Cell, Vol. 45, 113–126, April 11, 1986, Copyright © 1986 by Cell Press

Zygotically Active Genes That Affect the Spatial Expression of the *fushi tarazu* Segmentation Gene during Early Drosophila Embryogenesis

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Summary

The establishment of the segmental body pattern of Drosophila requires the coordinated functions of three classes of zygotically active genes early in development. We have examined the effects of mutations in these genes on the spatial expression of the *fushi tarazu* (*ftz*) pair-rule segmentation gene. Mutations in four gap loci and in three pair-rule loci dramatically affect the initial pattern of transverse stripes of *ftz*-containing nuclei. Five other pair-rule genes and several other loci that affect the larval cuticular pattern do not detectably affect *ftz* expression. No simple regulatory relationships can be deduced. Rather, expression of the *ftz* gene depends upon the interactions among the different segmentation genes active at each position along the anterior–posterior axis of the early embryo.

Introduction

Systematic genetic screens designed to identify genes active in the zygote that are essential to normal body pattern formation have identified about 20 loci that appear to control the number and orientation of body segments in Drosophila melanogaster (Nüsslein-Volhard and Wieschaus, 1980; Nüsslein-Volhard et al., 1984; Jürgens et al., 1984; Wieschaus et al., 1984a). The loci have been classified into three groups: the gap loci controlling the formation of several body segments; pair-rule loci affecting pattern formation in pairs of segments; and segment polarity loci affecting the development of pattern elements within each segment. Early pattern-forming regulatory events in the embryo are also dependent upon the proper function of maternal-effect loci that act during oogenesis to organize the major dorsal–ventral and anterior–posterior axes of the embryo (Nüsslein-Volhard, 1979; Anderson and Nüsslein-Volhard, 1984). Thus, a genetic network that involves both maternally and zygotically active genes initiates the determination of cell fate in the embryo. It is important to determine what interactions occur between the regulatory genes during development. The genes may function in an interconnected hierarchy, or may operate independently to establish cell fate. Direct analysis of the effects of mutations in each segmentation gene on the expression of the others will be necessary to understand the interactions required for normal development.

One well studied pair-rule locus is the *fushi tarazu* (*ftz*) gene of the Antennapedia Complex (ANT-C) (Kaufman et al., 1980; Wakimoto and Kaufman, 1981; Wakimoto et al., 1984). Embryos homozygous for null alleles of *ftz* form only about one-half the normal number of segments and die after producing cuticle. Molecular analysis of the *ftz* gene (Weiner et al., 1984; Kuroiwa et al., 1984) and the sequence of its coding region (Laughon and Scott, 1984) have revealed it to have a relatively small (1.95 kb) transcription unit that is expressed maximally at 2–4 hr of development. In situ hybridization of *ftz* DNA probes to sectioned embryos indicated that expression of the RNA transcript begins during the syncytial blastoderm stage of development and resolves into a pattern of seven transverse stripes even before the cell membranes are completed (Hafen et al., 1984). Immunological staining of whole embryos localized the *ftz* protein to the nuclei of the cells within the stripes (Carroll and Scott, 1985). After the *ftz* protein disappears from the embryo, during germ band extension, it reappears in a subset of the nuclei in every segment of the developing nervous system (Carroll and Scott, 1985).

How the temporal and spatial pattern of *ftz* expression is established is not known. It seems likely that the establishment of the spatial pattern of *ftz* expression involves other zygotically active segmentation genes that are expressed in distinct patterns that partially overlap *ftz* and each other (Gergen et al., 1985). We have analyzed the effect of mutations in other zygotically active segmentation genes on the pattern of *ftz* protein expression to detect interactions between these genes and *ftz*. Since the effect of segmentation gene mutations on the spatial expression of *ftz* is observed at the blastoderm stage, at the time of cell fate determination and hours before segments become visible, the patterns of *ftz* expression are not complicated by secondary events such as cell death or pattern regeneration. Furthermore, the use of antibody probes in whole mount embryos allows the patterns to be observed with single cell resolution. We find that seven of the previously identified zygotically active segmentation genes influence the pattern of *ftz* expression at the cellular blastoderm stage of embryogenesis, whereas seven other segmentation genes and three embryonic pattern formation genes do not affect the *ftz* protein patterns. The results support the idea of a hierarchy among the segmentation genes and reveal that the establishment of position-specific expression of the *ftz* protein involves combinations of segmentation genes that act in distinct partially overlapping domains along the anterior–posterior axis of the embryo.

Results

The Wild-Type Pattern

It is first possible to detect *ftz* protein after the thirteenth syncytial nuclear division, and during cellularization, as a series of seven evenly spaced transverse stripes each about four nuclei wide (Carroll and Scott, 1985; Figure 1a). At the initiation of gastrulation the stripes narrow to about three nuclei wide, whereas the areas lacking *ftz* protein in-

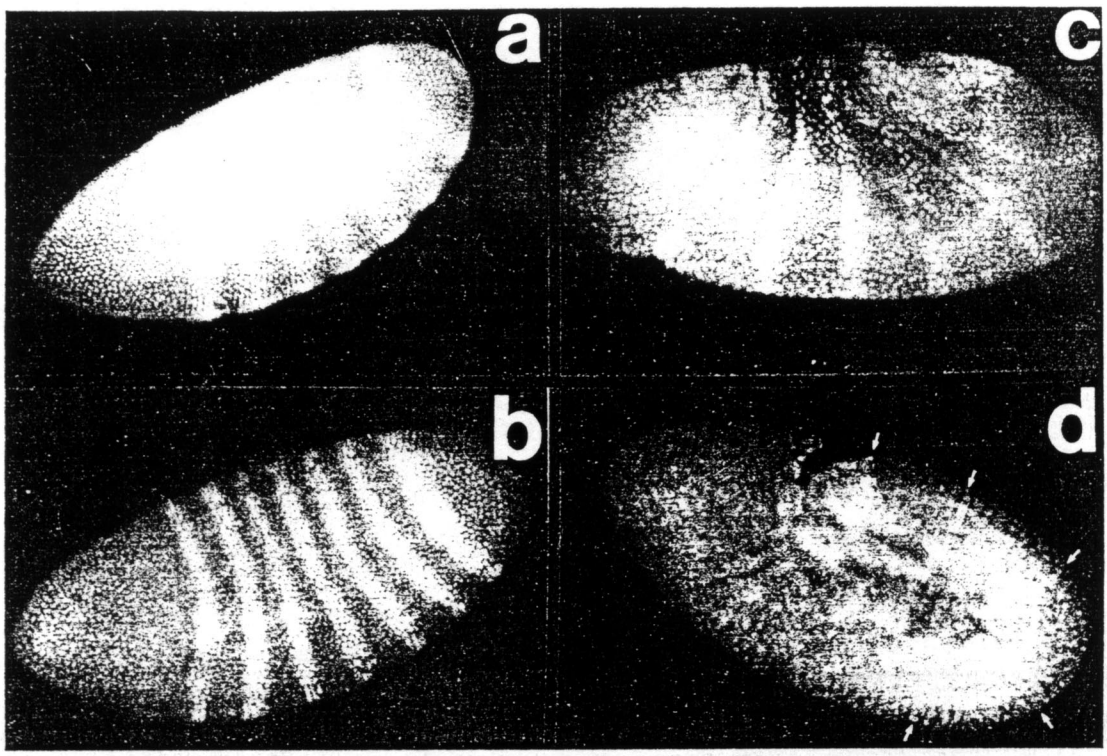


Figure 1. Expression of the *ftz* Protein in Wild-Type Embryos

In each photograph, the anterior end of the embryo is at the left and the ventral side, when visible, is at the bottom. Magnification is 50× for all photographs of entire embryos, higher magnification views are 75×. (a–d) Whole mount embryos incubated with anti-*ftz* antibodies and stained with fluorescein conjugated goat anti-rabbit antibody. (a) An embryo undergoing cellularization after the thirteenth nuclear division with a pattern of seven equally spaced transverse stripes. (b) An embryo at the onset of gastrulation when the stripes sharpen to about three nuclei in width. (c) An embryo undergoing germ band elongation when the stripes become wedge-shaped along the germ band. (d) An embryo near full germ band extension when very few nuclei contain *ftz* protein. The positions of the fading *ftz* stripes are noted with arrows.

crease to about five nuclei wide (Figure 1b). The most posterior stripe is initially about five nuclei wide, does not become narrower, and remains the widest *ftz*-containing stripe during gastrulation and germ band extension. As the germ band extends, the stripes become wedge shaped, about two to three nuclei wide on the dorsal side, and about four nuclei wide ventrally (Figure 1c). The *ftz* stripes persist until nearly the time of full germ band extension (Figure 1d) and then disappear.

