# Genetic Characterization of the Homeodomain-Independent Activity of the Drosophila *fushi tarazu* Gene Product

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ABSTRACT

The gene product of *fushi tarazu* (FTZ) has a homeodomain (HD)-independent activity. Ectopic expression of a FTZ protein that lacks half the HD in embryos results in the anti-ftz phenotype. We have characterized this FTZ HD-independent activity further. Ectopic expression of the HD-independent FTZ activity, in the absence of FTZ activity expressed from the endogenous *ftz* gene, was sufficient to result in the anti-ftz phenotype. Since the anti-ftz phenotype is a first instar larvae composed nearly entirely of FTZ-dependent cuticular structures derived from the even-numbered parasegments, this result suggests that expression of the HD-independent FTZ activity is sufficient to establish FTZ-dependent cuticle. Activation of FTZ-dependent Engrailed (EN) expression and activation of the *ftz* enhancer were HD-independent. The *ftz* enhancer element, *AE-1*, was activated by the HD-independent FTZ activity; however, the *ftz* enhancer element, *AE-BS2CCC*, which is the same as *AE-1* except for the inactivation of two FTZ HD DNA-binding sites, was not. Activation of the *ftz* enhancer by ectopic expression of FTZ activity was effective only during gastrulation and germ band extension. In the discussion, we propose an explanation for these results.

GENETIC screens for mutants have identified a large number of developmentally important genes (JÜRGENS *et al.* 1984). Molecular biology has identified potential roles and activities for the products of many of these genes. However, it is still necessary to return to the organism, using a variety of genetic approaches, to test if the proposed activity is the only activity of the gene product, and if it is the most important developmental activity. We have applied this approach to one of the most studied Drosophila pair-rule genes, *fushi tarazu* (*ftz*).

The *fushi tarazu* (FTZ) protein is a zygotically expressed factor involved in the control of embryonic segmental pattern formation. First, instar larva homozygous or hemizygous for null *ftz* alleles lack the cuticle derived from even-numbered parasegments (the denticle belts of segments T2, A1, A3, A5, A7 are missing) (JÜRGENS *et al.* 1984; WAKIMOTO *et al.* 1984; WEINER *et al.* 1984). The *ftz* gene encodes a homeodomain (HD)-containing gene product (LAUGHON and SCOTT 1984). *In vitro* the FTZ HD interacts sequence specifically with DNA (PERCIVAL-SMITH *et al.* 1990). Also in *in vitro* and in tissue culture cell transcriptional activation assays, FTZ can act as a DNA-binding activator of transcription, with the DNA binding being mediated by the HD (JAYNES and O'FARRELL 1988; OHKUMA *et al.* 1990; FITZPATRICK *et al.* 1992).

The expression pattern of FTZ protein and *FTZ* mRNA are temporally and spatially dynamic (HAFEN *et al.* 1984; CARROLL and SCOTT 1985). Because of the short half life of both FTZ protein and *FTZ* mRNA, their expression patterns follow each other quite closely (KARR and KORNBERG 1989; YU and PICK 1995). FTZ is expressed just before cellularization in a large domain. As cellular blastoderm development proceeds, a striped expression pattern of *FTZ* mRNA and FTZ protein appears. These stripes of FTZ expression are established in a specific order. At late cellular blastoderm, *FTZ* mRNA and FTZ protein are expressed at their highest level in seven, sharp, three- to four-cell-wide bands of cells that are perpendicular to the anterior posterior axis. The regions of FTZ expression at this stage correspond to the even-numbered parasegments, and it is the cuticle derived from these primordia that is missing in *ftz* mutant embryos (HAFEN *et al.* 1984; MARTINEZ-ARIAS and LAWRENCE 1985). There is a strong correlation between expression at this stage and the loss-of-function ftz phenotype. But is FTZ active at this stage? During gastrulation (stage 6) the stripes rapidly narrow in width. During germ band extension (stage 7) the FTZ protein and *FTZ* mRNA are still detectable, but their levels are decreasing. By the end of germ band extension, FTZ protein and *FTZ* mRNA are no longer detectable in most of the stripes. Central nervous system expression of FTZ commences in germ band extended embryos.

FTZ activity is required for wild-type regulation of *engrailed* and *wingless* expression (DINARDO and O'FARRELL 1987; INGHAM *et al.* 1988) and for autoactivation of the *ftz* gene (HIROMI and GEHRING 1987). Autoactiva-

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tion of the *ftz* gene requires the *ftz* enhancer, a *cis*-acting upstream element. Deletion of the *ftz* enhancer results in insufficient accumulation of FTZ protein for rescue of the ftz phenotype (HIROMI *et al.* 1985). The *ftz* enhancer fused to the *lac Z* reporter gene directs expression of β-galactosidase in seven bands of cells; this expression pattern is dependent on expression of FTZ activity (HIROMI and GEHRING 1987). Extensive analysis of the *ftz* enhancer has shown that FTZ HD DNA-binding sites are required for activation, and that FTZ is bound to these FTZ HD DNA-binding sites during activation (SCHIER and GEHRING 1992; SCHIER and GEHRING 1993a).

The ON/OFF pattern of FTZ expression across the anterior posterior axis is important for wild-type development. Ectopic expression of FTZ, that is expression in all cells, is achieved by heat-shocking embryos that contain a heat-shock promoter/*ftz*-coding region fusion gene. Ectopic expression of FTZ in gastrulating embryos results in development of larvae that consist of only cuticle derived from even-numbered parasegments (the denticle belts of segments T1, T3, A2, A4, A6, A8 are missing) (STRUHL 1985; ISH-HOROWICZ and GYURKOVICS 1988). This phenotype is referred to as the anti-ftz phenotype, because it is the reciprocal of the ftz phenotype. Even-numbered parasegmental cuticle is FTZ-dependent cuticle, as it is missing in *ftz* mutant larva.

Induction of the anti-ftz phenotype is HD-independent; the $FTZ^{\Delta 274\text{-}302}$ polypeptide, which lacks half the homeodomain and does not bind to a FTZ HD DNA-binding site, induces the anti-ftz phenotype when ectopically expressed (FITZPATRICK *et al.* 1992). One major problem in interpreting the anti-ftz phenotype is that the flies used carry two *ftz* genes: the *hsp ftz* fusion gene and the endogenous *ftz* gene. Since expression from the endogenous *ftz* gene changes as a result of the ectopic expression of FTZ from the *hsp ftz* fusion gene, it is possible that the HD-independent activity of FTZ just induces a subtle change in the expression of the endogenous *ftz* gene. This is essentially the model ISH-HOROWICZ used to explain the generation of the anti-ftz phenotype (ISH-HOROWICZ *et al.* 1989). This model predicts that expression of FTZ from the endogenous *ftz* gene is required for the induction of the anti-ftz phenotype.

Here we show that the endogenous *ftz* gene is not required for the induction of the anti-ftz phenotype by ectopic expression of FTZ. Also the FTZ HD was not required for the induction of the anti-ftz phenotype. Activation of Engrailed (EN) expression and the *ftz* enhancer was HD-independent as well. We propose a model to resolve the previously described importance of the FTZ HD with these results.

## MATERIALS AND METHODS

**Stock construction:** For a description of the genetic markers and balancer chromosomes used here see LINDSLEY and ZIMM (1992). In this study, we used *heat-shock promoter/ftz*-coding region fusion genes carried on *P* elements that had transposed onto the second chromosome (APS230A, APS231B, APS245B) to build our test stocks (FITZPATRICK *et al.* 1992) (Table 1; Figure 1A). When homozygous, these second chromosomes, carrying the various fusion genes, are weakly viable, male fertile, but female sterile; these chromosomes are present in the original $ry^{506}$ stock used for injection and are not a result of the insertion of the *P* elements carrying the various *hsp ftz* genes. Both $ftz^{11}$ and $ftz^{13}$ are protein minus *ftz* null alleles (HIROMI and GEHRING 1987; SCHIER and GEHRING 1993a).

