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Regulation and Function of the Drosophila Segmentation Gene *fushi tarazu*

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Summary

The Drosophila segmentation gene *fushi tarazu* (*ftz*) is expressed in a pattern of seven stripes at the blastoderm stage. Two *cis*-acting control elements are required for this expression: the zebra element, which confers the striped pattern by mediating the effects of a subset of segmentation genes; and the upstream element, an enhancer element requiring *ftz*$^+$ activity for its action. Fusion of the upstream element to a basal promoter results in activation of the heterologous promoter in a *ftz*-dependent striped pattern, supporting the idea that *ftz* regulates itself by acting through its enhancer. The upstream element can also confer expression patterns similar to that of the homeotic gene *Antennapedia*, suggesting that a similar element may play a role in the activation of *Antennapedia*.

Introduction

The process of segmentation in Drosophila is governed by a set of genes called the segmentation genes (Nüsslein-Volhard and Wieschaus, 1980). They can be subdivided into three classes according to the embryonic phenotypes of their mutants: the gap mutants, which cause deletions of continuous stretches of segments; the pair-rule mutants, which produce pattern deletions with double-segment periodicity; and the segment-polarity mutants, which delete part of every segment.

An important characteristic of the segmentation genes is that they are expressed in a spatially restricted manner during early embryogenesis. For example, all of the pair-rule genes analyzed are expressed in overlapping patterns of stripes of cells at the blastoderm stage (Hafen et al., 1984a; Ingham et al., 1985a; Kilchherr et al., 1986; Harding et al., 1986; Macdonald et al., 1986). Concerted action of several pair-rule genes appears to instruct the blastoderm cells to contribute to the normal pattern (reviewed by Gergen et al., 1986; Scott and O'Farrell, 1986). Normal patterns of expression are essential for the execution of proper function, because ectopic expression or overexpression leads to dominant phenotypes (Struhl, 1985; Gergen and Wieschaus, 1986). Therefore, the question of how the spatial pattern of expression of segmentation genes is achieved is one of the central problems for understanding development.

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The *fushi tarazu* (*ftz*) gene is one of the best characterized pair-rule genes (Wakimoto et al., 1984; Kuroiwa et al., 1984; Weiner et al., 1984). The *ftz* gene contains a homeobox of the *Antennapedia* (*Antp*) type and is located within one of the homeotic gene clusters, the Antennapedia complex (McGinnis et al., 1984; Laughon and Scott, 1984; reviewed by Gehring and Hiromi, 1986). Expression of the *ftz* gene occurs in two phases during embryonic development. At the blastoderm stage, *ftz* transcripts and protein accumulate in cells that form a pattern of seven stripes of double-segment periodicity (Hafen et al., 1984a; Carroll and Scott, 1985). Later, during neurogenesis, *ftz* is expressed in a segmentally repeated pattern in specific neuronal precursors and progeny in the developing central nervous system (CNS) (Carroll and Scott, 1985; Hiromi et al., 1985; Doe et al., unpublished). The difference in periodicity between expression in the stripes and in the CNS suggests the existence of different regulatory mechanisms for *ftz* expression during the two stages.

We have previously shown that normal *ftz* expression and function depend on several *cis*-acting control elements (Hiromi et al., 1985). Analysis of expression of *ftz/lacZ* fusion genes in transformant embryos has revealed three control elements in the 5′ flanking region: the "zebra element," which confers a striped pattern of expression; the "neurogenic element," which is involved in expression in the CNS; and the "upstream element," which has an enhancer-like effect on expression in the stripes. The location of these elements suggests that the regulation of expression is mainly, if not exclusively, at the transcriptional level. Measurement of transcript stability also argues against differential degradation of transcripts being the mechanism whereby the striped pattern is generated (Edgar et al., 1986).

Here, by analysis of *ftz/lacZ* gene expression in segmentation mutants, we have identified genes encoding factors that act via *ftz* control elements. We find that normal expression in the striped pattern requires products of a subset of segmentation genes whose action is mediated by different control elements. In particular, the product of the *ftz* gene itself appears to be a factor that is required for the enhancer action of the upstream element.

Results

The Upstream Element Is an Enhancer Required for Expression in the Stripes

We have constructed a set of *ftz/lacZ* genes to investigate the role of the upstream element in *ftz* transcription (Figure 1). These *ftz/lacZ* genes have the zebra element fused to the *lacZ* gene, and the upstream element is inserted at various positions and orientations. P elements containing *ftz/lacZ* genes were introduced into the genome by P element–mediated transformation, and several independent transformant lines were established. The pattern of expression of each fusion gene was determined as described in Experimental Procedures.

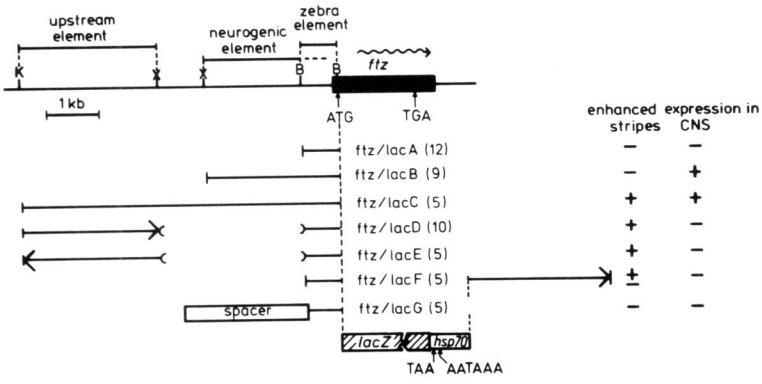

Figure 1. Schematic Diagram of *ftz/lacZ* Genes

The top line shows the structure of the *ftz* gene. The transcribed region is shown by a solid box, and three control elements in the 5′ flanking region are indicated. Restriction sites: K, KpnI; X, XbaI; B, BalI. Solid lines below represent *ftz* upstream sequences included in the fusion genes. The fusion point is within the second amino acid codon of the *ftz* gene. *ftz/lac*A through *ftz/lac*C constitute a simple 5′ deletion series. *ftz/lac*D and *ftz/lac*E have an internal deletion (shown by parentheses) in the 5′ flanking region including the neurogenic element, and the upstream element is fused to the zebra element in normal (*ftz/lac*D) and opposite (*ftz/lac*E) orientation. *ftz/lac*F has the upstream element inserted downstream of the transcription unit. The indicated sequences were inserted into the P-element vectors Carnegie 20 or cp20.1 such that the *rosy*$^+$ gene is located at the 5′ side of the *ftz/lacZ* genes. *ftz/lac*G has a 2.5 kb spacer sequence upstream of the zebra element. The distance between *rosy*$^+$ and the zebra element is therefore approximately the same in *ftz/lac*D, *ftz/lac*E, and *ftz/lac*G.

Numbers in parentheses indicate the number of independent transformant lines examined for each construct. Three of the *ftz/lac*A lines and five of the *ftz/lac*D lines were obtained by inducing secondary transpositions from original integration sites. Expression of each *ftz/lacZ* gene is summarized at right.

