Mutations affecting the stability of the ftz protein of Drosophila

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We present a molecular analysis of four dominant alleles of the pair-rule gene ftz. Three of these, the ftzUal alleles, cause anti-ftz segmentation defects and homeotic transformations of the first abdominal segment to the third. These alleles are shown to be missense changes affecting two nearby proline codons. Embryos homozygous for these mutations accumulate higher levels of ftz protein than wild type and show strong persistence of ftz protein, but not RNA. These effects appear to result from stabilization of the ftz protein, since ftz stripes decay much more slowly in mutant embryos than in wild type after injection of the protein synthesis inhibitor cycloheximide. We trace the origin of segmentation defects in ftzUal embryos to repression of the pair-rule gene even-skipped by excess ftz protein during stripe sharpening. Homeotic transformations are shown to be correlated with ectopic expression of the abd-A gene of the bithorax complex. A 12-amino-acid sequence containing the proline residues altered in the ftzUal mutants appears to be conserved in the proteins encoded by other segmentation genes and the vertebrate oncogene myc and may target these proteins for rapid degradation. The fourth allele examined, T(2;3)ftz bal (Rpl), also causes homeotic transformations and is a translocation broken within the ftz-coding region. Both ftz transcript and protein stripes are persistent in Rpl embryos, suggesting that the Rpl RNA is stabilized relative to wild type.

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The fushi tarazu (ftz) gene of Drosophila is a pair-rule segmentation gene [Nüsslein-Volhard and Wieschaus 1980] that is expressed in a series of seven stripes at blastoderm [Hafen et al. 1984; Carroll and Scott 1985]. These stripes play a key role in subdividing the embryo into parasegments, the fundamental units of insect segmentation [Martinez-Arias and Lawrence 1985; Patel et al. 1989a]. By regulation of the segment polarity genes engrailed [en] [Howard and Ingham 1986; DiNardo and O'Farrell 1987; Lawrence et al. 1987] and wingless [wg] [Ingham et al. 1988], ftz stripes define the boundaries of even-numbered parasegments. In ftz- embryos, these boundaries fail to form, and structures from even-numbered parasegments do not develop [Nüsslein-Volhard et al. 1985]. Odd-numbered parasegments are defined by the pair-rule gene even-skipped [eve], which is expressed in stripes that are complementary to those of ftz [Harding et al. 1986; Macdonald et al. 1986; Frasch and Levine 1987; Frasch et al. 1987]. In addition to definition of parasegment boundaries, ftz appears to play a central role in the parasegment-specific activation of the homeotic genes [Duncan 1986; Ingham and Martinez-Arias 1986; Ingham et al. 1986; Ish-Horowicz et al. 1989]. The ftz protein contains a homeo domain and binds to DNA [Laughon and Scott 1984; Desplan et al. 1988; Laughon et al. 1988].

In a previous report, [Duncan 1986], the genetic properties of three dominant gain-of-function alleles of ftz were described. These alleles have two major phenotypes: They cause segmentation defects that are out of phase (anti-ftz) with respect to those caused by ftz loss-of-function alleles, and they cause homeotic transformations of the anterior first abdominal segment (A1) to the third (A3). Because the latter transformation superficially resembles those caused by the Ultra-abdominal mutants of the bithorax complex [Lewis 1978], these ftz alleles were called Ultra-abdominal-like, or Ual, alleles. Two lines of genetic evidence indicate that the basic defect in the Ual mutants is hyperactivity or overproduction of the ftz protein. First, animals that carry extra doses of ftz+, but are otherwise wild type, show both anti-ftz segmentation defects [I. Duncan, unpubl.] and transformations of A1 to A3 [Duncan 1986]. Second, the Ual mutants are themselves dosage sensitive: Ual hemizygotes are virtually wild type in phenotype, Ual/+ animals show an intermediate phenotype, and Ual homozygotes are strongly affected.

Here, we present a molecular characterization of the Ual alleles. We describe the DNA sequence changes in the mutants and identify their primary defect as the stabilization of the ftz protein. We trace the origin of anti-ftz segmentation defects in the mutants to repression of eve by excess ftz protein during stripe sharpening. Although the underlying cause of homeotic transformations in the mutants is not identified, we show that the...

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Ual alleles cause ectopic activation of the homeotic gene abd-A in the anterior first abdominal segment. Finally, we present a molecular analysis of T(2;3)ftzRpl [Rpl], a translocation broken at the 3' end of the ftz homeo box (Laughon and Scott 1984) that also causes homeotic transformations. Our analysis suggests that the truncated Rpl transcript is stabilized relative to wild type.