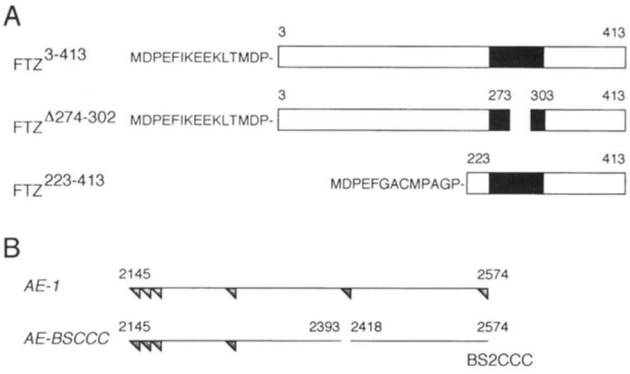


FIGURE 1.—Structure of the FTZ polypeptides and *ftz* enhancer elements. The structure of the three FTZ polypeptides used in this study (A). The amount of FTZ protein expressed from the various *hsp ftz* fusion genes is indicated above the diagrammatic representation of the structure; also the sequence of non-FTZ amino acids introduced by the DNA manipulations at the N terminus are indicated in single letter code (FITZPATRICK *et al.* 1992). The structure of the *AE-1* and *AE-BS2CCC* enhancer elements (B). The construction of these is described in (SCHIER and GEHRING 1993a). The FTZ HD DNA-binding sites are indicated by the filled triangles. The position of BS2CCC indicates which FTZ HD DNA-binding site was replaced by the inactive BS2CCC site.

For the study of the cuticular phenotypes (see Table 1), two stocks were constructed: DH51, which was used to derive the stocks DH101, DH102, and DH103; and DH52, which was used to derive the stocks DH201, DH202, and DH203 (*TM6B, P{w*alLy*}* chromosome was provided by G. GLOOR).

For the study of embryonic phenotypes, we obtained the stock PL101 from H. KRAUSE and used it to derive the stock DH61 (see Table 1). This stock was subsequently used to derive the stocks DH301, DH302, and DH303. After the DH100, 200, and 300 series of stocks were constructed, PCR was used to confirm that these stocks contained the correct *hsp ftz* fusion gene.

The stock DH502, used to assay *ftz* enhancer activation, was constructed as follows (see Table 1). The *P*-element construct *P{UPHZ50H,* $ry^{+}$*}*, (HIROMI and GEHRING 1987) was retransformed into $ry^{506}$ flies (RUBIN and SPRADLING 1982), and an insert on the first chromosome was isolated. This insert strain, DH501, was used to construct the stock DH502.

**Heat shock treatments:** Eggs were collected for 30 min at 25°. Embryos were heat shocked for 20 min at 36.5° 2.50–3.00 hr after egg laying (AEL) for the first instar larval cuticle experiments. Embryos were heat shocked for 17 min at 36.5° 2.50–3.00 hr AEL for both the EN and *ftz* enhancer activation experiments.

**Cuticle preparations:** The larvae were devitellinized by shaking in a 1:1 heptane: methanol mixture at 24–26 hr AEL. The devitellinized larvae were mounted in Hoyer's mountant

**TABLE 1**

**List of stocks**

| Stock | Genotype | Origin |
|---|---|---|
| APS245B | *P{hsp ftz$^{3\text{-}413}$ ry$^{+}$}/CyO; ry$^{506}$* | FITZPATRICK *et al.* (1992) |
| APS230A | *P{hsp ftz$^{\Delta 274\text{-}302}$ ry$^{+}$}/CyO; ry$^{506}$* | FITZPATRICK *et al.* (1992) |
| APS231B | *P{hsp ftz$^{223\text{-}413}$ ry$^{+}$}/CyO, ry$^{506}$* | FITZPATRICK *et al.* (1992) |
| DH51 | *y w; L/CyO; TM6B, P{w*allL*y}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH52 | *y w; L/CyO; TM6B, P{w*allL*y}/Ki ftz$^{11}$* | This work |
| PL101 | *Sp/CyO, P{hb-lacZThb8 w$^{+}$}; Ki hb/TM3, P{hb-lacZThb8 w$^{+}$}* | DRIEVER *et al.* (1989) |
| DH61 | *L/CyO, P{hb-lacZThb8 w$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH101 | *y w; P{hsp ftz$^{3\text{-}413}$ ry$^{+}$}/CyO; TM6B, P{w*allL*y}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH102 | *y w; P{hsp ftz$^{\Delta 274\text{-}302}$ ry$^{+}$}/CyO; TM6B, P{w*allL*y}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH103 | *y w; P{hsp ftz$^{223\text{-}413}$ ry$^{+}$}/CyO; TM6B, P{w*allL*y}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH201 | *y w; P{hsp ftz$^{3\text{-}413}$ ry$^{+}$}/CyO; TM6B, P{w*allL*y}/Ki ftz$^{11}$* | This work |
| DH202 | *y w; P{hsp ftz$^{\Delta 274\text{-}302}$ ry$^{+}$}/CyO; TM6B, P{w*allL*y}/Ki ftz$^{11}$* | This work |
| DH203 | *y w; P{hsp ftz$^{223\text{-}413}$ ry$^{+}$}/CyO, TM6B, P{w*allL*y}/Ki ftz$^{11}$* | This work |
| DH301 | *P{hsp ftz$^{3\text{-}413}$ ry$^{+}$}/CyO, P{hb-lacZThb8 w$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH302 | *P{hsp ftz$^{\Delta 274\text{-}302}$ ry$^{+}$}/CyO, P{hb-lacZThb8 w$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH303 | *P{hsp ftz$^{223\text{-}413}$ ry$^{+}$}/CyO, P{hb-lacZThb8 w$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work |
| DH501 | *P{UPHZ50H ry$^{+}$}; ry$^{506}$* | This work |
| DH502 | *P{UPHZ50H ry$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | This work and I. DUNCAN |
| AS142 | *P{AE-1-lacZ ry$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | SCHEIR and GEHRING (1993a) |
| AS147 | *P{AE-BS2CCC-lacZ ry$^{+}$}; TM3, P{hb-lacZThb8 w$^{+}$}/ftz$^{13}$ ry$^{506}$ e$^{s}$* | SCHEIR and GEHRING (1993a) |

and viewed under darkfield optics (WIESCHAUS and NÜSSLEIN-VOLHARD 1986).

The ftz phenotype is larva having the thoracic denticle belts T1 and T3; two sets of ventral pits and Keilin's organs between T1 and T3, T3 and A2; and four broad abdominal denticle belts in which the last denticle belt, A8, is juxtaposed with the anal pad. The anti-ftz phenotype is larva having one thoracic denticle belt, T2, and one set of ventral pits and Keilin's organs between T2 and A1; four abdominal denticle belts of which the first is narrow, A1, and the last, A7, is separated from the anal pads by naked cuticle.

**Immunolocalization:** Embryos were fixed at 4.5 hr AEL and immunostained for EN expression with a mouse monoclonal anti-Engrailed antibody supplied by S. COTÉ (PATEL *et al.* 1989). Where appropriate the embryos were coimmunostained for β-galactosidase expression with a mouse monoclonal anti-β-galactosidase antibody (Promega). The immunostaining procedure was as described in KELLERMAN *et al.* (1990).

***ftz* enhancer activation:** Embryos were fixed at 4.5 AEL, and β-galactosidase activity was visualized with the chromogenic substrate X-gal (BELLEN *et al.* 1989). Before mounting on slides, the vitelline membrane was removed by shaking the embryos in a 1:1 heptane:methanol mixture.

**Staging embryos:** Eggs were collected for 30 min, and the embryos were fixed at specific times AEL. The embryos were assigned stages as defined in (CAMPOS-ORTEGA and HARTENSTEIN 1985). Greater than 100 embryos were staged at each time point.

