-numbered *en* bands.

(c) In contrast, *en* is expressed in seven broad bands in h^- extended germ bands.

(d) An additional anterior band (arrow) is often apparent at this stage in h^- embryos; this probably corresponds to the wild-type band 1. The broader bands are not always coherent at this stage of development. In this example, the third broad band is clearly replaced by two narrower bands: compare (d) with (c). This seems best explained by fission of the single broad band.

Exposures: (a, c, and d), 33 days; (b), 43 days.

4c, and 4e). This modulation diminishes during gastrulation and is no longer apparent by the middle of germ band extension (Figure 4g).

The pattern of *en* expression in a late h^- blastoderm is shown in Figures 4b and 4d. In contrast to wild-type (Figures 4a and 4c) there are only seven bands of *en* transcript, each significantly broader than normal and of approximately equal intensity. The anteriormost lies just posterior to the cephalic fold and is slightly narrower than the other six (compare Figures 4a and 4b, 4e and 4f). This suggests that the novel pattern arises by enhancement of the strong wild-type bands and suppression of the weak wild-type bands. As gastrulation proceeds, evidence of much weaker intervening bands is occasionally detectable (see Figure 4f). In the majority of cases, however, the pattern of seven broad bands persists during germ band elongation (Figure 4h).

Additional bands of *en* expression sometimes appear in the extended germ band. In these cases, two closely spaced narrower bands appear to replace a single wide band (see Figure 5d). These may arise by the fission of the original broad band.

The normal pattern of *en* expression is also dependent on activity of the *ftz* gene. In ftz^- embryos at the extended germ band stage only six or seven bands of *en* transcript are apparent (Figure 5b). In contrast to the

broad bands typical of h^- embryos these are approximately the same width as those in wild-type though more widely spaced (Figure 5).

Discussion

The two pair-rule genes *fushi tarazu* and *hairy* exhibit similar patterns of expression in the late blastoderm embryo, with transcripts accumulating in alternate segment-wide regions along the anteroposterior embryonic axis (Hafen et al., 1984; Ingham et al., 1985a). According to a recently proposed model (Gergen et al., 1985) the differential spatial expression of each of the pair-rule genes generates an array of unique combinations of active genes, each combination specifying the fate of an individual blastoderm cell. In such a system each pair-rule gene would perform an equivalent function, namely to contribute to the combinatorial code of positional values. A subset of all blastoderm cells would thus be specified by a code consisting of the states of expression of *h* and some other genes. In this case, removal of h^+ would alter the codes of those cells that normally express it.

In the simplest case, these cells would fail to respond to the novel "nonsense" codes and would not contribute to the final pattern of the cuticle. The amorphic phenotype of *h* would then be deletion of pattern elements corresponding to the segment-wide domains in which *h* is expressed at the blastoderm stage. We have found, however, that embryos which lack the *h* gene can show deletions that span considerably less than a full segment. This observation might be explained if the code were redundant and other components could, occasionally, substitute for *h*. Alternatively, *h* might be involved in some process other than the direct labeling of cells and might therefore be affecting the pattern indirectly. Interestingly, the homeo-box sequence that is associated with many genes involved in cell labeling, including several homeotic loci and both *ftz* and *en* (McGinnis et al., 1984; Scott and Weiner, 1984; Poole et al., 1985; Fjose et al., 1985), is not present in the *h* coding sequence (C. A. Rushlow, personal communication).

The Interactions of *h*, *ftz*, and *en* Suggest a Hierarchical System

The pattern of *ftz* transcripts in *h* mutants shows that h^+ activity is required to organize *ftz* expression. There is a correlation between the stability of the pattern of *ftz* transcript at blastoderm stage and the final pattern of differentiated structures in *h* mutants. This suggests that the two are causally related—i.e., that the *h* phenotype is, at least in part, a consequence of the changes in *ftz* expression seen in h^- blastoderms. Thus, the regions between the third and fourth bands and between the fifth and sixth *ftz* bands are most stable in *h* blastoderms. These correspond to the primordia for the second and sixth abdominal segments, which do develop, albeit rarely, in h^- animals. Conversely, the region between the fourth and fifth *ftz* bands, which are most commonly fused in h^- blastoderms, corresponds to the fourth abdominal segment, which is frequently absent even in h^- heterozygotes (Ingham et al., 1985b).

The novel pattern of *en* expression at blastoderm in h^- embryos is similarly consistent with the *h* mutant phenotype. One broader *en* band replaces each pair of strong and weak bands typical of wild-type animals. This reflects the formation of half the normal number of segments and, therefore, of posterior compartments in h^- animals.

