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Autocatalytic *ftz* Activation and Metameric Instability Induced by Ectopic *ftz* Expression

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Summary

Inappropriate expression of the Drosophila pair-rule gene, *fushi tarazu* (*ftz*), causes cuticular pattern deletions apparently complementary to those in *ftz* larvae. We show that the two patterns actually originate similarly, in both cases affecting the even-numbered parasegmental boundaries. The reciprocal cuticular patterns derive from differing patterns of selector gene expression (homoeotic transformations). The primary effect of ectopic *ftz* activity is to broaden *ftz* domains by autocatalytic activation of endogenous *ftz* expression in an additional anterior cell. This activates *engrailed* (*en*) and represses *wingless* (*wg*) expression, consistent with their proposed combinatorial control by *ftz* (and other pair-rule genes) to define parasegmental primordia. We propose that the anterior margin of each *ftz* stripe is normally defined by the posterior *even-skipped* (*eve*) boundary.

Introduction

A major approach to the study of Drosophila segmentation genes has been the analysis of their expression patterns in mutant embryos, ranking genes into a hierarchy according to their interactions. Such analyses suggest that the segmentation genes act to subdivide the embryo into successively more precise spatial domains (Nüsslein-Volhard and Wieschaus, 1980; reviewed in Scott and O'Farrell, 1986; Akam, 1987; Scott and Carroll, 1987; Ingham, 1988). By the end of the blastoderm stage, the embryo is organized into an antero-posterior pattern of 14 reiterated parasegmental primordia, marked at their anterior and posterior margins by rows of cells expressing the segment-polarity genes *en* and *wg*, respectively (Figure 1a; see above reviews for references).

The establishment of these parasegmental domains is dependent on prior action of pair-rule segmentation genes that are expressed in patterns of overlapping stripes. The various hypotheses advanced to explain how pair-rule genes generate normal embryonic pattern tend to fall into two classes:

- models in which each blastoderm cell is assigned a specific "cell identity." In this view, each blastoderm cell acquires a positional value through the combinatorial activity of pair-rule genes expressed in overlapping domains (Gergen and Wieschaus, 1985; Gergen et al., 1986).
- models that emphasize the subdivision of the blastoderm into parasegmental primordia (Howard and Ingham, 1986; Ingham and Martinez-Arias, 1986; Lawrence et al., 1987; Ingham, 1988). Such models stress the establishment of autonomous parasegmental fields, whose boundaries are defined by stripes of *en* expression. Positional values arise secondarily within each parasegmental developmental field.

One line of support for cell identity models comes from experiments that aim to express pair-rule genes throughout the embryo. Struhl (1985) used a hybrid gene *HSF*, in which the pair-rule gene *fushi tarazu* (*ftz*) is controlled by the *hsp70* heat shock promoter, to show that ectopic *ftz* expression causes severe pattern defects. These take the form of cuticular deletions of alternate metameres, those that normally do not express *ftz* (Struhl, 1985; Ish-Horowicz and Gyurkovics, 1988). Thus, the *HSF* cuticular phenotype is roughly complementary to that of homozygous *ftz* embryos, i.e., "anti-*ftz* " (Figure 2). Similarly, an analogous *hsp70-hairy* (*HSH*) construct induces "anti-*hairy*" pair-rule pattern defects (Ish-Horowicz and Pinchin, 1987), and extra copies of the *runt* gene lead to "anti-*runt* " phenotypes (Gergen and Wieschaus, 1986).

The "cell identity" model interprets these cuticular phenotypes in terms of combinatorial codes of pair-rule genes (Gergen and Wieschaus, 1986). Nonsense codes, whether caused by a mutated pair-rule gene or by ectopic pair-rule expression, lead to the loss of positional values and thence to cell death. This model predicts that the pattern deletions in pair-rule mutations arise from the elimination of primordia in which the genes would normally be expressed. Similarly, it predicts that the reciprocal pattern deletions caused by inappropriate pair-rule gene expression is due to nonsense combinatorial codes in the alternate primordia. For example, the model implies that *HSF* embryos should be *ftz*-reciprocal (Struhl, 1985): as *ftz* embryos lack the even-numbered stripes, *HSF* embryos should lack the odd-numbered ones.

In contrast, parasegmental models explain mutant phenotypes in terms of establishing the wrong number of inappropriately sized primordia. For example, *ftz* mutant embryos establish only alternate (even-numbered) *en* bands and divide the embryo into half the normal number of metameres (Howard and Ingham, 1986; DiNardo and O'Farrell, 1987; Martinez-Arias and White, 1988). This class of model predicts that ectopic gene expression affects pattern by inhibiting the establishment of appropriate

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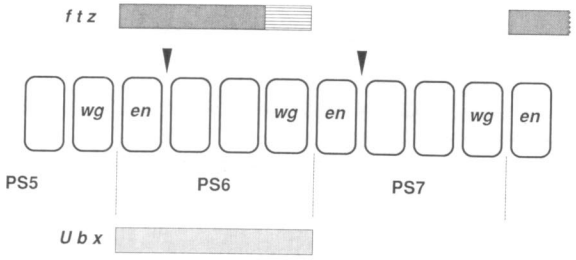

Figure 1. Diagrammatic Representation of Parasegmental Metameres, the Initial *en*- and *wg*-Expressing Cells, the *ftz* Stripe Domains, and the Parasegmental PS6 *Ubx* Domain

Differential shading of the posterior-most *ftz* cell reflects narrowing of the *ftz* stripes just before gastrulation (DiNardo et al., 1985). Arrowheads mark the segmental metamere, one cell posterior to the parasegment (Martinez-Arias and Lawrence, 1985).

metameric boundaries. Duncan (1986) has proposed a model for the *HSF* cuticular phenotype whereby uniform *ftz* expression eliminates anterior *ftz* margins and thereby the even-numbered *en* domains.