Based upon morphological analysis of *ftz*$^-$ larvae (Wakimoto and Kaufman, 1981; Jürgens et al., 1984), in situ hybridization analysis of *ftz* RNA expression (Hafen et al., 1984; Martinez-Arias and Lawrence, 1985), and immunofluorescent localization of *ftz* protein, it appears that *ftz* is expressed in stripes with nonsegmental phasing. Each stripe is approximately centered at a position where a segment boundary will later form, and therefore includes cells that are within two segment primordia. The locations of the seven stripes are the maxillary–labial, T1–T2, T3–A1, A2–A3, A4–A5, A6–A7, and A8–A9–caudal segment boundary primordia. The number, width, location, and spacing of the *ftz*-containing nuclear stripes are perturbed by the mutations that are discussed below. The late pattern of *ftz* expression in the developing ventral nervous system of mutant embryos will not be addressed here, since morphological distortions caused by aberrant gastrulation in the mutants make interpretation of late altered *ftz* patterns quite difficult.

Expression of *ftz* in Gap Mutant Embryos: All Four Loci Affect *ftz* Expression

Krüppel

Strong Krüppel (*Kr*) mutants lack thoracic and anterior abdominal segments; in their place a partial mirror image duplication of the posterior abdomen is observed (Wieschaus et al., 1984b; Priess et al., 1985). In embryos homozygous for the strong *Kr*^{1} allele, *ftz* expression is significantly altered (Figures 2a and 2c). Generally, there are four strong stripes of *ftz* expression and a faint fifth stripe between the first two strong ones (Figure 2a, lone arrow). The pattern can be compared to the cuticular phenotypes described by Wieschaus et al. (1984b). The first *ftz* stripe on a *Kr/Kr* embryo, which occurs at the proper position with respect to egg length, could correspond to a normal gnathal (maxillary–labial) segment, the second (faint) stripe to a partial thoracic segment, the third stripe to a posterior abdominal segment (part of the mirror image), the large gap (Figure 2a, opposing arrows) to the plane of the mirror image, and the two most posterior stripes to largely normal posterior abdominal segments. Therefore

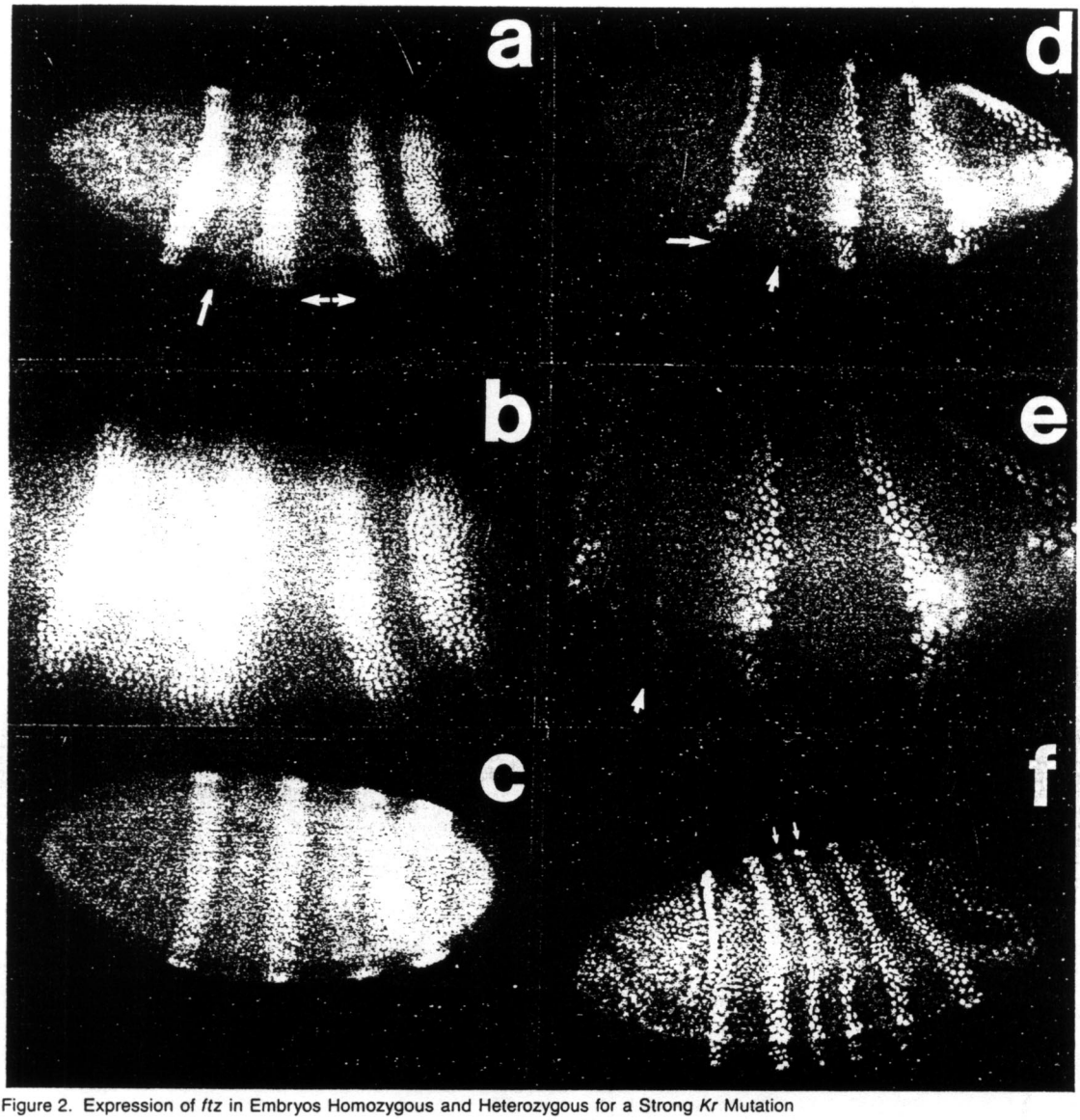


Figure 2. Expression of *ftz* in Embryos Homozygous and Heterozygous for a Strong *Kr* Mutation

(a) Homozygous Kr^1 embryo at the cellular blastoderm stage. Large arrow indicates putative thoracic region. Opposing arrows indicate a possible plane of mirror image. (b) Higher magnification (75×) view of (a). (c) Homozygous Kr^1 embryo. (d) Homozygous Kr^1 embryo undergoing germ band elongation. Long arrow indicates a point of dorsal–ventral shift. The short arrow indicates the position of a few ventral putative thoracic *ftz*-containing nuclei. (e) Higher magnification view of (d). (f) Heterozygous Kr^1 embryo. Arrows indicate the area of compression of normal spacing and the narrow third and fourth stripes.

the correlation between the cuticular defects seen late in development and the altered *ftz* stripes seen at the blastoderm stage seems to be reasonable. However, there is as yet no direct proof that the altered stripes do in fact correspond to segmental primordia in the way that we have described.

The fate of the faint second stripe is shown in Figures 2d and 2e. As the germ band elongates most *ftz* expression disappears dorsolaterally from the faint stripe; the signal remains in only a few ventral nuclei. The persistence of *ftz* protein in the ventral cells correlates well with the results of the analysis of in vivo culture of *Kr/Kr* mutant embryos where only ventral thoracic structures were found in the embryo cultures (Wieschaus et al., 1984b). This correlation is consistent with the idea that the second stripe is in fact located in a region of primordial thoracic cells.

The dominant phenotype of Kr^1 is seen in adult flies as defects in the thoracic appendages, particularly in the third thoracic segment. Defects are also seen in larvae: most commonly in the metathorax, but also in the mesothorax and in the second abdominal segment (Wieschaus et al., 1984b). A dominant effect on *ftz* expression in *Kr/+* embryos can also be seen. The width and spacing of the third and fourth *ftz* stripes is altered in *Kr/+* embryos (Figure 2f, arrows), which gives rise to a stained region that