**Results**

ftzUal alleles are missense mutations

The Ual mutants all appear normal by Southern blotting. No restriction fragment length differences within the known ftz regulatory or transcribed regions were seen between any of the mutants and their background alleles. Genomic libraries were constructed from stocks homozygous or hemizygous for each of the mutants and their wild-type background alleles and clones covering the ftz transcribed region were isolated. Sequence analysis of these clones reveals that each of the Ual mutations differs from its wild-type background allele by a single base-pair change. As shown in Figure 1, the Ual mutants are all C→T transitions that affect two nearby proline codons. Ual1 causes an inferred substitution of leucine for Pro215 (numbering from Laughon and Scott 1984), Ual2 causes a substitution of serine for this same proline, and Ual3 causes a substitution of serine for Pro217.

ftz protein expression in Ual mutant embryos

We followed ftz protein expression during embryogenesis of the Ual mutants and wild type using the mouse anti-ftz monoclonal antibody mAb DMftz.1 (see Materials and methods). Early intermediates in the resolution of the seven-striped pattern of ftz protein expression (Karr and Kornberg 1989) are seen easily after staining with this antibody and appear normal in the wild type. As in wild type, stripes are broad at first and have diffuse borders; these stripes then narrow and sharpen at their borders [see Fig. 2]. After sharpening, stripes in the Ual mutants are wider than in wild type at this stage. During germ-band extension, staining persists in the Ual mutants and does not undergo the rapid fading seen in wild type; indeed, ftz stripes appear to widen as germ-band extension proceeds. Epidermal staining in the mutants persists until well after the completion of germ-band extension. In Ual2, the strongest of the mutants, epidermal staining fades out during stage 11 (Campos-Ortega and Hartenstein 1985), ~2 hr later than in wild type. During stage 11, it becomes clear that the limits of persistent ftz stripes in the Ual mutants coincide precisely with parasegmental grooves.

In both mutant and wild-type embryos, neural expression of ftz protein (Carroll and Scott 1985) becomes visible at the beginning of stage 10. Although there is no detectable temporal overlap between epidermal and neural expression in wild type, these expression modes overlap for a period of ~1 1/4 hr in Ual2. Moreover, unlike wild type, Ual mutant embryos show a strong pair-rule modulation of early neural expression: Neural cells in even-numbered [ftz-expressing] parasegments stain precociously relative to those in odd-numbered parasegments. This observation suggests that ftz transcription in the nervous system may be enhanced by ftz protein persisting from the zebra-stripe stage [although previous tests of ftz autoregulation in the nervous system have been negative (Hiromi and Gehring 1987; Dearolf et al. 1989)]. In addition, an irregular pattern of large, darkly staining cells is seen in the epidermis of even-numbered parasegments in the Ual mutants [Fig. 2h, arrow]. Although the fate of these cells is not known, their relatively rapid disappearance from the epidermis suggests they may sink internally and participate in formation of the nervous system. Despite these early differences, the pattern of neural staining at later stages appears normal. At all stages, neural staining is more intense in the Ual mutants than in wild type.

ftz protein is more abundant in Ual mutant embryos than in wild type

The intense staining of Ual mutant embryos relative to wild type suggests that the mutants accumulate higher levels of ftz protein. To determine ftz protein amounts we used the polyclonal anti-ftz antisera of Krause et al. (1988) to probe Western blots of proteins from mutant and wild-type embryos [Fig. 3]. These experiments reveal that in 2.75- to 3.25-hr-old embryos, ftz protein levels are ~1.3-fold greater than wild type in Ual1 homozygotes, 2.7-fold greater in Ual2 homozygotes, and 2.0-fold greater in Ual3 homozygotes. Repeat experiments using independent embryo collections gave increases of 1.1-fold for Ual1, 2.9- and 2.7-fold for Ual2, and 1.7-fold for Ual3. These levels of ftz protein correlate well with the severities of the Ual mutants; Ual2 has the most extreme phenotype, and Ual1 has the weakest (Duncan 1986).
Figure 2. *ftz* protein staining in wild-type and *Ual2/Ual2* embryos. (a–d) Wild-type embryos; (e–h) *Ual2* homozygotes. At blastoderm, wild-type (a) and mutant (e) patterns are similar, although stripes stain more darkly and are slightly wider in the mutant. By mid-germ-band extension, stripes have largely faded in wild type (b) but continue to stain intensely in the mutant (f). Slightly later, stripes appear to widen in wild-type embryos relative to earlier stages (cf. arrowheads in f and g), whereas stripes in wild type continue to fade (c). At full germ-band extension, stripes fade out completely in wild type and expression in the nervous system begins (d). In *Ual2* embryos, stripes persist into the neural round of expression (h). Note the precocious development of neural staining in even-numbered (*ftz*-expressing) parasegments and the presence of darkly staining cells in the epidermal layer (arrow).