Clearly, such models for pair-rule function can only be distinguished by analyzing patterns of segmentation gene expression as well as cuticular phenotypes. We have previously shown that the *HSH* phenotype is not due to deletion of the *h*-reciprocal primordia. Rather, the pair-rule cuticular phenotype arises because the ectopic *h* represses *ftz* expression, leading to loss of the *ftz*-dependent *en* stripes and a failure to define alternate parasegmental boundaries (Ish-Horowicz and Pinchin, 1987). Although these results are consistent with parasegmental models, they do not exclude models in which *ftz* but not *h* acts in defining combinatorial cell identities.

In this paper, we analyze segmentation gene expression in heat-shocked *HSF* embryos to distinguish between the above models and to define the origin of the pair-rule defects. We show that the *HSF* "anti-*ftz*" cuticular phenotype is seriously misleading because *HSF* and *ftz* embryos do not delete complementary primordia. Rather, *ftz* and *HSF* are divided into similar metameric domains that develop reciprocal segmental characters due to different patterns of selector (homoeotic) gene expression. The effects of *HSF* on pattern are best explained by a model in which combinatorial action of *ftz* and other pair-rule genes establishes parasegmental boundaries defined by adjacent *en* and *wg* domains (Ingham, 1988).

Results

Altered *en* Patterns and Metameric Instability in Heat-Shocked *HSF* Embryos

In wild-type gastrulae, *en* protein is expressed in 14 evenly spaced bands along the antero-posterior axis (Figure 3B). Surprisingly, all 14 *en* stripes are also present in heat-shocked *HSF* (hs-*HSF*) embryos (Figure 3A), in contrast to the final pair-rule cuticular phenotype. However, the pattern is not normal, each even-numbered domain being broader and closer to their anterior neighbors. The stripe widths show that this is due to an extra anterior cell expressing *en* (see Experimental Procedures; Figure 8). The ectopic *en* expression is visible within 35 min of the heat shock, suggesting that it results directly from *HSF*-induced

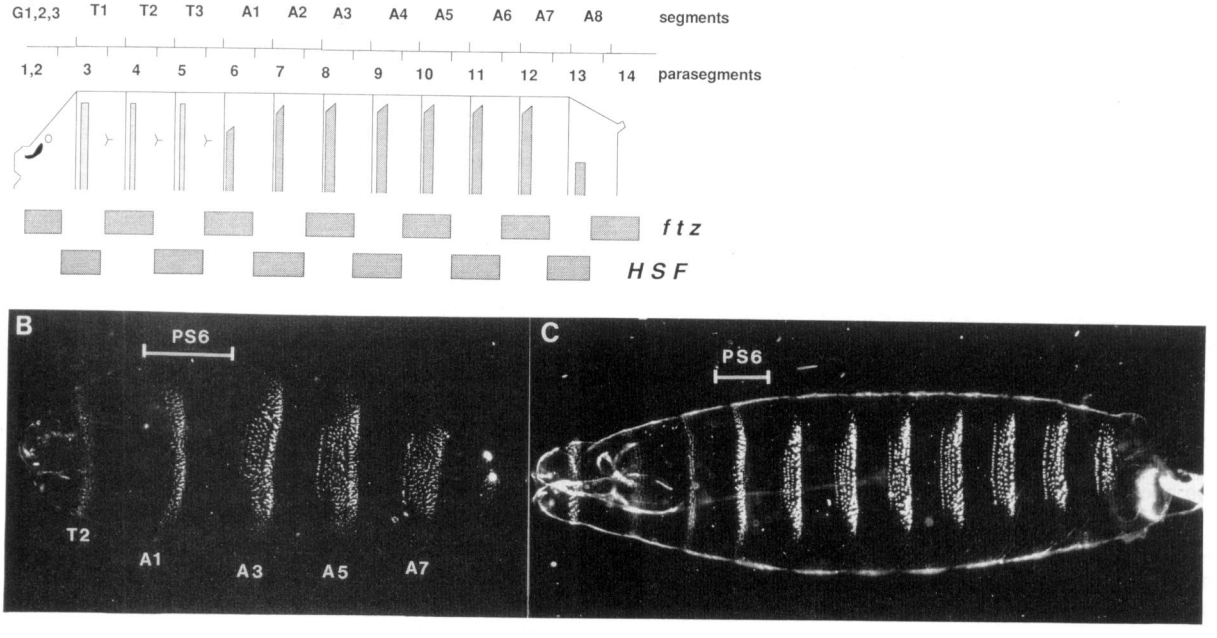

Figure 2. *HSF* Pair-Rule Phenotype

(A) The bars represent the regions phenotypically deleted in pair-rule *ftz* and *HSF* embryos (Wakimoto et al., 1984; Ish-Horowicz and Gyurkovics, 1988, respectively). Dark field views of (B) heat-shocked *HSF/HSF* and (C) +/+ ventral cuticles.

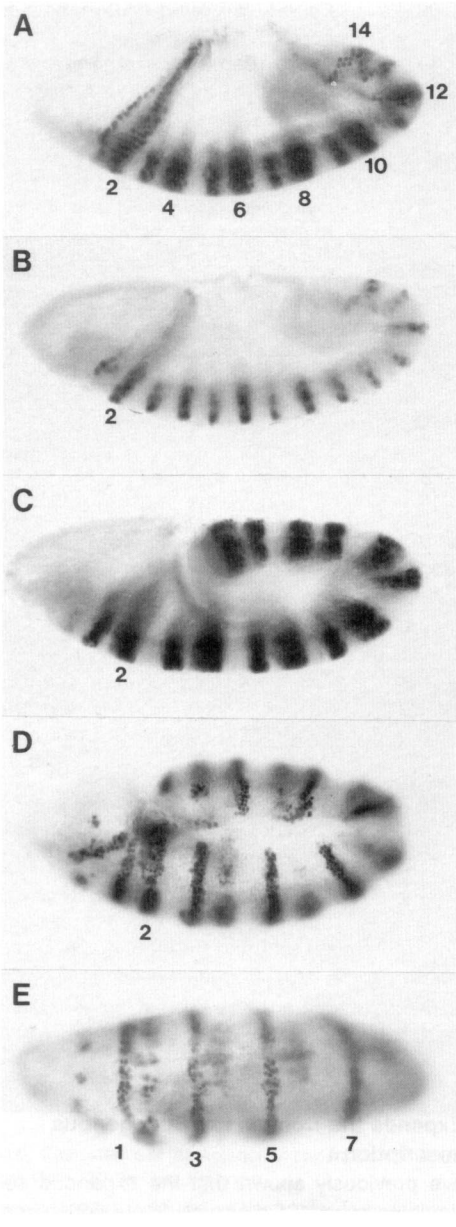

Figure 3. Patterns of *en* Expression in Heat-Shocked *HSF* (hs-*HSF*) and +/+ Embryos