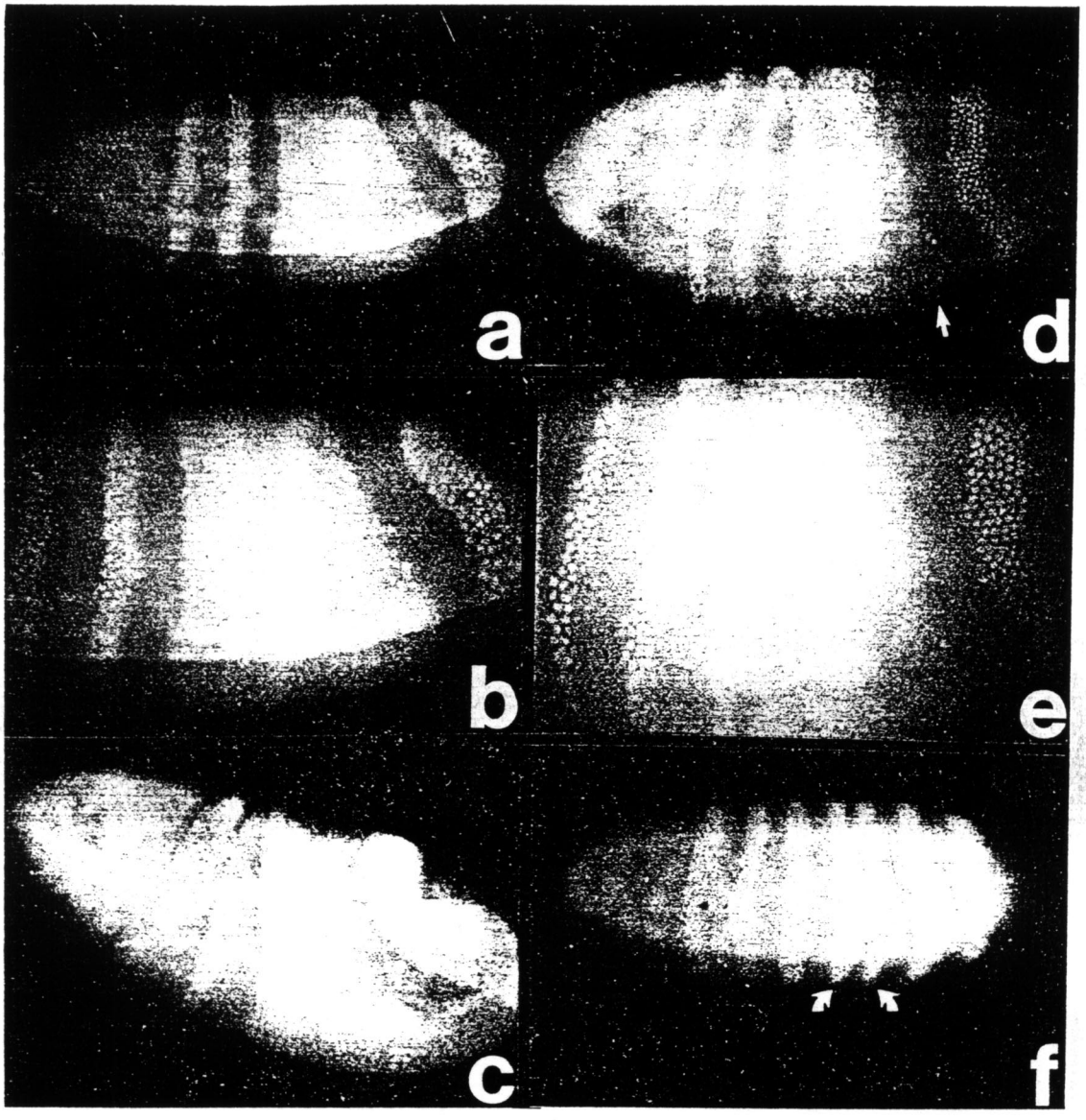


Figure 3. Expression of *ftz* in Homozygous and Heterozygous *kni* Embryos

(a) Homozygous kni^{IIE72} embryo undergoing the first steps of germ band elongation. (b) Higher magnification view of (a). (c) Homozygous kni^{IIE72} embryo later in germ band elongation, the wide *ftz* protein band remains strongly stained. (d) Homozygous kni^{IIE72} embryo at beginning of gastrulation. The arrow indicates the area at the posterior end of the wide *ftz*-containing band of cells that does not stain evenly and completely. (e) Higher magnification view of (d). (f) Heterozygous kni^{IIE72} embryo. The curved areas indicate the area of compression altering the spacing of the *ftz* stripes.

appears "compressed" with respect to the rest of the embryo.

knirps

knirps$^-$ (*kni*$^-$) embryos have one large fused abdominal segment where the normal A1–A7 segments would form (Nüsslein-Volhard and Wieschaus, 1980; Jürgens et al., 1984). Embryos homozygous for strong *kni* alleles exhibit a wide band of *ftz* staining that extends across the area where the third through sixth stripes normally form (Figure 3a–3d). Sometimes this is a uniform band (Figure 3a), whereas about one-third of the time the band is interrupted near its posterior end (Figure 3d, arrow). The area of abnormal *ftz* staining therefore correlates very well with pattern changes seen later in the larval cuticle. The wide band remains strongly stained throughout the normal period of *ftz* expression (Figure 3c) and disappears at the normal time. Like *Kr, kni* also has dominant effects on pattern in the adult fly in the second through fifth abdominal segments (R. Lehmann, C. Nüsslein-Volhard, and E. Wieschaus, personal communication), and in *kni*/+ embryos a "compression" in the area of the fourth and fifth *ftz* stripes occurs (Figure 3f, arrows).

hunchback

Embryos homozygous for strong *hunchback* (*hb*) mutations lack gnathal and thoracic segments and have defects in the eighth abdominal segment (Jürgens et al.,

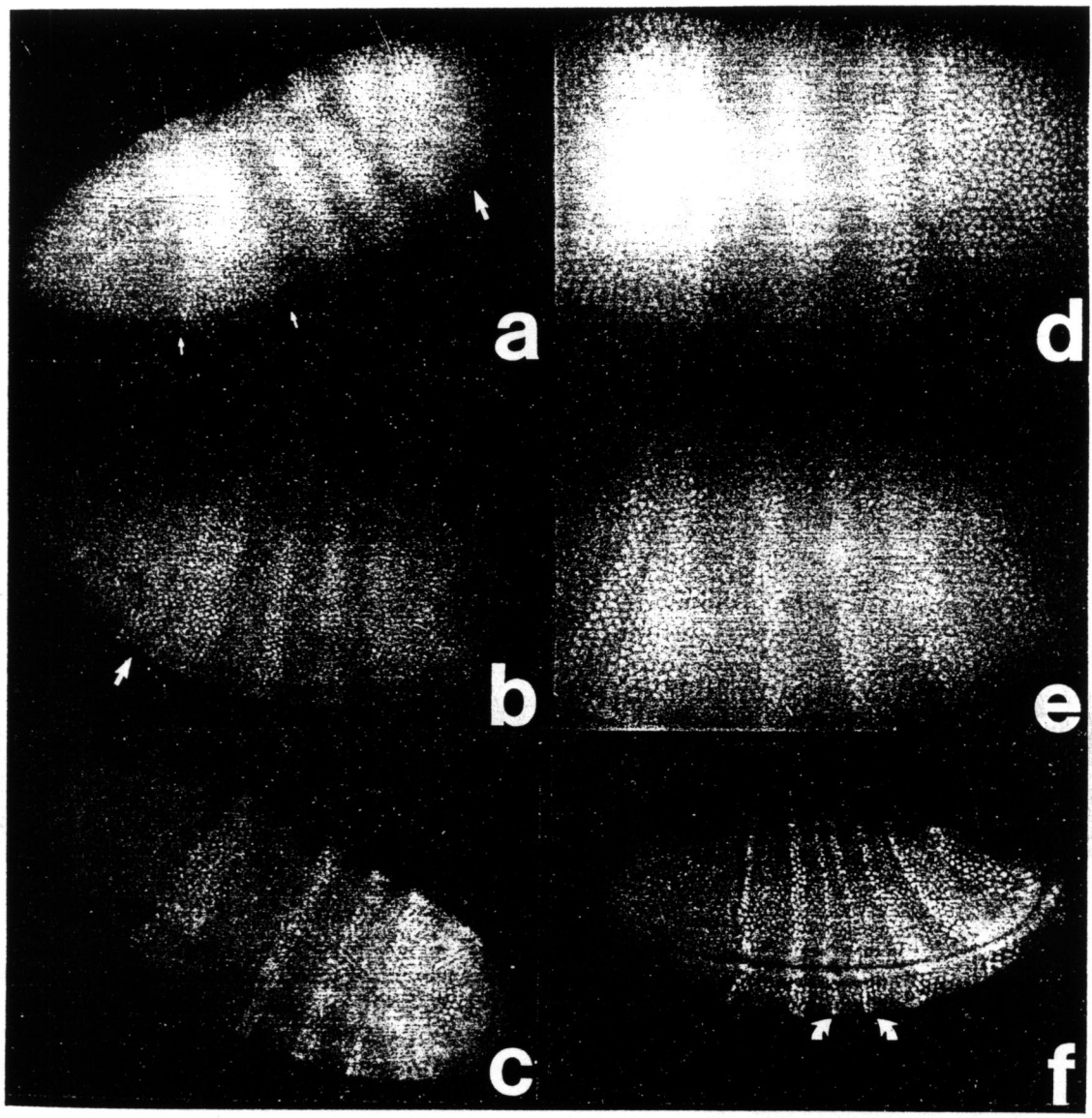


Figure 4. Expression of *ftz* in Embryos Homozygous and Heterozygous for Hypomorphic, Null, and Neomorphic Alleles of *hb*

(a) Homozygous *hb* deficiency embryo (Df(3R)p25) at the cellular blastoderm stage. The area between the two small arrows is entirely stained for *ftz*. The large arrow indicates the near fusion of the two most posterior *ftz* stripes. (b) Homozygous hb^{6N47} embryo at the cellular blastoderm stage. The arrow indicates the interruption in the pattern of *ftz*-containing nuclei at the anterior end of the embryo. (c) Homozygous *hb* deficient embryo with a smaller anterior band of *ftz* protein than (a). (d) Higher magnification view of (a). (e) Higher magnification view of (b). (f) Heterozygous *Rg pbx* embryo. The curved arrows indicate the area of compression in the third and fourth *ftz* stripes.

