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; Andoro 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) (Kauffman 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 (Weisler et al., 1984, Kuroiwa et al., 1984) and the sequence of its coding region (Laughon and Scott, 1984) have revealed that it has 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 (Gorgon et al., 1985). We have analyzed the effects 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 gene 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 50x for all photographs of entire embryos, higher magnification views are 75x. (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 wide. (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 (Hafernik 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 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
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 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^{P72} embryo undergoing the first steps of germ band elongation. (b) Higher magnification view of (a). (c) Homozygous kni^{P72} embryo later in germ band elongation, the wide ftz protein band remains strongly stained. (d) Homozygous kni^{P72} 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^{P72} 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 hb6N47 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 Rp 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 hb6N47 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 hbRNAi allele (Figure 4f) causes lesser cuticular defects within the tho-
Figure 5. Expression of ftz in Homozygous gt^{g82} Embryos

(a) Homozygous gt^{g82} 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^{g82} 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^{g82} embryo beginning germ band elongation. The black arrows point to areas of incomplete ftz expression.

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 h7H94 Embryos

(a) Homozygous h7H94 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.

stripes 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 run⁴⁵⁵ 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 runXK52 embryo at the cellular blastoderm stage. The arrow indicates the weak anterior ftz stripe. (b) Higher magnification view of (a). (c) Homozygous runYEg6 embryo. The arrow points to a large patch of irregularly shaped ftz+ nuclei. (d) Higher magnification view of (c). (e) Homozygous runXK5Z embryo. Arrow points to large anterior band of ftz-containing nuclei. (f) Homozygous runXK5Z embryo. Arrow points to very weak anterior ftz protein stripe. (g,h) Heterozygous runXK52 embryos. Arrows indicate reduced first, third, and sixth ftz protein stripes.
protein staining per nucleus, the amount of protein tapering off posteriorly (Figure 7b). The nuclei in embryos bearing the hypomorphic allele (run\textsuperscript{XK52}) are irregular in shape, but not as abnormal as those in run\textsuperscript{YE6} embryos. Heterozygous run\textsuperscript{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\textsuperscript{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\textsuperscript{YE6} 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\textsuperscript{YE6} embryos (Figure 7d, arrow).

**even-skipped**

Weak even-skipped\textsuperscript{−} (eve\textsuperscript{−}) 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\textsuperscript{−} (Df(2R)eve 1.27) embryos at the cellular blastoderm stage is surprising. In most eve\textsuperscript{−} 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\textsuperscript{−} 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\textsuperscript{−} 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

<table>
<thead>
<tr>
<th>Gene Class, Locus, and Alleles Studied</th>
<th>Larval Phenotype</th>
<th>Effect on ftz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KrippelKr'</td>
<td>thoracic and anterior abdominal segments missing, mirror-image duplication of posterior abdomen, dominant defects in T3 and A2</td>
<td>abnormal intensity and spacing of stripes in thoracic and anterior abdomen, dominant effects in third and fourth stripes</td>
</tr>
<tr>
<td>knirpsknirps abdominal segments A1-A7 fused, dominant defects in A2-A5</td>
<td>broad band of ftz protein in A1-A7 interval, dominant effects in fourth and fifth stripes</td>
<td></td>
</tr>
<tr>
<td>hunchbackhunchbackgnathal and thoracic segments deleted, A8 defects, dominant defects in T3 in Rg pbx</td>
<td>broad band of ftz expression anteriorly and posteriorly in ftz embryos, dominant effects in Rg pbx in third and fourth stripes</td>
<td></td>
</tr>
<tr>
<td>giantgiantlabial, anterior thoracic, and A5-A7 segment defects</td>
<td>inappropriate expression in labial and anterior thoracic segments, patchy loss of expression in A5-A7</td>
<td></td>
</tr>
<tr>
<td>Pair-Rule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hairyhairyabnormal intensity and spacing of stripes In thoracic and anterior abdomen, dominant effects in third and fourth stripes</td>
<td>broad increase in ftz expression in cells requiring hairy, periodicity complicated by pattern duplications</td>
<td></td>
</tr>
<tr>
<td>runrunX52, runYEG6reduction of ftz protein in anterior four stripes, abnormal expression in A5-A7 may reflect pattern duplication; abnormal nuclear morphology</td>
<td>initially six unevenly spaced stripes, premature loss of ftz expression during germ band elongation</td>
<td></td>
</tr>
<tr>
<td>even-skippedevenDN3, Df(2R)eve 1.27unsegmented lawn of denticle bands</td>
<td>initially six unevenly spaced stripes, premature loss of ftz expression during germ band elongation</td>
<td></td>
</tr>
<tr>
<td>odd-skippedodd1036partial deletion of odd denticle bands</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>pairedpaireddeletion of even denticle bands and odd naked cuticle</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>odd-pairedodd1031deletion of odd denticle bands and even naked cuticle</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>sloppy-pairedspl117partial deletion of odd naked cuticle</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>engrailed**en*, Df(2R)en28defects in even naked cuticle</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>Segment Polarity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hedgehoghedgehogdeletion of naked cuticle and fusion of denticle bands</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>patchedpatchedmirrored expression of all segment boundaries</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unpairedunpairedT2 and A5 defects</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>nakednakedcuticle</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>branchbranchincomplete fusion of denticle belts</td>
<td>no effect</td>
<td></td>
</tr>
</tbody>
</table>