Figure 2 shows the patterns of expression of *ftz/lacZ* genes at the germ-band extension stage. As previously reported, the *ftz/lacZ* genes that do not contain the upstream element (*ftz/lac*A and *ftz/lac*B genes) are expressed only weakly in the stripes compared with a gene that does contain this element (the *ftz/lac*C gene). Although the quantitative difference in the levels of β-galactosidase is only about 2-fold (Hiromi et al., 1985), there is a spatial difference in β-galactosidase localization. In transformant embryos carrying *ftz/lac*A or *ftz/lac*B genes, the expression in the mesoderm is rather strong, whereas staining is greatly reduced in the ectoderm, relative to the *ftz/lac*C transformants (Figures 2A–2C, 2H, and 2I). Addition of the upstream element in either orientation to the 5′ side of the zebra element (*ftz/lac*D and *ftz/lac*E genes) restores strong expression in the ectoderm (Figures 2D, 2E, and 2J). When the upstream element is inserted 3′ to the *lacZ* gene (in the *ftz/lac*F gene), expression in the ectoderm is partially restored, but the levels of expression are lower than with the *ftz/lac*D and *ftz/lac*E genes (Figure

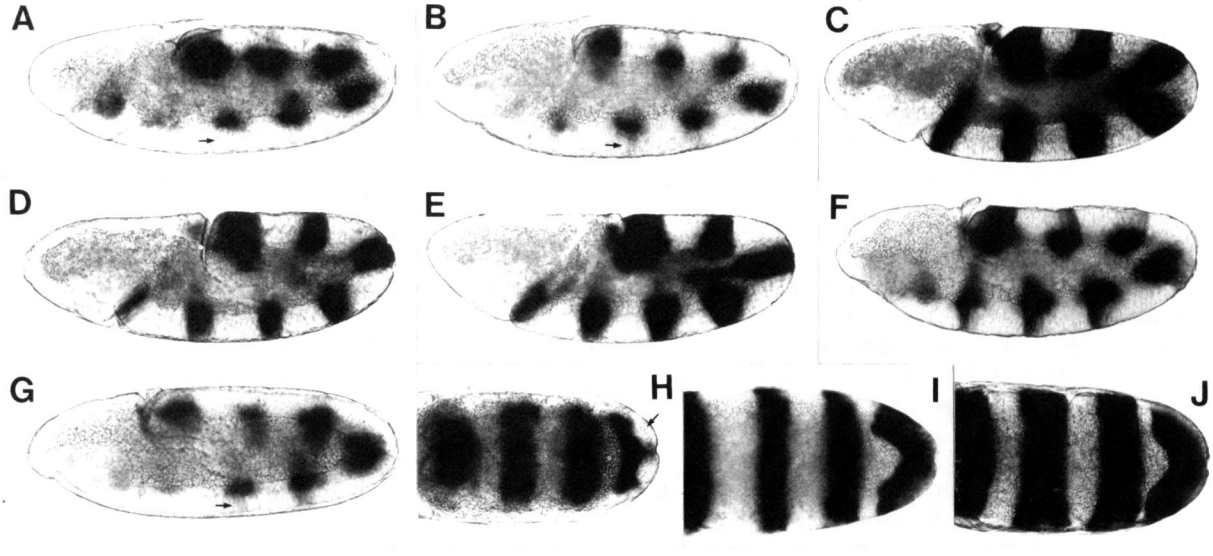

Figure 2. Expression of *ftz/lacZ* Genes in the Stripes

(A–G) Localization of β-galactosidase in transformant embryos carrying *ftz/lac*A through *ftz/lac*G genes, respectively. Whole-mount embryos were stained for β-galactosidase activity at the germ-band extension stage (4.5–6 hr of development). In *ftz/lac*A, *ftz/lac*B, and *ftz/lac*G transformant embryos, the staining appears mainly internally because of weak expression of these genes in the ectoderm (arrows in A, B, and G). (H–J) Ventral views of *ftz/lac*A (H), *ftz/lac*C (I), and *ftz/lac*D (J) transformant embryos in which the fourth stripe is almost parallel to the anterior–posterior axis of the embryo. The *ftz/lac*A gene is expressed mainly in the mesoderm (H), whereas *ftz/lac*C and *ftz/lac*D transformants show strong staining in both mesoderm and ectoderm (I, J). Note the absence of strong staining of the ectoderm in (H) (arrow). Deletion of the upstream element also results in expression of β-galactosidase in the region anterior to the cephalic furrow (Hiromi et al., 1985) (A, B, G, and also F). This expression is somewhat variable and is not discussed in this paper.

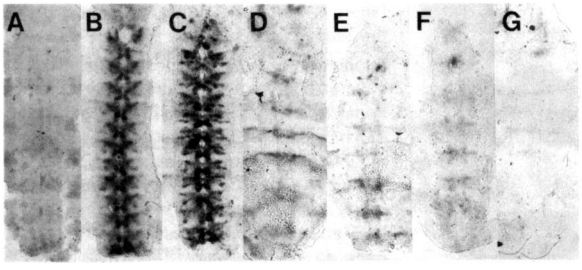

Figure 3. Expression of *ftz/lacZ* Genes in the CNS

Transformant embryos after the germ-band shortening (12–15 hr) were stained for β-galactosidase activity and were subsequently dissected to reveal the ventral nerve cord. (A–G) Embryos carrying *ftz/lac*A through *ftz/lac*G genes, respectively. Significant expression in the CNS is observed for *ftz/lac*B and *ftz/lac*C transformants only, which correlates with the presence of the neurogenic element. Residual staining in double-segment periodicity is due to expression in the stripes at earlier stages.

2F). The *ftz/lac*G gene is identical to the *ftz/lac*A gene except that a spacer sequence similar in length to the upstream element has been used to distance the zebra element from the flanking *rosy*$^+$ gene. Expression of this gene is indistinguishable from that of the *ftz/lac*A gene (Figure 2G), indicating that there is a requirement for a specific sequence to achieve enhanced expression, and not that the upstream element simply serves to separate the zebra element–*lacZ* gene from possible inhibitory effects from the flanking sequences. Since the upstream element acts at a distance and in both orientations, we conclude that it is an enhancer element required for ectodermal expression in the stripes.

Although the *ftz/lac*D and *ftz/lac*E genes are expressed strongly in the stripes, they show little, if any, expression in the CNS (Figures 3D and 3E). Strong expression in the CNS is achieved only with *ftz/lacZ* genes that contain the neurogenic element—namely, *ftz/lac*B and *ftz/lac*C (Figures 3B and 3C). Our results show that the actions of the upstream element and the neurogenic element are independent. Enhancement of expression in the stripes requires the presence of the upstream element but not the neurogenic element (*ftz/lac*D and *ftz/lac*E genes). Conversely, high levels of expression in the CNS require the neurogenic element but not the upstream element (*ftz/lac*B gene).

Mutations Affecting Expression in the Stripes

To identify *trans*-acting factors that interact with *cis*-acting elements, we have adopted a genetic approach to search for genes encoding *trans*-acting factors. Several of the segmentation mutations have been shown to change the distribution of the *ftz* protein and/or transcript (Carroll and Scott, 1986; Howard and Ingham, 1986). If such mutations also alter the expression of *ftz/lacZ* genes, this would indicate that the wild-type product of the gene acts through the sequences included in the fusion gene. The site of action could then be mapped using *ftz/lacZ* genes that have deletions in the control region.

We have used the *ftz/lac*C gene, which contains all three *cis*-acting elements, to search for mutations that alter its expression in the stripes. We find that all of the gap mutations and a subset of the pair-rule mutations affect the pattern of stripes at the blastoderm stage. Patterns of expression of the *ftz/lac*C gene in the gap mutant *hunchback* and in the pair-rule mutants *hairy* and *runt* are shown in Figures 4A, 4C, and 4E. The change in the pattern of β-galactosidase localization resembles the altered distribution of *ftz* transcripts and protein (Carroll and Scott, 1986; Howard and Ingham, 1986). We conclude that the wild-type products of these genes interact, either directly or indirectly, with one or more of the *cis*-acting control elements in the 5′ flanking region of the *ftz* gene.

To identify which control element is the site of interaction, we have analyzed expression of the *ftz/lac*A gene, which contains only the zebra element from the *ftz* gene. In all of the gap mutants and in pair-rule mutants *hairy* and *runt*, the pattern of expression of the *ftz/lac*A gene is altered in a way similar to the change in the expression pattern of the *ftz/lac*C fusion gene (Figures 4B, 4D, 4F, and data not shown). This indicates that the zebra element mediates the effect of the products of these *trans*-acting genes to establish the normal striped pattern.