## RESULTS

**Identification of *ftz* mutant embryos and larvae:** We wanted to determine whether the anti-ftz phenotype is dependent on FTZ expression from the endogenous *ftz* gene. *ftz* mutant alleles are maintained in heterozygous stocks because of their lethality. One-quarter of the embryos laid by these stocks are *ftz*, and we tested if these *ftz* embryos would be transformed toward an anti-ftz phenotype by ectopic expression of FTZ. However, the three-quarters of *ftz*$^{+}$ embryos would also be transformed toward the anti-ftz phenotype. To mark the *ftz* genotype independently of the ftz phenotype, we employed third chromosome balancers that carried dominant markers that could be scored during early embryogenesis and on the first instar larval cuticle. The quarter of *ftz* embryos laid by a heterozygous stock containing such a dominantly marked balancer chromosomes lack expression of the dominant marker; the *ftz* embryos lack the balancer chromosome and hence lack expression of the dominant marker. Two dominant markers were employed to score the presence of a *ftz*$^{+}$ balancer chromosome: a *hunchback promoter lacZ* fusion gene, *P{hb-lac Z Thb8, w$^{+}$}*, to mark *ftz*$^{+}$ embryos (W. DRIEVER *et al.* 1989) and the *yellow* (*y*) gene, *P{w*allL*y}*, to mark *ftz*$^{+}$ first instar larval cuticles (NASSIF *et al.* 1994).

**The endogenous *ftz* gene is not required for generation of the anti-ftz phenotype:** Loss-of-function *ftz* alleles result in the loss of cuticular structures derived from the even-numbered parasegments (Figure 2B). Ectopic expression of FTZ during gastrulation results in the reciprocal phenotype, the anti-ftz phenotype, where cuticular structures derived from the odd-number parasegments are missing (Figure 2C). The ISH-HOROWICZ model for the generation of the anti-ftz phenotype by ectopic expression of FTZ proposes that the transient expression of FTZ from the heat-shock promoter/ftz fusion gene widens the normal expression domain of the endogenous *ftz* gene, and it is this widened domain of FTZ expression from the endogenous *ftz* gene that results in the anti-ftz phenotype (ISH-HOROWICZ *et al.* 1989). We have tested

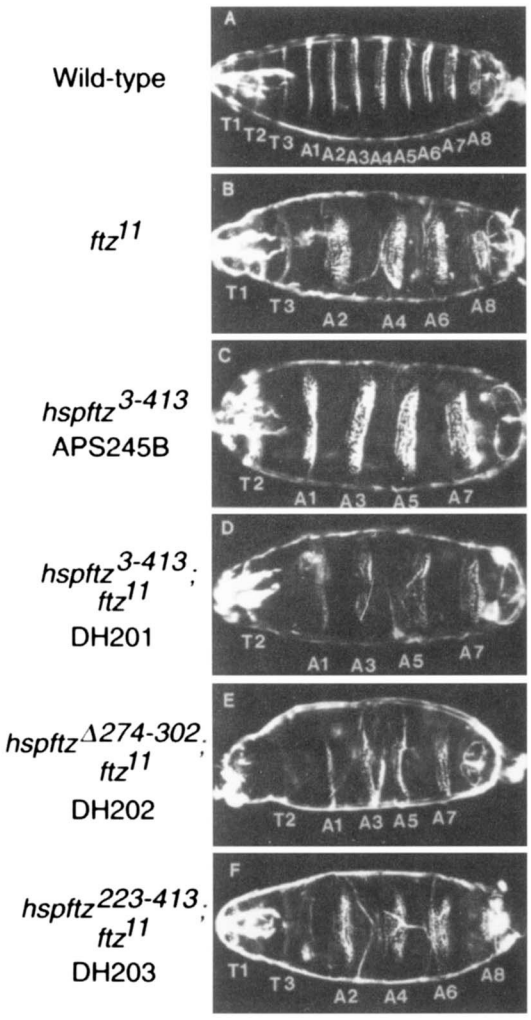


FIGURE 2.—Ectopic expression of $FTZ^{3\text{-}413}$ and $FTZ^{\Delta 274\text{-}302}$ induces the anti-ftz cuticular phenotype in the absence of endogenous FTZ activity. The important elements of the genotype are indicated on the left side of each panel: what *hsp ftz* fusion gene was expressed, and what *ftz* allele was used. Below the simplified genotypes are the stock designations of the flies that laid the embryos analyzed (see Table 1).

this model's genetic prediction that ectopic expression of FTZ in a *ftz* embryo would not transform to the anti-ftz phenotype, but would remain ftz.

A balancer chromosome carrying a $y^+$ gene (*TM6B, P{w*alL*y}*) was used to mark the $ftz^+$ genotype independently of the ftz phenotype (see MATERIALS AND METHODS). Administration of a heat shock to embryos carrying the *hsp* $ftz^{3\text{-}413}$ fusion gene resulted in a significant transformation of *ftz* embryos toward the anti-ftz phenotype (Figure 2D, Table 2). This transformation was independent of the *ftz* null allele used, as it occurs in both $ftz^{11}$ and $ftz^{13}$ larvae (Table 2). Thus, the strict involvement of the endogenous *ftz* gene in generation of the anti-ftz phenotype proposed by ISH-HOROWITZ is incorrect (ISH-HOROWICZ *et al.* 1989). However, the presence of an active endogenous *ftz* gene does increase the efficiency of the transformation toward the anti-ftz phenotype (Table 2).

In control experiments, a third of the cuticles laid by these stocks were y, of these 99–99.5% were ftz, and 0.5–1% were wild-type (Table 2). However, a y fly was never found in the stock. We have observed similar behavior with a *TM2, P{ry*$^+$*, y*$^+$*}* marked balancer chromosome (GEYER and CORCES 1987), except 10% of the y cuticles were wild-type. However, again a y fly was never found in the stock. This indicates that the penetrance of the $y^+$ genotype during embryogenesis is not complete; this incomplete penetrance does not increase upon administration of a heat shock. But since transformation to the anti-ftz phenotype is 15- to 40-fold greater than this incomplete penetrance, it does not affect the interpretation of our results.

**The HD is not required for generation of the anti-ftz phenotype:** A FTZ protein that lacks most of the HD, $FTZ^{\Delta 274\text{-}302}$ (Figure 1A), can induce the anti-ftz phenotype, even though it cannot activate FTZ-dependent transcription in Drosophila tissue culture cells (FITZPATRICK *et al.* 1992). The ISH-HOROWICZ model was used previously as a basis for interpreting this result. It was proposed that ectopic expression of the FTZ HD-independent activity in embryos widens the expression domain of the endogenous *ftz* gene, and it is the FTZ activity expressed from the endogenous *ftz* gene that generates the anti-ftz phenotype. We tested whether ectopic expression of $FTZ^{\Delta 274\text{-}302}$ was sufficient in a *ftz* embryo to induce the anti-ftz phenotype. Ectopic expression of the FTZ peptide, $FTZ^{\Delta 274\text{-}302}$, transformed $ftz^{11}$ embryos toward the anti-ftz phenotype (Figure 2E; Table 2). This transformation was also independent of the *ftz* null allele used, as it occurs with the $ftz^{13}$ allele (Table 2).

The activity of the $FTZ^{223\text{-}413}$ polypeptide was tested in *ftz* larvae. $FTZ^{223\text{-}413}$ is a strong activator of FTZ-dependent transcription in Drosophila tissue culture cells (FITZPATRICK *et al.* 1992). Induction of the *hsp* $ftz^{223\text{-}413}$ gene with a heat shock results in expression and nuclear accumulation of the $FTZ^{223\text{-}413}$ polypeptide (data not shown). Ectopic expression of $FTZ^{223\text{-}413}$ did not transform *ftz* larvae to the anti-ftz phenotype (Figure 2F; Table 2). There also was no alternative transformation; *ftz* larvae had a ftz phenotype. Hence, the $FTZ^{223\text{-}413}$ polypeptide had no activity in this assay.