1984). Three alleles at the *hb* locus that each confer a different phenotype were examined for their effects on *ftz* expression. Embryos homozygous for a deficiency of the *hb* locus (Df(3R)p25) show two equally frequent patterns of *ftz* expression. Some embryos have a wide band of *ftz* expression as the most anterior stripe and a small gap of unstained nuclei before the next most anterior stripe (Figures 4a and 4d). Other embryos have a smaller anterior *ftz* protein band (which is still wider than in wild-type embryos) and a larger unstained gap posterior to it (Figure 4c). All embryos that are homozygous for the *hb* deficiency have a complete or nearly complete fusion of the two most posterior stripes (Figure 4a, large arrow). hb^{-} larvae do have a posterior abdominal defect, which correlates with the abdominal *ftz* expression in this region. The anterior edge of the first stripe is in its normal position with respect to egg length in both patterns. *hb* is reported to have some maternal effect (M. Bender, F. Turner, and T. Kaufman, manuscript submitted) that may be responsible for the variability in *ftz* protein patterns and the extent of posterior thoracic segment deletions in hb^{-} embryos. Embryos homozygous for the hypomorphic hb^{6N47} allele have the same abnormal posterior pattern of staining, but the anterior band is interrupted by a stripe of 1–2 unlabeled nuclei (Figures 4b and 4e, arrow). The hb^{6N47} allele (Figure 4b) causes lesser cuticular defects within the tho-

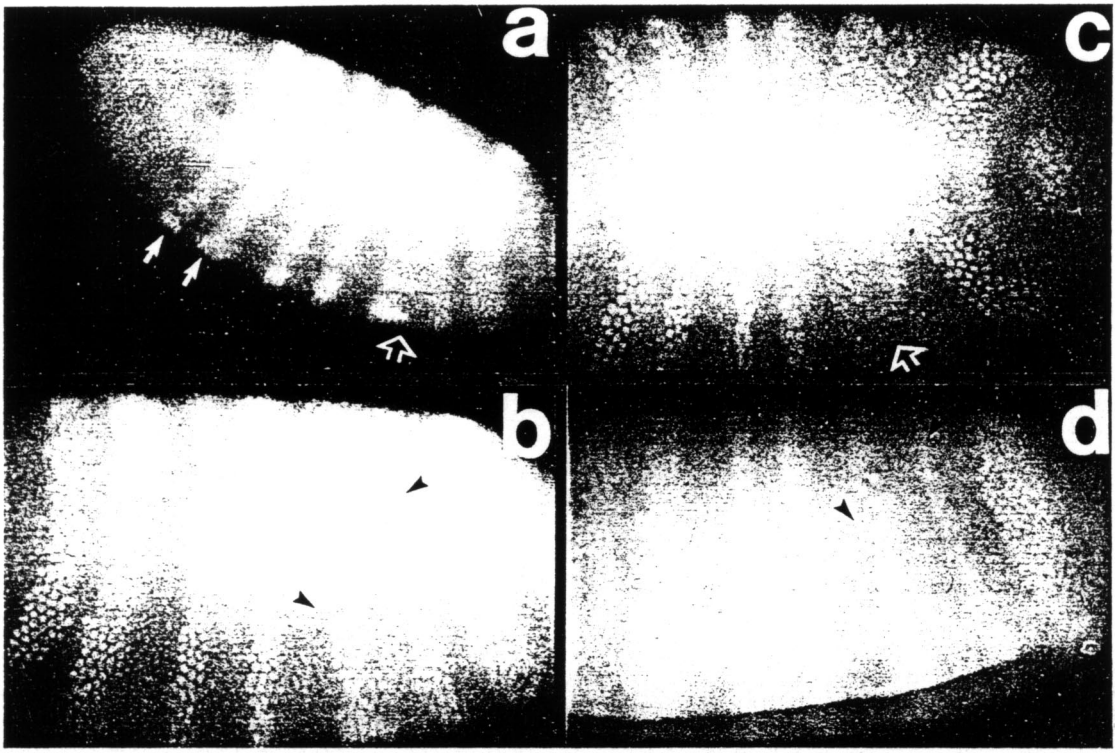


Figure 5. Expression of *ftz* in Homozygous gt^{YA82} Embryos

(a) Homozygous gt^{YA82} embryo at the cellular blastoderm stage. The pair of solid arrows points to the two anterior stripes that are wider and more closely spaced than normal. The large single arrow indicates the area of uneven and incomplete *ftz* expression in the fifth and sixth stripes. (b) Higher magnification view of (a). The large black arrows indicate areas of incomplete *ftz* stripes. (c) High magnification dorsal view of gt^{YA82} homozygous embryo undergoing germ band elongation. The arrow points to the area of fusion between the fifth and sixth stripes. (d) High magnification ventrolateral view of homozygous gt^{YA82} embryo beginning germ band elongation. The black arrows point to areas of incomplete *ftz* expression.

racic segments, which is consistent with the more normal gnathal precursor region *ftz* stripe in these animals.

Regulator of postbithorax (*Rg pbx*), a neomorphic dominant allele of *hb*, is a chromosomal inversion with one of its breakpoints within *hb* (Lewis, 1968; M. Bender, F. Turner, and T. Kaufman, manuscript submitted). Adult heterozygous flies exhibit a transformation of the posterior third thoracic segment to the posterior second thoracic segment (haltere to wing). *Rg pbx/+* heterozygous embryos show a *Kr*-like compression in the third and fourth stripes (Figure 4f), as do *Rg pbx/hb*$^{-}$ embryos (data not shown).

giant

Embryos homozygous for strong alleles of the *giant* (*gt*) locus exhibit abnormal labial segment formation, fusion of the first and second thoracic segments, and some abnormalities in the A5–A7 segments (Wieschaus et al., 1984a; J. P. Petschek, N. Perrimon, and A. P. Mahowald, manuscript submitted). The expression of *ftz* is abnormal in *gt*$^{-}$ embryos in the two most anterior and in the fifth and sixth stripes at the cellular blastoderm stage. The two anterior stripes are wider and more closely spaced than in wild-type embryos, and the fifth and sixth stripes are irregular in width, spacing, and the regularity of their borders (Figures 5a–5d). The most anterior *ftz*-containing nuclei are in a more anterior position than those in wild-type embryos. The areas altered in the larval cuticle by the loss of *gt* function therefore appear to correspond to the perturbations in the *ftz* protein pattern that are observed at the cellular blastoderm stage.

Pair-Rule Loci: Three Out of Eight Loci Affect *ftz* Expression

hairy

Embryos homozygous for strong alleles at the *hairy* (*h*) locus have greater than segment-sized deletions repeated at double segment intervals along the length of the embryo (Holmgren, 1984; Jürgens et al., 1984; Ish-Horowicz et al., 1985; Ingham et al., 1985). In the abdomen, for example, the posterior part of each odd-numbered segment and the anterior part of each even-numbered segment are missing. In strong alleles there is some duplication of the pattern elements that remain. Embryos homozygous for strong alleles of *h* display a striking alteration of *ftz* expression: *ftz* protein is found in nearly all of the nuclei where it is normally absent, except that no *ftz* protein is seen in the anterior 30% or posterior tip of the embryo, just as in wild-type embryos. There is some periodicity to the pattern, in the form of three to six narrow stripes of unlabeled nuclei (Figures 6a and 6b). Posterior to the first broadened