Weak persistence of the *Ual* mutant transcripts

To determine whether the changes in *ftz* protein expression described above are also seen at the RNA level, we characterized *ftz* transcripts in the *Ual* mutants by in situ hybridization. As shown in Figure 4, *ftz* transcript stripes are only slightly more persistent in the *Ual* mutants than in wild type, indicating that strong *ftz* protein persistence in these mutants does not result from increased mRNA stability or from continued transcription (cf. Fig. 2c,g with Fig. 4c,f). The slight increase in transcript persistence seen may result from autoregulation, as Hiromi and Gehring (1987) and Dearolf et al. (1989) demonstrated that *ftz* transcription is enhanced by *ftz* protein in epidermal cells. *ftz* transcript stripes also appear to be slightly wider in the *Ual* mutants than in wild type during germ-band extension. Pronounced pair-rule modulation of *ftz* transcript accumulation occurs during early neural expression in *Ual* embryos, although such modulation is also seen weakly in wild type (data not shown).

The *Ual* mutant proteins appear to have longer half-lives than wild type

To determine whether persistence of *ftz* protein in the *Ual* mutants results from reduced susceptibility to degradation, we followed *ftz* protein staining in blastoderm embryos after injection of the protein synthesis inhibitor cycloheximide. Blastoderm embryos were injected at mid-stage 5 (Campos-Ortega and Hartenstein 1985), incubated for varying times, and then fixed and stained for *ftz*, as described in Materials and methods. Stained wild-type and *Ual2* mutant embryos fixed at successive times after injection are shown in Figure 5. The visual impression is that *ftz* protein decay is biphasic in wild type; staining declines very rapidly immediately after injection, but is relatively stable after ~10 min, so that weak stripes are still present even 1 hr after injection. In contrast, *Ual2* mutant embryos show a gradual, apparently monophasic, decay in *ftz* protein staining after cycloheximide injection.

We quantitated the decay in *ftz* protein staining in embryos injected with cycloheximide by measuring optical densities of photographic negatives (Fig. 6). In Figure 6a, we demonstrate that the film used responds approximately linearly in the range of optical densities measured. Figure 6b–d shows semilog plots of *ftz* stripe optical density versus time after injection for wild type and each of the *Ual* mutants. In wild type, *ftz* stripe decay is biphasic; immediately after injection, the half-life of decay is ~6 min; however, the half-life increases to ~40 min 10–20 min after injection. The initial rapid decay rate is consistent with Edgar et al. (1987), who estimated that *ftz* protein decays with a half-life of <10 min. However, these authors did not see a second slow phase of decay, presumably because their antibody detection was not as sensitive as ours. In contrast to wild type, *ftz* stripes in the *Ual* mutants have half-lives of 38–42 min and decay in a monophasic fashion. It is un-
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likely that our results in these experiments are complicated by incomplete blockage of translation because essentially identical results have been obtained for wild type after injection of 10-fold greater amounts of cycloheximide (data not shown).

Origin of segmentation defects in the Ual mutants

Ual mutant larvae and adults show segmentation defects that are out of phase with those caused by ftz loss-of-function alleles (Duncan 1986). To determine when these defects first appear in embryogenesis, we stained Ual mutant embryos with antibodies against the eve and en proteins, as well as with anti-ftz antibodies. In normal development the eve protein is expressed in odd-numbered parasegments in a pattern that is essentially complementary to that of ftz (Frasch and Levine 1987; Frasch et al. 1987), whereas en expression is restricted to the posterior compartment (anterior edge) of each parasegment (DiNardo et al. 1985; Fjose et al. 1985; Kornberg et al. 1985).

The first evidence of abnormality seen in Ual mutant embryos is at late blastoderm during the sharpening of ftz and eve stripes. In normal development, ftz and eve stripes are broad prior to sharpening and have diffuse borders that overlap (see Fig. 7a). These stripes then narrow and develop sharp boundaries as the expression of each gene is extinguished in single rows of cells in the regions of overlap (Frasch and Levine 1987; Frasch et al. 1988) (Fig. 7b). In wild type, ftz and eve stripes narrow to the same extent during sharpening and so remain equal in width. However, in Ual2 homozygotes, eve stripes become much narrower than ftz stripes during sharpening (cf. Fig. 7c and d). Indeed, it would appear that stripe sharpening in Ual2 homozygotes occurs almost entirely by loss of eve expression, with ftz stripes remaining almost at their presharpening width. The spacing of en stripes during germ-band elongation in Ual2 embryos indicates that the parasegments defined by the sharpened eve stripes are also narrower than normal, whereas those defined by the ftz stripes are abnormally wide (see arrowheads in Fig. 8a). In situ hybridization experiments show that wg stripes, which are located just anterior to en stripes (Baker 1987; Ingham et al. 1988), are similarly displaced (data not shown).