(A) hs-*HSF* embryo fixed 35 min after the start of a 10 min/36°C heat shock; (B) heat-shocked +/+ embryos, fixed after 40 min; (C, D, and E) hs-*HSF* embryos fixed after 50 min (C), 120 min (D), and 2.75 hr (E), a ventral view. Note that initial intensity differences in wild-type embryos between even- and odd-numbered *en* stripes soon diminish (Weir and Kornberg, 1985; DiNardo et al., 1985). Unless otherwise indicated, embryos are orientated antero-dorsally at the upper left.

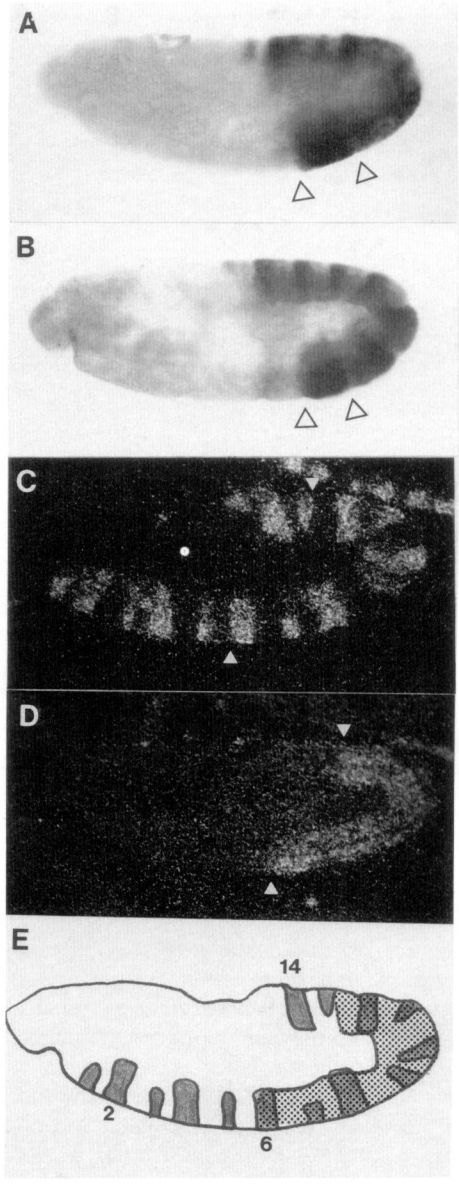

Figure 4. *Ubx* and *en* Expression in hs-*HSF* Embryos

Ubx protein staining in: (A) an hs-*HSF* embryo fixed 2 hr 40 min after heat shock, with the arrowheads indicating PS5/6; and (B) a non-heat-shocked +/+ embryo, with the arrowheads indicating PS6. (C and D) In situ hybridization of adjacent sections of an *HSF* embryo fixed 40 min after heat shock: (C) *en*; (D) *Ubx*. (E) Diagram indicating a superimposition of the *en* and *Ubx* domains from the above sections. The sections were drawn with a camera lucida, and the embryo outlines were aligned manually. Note broadening of the even-numbered *en* stripes, as seen with the *en* antibodies (Figure 3).

ftz expression and persists through germ band extension (Figure 3C).

Soon after hs-*HSF* embryos have completed germ band extension (5 hr postfertilization; 2 hr post–heat shock), *en* expression in the broader even-numbered stripes decays and is essentially extinguished by about 6 hr (Figures 3D and 3E). Such embryos now show only seven *en* stripes, and appear organized into seven double-sized metameres PS1/2, PS3/4, PS5/6, etc. Thus, the pair-rule metameric organization arises before the onset of germ band retraction.

Homeotic Transformations in *HSF* Embryos

Strikingly, in both hs-*HSF* and *ftz* mutant embryos, it is the odd-numbered *en* stripes that are retained, so that the fi-