The *ftz*$^+$ Product Is Required to Maintain Quantitative Levels of Expression in the Stripes

Using *ftz/lacZ* genes, it is possible to ask whether products of the *ftz* gene itself are necessary for normal expression of the *ftz* gene. In *ftz* mutant embryos the *ftz/lac*C gene is expressed in a periodic manner in seven stripes, but the accumulation of β-galactosidase in the ectoderm is greatly reduced compared with expression in the wild-type embryos (Figure 4G). This suggests that the *ftz*$^+$ product is required to maintain quantitative levels of expression of its own gene at the blastoderm stage.

Mutations in the *hairy* gene cause ectopic expression of the *ftz* gene in cells that normally do not express *ftz* (Howard and Ingham, 1986; Carroll and Scott, 1986; see Figure 4F). We investigated whether *ftz*$^+$ function is also required for ectopic expression of the *ftz* gene in *hairy* mutant embryos by examining *ftz/lac*C gene expression in embryos that lack both *hairy*$^+$ and *ftz*$^+$ activity. Although the *ftz/lac*C gene is expressed in a broadened pattern in the mesoderm, as in *hairy* mutant embryos, there is little staining in the ectoderm, which is characteristic of *ftz* mutant embryos (Figure 4I). Thus ectopic expression of the *ftz* gene in *hairy* mutant embryos is also dependent on *ftz*$^+$ activity.

To map the site of interaction between the *ftz*$^+$ product and control elements of the *ftz* gene, expression of the *ftz/lac*A gene in *ftz* mutant embryos was examined. In contrast to the other segmentation mutations affecting the pattern of the stripes, loss of *ftz*$^+$ activity had no effect on the expression of the *ftz/lac*A gene (Figure 4H). This indicates that there is no functional interaction between the *ftz*$^+$ product and the zebra element, and that the site of *ftz*$^+$ action has to be farther upstream. Since the neurogenic element is dispensable for expression in the stripes, the most likely candidate is the upstream element, which is an enhancer element required for expression in the

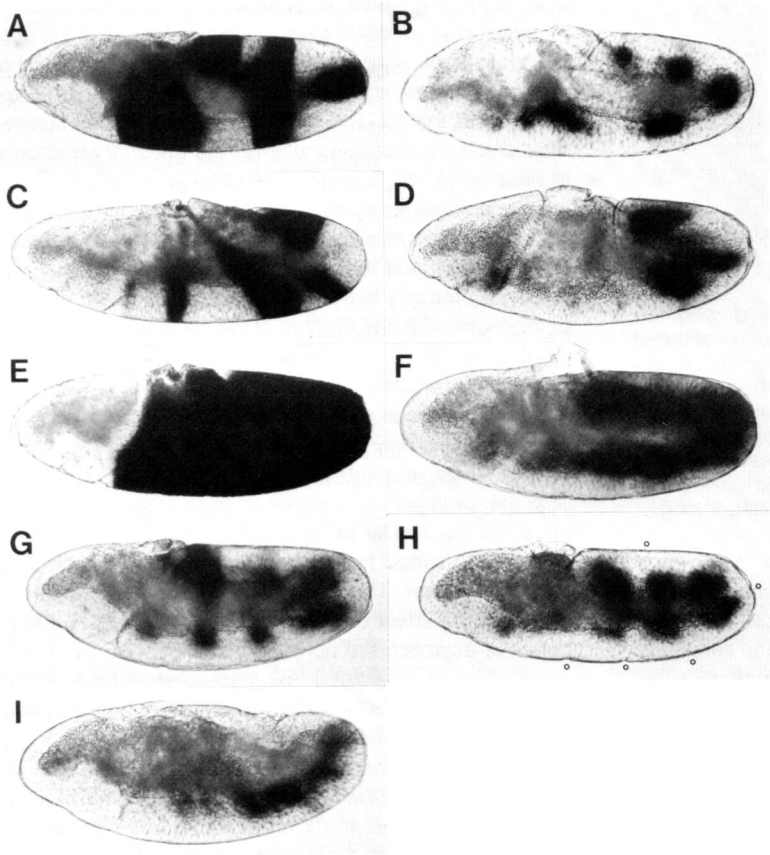

Figure 4. Mutations Affecting Expression in the Stripes

Expression of *ftz/lacZ* genes in segmentation mutant embryos was examined by crossing transformant lines carrying *ftz/lacZ* genes into mutant stocks. Embryos were stained in whole mounts for β-galactosidase activity. The left column (A, C, E, G, and I) shows the expression of the *ftz/lac*C gene, and the right column (B, D, F, and H) shows expression of the *ftz/lac*A gene. (A, B) *hunchback*^{14F21}. (C, D) *runt*^{YE96}. (E, F) *hairy*^{5H07}. (G, H) *ftz*^{9H34}. (I) *hairy*^{5H07} *ftz*^{9H34} double mutant. Compare with expression in wild-type embryos in Figures 2A and 2C. Homozygous mutant embryos were identified by alterations in the staining pattern except in (H), where we relied on morphological criteria (reduction in the number of the epidermal grooves, indicated by open circles).

In *hunchback* mutant embryos (A), the anterior three stripes are replaced by a broad stripe. In addition, the spacing between the last two stripes is narrower than in the wild-type embryos. Other gap mutations (*Krüppel*, *knirps*, and *tailless*) also alter the pattern of *ftz/lac*C gene expression in a way similar to the alteration in distribution of *ftz* transcripts and protein (Ingham et al., 1986; Carroll and Scott, 1986; data not shown). *runt* mutant embryos (C) have only five stripes of different widths. The missing stripes correspond to the second and the sixth stripes of wild-type embryos, because in hypomorphic *runt* alleles (e.g., XK52 and YC28; Gergen and Wieschaus, 1986) these stripes are present but are narrower than their wild-type counterparts (data not shown). In *hairy* mutant embryos (E), each stripe is broadened so that almost all cells in the region that normally gives rise to seven stripes are stained. The expression does not extend anterior to the cephalic furrow, which is the anterior boundary of the first stripe in wild-type embryos. In these mutants and also in other gap mutants, a pattern change similar to that found for expression of the *ftz/lac*C gene is observed for expression of the *ftz/lac*A gene (B, D, F, and data not shown). In *ftz* mutant embryos (G), the *ftz/lac*C gene is expressed only weakly in the ectoderm, similar to the expression of the *ftz/lac*A gene in wild-type embryos (compare with Figure 1A). Expression of the *ftz/lac*A gene is not affected by the *ftz* mutation (H). The *ftz* mutation also affects ectopic expression of the *ftz/lac*C gene in *hairy* mutant embryos (I).

stripes. Indeed, the expression of the *ftz/lac*A gene (which lacks the upstream element) in wild-type embryos is similar to the expression of the *ftz/lac*C gene (which includes the upstream element) in *ftz* mutant embryos (compare Figure 2A with Figure 4H). Thus, deletion of the upstream element has the same effect on expression as deletion of a putative *trans*-acting factor required for the action of the enhancer.

The Upstream Element Can Activate a Foreign Promoter in a *ftz*-Dependent Manner

Our results suggest that there is an interaction between the *ftz*$^+$ product and the upstream element to achieve enhanced expression in the stripes. If the *ftz*-dependent effect of the upstream element is independent of the other control elements of the *ftz* gene, this element should confer *ftz*-dependent enhancer activity when fused to a heterologous promoter. To test this possibility, we constructed a series of P elements containing *lacZ* fusion genes in which the upstream element is fused to a basal promoter of the Drosophila *hsp70* gene (Figure 5). The basic construct is HZ50, which contains the sequence of the *hsp70* promoter up to position −50 relative to the transcription initiation site. This sequence in HZ50 includes the TATA box and 17 nucleotides farther upstream but lacks all of the heat shock elements shown to be required for heat inducibility (reviewed by Pelham, 1985). Transcription from the initiation site of the *hsp70* gene would produce a fusion transcript containing the *hsp70* leader sequence and the sequence encoding the first seven amino acids of the hsp70 protein, the *lacZ* coding sequence, and an *hsp70* trailer sequence with the polyadenylation signal, thus having no contribution from the *ftz* transcript. The upstream element of the *ftz* gene was inserted at various positions and orientations as shown in Figure 5. These *ftz* upstream element–*hsp70* promoter–*lacZ* fusion genes are collectively called UPHZ genes. Transformant lines harboring these fusion genes were established by means of P element–mediated transformation.