**The intermediate transformations toward the anti-ftz phenotype form a graded series of phenotypes:** True anti-ftz phenotypes and intermediate phenotypes were observed with ectopic expression of both $FTZ^{3\text{-}413}$ and $FTZ^{\Delta 274\text{-}302}$. The intermediate class of phenotypes are *ftz* mutant larvae with both ftz and anti-ftz phenotypic characteristics. Many intermediates are ftz phenotypes with a slight rescue of even-numbered parasegments (Figure 3, B and C). Some display both cuticle derived from odd- and even-numbered parasegments (Figure 3D). These intermediate phenotypes and the reciprocal nature of the anti-ftz and ftz phenotypes (Figure 3, A and E) suggest that ectopic expression of either

**TABLE 2**

**Distributions of cuticular phenotypes**

| Phenotypes | *hsp ftz*$^{3\text{-}413}$; *ftz*$^{11}$ [a] (DH201) | | *hsp ftz*$^{3\text{-}413}$; *ftz*$^{13}$ (DH101) | | *hsp ftz*$^{\Delta 274\text{-}302}$; *ftz*$^{11}$ (DH202) | | *hsp ftz*$^{\Delta 274\text{-}302}$; *ftz*$^{13}$ (DH102) | | *hsp ftz*$^{223\text{-}413}$; *ftz*$^{11}$ (DH203) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked |
| (y$^+$ ftz$^+$) | | | | | | | | | | |
| Wild type | ND[e] | 129 | 545 | 158 | 286 | 189 | 248 | 173 | ND | ND |
| Anti-ftz | | 339 | 0 | 384 | 0 | 188 | 0 | 190 | | |
| Intermediate[b] | | 50 | 0 | 45 | 0 | 30 | 0 | 84 | | |
| Incomplete[c] | | 0 | 2 | 0 | 2 | 0 | 2 | 1 | | |
| ftz | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| (y ftz) | | | | | | | | | | |
| ftz | ND | 64 | 223 | 73 | 123 | 55 | 108 | 52 | ND | 84 |
| Wild type | | 0 | 3 | 4 | 1 | 0 | 0 | 3 | | 0 |
| Anti-ftz | | 37 (21) | 0 (0) | 45 (18) | 0 (0) | 12 (9) | 0 (0) | 23 (14) | | 0 (0) |
| Intermediate | | 47 (26) | 0 (0) | 63 (25) | 0 (0) | 42 (32) | 0 (0) | 43 (27) | | 0 (0) |
| Incomplete | | 31 | 0 | 63 | 0 | 23 | 0 | 42 | | 7 |
| Total | | 179 | 226 | 248 | 124 | 132 | 108 | 163 | | 91 |
| Ball[d] | | 31 | 18 | 40 | 15 | 38 | 12 | 52 | | 24 |

Values in parentheses are percentages.

[a] The *hsp ftz* fusion gene and the *ftz* allele are the only portions of the genotype indicated (see Table 1).

[b] Intermediate cuticles contain characteristics of both the ftz and the anti-ftz phenotype.

[c] Incomplete cuticles contain setal belts, but their identities could not be determined.

[d] Ball cuticles lack setals belts and thus their yellow phenotype was undeterminable.

[e] Not determined.

FTZ$^{3\text{-}413}$ or FTZ$^{\Delta 274\text{-}302}$ is rescuing FTZ-dependent cuticular structures. Hence, establishment of FTZ-dependent cuticle may depend solely on the FTZ HD-independent activity.

**Activation of FTZ-dependent EN expression is HD-independent:** EN is expressed at the anterior margin of every parasegment to give 14 stripes of expression. This EN expression pattern requires FTZ activity; in *ftz* mutant embryos only seven of the 14 stripes of EN expression are expressed (DINARDO and O'FARRELL 1987). This is called the ftz EN expression pattern (Figure 4B). Expression of these FTZ-dependent EN stripes are also affected by ectopic expression of FTZ activity. Induction of FTZ expression results in widened expression domains of the FTZ-dependent EN stripes (ISH-HOROWICZ *et al.* 1989). This is called the anti-ftz EN expression pattern (Figure 4C). We have tested whether activation of EN expression in the anti-ftz EN expression pattern requires expression of FTZ from the endogenous *ftz* gene, and whether it is HD-dependent.

The *ftz* embryos were marked using a *TM3* chromosome carrying a *hunchback promoter-lacZ* fusion gene *P{hb-lacZ Thb8 w*$^+$*}* (DREIVER *et al.* 1989). The *hb-lacZ* fusion gene is expressed in the head. Embryos that exhibit no head staining are *ftz*$^{13}$ embryos. The stocks used also contained a similarly marked *CyO* balancer chromosome, such that embryos that exhibit no head staining are also homozygous for the *hsp ftz* fusion gene. Embryos that had no head staining occurred at a ratio of 1:15; these embryos must have the genotype *hsp ftz; ftz*$^{13}$.

In all control experiments, all embryos that did not express β-galactosidase expressed EN in a ftz EN expression pattern (Table 3). This demonstrated faithful segregation of the *hb-lacZ* gene with the *ftz*$^+$ allele. Ectopic expression of either FTZ$^{3\text{-}413}$ or FTZ$^{\Delta 274\text{-}302}$ in *ftz*$^{13}$ mutant embryos resulted in a significant number of them expressing EN in an anti-ftz EN expression pattern (Figure 4, D and E; Table 3). Activation of the anti-ftz EN expression pattern was independent of the null *ftz* allele used, as it was observed in *ftz*$^{11}$ embryos (data not shown). Ectopic expression of FTZ$^{223\text{-}413}$ was unable to activate FTZ-dependent EN expression (Figure 4F). These results demonstrate that EN expression is activated by ectopically expressed FTZ in the absence of endogenous FTZ activity, and that this activation is FTZ HD-independent.

**Activation of the *ftz* enhancer is HD-independent:** The *ftz* enhancer is a *cis*-acting DNA element required for the rescue of the ftz phenotype (HIROMI *et al.* 1985). When fused to the bacterial *lacZ* gene that is expressed from a minimal promoter, the *ftz* enhancer directs β-galactosidase expression in seven stripes (Figure 5A). These seven stripes of β-galactosidase expression are completely dependent on expression of FTZ activity (FIGURE 5B) (HIROMI and GEHRING 1987). Ectopic expression of FTZ results in a widening of the β-galactosidase expression domains (Figure 5C). We tested whether ectopically expressed FTZ was sufficient to activate the *ftz* enhancer, and if activation required the FTZ HD.

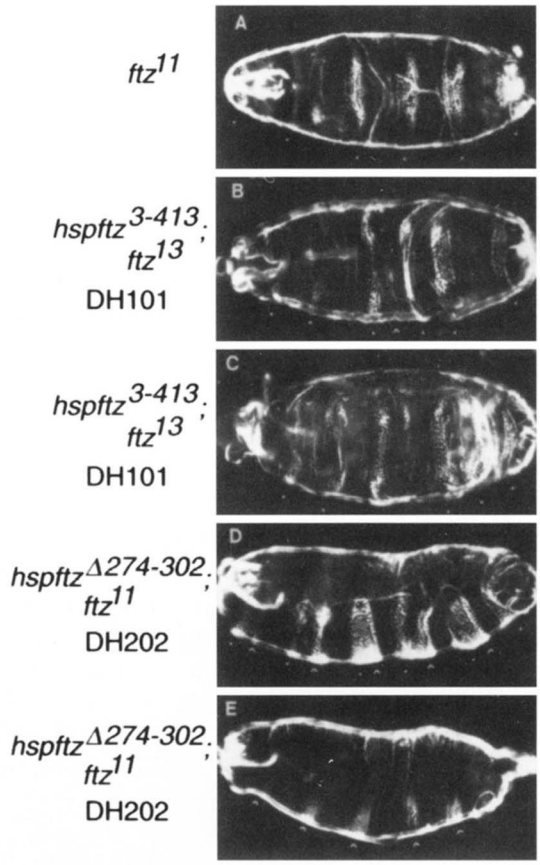


FIGURE 3.—Examples of intermediate phenotypes. The important elements of the genotype are indicated on the left side of each panel: what *hsp ftz* fusion gene was expressed, and what *ftz* allele was used. Below the simplified genotypes are the stock designations of the flies that laid the embryos analyzed (see Table 1). ftz and anti-ftz phenotypic characteristics are indicated by ■ and ⌒, respectively.