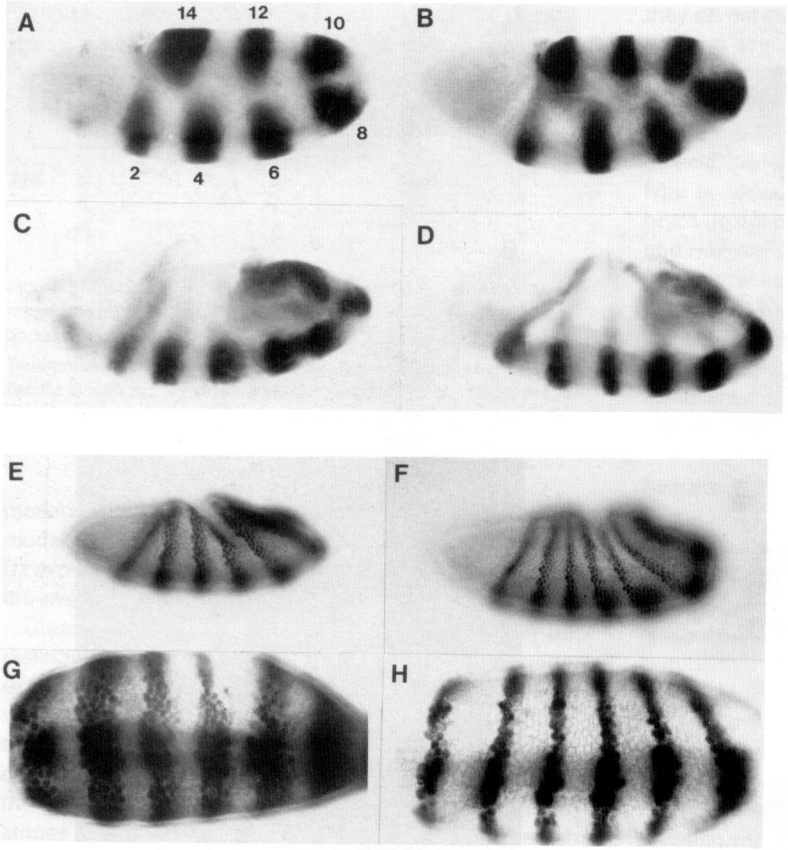

Figure 5. Broadened *ftz* Domains in hs-*HSF* Embryos

Even-numbered parasegments were marked with the *ftz-lacZ* construct (Hiromi et al., 1985; Lawrence et al., 1987; Carroll et al., 1988a). (A–D) *lacZ* expression in heat-shocked (A and C) and non-heat-shocked (B and D) *HSF;ftz-lacZ* embryos. (A) Fixed after 120 min; (C) fixed after 30 min of *ftz* protein staining. (E and G) Heat-shocked *HSF* embryos fixed after 35 min, and (F and H) non-heat-shocked embryos. The embryos in (C–H) would have been heat shocked at blastoderm.

nal metameric organizations of hs-*HSF* and *ftz* embryos are similar, not reciprocal. Their contrasting cuticular phenotypes suggest that the two genotypes must differ in their selector gene patterns. We therefore analyzed the expression of *Ubx* protein, which is expressed at characteristically high levels in PS6 but largely lacking in PS5 (Figures 1 and 4B; White and Lehmann, 1986). In extended germ band (5.5–6 hr) hs-*HSF* embryos, both PS5 and PS6 show the intense *Ubx* expression that is normally confined to PS6 (Figure 4A). This explains why the fused PS5/6 metamere develops as PS6 (Figure 2B). In contrast, *ftz* mutant embryos show little *Ubx* in PS5/6, corresponding to its development into a PS5 parasegment (Ingham and Martinez-Arias, 1986). The different segmental characters of PS5/6 in hs-*HSF* and *ftz* embryos are due to differential selector gene expression. The domains of *Sex-combs reduced*, a selector gene expressed in PS2, is extended in hs-*HSF* embryos (data not shown), indicating that the other metameres are similarly affected. Thus, the reciprocal cuticular phenotypes of *ftz* and hs-*HSF* embryos are not due to their subdivision into different metameric patterns but to differences in their patterns of selector gene expression.

Such inappropriate selector gene expression is not directly due to ectopic *ftz* expression. Forty minutes after the heat shock, when effects on *en* expression are already evident (Figures 3A and 4C), the anterior boundary of *Ubx* transcription has not yet encroached into PS5 and still corresponds to the sixth *en* band (Figures 4C, 4D, and 4E). Broadening of the *Ubx* domain arises subsequent to changes in segmentation gene expression, indicating that it is not a primary consequence of the ectopic *ftz* induction.

HSF Expands the Domains of Endogenous *ftz* Transcription

We have previously shown that the expanded selector gene domains in hs-*HSF* embryos do not derive from loss of the odd-numbered parasegmental primordia (Ish-Horowicz and Gyurkovics, 1988). We followed the fates of the even-numbered parasegments using a *ftz*-β-galactosidase (*ftz-lacZ*) fusion gene in which the *ftz* domains are marked by *lacZ* expression (Hiromi et al., 1985). This showed that hs-*HSF* embryos retain both unstained (odd PS's) and stained (even PS's) cells (Figure 5A; Ish-Horowicz and Gyurkovics, 1988).

However, the pattern of *lacZ* staining is not completely normal. In wild-type embryos, the stained domains are half as wide as the unstained domains (Figures 5B and 5D), reflecting narrowing of the *ftz* stripes during germ band extension (Carroll and Scott, 1985; Carroll et al., 1988a). In contrast, the *lacZ* stripes in pair-rule *HSF* embryos are equal to or broader than the unstained domains (Figures 5A and 5C). The *ftz-lacZ* staining reflects the pattern of endogenous *ftz* transcription (Hiromi et al., 1985; Hiromi and Gehring, 1987), so the enlarged *lacZ* stripes