We first analyzed expression of the HZ50 gene, which contains no sequence from the *ftz* gene. Embryos from 22 independent transformant lines were stained in whole mounts for β-galactosidase activity. Most of the lines showed little, if any, expression, whereas some lines exhibited various levels of β-galactosidase, the pattern varying from line to line (see Experimental Procedures). As ex-

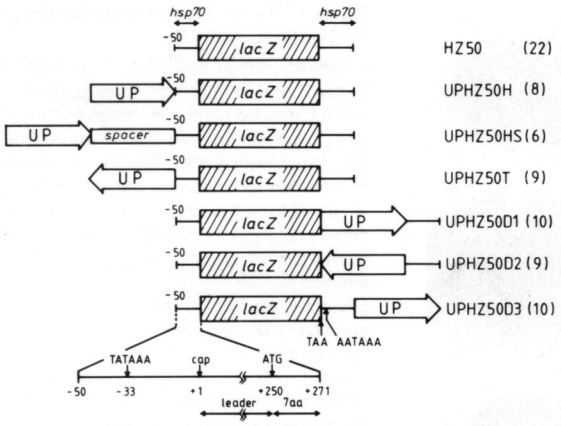

Figure 5. Schematic Diagram of UPHZ Genes

HZ50 is a control gene that has a promoter of the *hsp70* gene, containing sequences up to −50 from the cap site, fused in frame to the *lacZ* gene. At the 3′ side, trailer sequence and the polyadenylation signal are also provided by the *hsp70* gene. UPHZ genes have the *ftz* upstream element (indicated by an open arrow labeled UP) inserted at various positions and orientations. All fusion genes were inserted into the Carnegie 20 vector or its derivative such that the *rosy*$^+$ gene lies at the 5′ side. The numbers in parentheses indicate the number of independent transformant lines examined for each construct.

pected, expression was not affected by heat shock (data not shown).

If fusion of the *ftz* upstream element to a heterologous promoter confers *ftz*-dependent enhancer activity, we expected UPHZ genes to be active (or to show enhanced expression) in cells that contain the *ftz*$^+$ product, and inactive (or to show no enhancement of expression) in cells that do not express the *ftz* gene. The expression pattern of the UPHZ50H gene, in which the upstream element is fused directly to the *hsp70* promoter, is shown in Figure 6A. β-Galactosidase activity is localized in cells forming seven stripes, with little staining in the region between the stripes. The level of β-galactosidase varies considerably among the cells in the stripes, giving rise to a patchy appearance (Figure 6A; see also Figure 6C). These stripes appear to be in phase with the stripes of cells that express the *ftz* gene, since after germ-band retraction each stripe contributes to the posterior part of even-numbered abdominal segments and the anterior part of odd-numbered segments (Figure 6B). The UPHZ50HS gene, in which the upstream element is separated from the *hsp70* promoter by a spacer sequence of 2.5 kb, is also expressed in a similar pattern, although the expression was in general weaker than with the UPHZ50H gene (Figure 6C). Thus the *ftz* upstream element can activate a foreign promoter in a striped pattern at variable distances, consistent with our hypothesis that it is a *ftz*-dependent enhancer.

The enhancer effect of the upstream element could also be demonstrated when it was placed 3′ to the transcription unit. In the UPHZ50D1 and UPHZ50D2 genes, the upstream element is inserted between the translational stop codon and the polyadenylation signal. Transformant embryos carrying these fusion genes exhibited weak staining in a striped pattern (Figures 6D and 6E). Since these constructs alter the structure of the transcript, we cannot tell whether this weak staining is due to inability of the upstream element to function efficiently at the 3′ position, or to decreased stability of the transcript. When the upstream element was inserted about 0.6 kb downstream of the polyadenylation signal (in the UPHZ50D3 gene), we could not detect a striped pattern of expression in any of

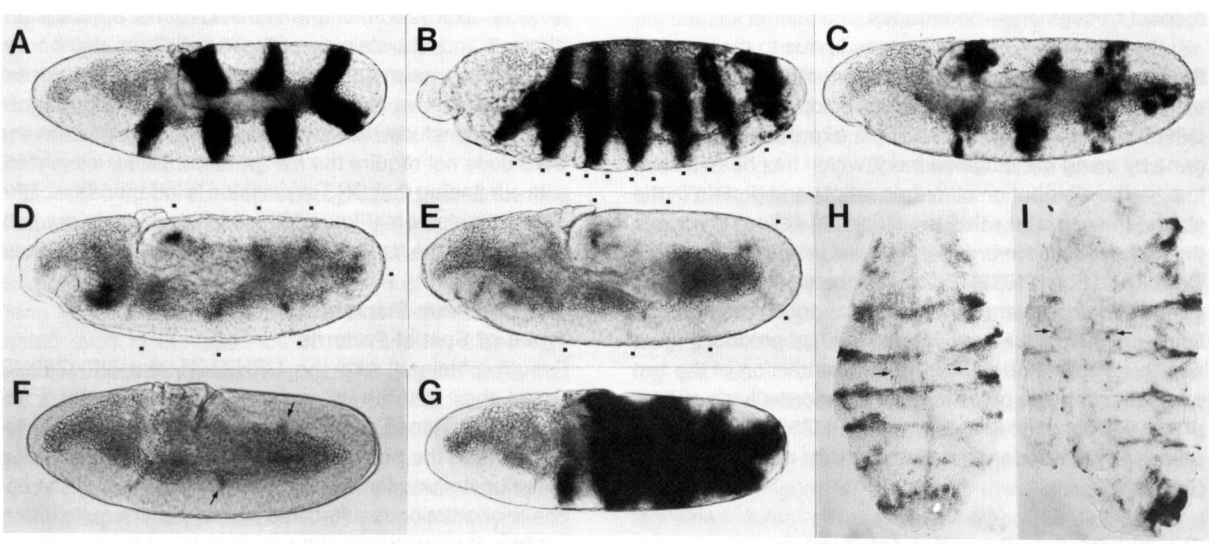

Figure 6. Expression of the UPHZ Genes

(A–E) Localization of β-galactosidase in transformant embryos carrying UPHZ genes in the wild-type background. (A, B) Expression of the UPHZ50H gene at the germ-band extension stage (A) or after germ-band shortening (B). Segmental boundaries are indicated by dots. (C) Expression of the UPHZ50HS gene. (D) Expression of the UPHZ50D1 gene. (E) Expression of the UPHZ50D2 gene. Positions of the weakly stained stripes are shown by dots in (D) and (E). (F) Expression of the UPHZ50H gene in a *ftz*^{9H34} mutant embryo; arrows point to weakly stained cells. (G) Expression of the UPHZ50H gene in a *hairy*^{5H07} mutant embryo. (H, I) Dissected nervous system from UPHZ50H (H) and UPHZ50HS (I) transformant embryos (12–15 hr) in a wild-type background; lateral boundaries of the CNS are indicated by arrows.