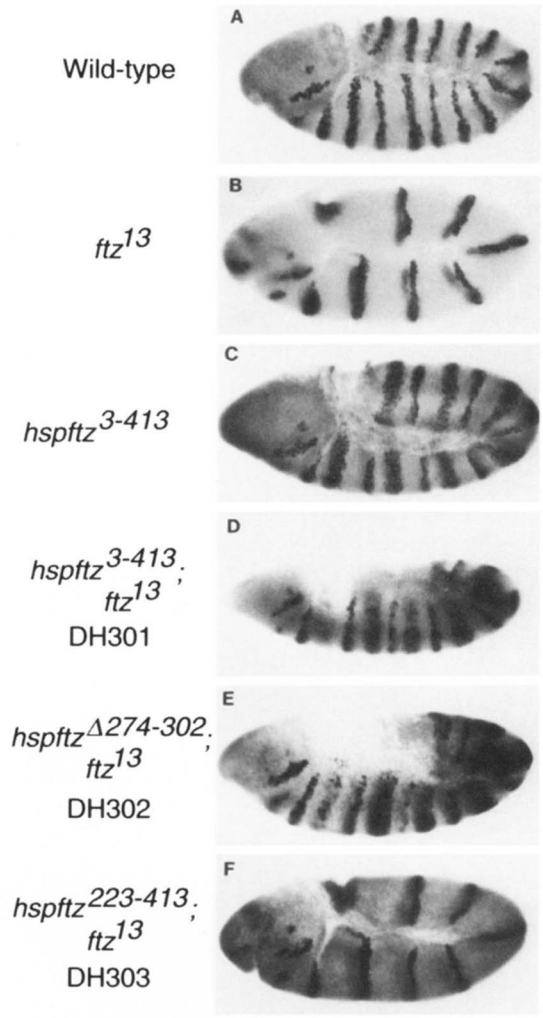


FIGURE 4.—Activation of EN protein expression is FTZ HD independent. The important elements of the genotype are indicated on the left side of each panel: what *hsp ftz* fusion gene was expressed, and what *ftz* allele was used. Below the simplified genotypes are the stock designations of the flies that laid the embryos analyzed (see Table 1).

We isolated an insertion of *P{UPHZ50H ry⁺}*, which carries the *ftz* enhancer *lacZ* fusion gene (HIROMI and GEHRING 1987), on the first chromosome. This stock (DH501) was used to construct the stock (DH502) that contains the *TM3* balancer chromosome carrying the *hb-lacZ* fusion gene over a chromosome carrying the $ftz^{13}$ allele (see Table 1). This stock laid embryos that stained very strongly for β-galactosidase expression or not at all (Figure 5B; Table 4). The non-β-galactosidase-staining embryos are *P{UPHZ50H ry⁺}; $ftz^{13}$*, because they lack the dominantly marked balancer chromosome and β-galactosidase expression from *P{UPHZ50H ry⁺}* is FTZ-dependent. To assay if the *ftz* enhancer could be activated by $FTZ^{3\text{-}413}$, $FTZ^{\Delta 274\text{-}302}$, and $FTZ^{223\text{-}413}$, males from the stocks DH301, DH302, and DH303 were crossed to virgin females of stock DH502, and the embryos of these crosses were heat shocked and stained for β-galactosidase activity.

Ectopic expression of $FTZ^{3\text{-}413}$ and $FTZ^{\Delta 274\text{-}302}$ strongly activated the *ftz* enhancer (Figure 5, D and E; Table 4); whereas, ectopic expression of $FTZ^{223\text{-}413}$ was unable to activate the *ftz* enhancer (Figure 5F; Table 4). This demonstrates that the *ftz* enhancer is activated by ectopically expressed FTZ in seven widened stripes in the absence of endogenous FTZ activity, and that *ftz* enhancer activation in this assay is HD-independent.

**Activation of the *ftz* enhancer by ectopically expressed FTZ requires HD DNA-binding sites:** The structure of the 2.6-kb DNA fragment originally defining *ftz* enhancer activity has been extensively studied (PICK *et al.* 1990; SCHIER and GEHRING 1992, 1993b). A subregion of this large *ftz* enhancer fragment, referred to as the *AE-1* element, has similar autoregulatory properties as the *ftz* enhancer and is expressed more weakly. The *AE-1* element has been used to define the role of the HD DNA-binding sites in the activity of the *ftz* enhancer (SCHIER and GEHRING 1992, 1993a). The *AE-1* element contains six FTZ HD DNA-binding sites, and removal of two of these sites strongly reduces the element's activity (SCHIER and GEHRING 1992). The *AE-*

**TABLE 3**

**Activation of engrailed expression**

| Engrailed expression patterns in $ftz^{13}$ embryos[b] | *hsp ftz*$^{3\text{-}413}$ [a] (DH301) | | *hsp ftz*$^{\Delta 274\text{-}302}$ (DH302) | | *hsp ftz*$^{223\text{-}413}$ (DH303) | |
|---|---|---|---|---|---|---|
| | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked |
| anti-ftz | 0 (0) | 47 (66) | 0 (0) | 15 (35) | 0 (0) | 0 (0) |
| ftz | 22 | 19 | 11 | 22 | 25 | 59 |
| Wild type | 0 | 0 | 0 | 0 | 0 | 0 |
| EN$^-$ [c] | 0 | 5 | 2 | 6 | 0 | 9 |
| Total | 22 | 71 | 13 | 43 | 25 | 68 |

Values in parentheses are percentages.
[a] The *hsp ftz* fusion gene is the only portion of the genotype indicated.
[b] As determined by the absence of β-galactosidase expression in the procephalon.
[c] EN$^-$ embryos showed no immunostaining for Engrailed.

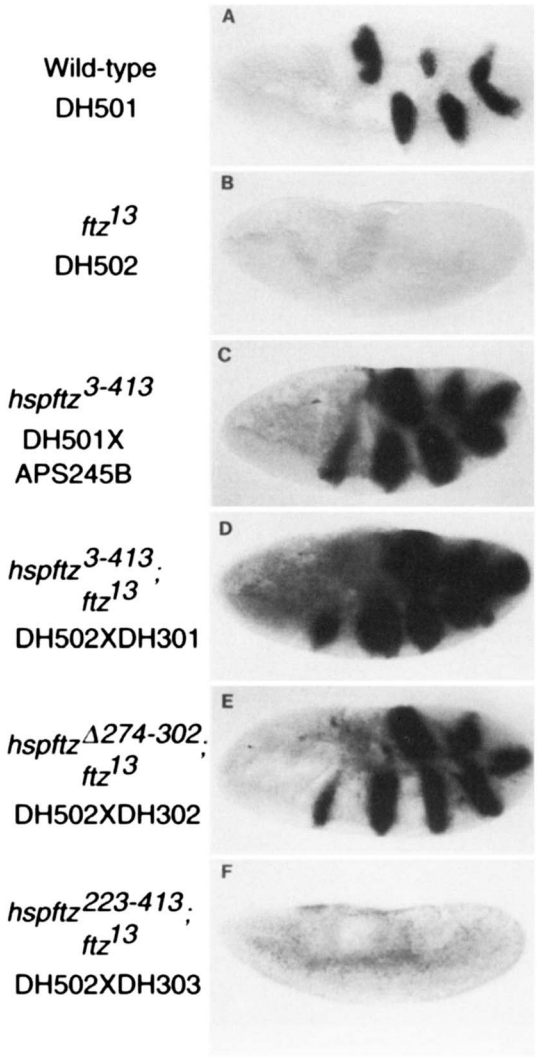


FIGURE 5.—Activation of β-galactosidase expression from a *ftz* enhancer *lac Z* fusion gene is FTZ HD independent. The important elements of the genotype are indicated on the left side of each panel: what *hsp ftz* fusion gene was expressed, and what *ftz* allele was used. Below the simplified genotypes are the stock designations of the females and males crossed to lay the embryos analyzed (see Table 1).