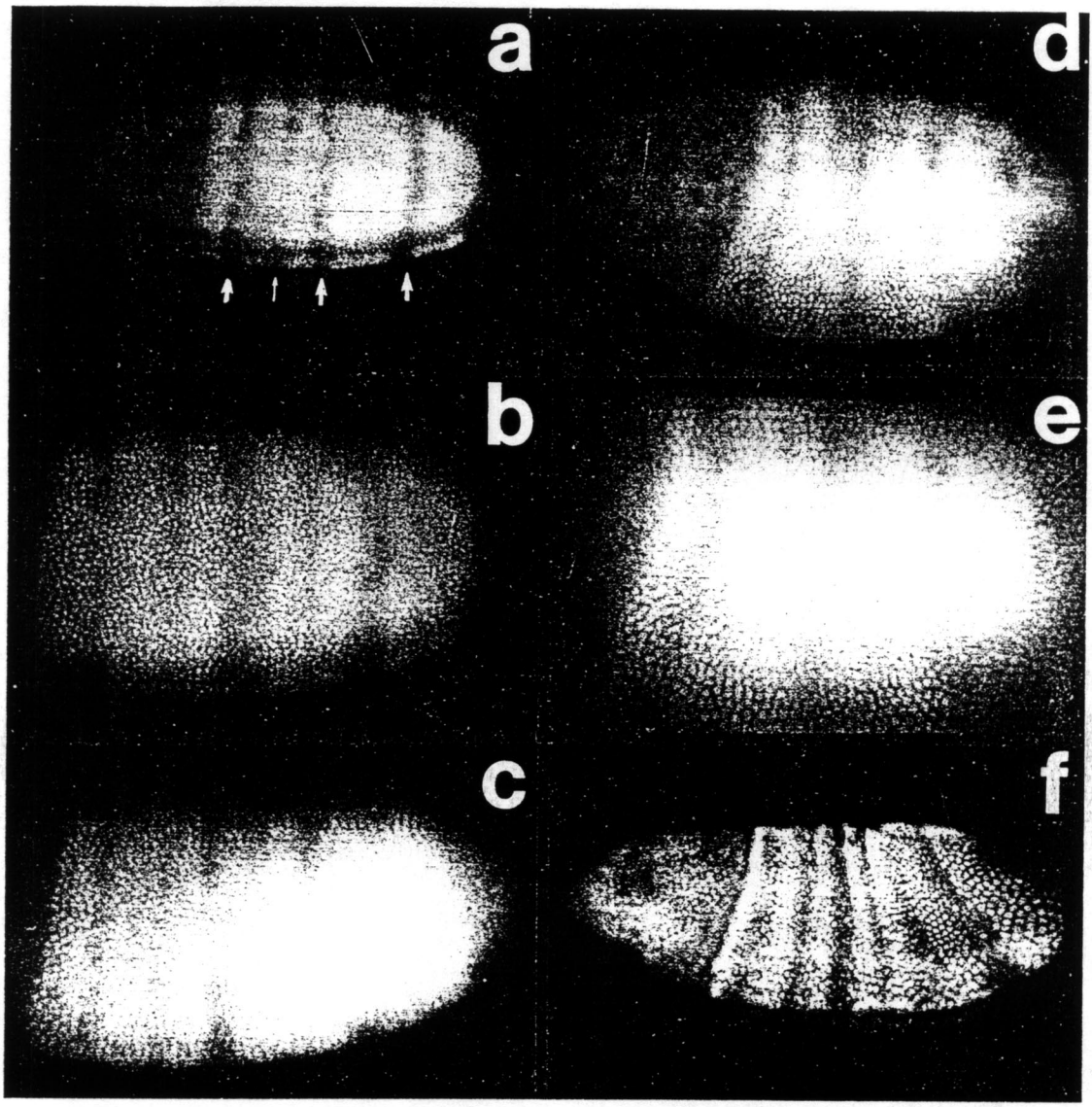


Figure 6. Expression of *ftz* in Homozygous Mutant h^{7H94} Embryos

(a) Homozygous h^{7H94} embryo at the cellular blastoderm stage. Large arrows indicate the position of larger spaces containing unstained nuclei. Small arrow indicates the small unstained space within the large *ftz* protein band. (b) Higher magnification view of (a). (c) High magnification view of a different homozygous embryo. (d) Homozygous embryo at cellular blastoderm. (e) Higher magnification view of (d). (f) Homozygous embryo undergoing germ band elongation. The spacing of unstained nuclei is more evident at this stage.

stripe there is a space about two nuclei in width; two more two-nucleus-wide spaces occur at intervals of 18 nuclei. Within the 18-nucleus-wide bands, there is often a thin stripe of unlabeled nuclei, which is about one nucleus in width (Figure 6a, small arrow). When the stripes become narrower as gastrulation proceeds, the unstained spaces become wider and more evident (Figure 6f). The pattern is reproducible; three examples are shown (Figures 6c–6e).

runt

In the cuticle of *runt*$^-$ (*run*$^-$) animals the denticle belts of the odd-numbered abdominal segments (and of corresponding alternate segments of the thorax and head), the naked cuticle anterior to the belts, and the posterior half of the remaining denticle belts are all deleted. Deleted regions appear to be replaced by mirror-image duplications of the pattern elements that remain (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus et al., 1984a). Embryos homozygous for mutant alleles of the *run* locus have complicated *ftz* expression patterns. The hypomorphic *run*^{XK52} allele exhibits several staining patterns (Figures 7a, 7b, 7e, 7f). In the majority of embryos there is a reduction in the width and intensity of the most anterior stripe (Figures 7a, 7b, 7f), while in about 10% of the embryos that stripe is brightly stained and extra-wide (Figure 7e). The spacing and width of the remaining stripes is also aberrant. In most embryos, the fifth stripe is extra-wide and appears as a gradient in the amount of *ftz* pro-

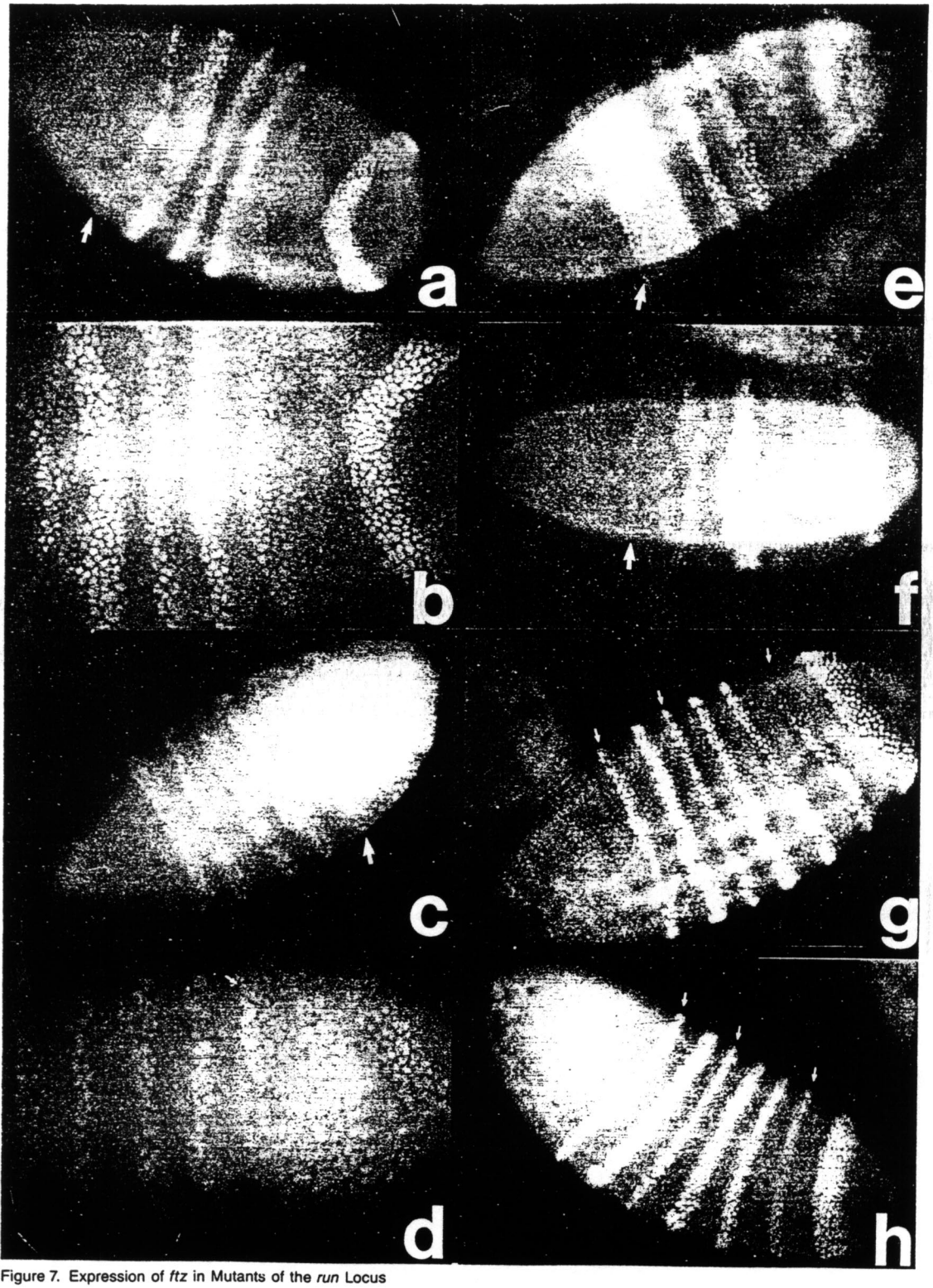


Figure 7. Expression of *ftz* in Mutants of the *run* Locus

(a) Homozygous *run*^{XK52} embryo at the cellular blastoderm stage. The arrow indicates the weak anterior *ftz* stripe. (b) Higher magnification view of (a). (c) Homozygous *run*^{YE96} embryo. The arrow points to a large patch of irregularly shaped *ftz*$^{-}$ nuclei. (d) Higher magnification view of (c). (e) Homozygous *run*^{XK52} embryo. Arrow points to large anterior band of *ftz*-containing nuclei. (f) Homozygous *run*^{XK52} embryo. Arrow points to very weak anterior *ftz* protein stripe. (g,h) Heterozygous *run*^{XK52} embryos. Arrows indicate reduced first, third, and sixth *ftz* protein stripes.