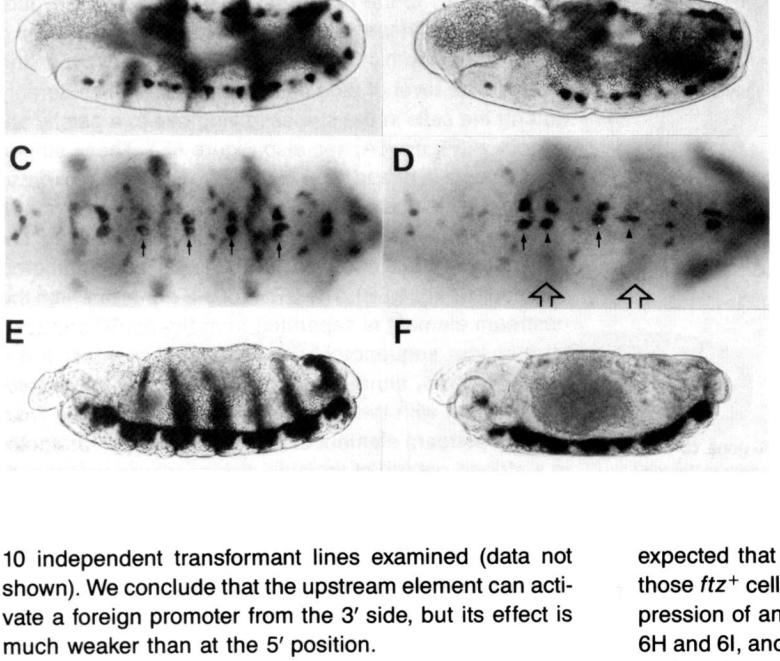

Figure 7. The *ftz*$^+$ Product Is Not Required for *ftz* Expression in the CNS

Expression of the *ftz*/*lac*C gene in wild-type (A, C, and E) and *ftz* mutant embryos (B, D, and F). (A–D) Extended germ band stage (~7 hr). (C) and (D) are ventral views of embryos in (A) and (B), respectively. Arrows indicate MP2s, a pair of identified *ftz*$^+$ neuronal precursors (Doe et al., unpublished), or their progeny. Arrowheads show ectopically formed MP2s residing in the stripes (open arrows). Detailed description and interpretation of the pattern alteration will be presented elsewhere. (E, F) Expression after germ-band shortening (~10 hr). Although the accumulation of β-galactosidase in the stripes is much reduced in *ftz* mutant embryos, cells in the CNS stain equally strongly, showing that *ftz*$^+$ neurons survive in the absence of *ftz*$^+$ activity.

10 independent transformant lines examined (data not shown). We conclude that the upstream element can activate a foreign promoter from the 3′ side, but its effect is much weaker than at the 5′ position.

A critical experiment to test our hypothesis was to examine the expression of UPHZ genes in the absence of the *ftz*$^+$ product. Figure 6F shows expression of the UPHZ50H gene in a *ftz* mutant embryo. The level of β-galactosidase in *ftz* mutants is drastically reduced compared to that in wild-type embryos, but is not completely eliminated. *ftz*-independent activity can be detected in a few cells, which are located roughly at the positions of *ftz*$^+$ stripes in wild-type embryos. However, because of weak staining and the small number of stained cells, it was not possible to determine the exact phase of expression with respect to segmental boundaries.

If the expression of UPHZ genes is due to response of the upstream element to the *ftz* product, ectopic expression of the *ftz* gene may lead to ectopic expression of UPHZ genes. We induced ectopic expression of the *ftz* gene by using the mutation *hairy*, which has been shown to alter the distribution of *ftz* transcripts and protein in the stripes in such a way that the stripes of cells that contain the *ftz*$^+$ product are broader (Howard and Ingham, 1986; Carroll and Scott, 1986). Ectopic expression of the *ftz*/*lacZ* genes in *hairy* mutant embryos is dependent on both the upstream element (Figure 4F) and the *ftz*$^+$ product (Figure 4I). This strongly suggests that the interaction of the two components takes place in cells that normally do not express the *ftz* gene. Indeed, the UPHZ50H gene is expressed in a broadened pattern in *hairy* mutant embryos (Figure 6G).

The *ftz*$^+$ Product Does Not Act through the Upstream Element in the CNS

Although these results are consistent with the idea that the upstream element responds to the distribution of the *ftz*$^+$ product, we found that the same does not hold true for *ftz* expression in the CNS. Since certain neuronal precursors and progeny in the CNS contain *ftz* protein, we expected that UPHZ genes would also be expressed in those *ftz*$^+$ cells in the CNS. However, we detected no expression of any of the UPHZ genes in the CNS (Figures 6H and 6I, and data not shown), indicating that *ftz* cannot activate itself through the upstream element in the CNS.

We therefore asked whether or not there is a requirement for *ftz*$^+$ activity for *ftz* expression in the CNS. Figure 7 shows the expression of the *ftz*/*lac*C gene in wild-type and *ftz* mutant embryos during neurogenesis. Even when *ftz*$^+$ activity is absent, we find that certain neuronal precursors and progeny express the *ftz*/*lac*C gene. The arrangement of these *ftz*$^+$ cells is altered, presumably because of the segmentation defect in the organization of the CNS (Wakimoto et al., 1984; Doe and Goodman, 1985). In spite of the pattern alterations, the quantitative level of expression of the *ftz*/*lac*C gene appears unaffected. Indeed, staining of these cells can also be detected after germ-band shortening, indicating that the cells can survive in the absence of *ftz*$^+$ activity (Figure 7F). We conclude that expression of the *ftz* gene in the CNS does not require the *ftz*$^+$ product. This is consistent with our finding that CNS expression is independent of the upstream element (Figure 3B), which appears to mediate the effect of the *ftz*$^+$ product for expression in the stripes.

The Upstream Element Can Confer Other Types of Spatial Patterns

Results obtained with the UPHZ50H and UPHZ50HS genes show that the upstream element can confer a *ftz*-dependent striped pattern of expression at variable distances from the promoter. However, we found that fusion of the upstream element to the *hsp70* promoter in the opposite orientation resulted in a spatial pattern quite different from *ftz*-like stripes. Figures 8A to 8C show the expression of the UPHZ50T gene. At the cellular blastoderm stage, β-galactosidase is mainly localized to cells that form a single stripe of a width of about three cells at approximately 55% egg length (0% is the posterior pole of the embryo) (Figure 8A). This stripe does not form a complete ring around the embryo; a region at about 25% in

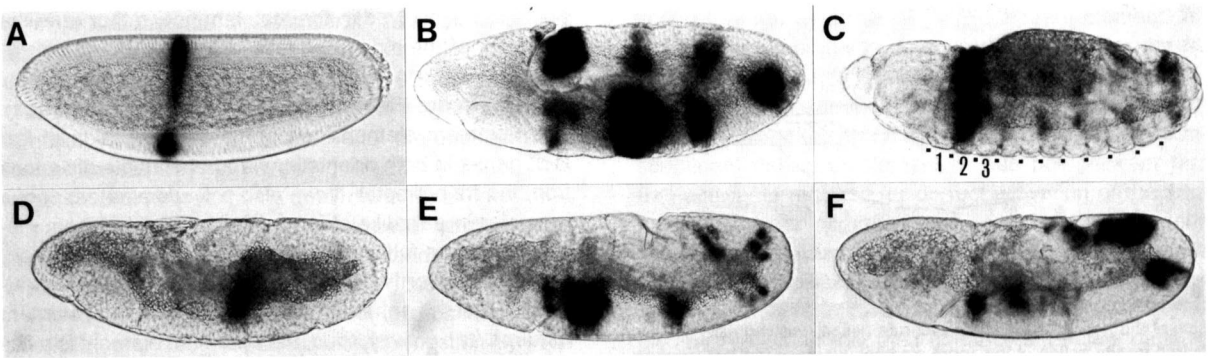

Figure 8. Expression of the UPHZ50T Gene

(A–C) Localization of β-galactosidase in transformant embryos carrying the UPHZ50T gene in a wild-type background. (A) Cellular blastoderm stage (3.5 hr). (B) Extended germ band stage (6 hr). Note that the anterior boundary of the second stripe coincides with the parasegmental groove. The stained cells in the weakly stained stripes are located around and dorsal to the tracheal pits. (C) Expression after germ-band shortening (10 hr). Segmental boundaries are indicated by dots. Three thoracic segments are numbered. (D–F) Expression of the UPHZ50T gene in ftz^{9H34} (D), $hunchback^{14F21}$ (E), and $Krüppel^{2}$ mutant embryos.

the dorsoventral dimension (0% is the ventral midline) stained only weakly. Cells at other positions also express the UPHZ50T gene at lower levels, giving rise to a pattern of seven stripes. This pattern is more easily seen during germ-band elongation, when the level of β-galactosidase increases (Figure 8B). Not all of the stripes are stained equally: the second stripe stains the strongest, followed by the seventh stripe, and the remaining five stripes show rather weak staining. Localization of β-galactosidase with respect to morphological landmarks of segmentation reveals that the most strongly stained (second) stripe corresponds to the posterior part of the first thoracic segment and the anterior part of the second thoracic segment, or parasegment 4 (PS4; Martinez-Arias and Lawrence, 1984), which is in phase with the second stripe of the *ftz* gene (Figures 8B and 8C). The second stripe of UPHZ50T transformant embryos is stained much stronger than the stripes of UPHZ50H transformants, indicating that the pattern of UPHZ50T gene expression is due, at least partly, to specific activation in PS4.