*BS2CCC* derivative has two HD DNA-binding sites removed: one removed in a short deletion and one substituted with an inactive HD DNA-binding site, BS2CCC (Figure 1B). The *AE-BS2CCC* element is inactive (SCHIER and GEHRING 1993a). Derivatives of the *AE-1* element have been used to show that FTZ binds via the HD to the HD DNA-binding sites for *ftz* enhancer activation. We tested if ectopic expression of FTZ$^{\Delta 274\text{-}302}$ could activate the *AE-1* and *AE-BS2CCC* elements in *ftz* mutant embryos.

Males of the stock AS142 or AS147 were crossed to virgin females collected from the stocks DH301 or DH302 (see Table 1). Ectopic expression of FTZ$^{3\text{-}413}$ and FTZ$^{\Delta 274\text{-}302}$ activated β-galactosidase expression in widened stripes from the *AE-1* element *lacZ* fusion gene (Figure 6; Table 5). This demonstrates that the full *ftz* enhancer element and the *AE-1* element behave the same way in our assay; activation of *AE-1* element is HD-independent. However, we found that neither ectopic expression of FTZ$^{3\text{-}413}$ nor FTZ$^{\Delta 274\text{-}302}$ was able to activate the *AE-BS2CCC* element (Figure 6; Table 5). The *AE-BS2BCD* element was also not activated by FTZ$^{\Delta 274\text{-}302}$ (SCHIER and GEHRING 1993a) (data not shown). These results indicate that although activation of the *AE-1* element is HD-independent, the HD-independent activation requires the sequences deleted in *AE-BS2CCC*, and these sequences overlap known HD DNA-binding sites.

**Ectopic expression of FTZ activates the *ftz* enhancer during gastrulation and germ band extension:** Expression of FTZ from the *hsp ftz* fusion gene is transient, because both *FTZ* mRNA and FTZ protein have very short half lives (EDGAR *et al.* 1986). Most of the FTZ protein is gone 30–40 min after the heat shock (ISH-HOROWICZ *et al.* 1989) (A. PERCIVAL-SMITH, unpublished observations). We determined when ectopically expressed FTZ activated the *ftz* enhancer, because our stocks allow us to assay ectopically expressed FTZ activity in the absence of endogenous FTZ activity.

Eggs were collected over 30-min intervals, and at specific times AEL the embryos laid by the DH502 × DH301 cross (see Table 1) were heat shocked and

**TABLE 4**

**Activation of the *ftz* enhancer is HD independent**

| *ftz* enhancer activity in *ftz*$^{13}$ embryos[b,c] | *UPHZ50H*[a] (DH502) | *hsp ftz*$^{3\text{-}413}$; *UPHZ50H*[a] (DH502 × DH301) | *hsp ftz*$^{\Delta 274\text{-}302}$; *UPHZ50H* (DH502 × DH302) | *hsp ftz*$^{223\text{-}413}$; *UPHZ50H* (DH502 × DH303) |
|---|---|---|---|---|
| Enhancer ON | 0 (0) | 102 (86) | 67 (94) | 0 (0) |
| Enhancer OFF | 70 | 17 | 4 | 95 |
| Total | 70 | 119 | 71 | 95 |

Values in parentheses are percentages.
[a] The *hsp ftz* fusion gene and enhancer reporter gene are the only portions of the genotype indicated.
[b] As determined by the absence of β-galactosidase staining in the procephalon.
[c] All embryos were heat shocked.

stained for β-galactosidase activity at 4.30 hr AEL. Also embryos collected in 30-min intervals at specific times AEL were fixed and staged (Figure 7). Administration of a heat shock at 2.20 hr and 2.35 hr AEL, when the majority of the embryos were at the cellular blastoderm stage (stage 5), resulted in little activation of the *ftz* enhancer. This does not seem to be a result of an inability to induce the heat-shock promoter, because high levels of FTZ protein are observed after administration of a 17-min heat shock at 2.35 hr AEL followed by a 15-min recovery (S. Desai and A. Percival-Smith, unpublished results). Heat shocks administered between 2.50 and 3.20 hr AEL strongly activated the *ftz* enhancer, but not at 3.35 hr AEL. Maximal competence for activation of the *ftz* enhancer preceeds gastrulation and germband extension by ~15 min. The embryos were heat shocked for 17 min, and it would take some time for the FTZ protein to accumulate before rapidly disappearing; hence, we conclude that the earliest activation of the *ftz* enhancer by ectopically expressed FTZ is at very late cellular blastoderm stage and is still activated during gastrulation and germ band extension stages, but not after the embryo has fully extended. We have observed a similar window of opportunity for activation of EN expression and establishment of FTZ-dependent cuticle by ectopically expressed FTZ (D. Hyduk, unpublished observations; G. Struhl 1984).

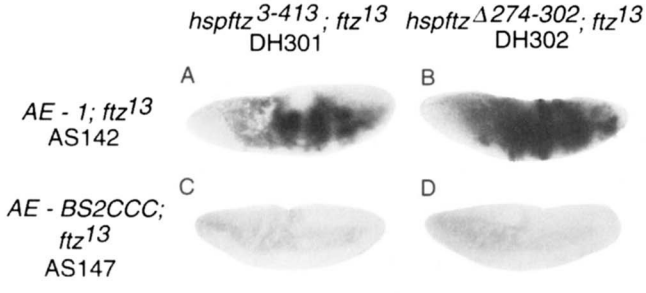


Figure 6.—Activation of β-galactosidase expression from a *ftz* enhancer *lac Z* fusion gene requires HD DNA-binding sites. The important elements of the genotype of the eggs are indicated across the top of the panels, and below these simplified genotypes is the stock designation of the female from which the egg was derived. The important elements of the genotype of the sperm are indicated on the left side of the panels, and below these simplified genotypes is the stock designation of the males from which the sperm was derived.

## DISCUSSION

**Summary of our results:** The model proposed by Ish-Horowicz explains how transient ectopic expression of FTZ during embryogenesis can result in the anti-ftz phenotype (Ish-Horwicz 1989). Central to the model is the proposal that ectopic expression of FTZ widens the normal domain of endogenous FTZ expression anteriorly. This widened domain of endogenous FTZ expression would then widen the expression domain of

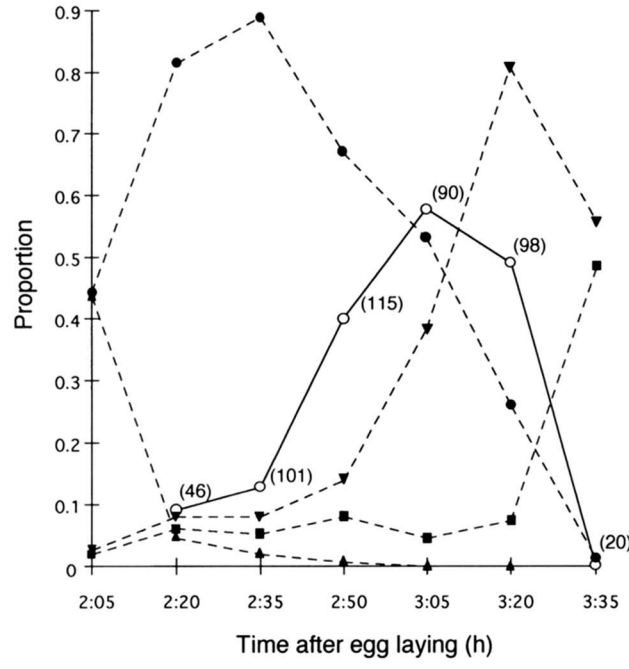


Figure 7.—The *ftz* enhancer is activated during gastrulation. The proportion of embryos at stage 4 (▲), stage 5 (●), stage 6 and 7 (▼), and stage 8 (■) at the various time points are indicated with - - -. The proportion of *UPHZ50H; hsp ftz*$^{3\text{-}413}$; *ftz*$^{13}$ embryos that express β-galactosidase at 4.30 hr AEL are indicated with (○) and straight lines. The number of *UPHZ50H; hsp ftz*$^{3\text{-}413}$; *ftz*$^{13}$ embryos examined is given to the right of each point. The x-axis indicates the time AEL that the embryos were either fixed for staging (- - -) or heat shocked to induce the *ftz* enhancer (—).