That both *ftz* and *en* expression are changed in h^- blastoderms in ways which correlate with the *h* phenotype suggests that *h* may regulate *en* via *ftz*. This is supported by our finding that in ftz^- embryos at the extended germ band stage, only six or seven widely spaced bands of *en* expression are seen. Taken together with the fact that *h* is normally expressed in ftz^- embryos these findings suggest a hierarchical mechanism in which *h* organizes *ftz*, which in turn regulates *en*.

If there were a transfer of information between *ftz* and *en* at a specific time during stage 14, we might expect to see a variation in the pattern of *en* expression reflecting the variability of the altered *ftz* pattern. Since the intervening weak *en* bands are barely detectable in wild-type animals, it is perhaps not surprising that we have not observed examples of such variability at this stage. Occasionally, however a weak *en* signal is detected between the enlarged bands during the late stages of gastrulation (see Figure 4f). This suggests that there is some variability in the *en* pattern in h^- embryos which parallels the variability in the *ftz* pattern and the phenotype of these animals.

Compartmentalization and Segmentation

We propose that the primary function of the pair-rule genes is to establish the appropriate compartmental organization of the embryo by regulating the expression of the *en* gene. Our suggestion that the pair-rule phenotype is due to incorrect compartmentalization of the embryo is, in some sense, analogous to the idea of "embryonic malpartition" postulated by Sander et al. (1980). The pair-rule modulation of *en* expression at the blastoderm stage (Weir and Kornberg, 1985; Di Nardo et al., 1985) seems to reflect its dependence on different (pair-rule) cues in alternate compartmental primordia.

It follows from our hierarchical model that, although superficially similar, the *h* and *ftz* mutant phenotypes are generated in contrasting ways. In the case of *ftz* we postulate that there is an autonomous requirement for the gene to establish alternate compartments by activation of *en*, with a corresponding *ftz*-like gene being responsible for the intervening compartments. This activation may occur in response to a threshold level of the activity of *ftz* and possibly other pair-rule genes. Alternatively, *ftz* and other genes may define a domain within which positional information to which *en* responds is generated. In the second case, there would be a segmental state that preceded the compartmentalization of the embryo.

In contrast, the *h* phenotype appears to be a consequence of the change in domains of activity of *ftz* and other pair-rule genes that then regulate *en* in the blasto-

derm. Thus, *ftz* becomes expressed in broader domains, presumably at the expense of its *ftz*-like counterpart. This results in a corresponding increase in size of the *en* domains defined by *ftz* activity and a suppression of the remaining seven *en* domains. This has the important consequence that some blastoderm cells which normally would not express *en*, and would, therefore, give rise to anterior compartments, now do express *en* and hence give rise to posterior compartments. One implication of this change in blastoderm cell fate is that *h* may also regulate, either directly or indirectly, the expression of the homeotic selector genes (Garcia-Bellido, 1975) that determine the developmental pathways followed by each compartment (see also Akam, 1985). The changes in *en* expression seen in *ftz*$^-$ embryos also imply changes of cell fate.

The *h ftz* double mutant phenotype can be explained in terms of changes in *en* expression. Removal of *ftz* from an *h*$^-$ blastoderm would prevent activation of *en* in the *ftz* regions without restoring expression of *en* in the intervening regions. Hence an embryo consisting of one large anterior compartment would form, which might, therefore, be expected to develop an aperiodic lawn of denticles. This is indeed the case (see Figure 2d). Some other pair-rule genes, those responsible for activation of *en* in the non-*ftz* frame, would not be expected to interact in this additive way with *h*. The major effect of such genes in double mutant combination with *h* would be to suppress the rare extra A2 and A6 pattern elements.

It has been suggested (Crick and Lawrence, 1975; Lawrence and Morata, 1976) that compartments have the ability to regulate their size. The unusually large anterior and posterior compartments formed in *h*$^-$ and *ftz*$^-$ embryos would then be expected to develop to approximately normal size. This regulative process could account both for the smaller size of *h* and *ftz* mutant larvae and for the cell death observed in *h* mutant embryos (Ingham et al., 1985a). Regulation might also account for the stronger *h* phenotypes, which do not have correlates in the patterns of *en* expression in *h*$^-$ blastoderms, and perhaps for the apparent fission of *en* bands seen in germ band *h*$^-$ embryos.