Since the amount of *ftz* product present in cells forming each stripe seems to be rather similar (Hafen et al., 1984a; Carroll and Scott, 1985), the ftz^{+} product alone cannot account for the PS4-specific activation of the UPHZ50T gene. Indeed, a similar pattern of expression can be detected even in the absence of ftz^{+} activity, albeit with greatly reduced intensity (Figure 8D). This indicates that the additional factor (or factors) responsible for the PS4-specific activation can by itself activate the UPHZ50T gene.

To search for genes encoding such factors, we have examined expression of the UPHZ50T gene in embryos carrying mutations in homeotic genes and segmentation genes known to be required in PS4. Among the homeotic genes, the *Antp* gene is expressed and required in PS4 from the blastoderm stage on (Schneuwly and Gehring, 1985; Levine et al., 1983; Martinez-Arias, 1986). Since the pattern of expression of the UPHZ50T gene at the blastoderm stage is similar to expression from the second promoter of the *Antp* gene (Ingham and Martinez-Arias, 1986), we wondered whether the $Antp^{+}$ product might be responsible for the PS4-specific activation of the UPHZ50T gene. However, we found no difference in expression of the UPHZ50T gene between wild-type and $Antp^{-}$ mutant embryos (data not shown).

Two of the gap mutations, *hunchback* and *Krüppel*, affect the cuticle structures of PS4 (Lehmann and Nüsslein-Volhard, 1987; Wieschaus et al., 1984), and the *Krüppel* gene is expressed in its primordium (Knipple et al., 1985; Jäckle et al., 1986). Since both of these mutations alter the distribution of the *ftz* gene product (Carroll and Scott, 1986; Ingham et al., 1986), we expected that the expression of UPHZ genes would be altered accordingly. In *hunchback* mutant embryos, the anteriormost three stripes of ftz^{+} cells are replaced by a broad stripe of cells expressing *ftz*. This broad stripe is sometimes split into a narrow stripe and a broader stripe, which are separated by a narrow stripe of ftz^{-} cells (Carroll and Scott, 1986; see also Figure 4A). The anterior three stripes of the UPHZ50T gene also show a similar change in pattern, but the broad band is clearly stained more strongly then other stripes, suggesting that the factor that discriminates the second stripe from others is still present in *hunchback* mutant embryos (Figure 8E). In *Krüppel* mutant embryos, the *ftz* product is localized in four stripes with variable spacing, and also in a weaker and narrow stripe between the first and the second stripe (Ingham et al., 1986; Carroll and Scott, 1986). Expression of the UPHZ50T gene in *Krüppel* mutant embryos mimics the distribution of the *ftz* product (Figure 8F). However, there is no stripe that is stained more strongly than others, indicating that $Krüppel^{+}$ activity is required for PS4-specific activation of the UPHZ50T gene.

Discussion

Spatially restricted patterns of expression of the *ftz* gene are likely to be mediated by specific interactions between

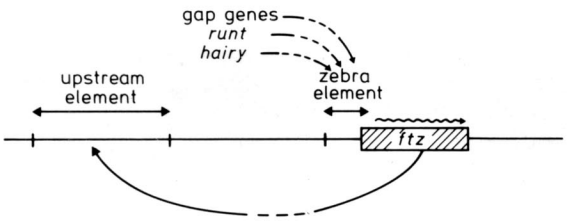

Figure 9. A Model for Regulation of *ftz* Expression in the Stripes

Normal expression of the *ftz* gene in the stripes requires two *cis*-acting control elements, the zebra element and the upstream element. Products of the gap genes and the pair-rule genes *runt* and *hairy* are required for the action of the zebra element, whereas the ftz^+ product acts through the upstream element. This model is not intended to show all factors interacting with the control elements, nor does it apply to *ftz* expression in the CNS. For example, interaction of the ftz^+ product and the upstream element does not take place in the CNS.

cis-acting control elements of the *ftz* gene and *trans*-acting factors. We have employed transformant flies carrying *ftz/lacZ* fusion genes containing all or a subset of the *ftz* control elements to identify such interactions. Normal expression in the stripes requires two control elements, the zebra element and the upstream element. We have shown that the products of the gap genes and of a subset of the pair-rule genes act through the zebra element, whereas the action of the upstream element requires the product of the ftz^+ gene itself (Figure 9).

Our strategy to identify interactions between *cis*-acting control elements and *trans*-acting gene products has several limitations. First, when a mutation changes the pattern of expression of a *ftz/lacZ* gene containing a particular control element, it shows that the wild-type gene product is required for the correct function of the control element, but does not provide any information about the nature of the interaction. The interaction could be direct binding of the gene products to regulatory sites within the control element, or more indirect effects via intermediate steps. Second, our assignment of the zebra element as the target sequence for the products of the gap genes and the pair-rule genes *hairy* and *runt* does not rule out the possibility that these gene products also interact with other control elements. Our data suggest that factors other than the ftz^+ product act through the upstream element, and one of them may be a gap gene product (see below).

A Positive Feedback Loop Is Involved in Expression in the Stripes

In *ftz* mutant embryos, the *ftz/lac*C gene is expressed only weakly, although a spatially repeated stripe pattern is still present. Thus, products of the ftz^+ gene appear to act on the *ftz* gene to achieve enhanced expression, implying a positive autoregulatory feedback mechanism. Mathematical models that involve autocatalytic feedback mechanisms have been proposed to explain spatially repeated gene activation (Meinhardt and Gierer, 1980). We note, however, that the striped pattern per se does not require the feedback loop, because it can be established in the absence of either the upstream element or the ftz^+ product, or both. A positive feedback mechanism therefore appears to be involved in the maintenance, rather than the initiation, of the striped pattern.

The Upstream Element Is a *ftz*-Dependent Enhancer

The upstream element can enhance expression of *ftz/lacZ* genes in both orientations and at variable distances from the *ftz* promoter. It can also activate a heterologous promoter in a *ftz*-like striped pattern. The upstream element therefore fulfills the criteria of an enhancer element (reviewed by Serfling et al., 1985). Our genetic analysis indicates that the enhancer action of the upstream element requires ftz^+ activity; thus, the upstream element is a *ftz*-dependent enhancer.

Unlike expression in the stripes, expression of the *ftz* gene in the CNS requires neither the upstream element nor the ftz^+ product. Conversely, the upstream element does not exhibit *ftz*-dependent enhancer activity in the CNS. The simplest interpretation of these results is that the enhancer action of the upstream element depends not only on the ftz^+ product but also on another factor (or factors) not present in the ftz^+ cells in the CNS. Such additional factors may act as cofactors by interacting with the upstream element, may modify the *ftz* product to enable it to function as a *trans*-acting factor, or may be involved in mediating the enhancer effect to the promoter.

The upstream element has been shown to contain an attachment site to the nuclear scaffold (or nuclear matrix) (Gasser and Laemmli, 1986). Factors involved in scaffold attachment may influence transcription efficiency (Mirkovitch et al., 1984; Cockerill and Garrard, 1986) and are thus candidates for the additional factor required for the enhancer action.