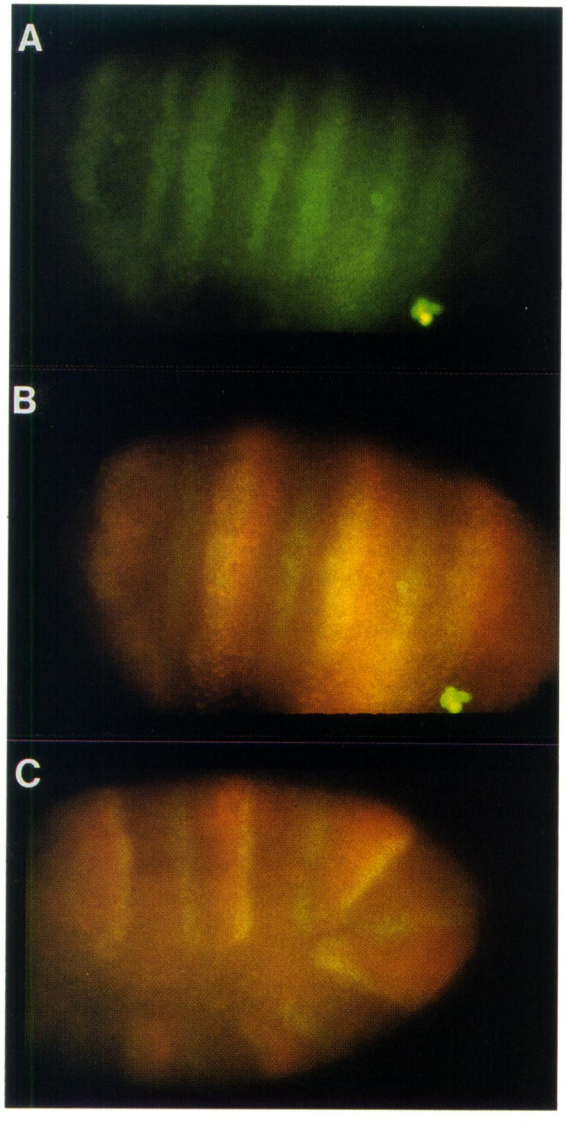

Figure 6. Coincidence of the Anterior *en* and *ftz* Domains in Wild-Type and Pair-Rule *HSF;ftz-lacZ* Embryos

The common anterior *ftz* and *en* boundaries indicate that each advance by a single cell. (A) A pair-rule *HSF* embryo illuminated to visualize its pattern of alternate broadened *en* domains (fluorescein); (B) a double exposure showing both *en* (fluorescein) and *lacZ* (rhodamine—marking the *ftz* domains). Overlap of the domains are seen as orange. (C) A wild-type pattern photographed as in (B).

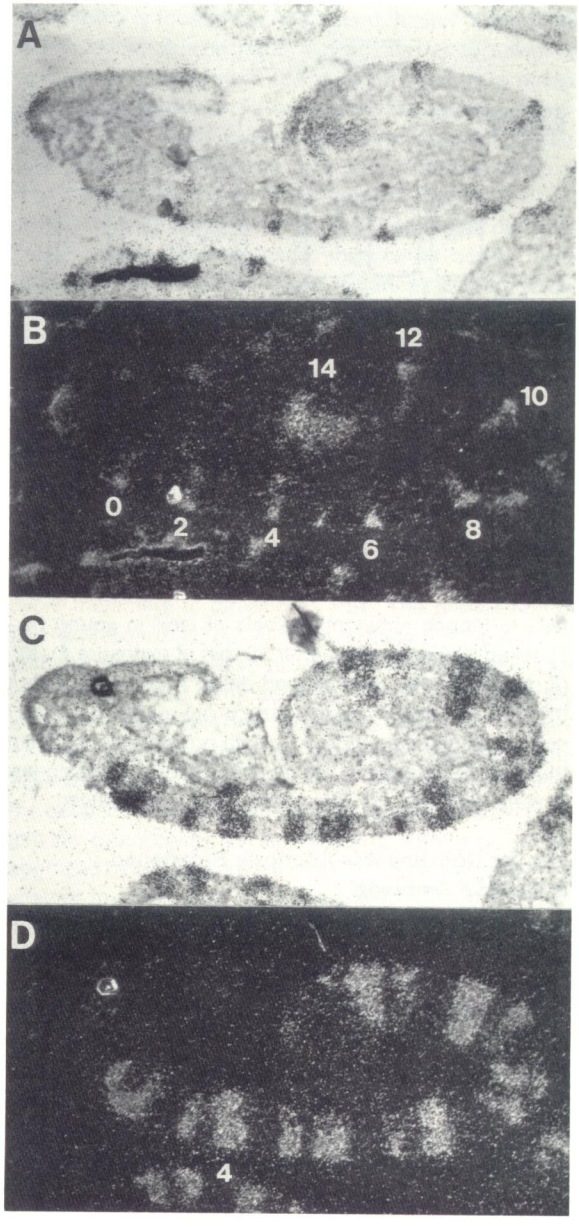

Figure 7. In Situ Hybridization of *wg* and *en* Transcription in Heat-Shocked *HSF* Embryos

(A) and (B) show *wg* transcription, fixed after 60 min; (C) and (D) show *en* transcripts in an adjacent section of the same embryo. (A and C) Bright field; (B and D) dark field.

indicate broadened activity of endogenous *ftz* promoters. Such activation is evident within 30 min of the heat shock (Figure 5C), arguing that it is directly due to the effect of *HSF*-encoded *ftz*.

The ectopic activation of the *ftz* promoter revealed by the *ftz-lacZ* fusion gene is mirrored by an alteration in *ftz* protein distribution. The stained domains broaden so that they are at least equal to the unstained domains (Figures 5E and 5G), contrasting with the narrower wild-type *ftz* stripes (Figures 5F and 5H). Thus, the *HSF* gene induces high-level *ftz* expression in a spatially restricted set of cells.

The broadened bands of *ftz* protein is not due to periodic expression from the *hsp70* promoter because in situ hybridization of heat-shocked *HSF* embryos shows transient *ftz* mRNA induction throughout the embryo (I. Davis, unpublished data). They are due to activation in specific cells of the endogenous *ftz* promoter, i.e., autocatalysis.

The hs-*HSF* embryos still show a clear striped pattern, showing that interstripe *ftz* levels are well below those from the endogenous gene (Figure 5E). Thus, the *hsp70* promoter must be relatively poorly induced at blastoderm by our mild heat shocks. Similarly, an *hsp70-lacZ* gene