**TABLE 5**

**Activation of the *ftz* enhancer requires HD DNA-binding sites**

| *ftz* enhancer activity in $ftz^{13}$ embryos[b] | *hsp* $ftz^{3\text{-}413}$; *AE-1*[a] (DH301 × AS142) | | *hsp* $ftz^{\Delta 274\text{-}302}$; *AE-1* (DH302 × AS142) | | *hsp* $ftz^{3\text{-}413}$; *AE-BS2CCC* (DH301 × AS147) | | *hsp* $ftz^{\Delta 274\text{-}302}$; *AE-BS2CCC* (DH302 × AS147) | |
|---|---|---|---|---|---|---|---|---|
| | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked | Control | Heat shocked |
| Enhancer ON | 0 (0) | 47 (30) | 0 (0) | 15 (16) | ND[c] | 0 (0) | ND | 0 (0) |
| Enhancer OFF | 24 | 111 | 72 | 76 | | 38 | | 45 |
| Total | 24 | 158 | 72 | 91 | | 38 | | 45 |

Values in parentheses are percentages.
[a] The *hsp ftz* fusion gene and *AE* reporter gene are the only portions of the genotype indicated.
[b] As determined by the absence of β-galactosidase staining in the procephalon.
[c] Not determined.

FTZ-dependent EN expression and repress FTZ-regulated *WG* expression. This model was also used to explain how a FTZ protein lacking half the HD is able to induce the anti-ftz phenotype; it widens the expression domain of the endogenous *ftz* gene, and the FTZ activity expressed from the endogenous *ftz* gene, which has an active HD, generates the anti-ftz phenotype. This interpretation relegates the HD-independent FTZ activity to a minor role, potentially to just an artifact of ectopic expression. We tested the prediction of this model that endogenous FTZ activity is required for ectopic expression of FTZ to generate the anti-ftz phenotype. We found that generation of the anti-ftz phenotype by ectopically expressed HD-independent FTZ activity did not require FTZ activity expressed from the endogenous *ftz* gene. Since the anti-ftz phenotype is the reciprocal of the ftz loss-of-function phenotype, this result demonstrates that the HD-independent FTZ activity is sufficient to establish FTZ-dependent cuticle.

We also demonstrated that FTZ-dependent EN expression is activated by the HD-independent FTZ activity. The *ftz* enhancer is also activated by the HD-independent FTZ activity. The *ftz* enhancer is proposed to be responsible for maintaining, through autoactivation, the seven bands of high FTZ expression observed at late cellular blastoderm stage (HIROMI and GEHRING 1987; SCHIER and GEHRING 1993a). Indeed, expression of the *ftz* enhancer *lacZ* fusion gene is first detected at midcellular blastoderm stage and remains ON through gastrulation (YU and PICK 1995). However, we found that ectopic expression of FTZ activated the *ftz* enhancer strongly during gastrulation and germ band extension. This activation of the *ftz* enhancer by the HD-independent FTZ activity required HD DNA-binding sites or DNA sites overlapping these HD DNA-binding sites.

**The conflicting results:** The results of our analysis of the HD-independent FTZ activity are surprising when considered against evidence that suggests the FTZ HD is required for establishment of FTZ-dependent cuticle, activation of EN expression, and activation of the *ftz* enhancer. Two hypomorphic *ftz* alleles are mutations located within the HD: a temperature-sensitive allele, $ftz^{5}$, is a single amino acid change at position 35 of the homeodomain, and $ftz^{Rpl}$ is a deletion of the COOH terminus starting within the HD (LAUGHON and SCOTT 1984). Also, the same homeodomain deletion used in our experiments, $FTZ^{\Delta 274\text{-}302}$, when expressed from *ftz* regulatory sequences, does not rescue the ftz cuticular phenotype at all (FURUKUBO-TOKUNAGA 1992). Analysis of an extensive series of *ftz* HD mutations showed a strong correlation between rescue of the ftz phenotype and affinity of DNA binding (FURUKUBO-TOKUNAGA 1992; SCHIER and GEHRING 1993a). However, the level of expression of FTZ proteins with defective homeodomains from *ftz* regulatory sequences is lower than the level of expression of the wild-type FTZ protein from *ftz* regulatory sequences (FURUKUBO-TOKUNAGA 1992; SCHIER and GEHRING 1993a). The lower level of expression of FTZ with defective homeodomains is likely due to a lower level of autocatalytic activation of the *ftz* gene by these FTZ proteins. Hence, relative comparison of FTZ activity cannot be made in these studies, because the expression levels of these FTZ proteins with defective HDs is not the same as wild-type FTZ protein.

The strongest evidence for a role of the HD in FTZ activity comes from the analysis of the *ftz* enhancer. A *ftz* enhancer constructed with HD DNA-binding sites that can be recognized by only a Bicoid HD, and not the FTZ HD, is inactive in the presence of normal FTZ protein. However, this altered *ftz* enhancer is active when supplied with a FTZ molecule that can recognize the BCD HD DNA-binding sites, $FTZ^{Q50K}$ (SCHIER and GEHRING 1992). This demonstrates that HD DNA-binding sites are required for enhancer activation, and FTZ is bound directly to them via the FTZ HD.

**A model that reconciles the conflicting results:** We postulate that the two activities of FTZ HD-dependent transcriptional activation and HD-independent transcriptional activation operate at different times during development (Figure 8). HD-dependent transcriptional activation operates before late cellular blastoderm stage