In wild type, ftz stripes fade rapidly from their posterior edges during germ-band extension (Fig. 2b). However, in Ual2 homozygotes ftz protein staining appears...
Figure 5. *ftz* staining of embryos at successive times after injection of the protein synthesis inhibitor cycloheximide. Wild-type and Ual2 embryos were injected with cycloheximide at blastoderm and fixed at 0, 10, 20, 40, and 60 min after injection, as indicated. Note that in wild type, staining declines rapidly during the first 10 min after injection and then stabilizes; in Ual2 embryos, staining shows a more uniform gradual decline. The regular cortical arrangement of blastoderm nuclei breaks down between 10 and 20 min after injection, resulting in a grainy appearance of the *ftz* stripes.

to increase in the posterior of each stripe at this stage. As a result, *ftz* staining becomes uniform, or nearly so, within even-numbered parasegments [cf. Figs. 2g and 8b]. One explanation for this apparent increase in staining at the posterior of each stripe could be that, in Ual2 homozygotes, residual *ftz* protein remains throughout each even-numbered parasegment at the start of germ-band extension. Because *ftz* is known to be autoregulated in epidermal cells (Hiromi and Gehring 1987; Dearolf et al. 1989), this residual protein could cause abnormal activation of further *ftz* transcription during germ-band extension. If autoregulation were to play such a role, we reasoned that a transformed *ftz*/*β*-galactosidase reporter construct should be similarly activated in a Ual2 mutant background. To test this, we constructed *ftz*+ and Ual2 stocks homozygous for the P[ry+, *ftz*/lacC] construct of Hiromi et al. (1985) inserted into the second chromosome. We were surprised to find that after germ-band extension, β-galactosidase stripes expand to fill even-numbered parasegments in both *ftz*+ and Ual2 embryos [data not shown].

An additional abnormality seen in Ual2 homozygotes is the formation, after germ-band extension, of partial ectopic *en* stripes in odd-numbered [*eve-expressing*] parasegments [Fig. 8c, arrowheads]. These ectopic stripes, which never occur in wild type, usually form dorsally and laterally, although occasionally they are complete ventrally as well. They are highly dynamic and, in late germ-band-extended embryos, often fuse partially with posterior-adjacent [even-numbered] *en* stripes, especially in lateral and dorsal regions. By late germ-band extension, parasegment sizes appear to be largely normalized. However, at this stage, gaps are often seen dorsally in even-numbered *en* stripes. Although we do not have a clear picture of how these gaps arise, possible intermediates suggest that they may occur when ectopic *en* stripes fuse with both anterior- and posterior-adjacent neighbors. By germ-band retraction, many embryos are nearly normal with respect to *en* staining, although many ectopic stripes and gaps persist [see Fig. 8d] and are presumably responsible for the segmentation defects seen in Ual mutant larvae and adults [Duncan 1986].

Homeotic transformations

The major segmental transformation seen in the Ual mutants is of the anterior first abdominal segment [located in parasegment (PS) 6] to anterior third abdominal segment [located in PS8] [Duncan 1986]. Because PS8 identity is defined by the *abd-A* gene of the bithorax complex (Sánchez-Herrero et al. 1985; for review, see Duncan 1987), it seemed likely that this transformation
dermal labeling is never seen in the anterior compartment of PS6. The early pattern of abd-A expression in Ual2 homozygotes appears normal. However, after germ-band retraction, most embryos show some abd-A staining in the epidermis of both anterior and posterior compartments of PS6 (Fig. 9c). Thus, Ual2 causes ectopic expression of abd-A in PS6, although at a much later time in development than envisioned previously (Duncan 1986). The expression of abd-A in PS6 in Ual2 is primarily lateral, perhaps explaining why we do not see transformation of the A1 ventral setal belt in Ual mutant embryos. The posterior limit of abd-A expression appears to be normal in Ual2 homozygotes.

T(2;3)ftz<sup>ab</sup>; abnormal persistence of ftz RNA

The basic properties of T(2;3)ftz<sup>Rpl</sup> (Rpl) have been described previously (Weiner et al. 1984; Duncan 1986). When heterozygous with wild type, Rpl frequently causes patchy transformations of posterior haltere to posterior wing. Rarely, patchy transformations of the first abdominal segment to a more posterior segment are also seen. Rpl homozygotes die as mature embryos that show defects in even-numbered (ftz-expressing) parasegments. The Rpl translocation truncates the ftz-coding sequence near the carboxy-terminal end of the homeo box and, in the inferred protein sequence, replaces the carboxy-terminal 100 amino acids of the wild-type protein with a sequence of 10 amino acids encoded by unrelated DNA (Laughon and Scott 1984). It has been suggested (Duncan 1986) that the transformation of posterior haltere to wing may occur because the Rpl protein is defective and occasionally fails to activate high-level expression of the homeotic gene Ubx.