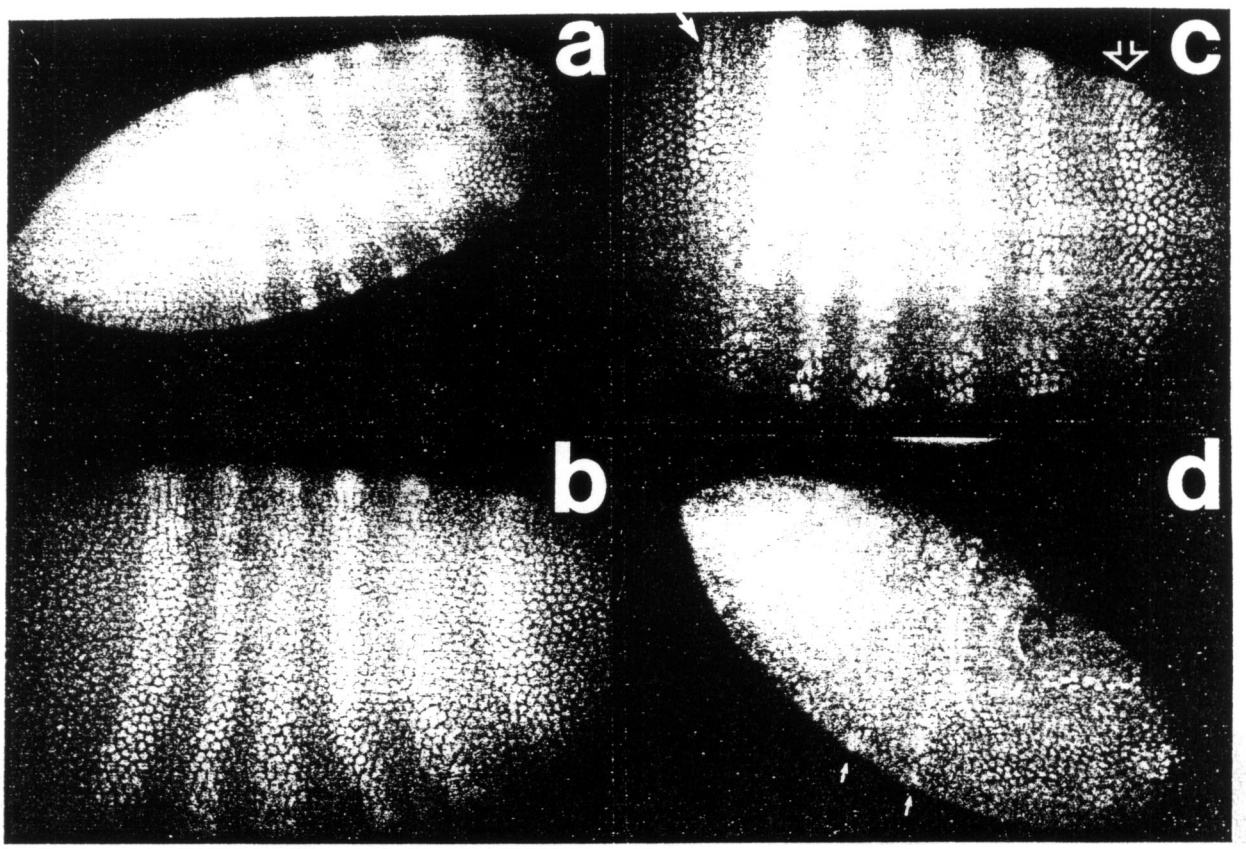


Figure 8. Expression of *ftz* in Homozygous *eve*$^-$ Embryos

(a) Homozygous Df(3R)*eve* 1.27 embryo. (b) Higher magnification view of (a). (c) High magnification view of homozygous *eve*$^-$ embryo. The solid arrow indicates the position of a partial anterior stripe. The open arrow points to the thinner posterior stripe. (d) Homozygous *eve*$^-$ embryo undergoing germ band elongation. The arrows point to the two more complete *ftz* stripes, *ftz* protein expression is prematurely diminished in the other stripes.

tein staining per nucleus, the amount of protein tapering off posteriorly (Figure 7b). The nuclei in embryos bearing the hypomorphic allele (*run*^{XK52}) are irregular in shape, but not as abnormal as those in *run*^{YE96} embryos. Heterozygous *run*^{XK52}/+ embryos have reduced first, third, and sixth stripes (Figures 7g and 7h), with the sixth stripe being the most reduced (Figure 7g). Heterozygous *run*^{XK52}/+ adult flies have their most severe abnormalities in A6–A7, probably corresponding to the area affected by the sixth stripe of *ftz* protein. The *runt* gene is sensitive to dosage effects which cause dominant segmentation abnormalities (Gergen and Wieschaus, 1986). Embryos homozygous for the stronger *run*^{YE96} allele (the strongest allele known) have an invariant pattern of four irregular weakly stained anterior stripes, then a large patch of stained nuclei, a small row of unstained nuclei, and the posterior stripe (Figures 7c and 7d). Note the irregularly shaped nuclei in *run*^{YE96} embryos (Figure 7d, arrow).

even-skipped

Weak *even-skipped*$^-$ (*eve*$^-$) mutant alleles result in larval cuticles that exhibit pair-rule deletions in even-numbered segment denticle belts, while extreme alleles result in embryos that have an unsegmented lawn of denticles (Nüsslein-Volhard et al., 1984). In light of the cuticular phenotype, the pattern of *ftz* expression in extreme *eve*$^-$ (Df(2R)eve 1.27) embryos at the cellular blastoderm stage is surprising. In most *eve*$^-$ embryos six stripes of labeled nuclei appear (Figures 8a and 8b), but the stripes do not show the proper width and spacing. The first and last stripes are the widest, but the posterior stripe is not as wide as in wild-type embryos. The most anterior stripe occurs at a position about where the space between the two most anterior stripes occurs in wild-type embryos, based upon measurements of egg lengths. Unstained spaces vary from approximately three to six nuclei wide, instead of the normal five-nucleus-wide gaps. In about 10%–15% of embryos, part of a seventh stripe is formed anteriorly only on the dorsal side (Figure 8c, solid arrow). In contrast to wild-type embryos, *ftz* expression in *eve*$^-$ embryos diminishes well before full germ band extension (compare Figure 8d to Figure 1d). This may be due in part to the slower and defective germ band extension in these embryos. The disappearance of *ftz* protein from the nuclei is uneven in contrast to wild-type embryos; in *eve*$^-$ embryos the two most anterior stripes retain *ftz* protein longer than the others do (Figure 8d).

Loci That Affect Pattern Formation but Do Not Alter *ftz* Protein Expression

In Table 1 is a list describing the *ftz* expression patterns of embryos carrying mutations at the loci we have examined for effects on early *ftz* expression. Five pair-rule loci:

Table 1. *ftz* Expression in Zygotic Mutants

Gene Class, Locus, and Alleles Studied	Larval Phenotype	Effect on *ftz*
Gap		
Krüppel Kr^{1}	thoracic and anterior abdominal segments missing, mirror-image duplication of posterior abdomen, dominant defects in T3 and A2	abnormal intensity and spacing of stripes in thoracic and anterior abdomen, dominant effects in third and fourth stripes
knirps kni^{IIE72}, kni^{7G75}	abdominal segments A1–A7 fused, dominant defects in A2–A5	broad band of *ftz* protein in A1–A7 interval, dominant effects in fourth and fifth stripes
hunchback hb^{6N47} Df(3R)p25 In(3R) Rg pbx	gnathal and thoracic segments deleted, A8 defects, dominant defects in T3 in Rg pbx	broad band of *ftz* expression anteriorly and posteriorly in hb^{-} embryos, dominant effects in *Rg pbx* in third and fourth stripes
giant gt^{YA82}	labial, anterior thoracic, and A5–A7 segment defects	inappropriate expression in labial and anterior thoracic segments, patchy loss of expression in A5–A7
Pair-Rule		
hairy h^{7H94}	deletion of even denticle bands and odd naked cuticle*	broad increase in *ftz* expression in cells requiring hairy, periodicity complicated by pattern duplications
runt run^{XK52}, run^{YE96}	mirror-image duplication of abdominal denticle bands 2,4,6,8, deletion of odd numbered abdominal bands, dominant defects in T3, A1 and A6	reduction of *ftz* protein in anterior four stripes, abnormal expression in A5–A7 may reflect pattern duplication; abnormal nuclear morphology
even-skipped eve^{ID19} Df(2R)eve 1.27	unsegmented lawn of denticle bands	initially six unevenly spaced stripes, premature loss of *ftz* expression during germ band elongation
odd-skipped odd^{IIID36}	partial deletion of odd denticle bands	no effect
paired prd^{IIB42}	deletion of even denticle bands and odd naked cuticle	no effect
odd-paired opa^{IIC71}	deletion of odd denticle bands and even naked cuticle	no effect
sloppy-paired slp^{IIM105}	partial deletion of odd naked cuticle	no effect
*engrailed*** en^{4}, Df(2R)en28	defects in even naked cuticle	no effect
Segment Polarity		
hedgehog hh^{6N16}	deletion of naked cuticle and fusion of denticle bands	no effect
patched ptc^{IN108}	mirror image duplications of all segment boundaries	no effect
Unclassified		
unpaired upd^{C43}	T2 and A5 defects	no effect
naked nkd^{YE88}	naked cuticle	no effect
branch bch^{10E113}	incomplete fusion of denticle belts	no effect

* Odd and even refer to the numbering of abdominal denticle bands; the corresponding thoracic segments (even and odd) are also affected.
** *en* is classified as a pair-rule locus based on some mutants; it also has segment polarity properties.

odd-skipped, *paired*, *odd-paired*, *sloppy-paired*, and *engrailed*, do not affect the early *ftz* pattern. Two segment polarity loci, *hedgehog* and *patched*, and three other genes that affect larval cuticular patterns, *unpaired*, *naked*, and *branch*, also have no effect. In all cases, we examined null alleles of the loci or the most extreme alleles available.