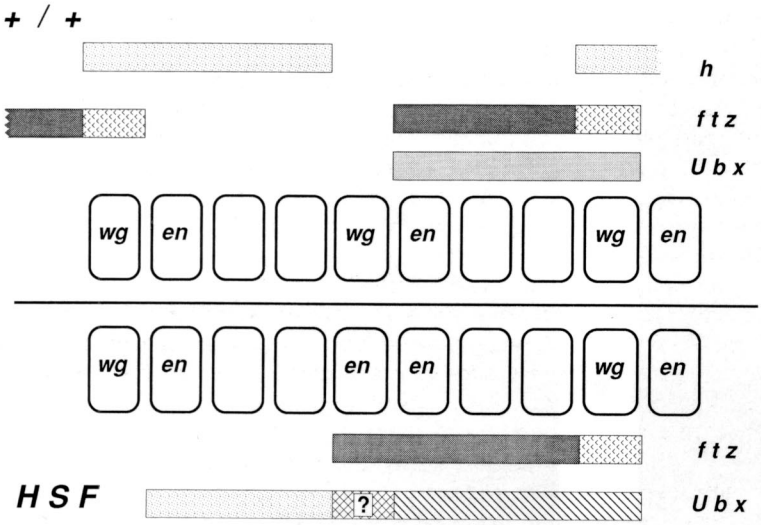

Figure 8. Diagram of the Effects of Heat Shock on Segmentation Gene Expression in Pair-Rule *HSF* Embryos

One cell/double metamere expresses neither *h* nor *ftz* (Ish-Horowicz et al., 1985; Ingham et al., 1985; Carroll et al., 1988b; X. Hooper and D. Ish-Horowicz, unpublished data).

fails to induce substantial levels of *lacZ* in young heat-shocked embryos, even after 20 min of exposure to 36°C (S. M. Parkhurst, unpublished data). Protein products of the *HSF* and *hsp70-lacZ* constructs are readily detected in older heat-shocked embryos (data not shown), indicating that the inefficiency of the *hsp70* promoter at blastoderm is stage-specific, not due to the fusion constructs.

The *ftz* Domains Are Interiorly Broadened in hs-*HSF* Embryos

In wild-type embryos, the anterior margins of *ftz* and *en* expression coincide, arguing that *ftz* defines the anterior extent of the even-numbered *en* stripes (Lawrence et al., 1987; Carroll et al., 1988a). Thus, the alternate broadened *en* stripes in hs-*HSF* embryos could be due to a similar anterior expansion of the *ftz* domains. This is confirmed by covisualizing *en* (green) and *ftz* (red) domains by immunofluorescence (Figure 6). The *ftz* domains undergo dramatic narrowing during gastrulation, when *en* is first expressed, so we viewed them indirectly using the β-galactosidase of the *ftz-lacZ* gene. The broadened *en* domains in hs-*HSF* embryos stain orange (green plus red), showing that they also express *ftz*, whereas the cells anterior to the orange stripes are unstained, indicating that the anterior *en* and *ftz* borders coincide (Figure 6A). As even-numbered *en* stripes broaden anteriorly by a single cell, the enlarged *ftz* domains in hs-*HSF* embryos must be due to *ftz* expression in an extra anterior cell.

Broadened *en* and *ftz* Domains Are Associated with *wg* Repression

At the onset of gastrulation, cells expressing *wg* lie immediately anterior to those that express *en* (Ingham et al., 1988). Thus, the cells in which ectopic *ftz* and *en* are induced correspond to the odd-numbered *wg* stripes (Figure 1). *ftz* behaves as a repressor of *wg* (Ingham et al., 1988), so broadened *ftz* expression might be expected to suppress alternate *wg* stripes. Analysis of *wg* transcription in pair-rule *HSF* embryos by in situ hybridization confirms this prediction, with such embryos showing only seven domains of *wg* transcripts (Figures 7A and 7B). Hybridizing adjacent sections with an *en* probe shows that the even-numbered *en* stripes are broadened and confirms that the missing *wg* bands correspond to the domains of ectopic *en* expression (Figures 7C and 7D). Indeed, lack of adjacent *wg* bands can explain the subsequent instability of the broadened *en* bands, *wg* being required for the maintenance of *en* expression (DiNardo and O'Farrell, 1987; Martinez-Arias et al., 1988).

Discussion

The *HSF* Phenotype Is Not Reciprocal to That of *ftz*

Our analysis provides a clear explanation of the hs-*HSF* cuticular phenotype. The primary consequence of heat shock–induced *ftz* is an expansion of the *ftz* domains by an extra anterior cell (Figures 5E, 5G, 6, and 8). This causes the most posterior cell of each odd-numbered parasegment to express *en* instead of *wg*, thereby becoming incorporated into the even-numbered *en* stripes (Figures 3, 7, and 8). These stripes fade prematurely towards the end of germ band extension, halving the number of metameres (Figure 3E). At the same time, selector gene domains enlarge, causing homoeotic transformations of the fused pair-rule metameres into even-numbered parasegments (Figures 4A and 8). Thus, a *ftz*-like metameric organization is converted by homoeotic transformations into an apparently reciprocal cuticular phenotype.

The rapid changes in *en* and *wg* expression suggest that their regulation by *ftz* is direct. This is in accordance with the previous evidence that *ftz* behaves as an activator of the even-numbered *en* bands and represses *wg* (Howard and Ingham, 1986; Ingham and Martinez-Arias, 1986; DiNardo and O'Farrell, 1987; Martinez-Arias and White, 1988; Ingham et al., 1988). *wg* repression is slightly delayed relative to the appearance of ectopic *en*, being partially evident after 40 min (data not shown) and complete within 60 min (Figures 7A and 7B). This may reflect

temporary persistence of *wg* transcripts made before the onset of ectopic *ftz* expression.

The above observations fit easily into a model for segmentation in which *ftz* and other pair-rule genes act combinatorially to establish adjacent *en* and *wg* domains that define parasegmental primordia (Ingham, 1988). In this model, parasegmental boundaries arise only between adjacent *en* and *wg* domains, each of these segment-polarity genes being required to sustain the other's expression (Martinez-Arias et al., 1988; DiNardo and O'Farrell, 1987). In hs-*HSF* embryos, the ectopic *ftz* expression directly activates *en* and represses *wg*, leading to the broadened *en* bands. The pair-rule phenotype arises because the even-numbered *en* bands in hs-*HSF* embryos are unsupported by *wg* cells and unable to form parasegmental boundaries.