The Upstream Element May Also Respond to Stripe-Determining Signals

The fact that the upstream element alone can give rise to a striped pattern raises a possibility that the upstream element is a second zebra element: i.e., this element confers the striped pattern by directly responding to the cues produced by the *trans*-acting genes such as the gap genes and the pair-rule genes *hairy* and *runt*. Indeed, mutations in these genes do alter the striped expression pattern of the UPHZ50H gene, which contains only the upstream element from the *ftz* gene (Figure 6G and data not shown). Given the *ftz*-dependent enhancer effect of the upstream element, this result can also be interpreted as a pattern change caused indirectly by the altered distribution of the ftz^+ product. However, the apparently spatially restricted pattern of *ftz*-independent expression of the UPHZ50H gene (Figure 6F) suggests that the upstream element may in fact respond to some, if not all, of the stripe-determining signals whose effects are enhanced by the action of the ftz^+ product.

The pattern of expression of the UPHZ50T gene also supports the idea that the upstream element and the zebra element respond to an overlapping set of signals. We have shown that PS4-specific activation of the UPHZ50T gene requires *Krüppel*$^+$ activity (Figure 8F). The broadened strong stripe in *hunchback* mutant embryos can be explained by anterior extension of the *Krüppel* do-

main in the absence of *hunchback*$^+$ function (Jäckle et al., 1986). Since PS4-specific expression also occurs in the absence of the *ftz*$^+$ product (Figure 8D), the observed effect of *Krüppel* on the UPHZ50T gene may be due to interaction of the *Krüppel*$^+$ product with the upstream element in a pathway not involving the *ftz*$^+$ product. This suggests that *Krüppel* has a dual role in *ftz* expression—first in establishment of the pattern of seven stripes by acting through the zebra element, and second in PS4-specific activation, which is mediated by the upstream element.

The difference in the pattern of expression of the UPHZ50T and *ftz*$^+$ genes leads us to question whether the interaction between the *Krüppel* product and the upstream element is relevant for expression of the chromosomal *ftz* gene. If such an interaction is indeed required for the expression of the *ftz* gene in the second stripe, it is likely that the other six stripes would also require other factors for their activation. Such factors may be responsible for weak *ftz*-independent expression of the UPHZ50H gene.

How the upstream element confers different patterns of expression in the UPHZ50H and UPHZ50T genes is an open question. It has been proposed that the action of enhancer elements is critically dependent on protein–protein interactions between factors bound to enhancers and other transcriptional control elements (Takahashi et al., 1986). If different factors interact with the upstream element in different stripes, the physical configuration of the upstream element with respect to the promoter may have hampered the efficient action of some of the factors by steric hindrance. Lack of PS4-specific activation in the *ftz*/*lac*E gene (Figures 1 and 2E) suggests that the critical information is not the orientation of the upstream element. Preliminary evidence indicates that a more normal *ftz*-like pattern can be obtained when the distance between the *hsp70* promoter and the inverted upstream element is increased (unpublished observation).

Mode of Action of Homeodomain-Containing Proteins

Several lines of genetic evidence indicate that genes containing a homeobox are involved in regulation of gene activity (Garcia-Bellido, 1977; Lewis, 1978; Hafen et al., 1984b; Struhl and White, 1985; Howard and Ingham, 1986; Carroll and Scott, 1986; Harding et al., 1986; Macdonald et al., 1986). The structural homology of a part of the homeodomain to the DNA-binding domain of prokaryotic and yeast regulatory proteins has led to the suggestion that homeobox-containing genes encode regulatory proteins that effect gene expression via sequence-specific DNA binding (Shepherd et al., 1984; Laughon and Scott, 1984). Nuclear localization of homeodomain-containing proteins (see, for example, White and Wilcox, 1984; Carroll and Scott, 1985) and sequence-specific DNA binding of an *engrailed* homeodomain fusion protein in vitro (Desplan et al., 1985) support this view. However, little is known about how such proteins influence the transcriptional activity of their target genes.

Our results shed some light on the mode of action of a homeodomain-containing protein. We have shown that the *ftz*$^+$ product regulates the activity of its own gene by acting through its enhancer, the upstream element. Although our experiments do not indicate whether the *ftz* protein directly binds to the upstream element, they provide functional evidence that a role of the active *ftz*$^+$ product (most likely the *ftz* protein) is to activate a transcriptional enhancer element. It has been shown that *ftz*$^+$ activity is required for correct activation of the segmentation gene *engrailed* and some of the homeotic selector genes (Howard and Ingham, 1986; Ingham and Martinez-Arias, 1986; Duncan, 1986). We suggest that the *ftz* protein may regulate expression of these target genes also by interacting with their enhancers. Since a putative interaction with the enhancer elements would likely be mediated via the homeodomain, we can speculate that other homeobox-containing gene products have similar functional specificity in their target sequences, and act on enhancer elements. This model does not imply that all homeodomain-containing proteins act by activating enhancers; they may also repress transcription by competing for activating factors for the binding sites in the enhancer element. These hypotheses are testable by identifying enhancer elements through construction of *lacZ* fusion genes such as UPHZ genes and testing whether their expression is dependent on the activity of the relevant gene.

The Role of the *ftz* Gene in Expression of the Homeotic Genes

The expression patterns of the UPHZ genes provide some clues to the problem of regulation of the homeotic selector genes such as *Ultrabithorax* (*Ubx*), *Antp*, and *Sex comb reduced* (*Scr*). These genes are expressed in a spatially restricted manner from the blastoderm stage on. The highest levels of expression usually occur in the region that gives rise to segments affected the most by mutation in the locus (PS6 for *Ubx*, PS4 for *Antp*, PS2 for *Scr*) (Akam and Martinez-Arias, 1985; Levine et al., 1983; Kuroiwa et al., 1985). In addition to these peaks of activity, these genes are expressed in transient striped patterns of double-segment periodicity, a pattern termed pair-rule modulation (Akam, 1985; Ingham and Martinez-Arias, 1986). This has been interpreted to mean that pair-rule genes are involved in providing the correct frame of expression (Akam, 1985). Indeed, it has been shown recently that the spatial pattern of expression of the homeotic selector genes is critically dependent on *ftz*$^+$ activity (Ingham and Martinez-Arias, 1986).

We offer a model to explain how the *ftz*$^+$ product is involved in the expression of the homeotic genes. We propose that the expression of homeotic genes *Antp* and *Scr* at the blastoderm stage is mediated by a sequence element that has homology to the *ftz* upstream element and has the properties of a *ftz*-dependent enhancer. Since the fusion of the upstream element to a promoter with no spatial specificity can result in *ftz*-dependent activation of the promoter in a pattern similar to *Antp* or *Scr* expression, this model accounts not only for *ftz* dependence but also for the spatial specificity of both genes.

Genes containing the homeobox are believed to have evolved by duplications of an ancestral gene. If there is a

functional interaction between the homeodomain and the upstream element, it is conceivable that such duplication events have conserved not only the homeobox but also some regulatory sequence required for its expression. This would create a regulatory interaction between the duplicated genes, such as those found among segmentation/homeotic genes. Alternatively, the upstream element of the chromosomal *ftz* gene itself may exert an enhancer effect in *cis* to the flanking homeotic genes *Antp* and *Scr*. It should be possible to distinguish these possibilities by providing ftz^+ function in *trans* using *ftz* transformant lines (Hiromi et al., 1985) and asking whether it can activate the *Antp* and *Scr* genes in the physical absence of the *ftz* upstream element.