The Function of the *h* Gene

We find that *h* plays a primary role in organizing *ftz* expression. Since *ftz* expression is structured in all *h*$^-$ animals and is relatively normal at later stages of development, *h* can be only one of the components of the mechanism influencing *ftz* expression. We can discount the possibility that *h* simply represses *ftz* at the end of the blastoderm stage. If this were so, the novel *ftz* pattern in *h*$^-$ could not extend beyond the sum of *h* and *ftz* domains in wild-type animals. In fact this summed *h* and *ftz* pattern in wild type is one of eight distinct stripes at developmental stages when *ftz* can be almost completely uniform in *h*$^-$ animals (Ingham et al., 1985a). Before *h* and *ftz* are expressed in stripes, both transcripts are present uniformly in large regions of the blastoderm. We must suppose that interactions between *h* and *ftz* and, presumably, other segmentation genes occur at this time and that they are, in part, responsible for generating the periodic pattern.

It could be that the changes in *ftz* pattern in *h*$^-$ embryos were due simply to a retardation of the normal pattern-forming mechanism in the absence of *h*. In this case the intermediate states we see might be present transiently in wild-type embryos. Sometimes early wild-type embryos do show a four-segment periodic state (Weir and Kornberg, 1985; see also Figure 3h and Figure 3i in Ingham et al., 1985a), suggesting that *h* may be involved in determining the kinetics of the patterning mechanism.

If we are correct in suggesting that the primary function of *h* is to organize compartments but that it does not directly regulate *en*, then the periodicity of *h* itself seems gratuitous unless necessary for the pattern to form. Two possibilities seem plausible: *h* may mediate between some underlying periodic information and *ftz*, perhaps increasing the resolution of that information in the process; alternatively, *h* may be involved in the generation of that information.

Theoretical considerations (Meinhardt, 1984) suggest that some long range interaction between components of the periodic structure is necessary to form the normal number of segments with the regularity characteristic of the wild-type animal. *h* could be a component of such a nonautonomous mechanism. Since the embryo is a syncytium at the time the pattern forms these effects could be mediated by the direct transfer of macromolecules, RNA or protein, between different regions of the syncytial blastoderm. We note that *h* is also involved in bristle patterning (Bridges and Morgan, 1923) and that this process may also require nonautonomous cellular behavior (Wigglesworth, 1940; Lawrence, 1973; Richelle and Ghysen, 1979).

Experimental Procedures

Drosophila Mutants

h^{k1}, and h^{5H07}, and *Df(3L)*h^{i22} are homozygous lethal and exhibit the pair-rule segmentation phenotype. They have been classified as apparent amorphs (see Ingham et al., 1985b). ftz^{9H34} and *Df(3R)4Scb* are homozygous lethal and exhibit a strong *ftz* phenotype. *Df(3R)4Scb* is also genetically *Scr*$^-$ and *Antp*$^-$ (Jurgens et al., 1984). These latter two homeotic loci have no effect on segment number but cause intersegmental transformations (see Wakimoto and Kaufman, 1981).

Double mutant combinations of the constitution of h^{5H07} ftz^{9H34}, *Df(3L)*h^{i22} *Df(3R)4Scb*, and *Df(3L)*h^{i22} Ubx^1 were generated by stranded breeding procedures. A description of the Ubx^1 phenotype can be found in Lewis (1978). *TM1* is a standard balancer chromosome (see Lindsley and Grell, 1968).

Flies were reared at room temperature (22° ± 1°C) on standard Drosophila medium.

Histology

Probes were synthesized using the SP6 transcription system as described previously (Ingham et al., 1985a). The template sequences were as follows: *en*, the 2.0 kb EcoRI fragment of the *en* cDNA clone C-2.4 described in Poole et al., 1985; *ftz*, the 1.2 kb EcoRI-SalI fragment of the *ftz* genomic sequence lying between coordinates 662 and 1813 in Figure 3 of Laughon and Scott, 1984; *h*, a 1.7 kb EcoRI-HindIII fragment of the *h* genomic sequence lying between coordinates 0 and 1.7 in Ish-Horowicz et al., 1985, which corresponds to the 1.7 kb of sequence lying to the right of the HindIII site in λ04 of Holmgren, 1984 (where transcription is from left to right). The antisense strands of these sequences were labeled with ^{3}H (*ftz* and *en*) or ^{35}S (*h*) to specific activities of 1.1 × 10^8 and 1.4 × 10^9 dpm μg^{-1} respectively.

Fixation, in situ hybridization, washing, and autoradiography were as described previously (Ingham et al., 1985a).

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