Discussion

Of the 17 genes examined that visibly affect embryonic pattern formation, seven have been shown to influence the initial pattern of *ftz* protein expression at the cellular blastoderm stage of embryogenesis. The ten genes that did not affect *ftz* expression, including some pair-rule and

segment polarity genes, provide evidence that *ftz* operates in the segmentation gene hierarchy below or in parallel with the four gap genes and three of the pair-rule genes, but above or independently of the other pair-rule genes and segment polarity genes. In all mutants except *eve*, the temporal aspects of *ftz* protein expression were normal even in cells where *ftz* expression was ectopic, which suggests that the major effect of most of the zygotically active genes is on the spatial rather than the temporal aspects of *ftz* expression. The pattern of *ftz* expression depends upon the interactions between segmentation genes active within each cell along the anterior–posterior axis of the embryo. No gene appears to act as a simple repressor or activator of *ftz* expression.

Abnormal Expression of *ftz* at the Cellular Blastoderm Stage and the Relationship of the Patterns to the Larval Cuticular Patterns

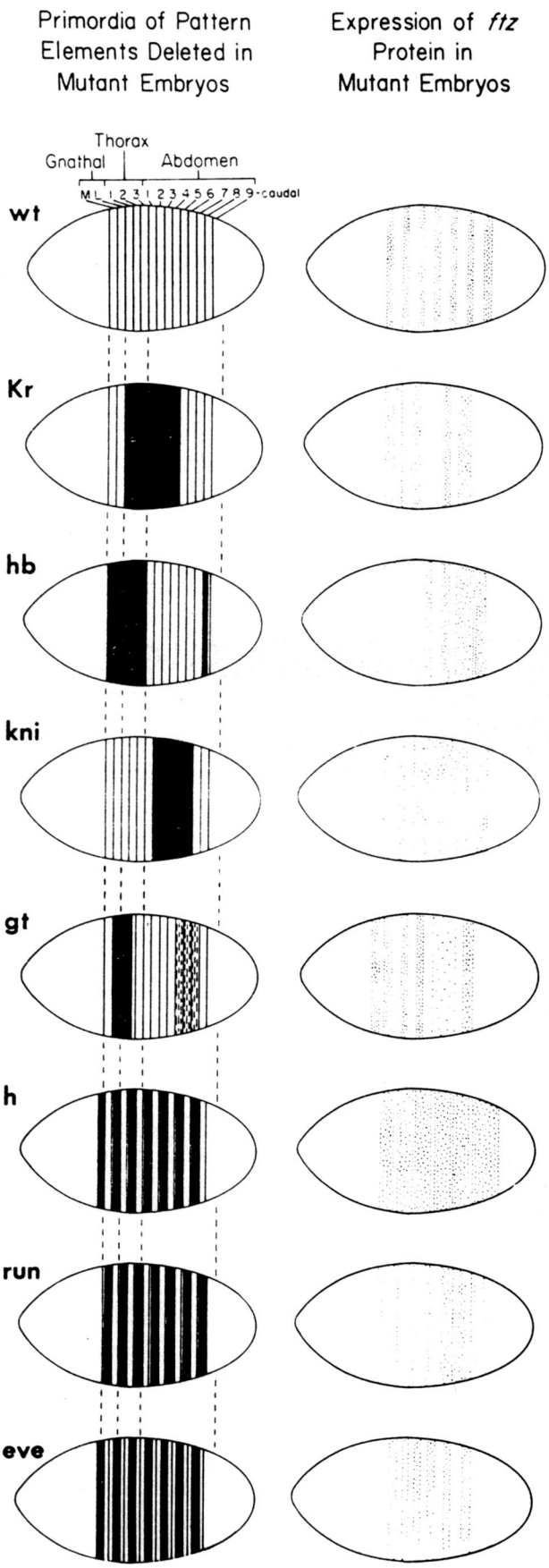
The description and classification of segmentation genes has been largely based on the interpretations of pattern elements present in the larval cuticle of wild-type and mutant animals. It is sometimes assumed that the pattern elements affected by the absence of a particular gene are those produced by the descendants of cells that initially express that gene, but this has not been directly shown for most of the genes. From observing *ftz* expression in mutants that perturb the wild-type pattern, we can assess whether the position of cells where *ftz* expression is abnormal corresponds to the position of primordia for pattern elements that are defective in the larval cuticle of these mutants.

A rough fate map of the embryo at the cellular blastoderm stage is diagrammed (Figure 9), indicating the positions of regions of the embryo that will give rise to the segments of the animal. For each gene affecting *ftz* expression, the segments or parts of segments that require that gene's activity to be properly established are indicated in black (left-hand column of Figure 9). The affected primordia are inferred from morphological descriptions of the pattern elements that are deleted from the larval cuticle of mutant animals (see Gergen et al., 1985). The locations of *ftz* protein in wild-type embryos and in mutant embryos that have changed patterns are also diagrammed (right-hand column of Figure 9). Comparison of each gene's predicted regional influence to the observed blastoderm-stage *ftz* protein pattern reveals that for *hb*, *kni*, and *gt*, there is good correspondence between the areas affected by the mutations and the places where *ftz* expression is abnormal. In embryos homozygous for *Kr*, *h*, *run*, and *eve* mutations there is abnormal *ftz* expression in areas outside those simply predicted by the cuticular pattern deletions. The abnormal expression in these areas may reflect the fact that there are pattern abnormalities (e.g., duplications) in the cuticle of these animals outside of the deleted regions. The diagram (Figure 9) does not incorporate any pattern duplication effects. Thus, while primordia affected by mutations in each gene roughly correspond to areas of abnormal expression, more cells than those within the eventually deleted pattern element show aberrant *ftz* expression. Some of the genes appear to affect cells in which they may not be expressed (see below), and the resultant respecification of the identity of those cells to form duplicated elements appears to occur very early in embryogenesis.

In *even-skipped*$^-$ embryos the initial expression of *ftz* protein is in six complete transverse stripes of abnormal width and spacing. This pattern does not appear to have any relationship to the larval cuticular pattern: an unsegmented "lawn" of denticle bands (Nüsslein-Volhard et al., 1984). However, the premature loss of *ftz* protein during germ band elongation suggests that one reason for the *eve*$^-$ larval phenotype may be the absence of *ftz* protein at a time when it is still needed. The absence of *ftz* protein during germ band elongation is insufficient to account for the complete absence of segmentation in *eve*$^-$ embryos, since *ftz*$^-$ embryos retain some segmental divisions, and therefore other genes required for segmentation may also be affected by the absence of *eve* function. Perhaps *eve* plays a role in the maintenance of the patterns of expression of many or all of the segmentation genes, and the absence of *eve*$^+$ eliminates segmentation. Partial *eve*$^+$ activity would permit the genes least dependent on *eve*$^+$ function to work well enough to generate the odd-numbered segments, thus giving the *eve* hypomorphic phenotype of the absence of even segments. An alternative possibility is that *eve*$^+$ is required for formation of even-numbered segments and for function of *ftz*$^+$ in the odd-numbered segments. The removal of *eve*$^+$ function would therefore lead to the absence of segmental boundaries.

Four genes of the seven affecting the *ftz* pattern have alleles that cause dominant effects in adult flies and on the blastoderm stage *ftz* expression. There is excellent spatial correspondence between the dominant segmentation defects in adult *Kr*/+, *kni*/+, *run*^{XK52}/+, and *Rg pbx*/+ flies, and the *ftz* patterns in embryos. For *run*^{XK52}/+ and *Rg pbx*/+ the dominant effects are not caused by haploinsufficiency at the locus, since these are not null alleles. Thus, the abnormal products of individual alleles, or the altered pattern of expression of normal products, affect the cues that specify *ftz* patterns. All dominant effects are characterized by compression of *ftz* stripes in a discrete region; both stripes and the gaps between them are reduced in width. Decreases in the width of *ftz* protein-containing stripes are not accompanied by increases in the width of adjoining unstained stripes (Figure 7h). Therefore, there must be a change in the total length of the striped region in order for the compressed pattern to occur. Without other precise anterior or posterior markers on the cellular blastoderm stage embryo, it is not possible to determine where simple, perhaps single cell wide, shifts in spacing have occurred. It will be useful to know whether the actual sizes of some segment primordia change in heterozygous dominant mutant embryos. The use of probes for *engrailed* protein (DiNardo et al., 1985) as cell markers for the posterior compartment may help to resolve where shifts in spacing have occurred.