Other models do not account for segmentation gene patterns in hs-*HSF* embryos. The cell identity model predicts deletion of the odd-numbered parasegments (Struhl, 1985; Gergen et al., 1986; Gergen and Wieschaus, 1986), but staining of these parasegments with *lacZ* shows that they are still present in hs-*HSF* embryos (Figure 5A; Ish-Horowicz and Gyurkovics, 1988). Nor does this model explain why expanding *ftz* domains by one cell should give rise to extensive pair-rule deletions.

Models that propose that parasegments are defined by the margins of pair-rule domains also fail to explain the hs-*HSF* phenotype. Lawrence (1987) has proposed that parasegmental boundaries are defined solely by anterior margins of *eve* and *ftz* expression. According to this model, shifting the *ftz* domain by an anterior cell should displace the margins (as indeed occurs for *en*), but the parasegments should then size-regulate and develop normally, not give a pair-rule phenotype.

Duncan (1986) correctly described the hs-*HSF* cuticular phenotype in terms of homoeotically transformed double parasegments (e.g., PS5/6). However, his model predicts that individual parasegments (e.g., PS5, PS6) would never form, whereas the establishment of all 14 *en* stripes shows that PS5 and PS6 are initially distinguished. The initial expression of *Ubx* in PS6 but not PS5 confirms that the hs-*HSF* phenotype is not due to a failure to subdivide double parasegments.

Local Action of the *wg* Protein

The even-numbered *en* bands decay at the end of germ band extension, at the same time that *en* expression is lost in *wg* mutant embryos (Martinez-Arias et al., 1988; DiNardo et al., 1988). This suggests that the decay of *en* expression in the broadened stripes is due to the lack of adjacent *wg*-expressing cells. This action of the *wg* gene-product must be strictly localized, as the remaining *wg* stripes do not rescue *en* expression in the enlarged stripes that are only four cells away (Figure 8; by the end of germ band extension, a postblastoderm mitosis doubles the number of intervening cells; Hartenstein and Campos-Ortega, 1985).

The *wg* protein is closely related to the protein encoded by the *int-1* oncogene and has the characteristics of a secreted protein, perhaps a growth factor (Rijsewijk et al., 1987). If *wg* is indeed secreted, its diffusion range must be low, either due to rapid degradation or to selective retention by targets near the cells in which it is made. Short-range *wg* action is consistent with models for intrametameric pattern involving localized cell–cell interactions rather than long-range gradients (Martinez-Arias et al., 1988; DiNardo et al., 1988; Ingham, 1988). Localized action of growth factors has significance for other developmental systems that demand short-range order. Thus, compartmentalization of growth factors by binding to extracellular heparan sulphate is indicated for fibroblast-derived growth factors, granulocyte/macrophage colony stimulating factor, and interleukin-3 (Folkman and Klagsbrun, 1987; Roberts et al., 1988).

Segment-Polarity Genes Affect Selector Gene Domains

The homoeotic transformations in hs-*HSF* embryos are due to inappropriate selector gene expression (Figure 4A). Thus, ectopic *Ubx* expression in PS5 leads the fused PS5/6 metamere to develop as PS6. The broadening of *Ubx* expression is not directly induced by the *HSF*-encoded *ftz*, as it arises at least 60 min after the heat shock (Figure 4E). This does not preclude *ftz* normally acting to define selector expression domains in even-numbered parasegments as has been previously suggested (Ingham and Martinez-Arias, 1986; Duncan, 1986). The heat-shocked *HSF* gene may induce insufficient *ftz* to activate *Ubx* directly, or its effects in PS5 may be antagonized by *eve* protein. It will be interesting to determine whether the high-level *ftz* in the posterior-most PS5 cell causes elevated *Ubx* expression (Figure 8), but a single-cell expansion of the PS6 *Ubx* domain is beyond the resolution of the in situ hybridization technique, and the *Ubx* domain expands into PS5 before *Ubx* protein is detectable.

It is tempting to ascribe the indirect destabilization of selector gene domains seen in hs-*HSF* embryos to altered patterns of segment-polarity expression (e.g., *wg*) induced by the ectopic *ftz*. Although we cannot yet provide a mechanism for such effects, a role for segment-polarity genes in maintaining selector gene domains provides an attractive basis for refining their extents and ensuring accurate coregistration with metameres, as well as allowing the elaborate pattern of *Ubx* expression that becomes evident later in development (White and Wilcox, 1984; Beachy et al., 1985).

Autoactivation of the Endogenous *ftz* Promoter by *HSF*

The primary consequence of heat shock–induced *ftz* expression is to activate the endogenous *ftz* promoter in particular cells, as shown by the altered *lacZ* patterns in heat-shocked *HSF;ftz-lacZ* embryos (Figures 5A and 5C). Thus, the broadened *ftz* bands in hs-*HSF* embryos are due to low levels of *HSF*-encoded *ftz* protein stimulating the endogenous *ftz* promoter, i.e., through autocatalytic *ftz* activation in specific cells. Hiromi and Gehring (1987) showed that the *ftz* promoter includes a *ftz*-dependent transcriptional enhancer and that *ftz-lacZ* expression is

strongly reduced or abolished in *ftz* mutant embryos. Our demonstration of autoactivation of *ftz* expression in hs-*HSF* embryos confirms their proposal of positive feedback control of *ftz* transcription.

Duncan (1986) has described *ftz* alleles (*ftz*^{Ua1}) causing segment defects and homoeotic transformations that he suggested might be due to broadened *ftz* domains. Such alleles appear to encode abnormally stable proteins (I. Duncan and S. B. Carroll, personal communication) that could result in broadened stripes through *ftz* autoactivation as seen for hs-*HSF*. We have found that heat shock of postblastoderm *HSF* embryos induces a high frequency of homoeotic transformations in surviving adult flies (H. G. Gyurkovics and D. Ish-Horowicz, unpublished data), indicating that deformation of metameric boundaries can lead to local perturbations of selector gene expression.