* 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 oper-
tors in the segmentation gene hierarchy below or in paral-
lel with the four gap genes and three of the pair-rule
genres, but above or independently of the other pair-rule
genres and segment polarity genes. In all mutants except
eve, the temporal aspects of ftz protein expression were
ormal even in cells where ftz expression was ectopic,
which suggests that the major effect of most of the zy-
gotic activity genes is on the spatial rather than the
temporal aspects of ftz expression. The pattern of ftz ex-
pression depends upon the interactions between seg-
mentation genes active within each cell along the an-
terior–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 mu-
tant animals. It is sometimes assumed that the pattern ele-
ments 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 mu-
tants that perturb the wild-type pattern, we can assess
whether the position of cells where ftz expression is ab-
normal corresponds to the position of primordia for pat-
tern elements that are defective in the larval cuticle of
these mutants.

A rough fate map of the embryo at the cellular blas-
toderm stage is diagrammed (Figure 9), indicating the po-

tions of regions of the embryo that will give rise to the
segments of the animal. For each gene affecting ftz ex-
pression, the segments or parts of segments that re-
quire 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 de-
scriptions 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). Com-
parison 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 expres-
sion in areas outside those simply predicted by the cuticu-
lar pattern deletions. The abnormal expression in these
areas may reflect the fact that there are pattern abnor-
malities (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 be-
low), and the resultant represcription of the identity of
those cells to form duplicated elements appears to occur
very early in embryogenesis.

In even-skipped 1 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 unseg-
mented “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 1 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 1 embryos,
since ftz 1 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 expres-
sion of many or all of the segmentation genes, and the ab-
sence of eve + eliminates segmentation. Partial eve + activ-
ity would permit the genes least dependent on eve +
function to work well enough to generate the odd-
numbered segments, thus giving the eve hypomorph.
phenotype of the absence of even segments. An alterna-
tive 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 segmenta-
tion defects in adult Kr/+, kni/+, runXX52+/+, and Rg
pxb/+ flies, and the ftz patterns in embryos. For runXX52+/+
and Rg pxb/+ 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 dis-
crete 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 af-
fact early ftz expression. The lack of perturbation of
the ftz pattern may reflect either the temporal, spatial, or
Primordia of Pattern Elements Deleted in Mutant Embryos

Expression of \( ftz \) Protein in Mutant Embryos

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 \( runX52 \) 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

1 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 \( gf \) 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 \( ftz \) activity. 

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, sip, 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., 1995). 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 ovo 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, homeotic 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 homeotic 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 homeotic gene programs comes from the homeotic 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 ftzRg allele (I. Duncan, unpublished results; Weiner et al., 1984; Laughon and Scott, 1984). Both alleles cause the dominant transformation of posterior halteres 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 homeotic 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 embryonic 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 assay of embryonic genotypes was based upon the fraction of embryos exhibiting an altered ftz pattern. This was roughly 1/4 for homozygous mutant ovo embryos and 1/2 for heterozygotes in all cases.

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