In situ hybridization and antibody staining reveal that stripes of both ftz RNA and protein are strongly persistent in Rpl homozygotes (data not shown). Although staining of RNA and protein fades from the posterior of each stripe during germ-band elongation, some ftz protein is detectable throughout each even-numbered parasegment even after the completion of extension. Epidermal staining of ftz protein does not persist as long as that in Ual embryos and fades out just prior to the start of ftz expression in odd-numbered parasegments in the nervous system. As in the Ual mutants, neural expression is precocious in even-numbered parasegments, perhaps because of autoregulatory activation by residual ftz protein. Western blotting experiments show that the Rpl protein is about fourfold more abundant than ftz protein in 2.75- to 3.25-hr embryos from Rpl<sup>+</sup> parents (see Fig. 3).

Probably the simplest explanation for persistence of both ftz RNA and protein in Rpl embryos is that the Rpl transcript is stabilized relative to wild type. The ftz<sup>+</sup> RNA is highly unstable, having a half-life of between 7 and 14 min during nuclear cycles 13 and 14 (Edgar et al. 1986). Although the sequences targeting the ftz transcript for rapid degradation are not known, the 3' end (see Laughon and Scott 1984) contains a match at 7 of 8
nucleotides to the UUAUUUAU consensus sequence described by Caput et al. (1986), which may target a number of mammalian transcripts for rapid degradation (Shaw and Kamen 1986; Wilson and Treisman 1988). The Rpl transcript must lack this sequence because the translocation breakpoint lies in a more 5' position. Although increased RNA stability seems to be the most likely explanation for the persistent striping in Rpl embryos, we cannot rule out the possibility that ftz transcription is prolonged in Rpl or that ftz protein stability is also increased.

In addition to persistence of ftz stripes during germ-band extension, we find that the evolution of the ftz striping pattern at blastoderm is much retarded in Rpl embryos. For similar stages of membrane ingrowth, ftz protein and RNA stripes are far less resolved in Rpl than in wild type. Because we do not see any marked delay in stripe resolution in the Ual mutants, which stabilize the ftz protein, these observations suggest that rapid turnover of RNA, and not protein, is of key importance during stripe formation.

Rpl embryos also show pattern abnormalities not obviously attributable to increased RNA stability. In homozygotes, the first and sixth stripes are significantly reduced compared to the other stripes and, in both homozygotes and heterozygotes, we see bilateral patches of ftz protein expression dorsolaterally in the head region. It is not clear whether these head patches result from altered transcriptional control of ftz or are a normal aspect of ftz expression not detected previously because of the extreme instability of the ftz RNA (Edgar et al. 1986). Other pair-rule genes are expressed in the same region (Ingham et al. 1985; Kilchherr et al. 1986; Gergen and Butler 1988).

Discussion

Ual alleles stabilize the ftz protein

In this report, we show that the Ual alleles are all missense mutations affecting two proline residues located 5 amino acids apart in the 413-amino-acid ftz protein. Two of the alleles cause proline to leucine changes, whereas one causes a proline to serine change. Two lines of evidence indicate that the basic defect in these mutants is the stabilization of the ftz protein. First, we find that the epidermal ftz protein persists ~2 hr longer in the Ual mutants than in wild type, although ftz transcripts disappear at a normal rate. Second, we find that after injection of the protein synthesis inhibitor cycloheximide, ftz staining decays severalfold slower in the Ual mutants than it does in wild type.

Figure 7. Stripe sharpening in wild-type (a, b) and Ual2 (c, and d) embryos. Embryos are double-labeled for ftz protein (orange) and eve protein (purple). In wild type, ftz and eve stripes are equal in width both before (a) and after (b) stripe sharpening. Note that stripes overlap in a and that ftz and eve appear to be extinguished in regions of overlap during sharpening. In Ual2 embryos, the early striping pattern (c) appears to be normal. However, after sharpening (d), ftz stripes are wider and eve stripes are narrower than in wild type. The white vertical bars in b and d mark the sharpened anterior edges of ftz and eve stripes. Note the equal spacing in b and the unequal spacing in d.
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Figure 8. Segmentation in Ual2 embryos after gastrulation. All embryos are double-labeled for ftz (orange) and en (gray). The en stripes identify the anterior edge of each parasegment. (a) An embryo early in germ-band extension. As indicated by arrowheads, even-numbered (ftz-expressing) parasegments are wider than odd-numbered parasegments. ftz staining is strong and uniform in the anterior ¼ of each even-numbered parasegment but is weaker in the posterior quarter. In embryos nearing the completion of germ-band extension (b), ftz staining becomes uniform, or nearly so, within even-numbered parasegments. In fully extended embryos (c), ectopic en stripes often form medially in odd-numbered parasegments (see arrowheads). During and after germ-band retraction (d), these ectopic en stripes usually fuse with posterior adjacent (even-numbered) en stripes (see arrowheads). Gaps often form in the even-numbered en stripes (arrow), perhaps as a result of fusion with ectopic en stripes.