Our results show that only some cells activate their endogenous *ftz* promoters in response to ectopic *ftz* expression. The specificity is explained by the pattern of expression of *h*, a negative regulator of *ftz* expression (Howard and Ingham, 1986; Carroll and Scott, 1986; Ish-Horowicz and Pinchin, 1987). In normal embryos, the *h* and *ftz* domains overlap such that one stripe of cells per double-segment expresses neither *h* nor *ftz* (Ish-Horowicz et al., 1985; Ingham et al., 1985; Carroll et al., 1988b; Hooper and Ish-Horowicz, unpublished data). It is these cells that activate *ftz* in hs-*HSF* embryos, indicating that *h* prevents *ftz* autocatalysis in the other cells (Figure 8). Nonresponding cells near the anterior and posterior poles are under the influence of "polar repressors" that inhibit normal *ftz* expression at the egg termini (Hiromi et al., 1985; Edgar et al., 1986).

The Anterior *ftz* Boundary Is Defined by *eve*

Why do the set of cells that are sensitive to *HSF* induction not express *ftz* constitutively in wild-type embryos? It is not because they previously lacked *ftz* protein to activate their promoters, because all nuclei between 15% and 65% egg-length express *ftz* early in nuclear cycle 14 (Hafen et al., 1984; Weir and Kornberg, 1985; Karr and Kornberg, submitted). The *HSF*-sensitive cells must express an inhibitor that prevents positive feedback and continued *ftz* expression. Either the repressor is initially present in all cells (and active only in some for the *ftz* pattern to evolve) or it is expressed with pair-rule periodicity, i.e., is itself the product of a pair-rule gene. Moreover, this repressor must have decayed in these cells by the end of the blastoderm stage, when the embryos become sensitive to *HSF*-encoded *ftz*.

One known pair-rule gene fulfills these criteria: *even-skipped* (*eve*). It is initially expressed in adjacent and complementary stripes to those of *ftz* but, like *ftz*, it retracts from four to three cells before the end of blastoderm (Figure 8; Harding et al., 1986; MacDonald et al., 1986; Frasch et al., 1987). If *eve* acts to antagonize *ftz* transcription at the anterior *ftz* boundary, the *ftz* domain should extend more anteriorly in *eve* embryos. This indeed appears to be the case. The relative phasings of *eve* and *ftz* protein domains appear perturbed in *eve* embryos, consistent with anterior extension of the *ftz* domain (Frasch et al., 1988). Moreover, *ftz*^{Ua1}/+ and *eve*/+ interact to give an *HSF*-like pair-rule phenotype, as might be expected if heterozygous *eve* embryos are unable to repress the more stable *ftz*^{Ua1} protein (I. Duncan, personal communication). Finally, direct observation of *ftz* domains in embryos mosaic for *eve* nuclei show anteriorly broadened *ftz* stripes (P. A. Lawrence, personal communication). These observations support the notion that *eve* plays a role in defining the parasegmental *ftz* metamere.

Indeed, one simple model for the interaction is that *eve* interferes with *ftz* positive feedback at the upstream activating site (Hiromi and Gehring, 1987), perhaps through common binding sites (Desplan et al., 1985; Hoey and Levine, 1988). Autoregulatory positive feedback of pair-rule transcription may serve not only to ensure discrete high-level expression of pattern-forming genes but also as a mechanism to define precise boundaries between adjacent metameres (Meinhardt, 1982).

Experimental Procedures

The *HSF* flies were the generous gift of Gary Struhl. Both *HSF2* and *HSF3* stocks behave similarly (Struhl, 1985). Experimental procedures were essentially as previously described (Ish-Horowicz and Pinchin, 1987). To summarize, embryos were staged manually under halocarbon oil and subjected for 10 min at 36°C. Such a heat shock of wild-type embryos causes no pattern defects, nor does it affect segmentation gene expression. The heat-shocked *HSF* embryos were either fixed for immunohistochemical staining or allowed to develop for cuticular analysis. In situ hybridization to transcripts were performed using single-stranded RNA probes as previously described (Ingham et al., 1985).

Essentially all *HSF* embryos that gastrulate 0–40 min from the start of the heat shock develop a prototype pair-rule cuticular phenotype. The phenotypes of embryos that enter gastrulation before the heat shock are similar, although expressivity is somewhat reduced. Indeed, some *HSF* embryos that gastrulate 30 min before the heat shock still show weak segmentation defects (unpublished data). (*HSF*/+ embryos behave similarly, although phenotypic expression and penetrance is slightly weaker than for homozygotes.)

Initial experiments used cohorts of manually staged pair-rule embryos, aged to ensure that they are all pair-rule. In situ hybridization and double-label immunofluorescence were done with 60 min egg-lays, and pair-rule embryos were identified from their patterns of *en* expression (see text).

Antibodies to *en*, *Ubx*, and *ftz* were described previously (DiNardo et al., 1985; White and Wilcox, 1984; Krause et al., 1988). β-galactosidase protein was detected using either monoclonal antibody 4C7 (gift of H. Durbin) at 1:5 or a monoclonal anti-β-galactosidase provided by Dr. C. Doe. The double-label immunofluorescence used rabbit polyclonal anti-*en* antibody and monoclonal anti-β-galactosidase antibody, followed by fluorescein-labeled goat anti-rabbit and rhodamine-coupled horse anti-mouse secondary antibodies. The embryos were photographed with appropriate filters using Fujichrome 400D film.

Estimating *en* Band Widths

The widths of *en* bands in wild-type and heat-shocked *HSF* embryos were estimated by measuring the widths of the stained and unstained domains using an eyepiece graticule. We corrected for the irregularity of the *en* boundaries (DiNardo et al., 1985) by normalizing the staining pattern to a wild-type stained:unstained ratio of 1:3. For the affected metameres in pair-rule *HSF* embryos, this gave a ratio of 2:2, i.e., an extra anterior *en*-expressing cell.

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