Ten genes have been studied that do not detectably affect early *ftz* expression. The lack of perturbation of the *ftz* pattern may reflect either the temporal, spatial, or



regulatory independence of *ftz* from these genes. Genes acting after *ftz* cannot affect its pattern. This may be the reason why *ftz* expression is not altered in *engrailed* (*en*) embryos. Studies on the pattern of *en* protein expression have shown that the major accumulation of *en* protein occurs later than *ftz* expression (DiNardo et al., 1985). Also, genes that do not influence the cues that *ftz* responds to, regardless of their spatial and temporal realms of activity, will not change the *ftz* pattern. Each of the genes that does alter the *ftz* pattern affects pattern elements more than one segment in length in the larval cuticle. The five pair-rule genes that do not alter the *ftz* pattern affect smaller elements. The morphological data and the effects on the *ftz* pattern reported here may therefore distinguish two functional classes of pair-rule genes.

The Regulation of *ftz* Expression: An Interacting Network of Zygotically Active Genes

A key observation about *ftz* regulation derived from these studies is that individual genes do not influence *ftz* expression in the same way in all affected cells. Simple activation/repression relationships cannot be drawn between any gene and *ftz*. For example, in hb^- embryos, *ftz* protein is present in certain prothoracic nuclei where it is normally absent, and absent from some metathoracic nuclei where it is normally expressed. In gt^- embryos, *ftz* protein is expressed in some anterior nuclei where it is normally absent, and absent in some abdominal nuclei where it is normally present. In Kr^- embryos, *ftz* protein is missing from some normal locations and expressed inappropriately in others. In run^{XK52} embryos, *ftz* expression in some anterior cells is reduced in some embryos and excessively expressed in others. In eve^- embryos, the shifts in *ftz* stripe spacing mean that some cells express *ftz* that would normally not have, while *ftz* protein is absent from other cells that should have produced it. In h^- embryos, *ftz* expression is increased in many cells. However, *h* and *ftz* expression normally overlap in some cells (see below) and thus h^+ cannot be a simple repressor of *ftz*; its effect must be dependent upon position. It is clear that along the length of the embryo, *ftz* expression is not under the con-

Figure 9. Correlation between the Anlagen of Pattern Elements Deleted in Segmentation Mutants and Abnormal Patterns of *ftz* Protein Expression

Left column: the approximate position of segment primordia in the Drosophila cellular blastoderm stage embryo. The black areas show the positions of the primordia for pattern elements that are absent from the larval cuticle of mutant animals. The defects in the gnathal region of *h*, *run*, and *eve* animals are inferred from the pattern of defects in the thorax and abdomen. Only the elements that are deleted from the larval cuticle are indicated; in *Kr*, *h*, *run*, and *eve* embryos there are abnormalities in pattern formation (for example, pattern duplication and mirror images) in areas outside of those indicated here (Gergen et al., 1985). Right column: schematic representation of the approximate position of *ftz* protein patterns in wild-type and mutant embryos at the cellular blastoderm stage. Note that the most anterior (in *gt* and *eve*) and posterior (in *Kr*, *hb*, *h*, and *eve*) *ftz* protein stripes are not always found at their normal positions along the anteroposterior axis. The patterns of *ftz* expression shown are for the most extreme alleles examined. They may not in all cases represent the complete absence of gene activity.

trol of a single gene. Rather, the expression of *ftz* is regulated by the array of other segmentation genes that are active or inactive in each cell of the embryo. Thus, cells in particular positions along the anterior–posterior axis each activate a certain set of genes, and keep another set inactive: the differences in the gene activation patterns along the axis are primary events in the determination of cell fates. It is possible that the genes regulating *ftz* also control the expression of each other; the observed changes in *ftz* expression could therefore be the outcome of interactions between the mutant gene and other segmentation genes, and may not simply reveal direct effects on *ftz*.

The Combinatorial Functions of Segmentation Genes

The four gap loci and nine pair-rule loci probably comprise the majority of zygotically active genes that establish the proper number and size of body segments. A key feature in understanding how the genes function to specify positional information is that they appear to act in nonidentical, partially overlapping regions of the embryo (Nüsslein-Volhard and Wieschaus, 1980; Gergen et al., 1985). It is probable that more than one gene is active in each blastoderm cell and that cells anterior or posterior to any individual cell express a different combination of segmentation genes. For example, Gergen et al. (1985) have pointed out that cells making the most anterior row of denticles in the second abdominal segment require *eve*, *prd*, *h*, *slp*, and *en*; to these *Kr* and *kni* could be added.

There is molecular evidence that *ftz*, *hairy*, *engrailed*, and *Krüppel* are expressed in different partially overlapping frames. *hairy*, like *ftz*, is expressed in stripes at the blastoderm stage, but the *hairy* stripes are offset from the *ftz* stripes by approximately two cells (Ish-Horowicz et al., 1985). Therefore some cells express both *ftz* and *hairy*, some cells express either *ftz* or *hairy*, and some cells express neither gene. From studies of the expression of the *engrailed* locus (Kornberg et al., 1985; DiNardo et al., 1985), it can be inferred that some *h* expressing cells and some *ftz* expressing cells also express *en*. Recently, expression of the *Kr* gene has been analyzed and, although the pattern is more complex than could be predicted, it is clear that in some cells *Kr* expression overlaps that of the pair-rule genes (Knipple et al., 1985).

The expression of partially overlapping sets of segmentation genes may provide the positional information necessary for boundary formation and the position-specific expression of other genes. While adjacent cells may be distinguished from each other by differential segmentation gene expression, segmental boundaries are defined with a periodicity of a larger number of cells. One can imagine that it is the juxtaposition of two different positional values between adjacent cells that "induces" the segmental boundary, with cells on one side of the boundary expressing a different set of segmentation genes than those immediately on the other side. Cell lineage groups (compartments) could be established in a similar fashion. The position of the initiation of certain gastrulation movements may also be defined by unique combinations of segmentation gene activities. For example, the position of cephalic furrow formation is affected by mutations in *eve* and the length of the germ band is affected by most of the segmentation genes.

The position-specific expression of later genetic programs may also be set by segmentation gene functions. For example, homoeotic gene expression is characterized by parasegment- or segment-specific patterns of activation (Akam and Martinez-Arias, 1985; Martinez-Arias and Lawrence, 1985). The anterior or posterior limits of homoeotic gene activation could be established by the segmentation gene network. In fact, expression of both *Antennapedia* and *Ultrabithorax* is dramatically extended anteriorly in hb^{-} embryos (Carroll and Scott, unpublished data). Another clue to the relationship between the segmentation and homoeotic gene programs comes from the homoeotic phenotypes of alleles of two segmentation genes, the *Rg pbx* allele of *hb* (E. B. Lewis, unpublished results; M. Bender, F. Turner, and T. Kaufman, manuscript submitted), and the ftz^{Rpl} allele (I. Duncan, unpublished results; Weiner et al., 1984; Laughon and Scott, 1984). Both alleles cause the dominant transformation of posterior haltere into posterior wing. Perhaps the dominant influence of these segmentation gene alleles on the establishment of cell determination within or near the third thoracic segment results from positional changes in homoeotic gene expression that lead to segmental transformations.

Until molecular probes become available for studying the expression of each of the segmentation genes, the patterns of segmentation gene activity within each segment and each cell cannot be established definitively. However, the evidence is compelling that specific combinations of segmentation genes do act in each cell. Regulatory relationships and the elucidation of the entire zygotic segmentation gene hierarchy will require analysis of gene expression with single cell resolution in wild-type and mutant embryos. This information should lead to a molecular analysis of the mechanisms through which position-specific segmentation gene expression is established.

Experimental Procedures

Staged embryo collections from balanced mutant stocks were fixed and stained with affinity purified antibody to a *ftz* hybrid protein as described in Carroll and Scott (1985). The assessment of embryonic genotypes was based upon the fraction of embryos exhibiting an altered *ftz* pattern. This was roughly 1/4 for homozygous mutant embryos and 1/2 for heterozygotes in all cases.

Acknowledgments

We would like to thank Eric Wieschaus, Christiane Nüsslein-Volhard, and Ian Duncan for providing mutant stocks. We are indebted to Eric Wieschaus for many thoughtful discussions and special insight, for providing unpublished information, and for his and Trudi Schüpbach's hospitality to S. B. C. We thank C. Nüsslein-Volhard for several helpful suggestions; Vern Twombly for meticulous care of the stocks; Drs. Susan Dutcher, Joan Hooper, Jim Kennison, Allen Laughon, John Tamkun, and Bill Wood for criticism of the manuscript; Dr. Michael Klymkowsky for generous use of his microscopes; Maureen Gaines for artwork; and Karen Brown and Cathy Inouye for secretarial help. This work was supported by grant number HD18163 from the National Institutes of Health to M. P. S., by Damon-Runyon Walter Winchell Postdoc-

toral Fellowship DRG-659 and N. I. H. Postdoctoral Fellowship GM-09756 to S. B. C., and by a Biomedical Research Support Grant from N. I. H. to the University of Colorado at Boulder.

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Received November 25, 1985; revised January 23, 1986.

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