Consistent with an increase in stability, higher levels of ftz protein accumulate at blastoderm in the Ual mutants than in wild type.

Figure 9. Expression of abd-A protein in wild-type and Ual2 embryos. All embryos are double-labeled for abd-A (brown) and en (gray). (a) Wild-type embryo during germ-band retraction. The anterior limit of abd-A expression coincides precisely with the en stripe of PS7. (b) Wild-type embryo after germ-band retraction. The anterior limit of abd-A expression is still coincident with the PS7 en stripe (the apparent extension of abd-A staining into PS6 in this embryo is due to internal, not epidermal, labeling). In both wild-type and Ual2 embryos, a group of cells within the en stripe of PS6 (marked by arrowheads) often begins to stain for abd-A after germ-band retraction (see arrow). (c) Ual2 embryo after retraction. As in most Ual2 homozygotes at this stage, abd-A staining is apparent in the epidermis of PS6 (arrowheads).

The ftz proteins encoded by the Ual mutants are not seriously impaired in essential functions; Ual mutant hemizygotes are almost wild type in phenotype and have full viability. This suggests that the region of the ftz protein affected by the Ual mutations may function specifically to target the ftz protein for rapid degradation. This region is very rich in the amino acids proline, glutamic acid, serine, and threonine. Rogers et al. (1986) and Rechsteiner (1988) have proposed that such "PEST" re-
regions target eukaryotic proteins for rapid degradation. That all three of the Ual mutants alter one of the PEST amino acids would seem to support the PEST hypothesis. However, because most of the amino-terminal half of the ftz protein is rich in the PEST amino acids, it is not at all clear according to the hypothesis why alteration of the prolines affected by the Ual mutants should have such a large effect on stability. Moreover, one of the mutants (Ual3) substitutes one PEST amino acid (serine) for another and would be expected to have little effect on stability.

Rather than amino acid composition, it may be the specific amino acid sequence in the region affected by the Ual mutants that determines ftz protein stability. Computer searches indicate that a 12-amino-acid sequence containing the prolines altered in the mutants is conserved in the inferred protein sequences of at least three other Drosophila segmentation genes (see Fig. 10). These are the pair-rule genes eve [Macdonald et al. 1986; Frasch et al. 1987] and paired [prd] [Frigero et al. 1986], and the gap gene hunchback [hb] [Tautz et al. 1987]. A region showing similarity to the ftz sequence is also present in the protein encoded by the myc oncogene [Watson et al. 1983]. The best match is with the eve sequence, which is identical to ftz at 8 of 12 positions and, like the ftz sequence, is located amino-terminal to the homeo domain. Close matches to the ftz sequence are also present in hunchback (hb) and myc, whereas paired is more closely related to eve than it is to ftz. Both of the prolines affected by the Ual mutants are at conserved positions within these sequences. Although stability measurements have been made only for ftz [Edgar et al. 1987; this paper] and myc [Ramsay et al. 1984], rapidly changing expression patterns for the other genes suggest that all encode unstable proteins. Thus, the conserved region may function to target these proteins for rapid degradation. Alternatively, because all of the proteins in Figure 10 are also likely to be transcription factors, the conserved region may play some role in interacting with other nuclear proteins or DNA. It is worth noting that the hb and myc sequences shown include matches to the cdc2 protein kinase phosphorylation site consensus sequence [Moreno and Nurse 1990], suggesting that Ual alleles might stabilize the ftz protein by altering its phosphorylation [see Krause and Gehring 1989].

The stabilization of the ftz protein by the Ual alleles could be quite indirect. For example, the Ual mutations could stabilize ftz protein by increasing its binding affinity for other molecules in the nucleus (for discussion of this mechanism, see Rechsteiner et al. 1987). The stabilization of ftz protein by such interactions may provide an explanation for our observation that decay of ftz protein staining in wild type is biphasic after cycloheximide injection: A subpopulation of ftz+ protein molecules could be protected from proteinase by binding to other proteins or DNA. Alternatively, biphasic decay may occur because the degradation system responsible for rapid decay is itself unstable.