Experimental Procedures

Drosophila Mutant Strains

The following mutant alleles of segmentation genes (Nüsslein-Volhard and Wieschaus, 1980) were used in this study: *hunchback*, hb^{14F21}, a class I allele (Lehmann and Nüsslein-Volhard, 1987); *Krüppel*, Kr^2 (Wieschaus et al., 1984); *hairy*, h^{5H07} and h^{IL79K}, class IV alleles (Ingham et al., 1985b); *runt*, run^{YE96} and Df(1)$runt^{III2}$ (Gergen and Wieschaus, 1986); *fushi tarazu*, ftz^{9H34}, ftz^{w20}, and Df(3R)*4scb* (Jürgens et al., 1984; Wakimoto et al., 1984). All of the alleles are either amorphic or strong hypomorphic alleles. In particular, the two *ftz* alleles used (ftz^{9H34} and ftz^{w20}) have been shown to produce no protein product detectable by antibody directed against the *ftz* protein (Carroll and Scott, 1985; S. Carroll and C. Doe, personal communication). Df(3R)*4scb* deletes the entire *ftz* gene as well as parts of the flanking homeotic genes *Antp* and *Scr* (Kuroiwa et al., 1985). ftz^{9H34} was used in most experiments. A chromosome carrying both ftz^{9H34} and $hairy^{5H07}$ was created by recombination. Embryos lacking $Antp^+$ function were generated by making *trans* heterozygotes of the deficiencies Df(3R)*4scb* and Df(3R)Ns^{+RC7}. The latter deficiency deletes most of the *Antp* gene but leaves *ftz* intact (Kuroiwa et al., 1985).

Construction of *lacZ* Fusion Genes

We have constructed two series of P-element vectors containing *lacZ* fusion genes. The first series consists of those that contain "transcriptional fusion" genes, in which the 5′ flanking sequence and leader sequence of the *ftz* gene are fused, after the first methionine codon, to the Escherichia coli *lacZ* gene (Figure 1). These fusion genes are collectively called *ftz/lacZ* genes. The second series of P-element vectors contain "enhancer fusion" genes, in which the *ftz* upstream element is fused to the *hsp70/lacZ* transcriptional fusion gene (Figure 5). These fusion genes are called UPHZ genes. All P-element constructs were made in the transformation vectors Carnegie 20 (Rubin and Spradling, 1983) and cp20.1 (Simon et al., 1985). In all cases, the $rosy^+$ marker gene is located at the 5′ side of the fusion gene. For simplicity, we refer to both the P elements containing the fusion genes and the plasmid constructs including the P-element vectors by the names of the fusion genes.

Three *ftz/lacZ* constructs with simple deletions of the 5′ flanking sequences (*ftz/lac*A to *ftz/lac*C) have been described previously (Hiromi et al., 1985). Four new *ftz/lacZ* constructs (*ftz/lac*D to *ftz/lac*G) can be considered derivatives of the *ftz/lac*A construct, which has the *ftz* zebra element fused to the *lacZ* gene. They were constructed by cloning the *ftz* upstream element into either the 5′ (*ftz/lac*D and *ftz/lac*E) or 3′ (*ftz/lac*F) side of the *ftz/lac*A construct. The *ftz/lac*G construct has a DNA fragment with a length similar to that of the upstream element (2.5 kb XbaI–SacI fragment from the *Antp* eighth exon; Schneuwly et al., 1986) at the 5′ side of the *ftz/lac*A gene, thus separating the zebra element from the $rosy^+$ gene by 2.5 kb.

Six UPHZ constructs and a control construct, HZ50, were made as follows. UPHZ50T and UPHZ50D3 were made by exchanging the zebra element–*lacZ* portion of the *ftz/lac*E and *ftz/lac*F constructs with a *hsp70/lacZ* fusion gene that has sequences of the *hsp70* gene to −50 from the cap site (derived from plasmid 31Nru; Amin et al., 1985; R. Voellmy, personal communication). HZ50 was made by deleting the *ftz* upstream element from UPHZ50D3. The UPHZ50D1 and UPHZ50D2 genes were made by inserting the upstream element at the unique SalI site located between the stop codon and the polyadenylation signal of HZ50. To construct UPHZ50H, the upstream element in UPHZ50T was first replaced with a polylinker (XbaI–BamHI–NotI–KpnI, made by inserting a NotI linker [New England Biolabs] into the SmaI site of pUC18), resulting in HZ50PL. This plasmid has three unique cloning sites (XbaI, NotI, and KpnI) in front of the *hsp70* promoter. UPHZ50H was made by inserting the upstream element in normal orientation into the XbaI site of HZ50PL. UPHZ50HS has the same DNA fragment from the *Antp* gene as was used in the *ftz/lac*G gene, inserted at the NotI site of UPHZ50H located between the upstream element and the *hsp70* promoter. A detailed protocol of the plasmid construction is available on request.

Germ-Line Transformation and Analysis of Expression of *lacZ* Fusion Genes

P element–mediated germ-line transformation and establishment of balanced transformant stocks were carried out as previously described (Rubin and Spradling, 1982; Karess and Rubin, 1984; Hiromi et al., 1985), using pπ25.7wc as the helper P element and $rosy^{506}$ (ORM) as the recipient strain. All but two of the *ftz/lacZ* gene transformant lines have single-copy insertions of P elements as determined by Southern blot hybridization with a *rosy* gene probe (data not shown). One line each of *ftz/lac*B and *ftz/lac*G elements had insertion of two P elements on the same chromosome. The copy number of P elements in UPHZ gene transformants was not determined.

Transformant embryos carrying *ftz/lacZ* fusion genes were fixed and processed for staining as previously described (Hiromi et al., 1985). To visualize the expression of UPHZ genes, a staining solution with lower pH (Simon et al., 1985) was used, and staining was overnight at 37°C. This method had higher sensitivity but also resulted in staining of endogenous β-galactosidase activity in late embryos.

To assess the expression pattern of each fusion gene, 5–10 independent transformant lines per P-element construct were examined at various stages during the establishment of the balanced stocks for β-galactosidase activity, using staining of whole-mount embryos. We found that quantitative levels of β-galactosidase can vary somewhat among different lines of a given construct (see also Hiromi et al., 1985). However, with the exceptions described below, no significant difference in the spatial and temporal patterns of β-galactosidase activity was observed. We consider the β-galactosidase staining pattern common to most, if not all, of the independent transformant lines to reflect the pattern of expression conferred by the sequences within the P element, and have described that pattern as the pattern of expression of the fusion gene. We cannot rule out the possibility that sequences in the Carnegie 20 transformation vector have some influence on the expression.

For *ftz/lacZ* gene transformants, we found that a fraction (~10%) of the transformant lines exhibit staining in the CNS in a pattern that was not seen in other lines of the same construct. Such expression also occurred with fusion genes that lack the neurogenic element. In two such cases we have mobilized the P element to different chromosomal sites by injection of pπ25.7wc DNA into transformant embryos (Levis et al., 1985). Lines that lost the original integrated element and have the same P element inserted at new chromosomal sites did not show the abnormal pattern, indicating that the exceptional pattern of expression is not due to rearrangements within the P element but is due to chromosomal position effects exerted by the sequences near the integration point.

Some line-dependent expression was also observed for the HZ50 gene and UPHZ genes. Such expression usually occurred during late embryogenesis; however, some lines showed weak staining along the ventral midline and/or mesodermal anlage at the germ-band extension stage. The striped expression pattern described for the UPHZ50H, UPHZ50HS, and UPHZ50T genes was observed for all transformant lines of each construct. Expression of the UPHZ50D1 and UPHZ50D2 genes was rather weak, and the striped pattern could be detected only in 7 out of 10 UPHZ50D1 lines and 5 out of 9 UPHZ50D2 lines.

To analyze the expression of *lacZ* fusion genes in mutant embryos, transformant lines having a P-element insertion on a chromosome different from the chromosome on which the mutation resides were

used to cross the *lacZ* fusion genes into the mutant strain. Since products of the *lacZ* fusion genes appear to be much more stable than *ftz* transcripts and protein (compare Hiromi et al., 1985 with Hafen et al., 1984a; Carroll and Scott, 1985; also see Edgar et al., 1986), it is possible to detect β-galactosidase when morphological landmarks of segmentation, such as epidermal grooves, form. In wild-type embryos at stage 10 (Campos-Ortega and Hartenstein, 1986), the anterior boundaries of each stripe are sharp and coincide with the epidermal grooves. The posterior boundaries are rather diffuse and do not reach the next groove. Thus the stripes are narrower than one segment wide at this stage. Homozygous mutant embryos were identified by characteristic morphological changes, and if such embryos had altered patterns of β-galactosidase localization, embryos having the same pattern change were traced back to earlier stages.

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