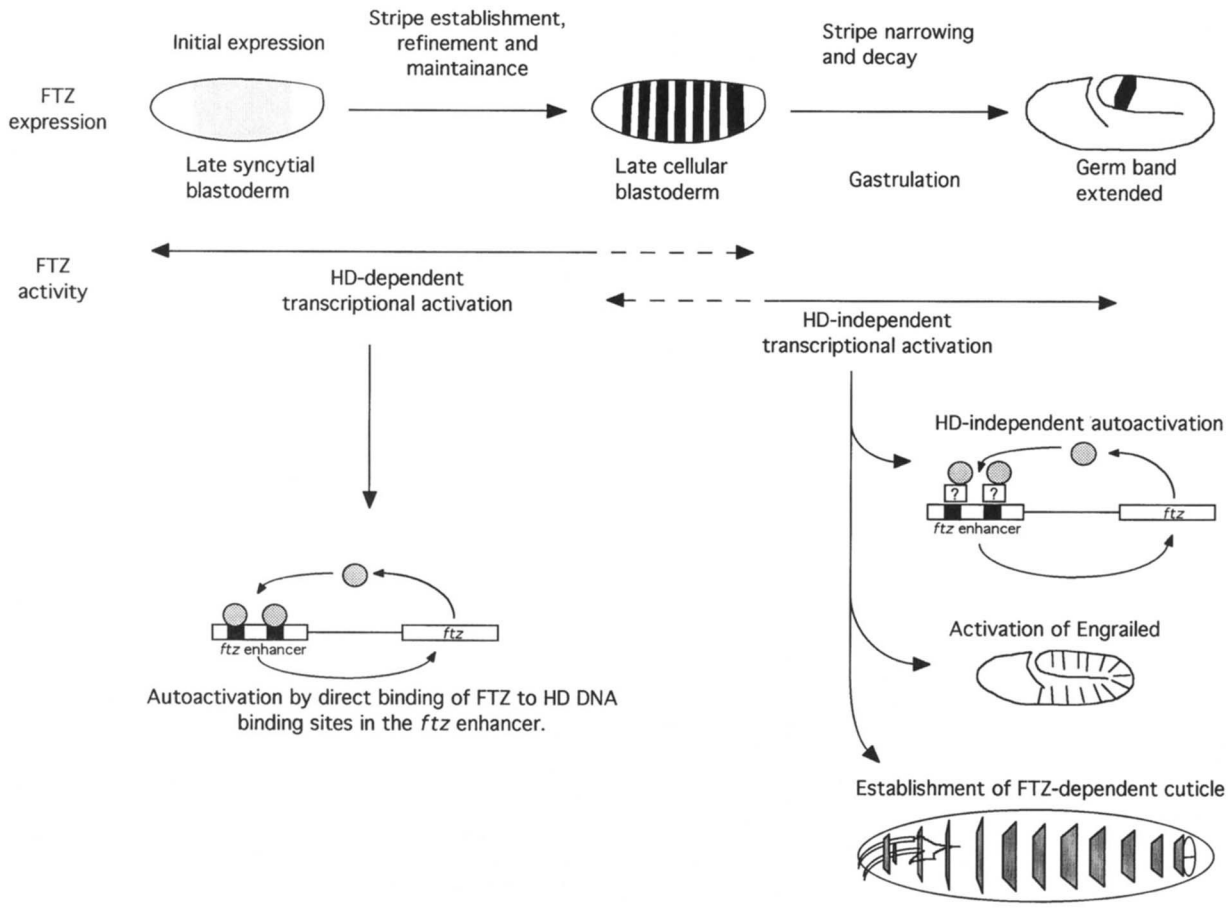


FIGURE 8.—A model that reconciles the known requirement of the FTZ HD for rescue of the ftz phenotype and our results demonstrating that the HD is dispensable for many functions of FTZ. Along the top of the figure are the phases of FTZ expression that are important during segmentation. Below are the proposed time periods that the HD-dependent and HD-independent FTZ activities operate. On the lower portion of the figure are the proposed roles of these two FTZ activities.

and is required to establish high levels of FTZ expression via autoactivation. HD-independent transcriptional activation operates during gastrulation and is required for activation of FTZ-dependent EN expression, HD-independent *ftz* enhancer activation, and ultimately establishment of FTZ-dependent cuticle (Figure 8).

This model proposes an indirect requirement for the FTZ HD in activation of FTZ-dependent EN expression and establishment of FTZ-dependent cuticle. The FTZ HD is required for maximal autocatylytic accumulation of the FTZ protein, and hence HD-independent FTZ activity, at late cellular blastoderm stage. This proposal has independent support. Reversions of the dominant $ftz^{Ual2}$ allele, which encodes a more stable FTZ protein, has generated a large set of new *ftz* alleles (DUNCAN 1986; KELLERMAN *et al.* 1990). Some of these $ftz^{Ual2}$ revertants exhibit a wild-type phenotype over a *ftz* null allele, and many of these are amino acid changes in the HD that would be expected to abolish HD function (I. DUNCAN, personal communication). Also, $FTZ^{Q50K}$ can rescue FTZ-dependent cuticle in the stripes where it is expressed highly, 4 and 7 (SCHIER and GEHRING 1993a). FTZ HD function is dispensable in two situations: when FTZ protein is expressed at the correct time and at high levels from a heat-shock promoter and when the FTZ protein is more stable, bypassing the need for autoregulation to accumulate high levels of FTZ HD-independent activity.

Expression of the *ftz* enhancer *lacZ* fusion gene is first detected at midcellular blastoderm, and this expression closely mimics the temporal and spatial accumulation of the FTZ protein (YU and PICK 1995). In *ftz* mutants the *ftz* enhancer is not active, and the domain of *ftz* enhancer activity is widened in *hairy* mutants due to ectopic expression of FTZ (HIROMI and GEHRING 1987). We found that ectopically expressed FTZ could only activate the *ftz* enhancer strongly during gastrulation and germ band extension. However, analysis of FTZ protein accumulation from transgenes expressing HD mutant FTZ proteins from *ftz* regulatory sequences shows a low accumulation at late cellular blastoderm stage, suggesting FTZ is required before late cellular blastoderm stage for autoregulation (FURUKUBO-TOKUNAGA 1992; SCHIER and GEHRING 1993a). Then why do we see no activation of the *ftz* enhancer by ectopic expression of FTZ during the cellular blastoderm stage

(Figure 7)? We suspect that transient accumulation of HD-dependent FTZ activity expressed from the *hsp ftz* fusion gene is not sufficient to activate the *ftz* enhancer during the cellular blastoderm stage, or a heat shock during the cellular blastoderm stage stalls development such that even though the FTZ polypeptide accumulates transiently, it still is unable to activate the *ftz* enhancer. But, the transient accumulation of HD-independent FTZ activity during gastrulation and germ band extension is sufficient to activate the *ftz* enhancer. In the experiments of SCHIER and GEHRING, we propose that they were detecting the HD-dependent FTZ activity expressed during cellular blastoderm stage on *ftz* enhancer activation, either because endogenous FTZ is continually produced during the cellular blastoderm stage or because no heat shock had to be administered.

**The biochemical nature of the HD-independent FTZ activity:** Our results demonstrate that FTZ has an important HD-independent activity. Although a DNA-protein interaction mediated by a DNA-binding activity other than the homeodomain may be involved, no DNA-binding activity other than the FTZ HD is detectable (ANANTHAN *et al.* 1993). The HD-independent FTZ activity may involve a protein-protein interaction. This protein-protein interaction would not involve any part of the HD, as a $FTZ^{\Delta 257\text{-}316}$ polypeptide, which completely lacks the HD, can still induce the anti-ftz phenotype (J. HO and A. PERCIVAL-SMITH, unpublished results). A FTZ HD-independent activity is able to activate expression of a reporter gene containing elements derived from the *en* gene in Drosophila S3 tissue culture cells (ANANTHAN *et al.* 1993).

Interaction with the *cis*-regulatory sequences would be mediated by another factor(s) to which FTZ would bind. Our results suggest some properties of this FTZ cofactor(s). The results of the analysis of the *AE-BS2CCC ftz* enhancer element suggest that this FTZ cofactor(s) may recognize the HD DNA-binding site or sites overlapping the HD DNA-binding sites. Hence, the cofactor(s) may be a HD-containing protein; however, the concern that the cofactor may recognize an overlapping DNA-binding site is not trivial, as the small deletion in *AE-BS2CCC* removes a Tramtrack and a FTZ-F1-binding site as well as a FTZ HD DNA-binding site (HAN *et al.* 1993). Also *ftz* enhancer and FTZ-dependent EN expression are activated in spatially restricted domains when FTZ is ectopically expressed suggesting that the activity of the FTZ cofactor(s) is spatially restricted. If the cofactor was one polypeptide and expression was the only way to regulate its activity, a FTZ-binding, HD-containing protein expressed during gastrulation in seven stripes that overlap FTZ-regulated *WG* and FTZ-dependent EN expression domains would satisfy the criteria for the FTZ cofactor.

The *ftz* enhancer can be activated by both FTZ activities, why? A possibility is that the switch from the HD-dependent FTZ activity to the HD-independent FTZ activity is required for the narrowing of the FTZ expression domains during gastrulation. Because the FTZ HD-independent activity may depend on a cofactor(s) whose activity is spatially restricted, the *ftz* enhancer would drive *ftz* expression in a narrower stripe coincident with the FTZ-dependent EN expression domain during gastrulation (LAWRENCE *et al.* 1987).

In previous discussions of FTZ activity, the $\alpha 2$ gene product of the yeast mating type locus *MAT*$\alpha$ was used as a precedent for a combination of DNA-protein and protein-protein interactions in the control of gene expression (SCHIER and GEHRING 1993a). The example cited is the cooperative interaction between the MCM1 and the MAT $\alpha 2$ gene products (KELEHER *et al.* 1988). However, recent investigation of the cooperative interactions between $\alpha 2$ and **a**1 has shown that this protein complex has wild-type activity when the $\alpha 2$ protein can no longer recognize its HD DNA-binding site, and it is proposed that the HD of **a**1 provides a majority of the protein-DNA interaction activity of the $\alpha 2$ **a**1 complex (VERSHON *et al.* 1995). We propose a similar interaction between the HD-independent FTZ activity and an, as yet, unidentified cofactor that may contain a HD.

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