Although the proteolysis system involved in ftz degradation has not been identified, rapid degradation of ftz, like most protein turnover, appears to be energy dependent (for review, see Rechsteiner 1987). We found [I. Duncan and D. Mattson, unpubl.] that injection of 2-deoxy-d-glucose and sodium azide, which together cause ATP depletion in other systems [Hochster 1963; Fagan et al. 1986; Gronostajski et al. 1985], along with cycloheximide, blocks rapid ftz protein degradation in wild type, although gradual decay continues to occur. That all three Ual mutations alter proline codons suggests that a proline endopeptidase [Andrews et al. 1982; Mentlein 1988, Yoshimoto et al. 1987; Pratt et al. 1987] might be involved in the rapid phase of ftz degradation.

Finally, it should be noted that although the close proximity of the Ual alleles and their lack of effect on essential functions of the ftz protein are consistent with the idea that these mutants identify an instability domain, we have not ruled out the possibility that many amino acid changes stabilize the ftz protein and that the Ual alleles are specific only in their lack of effect on essential functions.

Figure 10. Sequence similarities between the region of ftz protein affected by the Ual mutants and regions of the proteins encoded by the Drosophila segmentation genes hb, eve, and prd, and the vertebrate oncogene myc. Computer searches reveal that in terms of amino acid identities, the best match in the NBRF protein data base to the ftz sequence shown is in the eve protein. The eve sequence shows identity at 8 of 12 positions and, as in ftz, is located amino-terminal to the homeo domain. The two proline residues altered by the Ual mutations (see arrowheads) are at shared positions in these sequences. Many other proteins in the database have regions showing identity at seven or fewer positions in the ftz sequence. Because the sequence TPPXSP is present in the ftz and eve sequences and contains the two prolines altered by the Ual mutations, we searched the data base for other proteins containing this sequence. Ten proteins (counting homologs as single proteins) were found. Of these, the three closest matches to the 12-amino-acid ftz sequence are in the eve, hb, and myc proteins. The remaining proteins in this group, most of which associate with nucleic acids, are the Antennapedia protein, jun, a fragment of the gag protein from baboon endogenous virus, human uracil-DNA glycosylase, the DNA-binding domain of bovine fibronectin, the E2 protein of human papillomavirus, and the tau microtubule-associated protein. Although prd shares only 5 amino acid identities with the ftz sequence, it is included because of its similarity to the eve sequence (6 of 12 positions identical with two conservative differences) and because, like ftz and eve, it is a pair-rule gene of Drosophila. The amino acid carboxy-terminal to the glutamine (Q) in the hb sequence is serine (S), so that even if a gap is not included, it is identical to the ftz sequence at 7 of 12 positions. The hb sequence shown includes residues 184-194 [Tautz et al. 1987], the myc sequence includes residues 61-72 from the chicken c-myc gene [Watson et al. 1983], the eve sequence includes residues 40-51 [Macdonald et al. 1986; Frasch et al. 1987], and the prd sequence includes residues 411-422 [Frigero et al. 1986].
Segmentation defects

Stripe sharpening  The first, and probably primary, segmentation defect we see in Ual mutant embryos is at blastoderm during ftz and eve stripe sharpening. In wild type, ftz and eve stripes narrow to the same extent so that they remain equal to one another in width. In Ual2 embryos, however, eve stripes narrow to a greater extent than ftz stripes do. Because Ual2 causes the accumulation of approximately threefold higher levels of ftz protein at blastoderm than wild type, this bias suggests that normal stripe sharpening depends on a balance between the amounts of ftz and eve protein. Data to be presented elsewhere indicate that stripe sharpening involves the mutual repression of ftz and eve in the regions of stripe overlap. Thus, genotypes in which ftz protein is in excess relative to eve protein (such as the Ual mutants) show widening of ftz stripes during sharpening, whereas genotypes in which eve protein is in excess show widening of eve stripes. A bias in favor of ftz stripes similar to that seen in the Ual mutants appears to occur in embryos homozygous for weak eve alleles (Frasch et al. 1988). Very likely, this bias results from the reduced ability of the proteins encoded by these alleles to repress ftz during sharpening. A requirement for eve+ in the sharpening of ftz stripes has been noted previously by Lawrence and Johnston (1989a,b).

Examination of the stages of membrane ingrowth at blastoderm reveals that stripe sharpening is not significantly delayed in Ual mutants. Initially, this observation seemed paradoxical because stabilization of the ftz protein was expected to delay sharpening. The explanation appears to be that stripe sharpening in the Ual mutants occurs primarily by decay of eve, and not ftz, protein. As a consequence, ftz stripes remain at approximately their presharpening width, whereas eve stripes become abnormally narrow. In both wild-type and Ual2 embryos, sharpening is a two-step process, with gaps forming anterior to eve stripes before they form anterior to ftz stripes (Frasch et al. 1988). We have not determined whether both of these phases are affected in the Ual mutants.

Anti-ftz segmentation defects superficially similar to those caused by the Ual mutants occur when ftz is expressed indiscriminately in the early embryo under the control of a heat-shock promoter (Struhl 1985; Ish-Horowicz and Gyrkovics 1988; Ish-Horowicz et al. 1989). Although the end result is similar, Ish-Horowicz et al. (1989) have shown that the primary defect in heat-shock ftz embryos is quite different and involves alterations in en and wg expression not seen in the Ual mutants.

Germ-band extension  In wild type, ftz stripes become graded during gastrulation and germ-band extension so that the anterior of each stripe stains strongly and is sharply defined, whereas the posterior stains weakly and is of indefinite extent [Lawrence 1987; Lawrence et al. 1987, Lawrence and Johnston 1989a]. Much significance has been attached to this gradient. For example, Frasch et al. (1988) have suggested that the segment polarity gene en is regulated so that it becomes expressed only at the high point of the gradient, at the anterior edge of each ftz stripe. Lawrence and Johnston (1989a) have speculated that the ftz gradient may provide positional information to other segment polarity genes as well. Our observations argue against these models because we find that in the Ual mutants en is expressed at the anterior edge of each ftz stripe, just as in wild type, even though a normal gradient of ftz protein accumulation is not present. Although ftz staining in Ual2 embryos is transiently reduced in the posterior quarter of each stripe during sharpening, staining is maintained at a high level in the anterior ¼ of each stripe. During germ-band extension, staining increases in the posterior quarter and becomes uniform throughout each even-numbered parasegment. Because most even-numbered parasegments develop normally in the Ual mutants, our results suggest that the ftz gradient may be entirely dispensable.

Although the distributions of ftz protein and RNA are similar in Ual2 embryos at blastoderm and early germ-band extension, the increase in protein staining at the posterior of each stripe at later stages is not accompanied by an observable increase in ftz RNA. Likely, ftz transcripts are present here but at levels too low to detect. The posterior increase in ftz protein staining appears to require functional ftz protein, since a large number of ftz loss-of-function alleles recovered as revertants of Ual2 show persistent but narrow ftz protein stripes after germ-band extension (data not shown). This suggests that autoregulation may be an important component of posterior ftz protein increase and argues against the possibility that diffusion of ftz protein is responsible. We find that β-galactosidase staining in a ftz/β-gal transormant also increases to fill even-numbered parasegments during germ-band extension in both ftz+ and Ual2 backgrounds. Thus, a posterior increase in ftz protein during germ-band extension may be an aspect of normal development that has gone undetected because of the extreme instability of the wild-type protein and RNA. The posterior increase in staining also suggests that entire parasegments are domains of ftz expression and lends support to models which specify that ftz protein is involved in directing cell fates throughout even-numbered parasegments (e.g., Duncan 1986, Ingham and Martinez-Arias 1986, Ingham et al. 1988).

The extended germ band and germ-band retraction  During germ-band extension, en expression appears to be essentially normal in Ual2 embryos, with the exception that stripe spacing is altered because of the widening of even-numbered parasegments. However, after the completion of extension, ectopic en stripes frequently appear dorsally in odd-numbered parasegments. The cause of these ectopic stripes is not clear. However, because ftz does not seem to be expressed in odd-numbered parasegments (except at very early stages), it seems likely that they are an indirect consequence of parasegment narrowing. One possibility is that odd-numbered parasegments are often too small in the Ual mutants to accommodate a complete repertoire of segment polarity gene expression patterns. Because ectopic
en stripes occur in every parasegment in embryos mutant for the segment polarity gene patched (DiNardo et al. 1988; Martinez-Arias et al. 1988), one possibility is that ectopic en stripes arise in Ual2 embryos because of defective patched expression in narrowed parasegments. Like the ectopic en stripes in patched− embryos (DiNardo et al. 1988), those in Ual embryos are dynamic and fuse with adjacent stripes. The expression of wg, which may be expected to show ectopic activation in concert with en (DiNardo et al. 1988; Martinez-Arias et al. 1988), has not been examined at the extended germ-band stage in Ual mutants.

**Homeotic transformations**

In addition to segmentation defects, Ual mutants cause homeotic transformations of the anterior first abdominal segment (in PS6) to anterior third abdominal segment (in PS8) in the adult cuticle. In Ual2 homozygotes this transformation has almost 100% penetrance and expressivity. Because PS8 identity is controlled by the abd-A gene of the bithorax complex (for review, see Duncan 1987), we examined abd-A protein expression in Ual2 mutant embryos. We find that in germ-band-extended embryos, abd-A protein expression is limited anteriorly by the PS7 anterior boundary, as in wild type. However, after germ-band retraction, variable expression of abd-A is initiated in the anterior compartment of PS6 in Ual2 embryos but not in wild type. This ectopic expression of abd-A is presumably responsible for the homeotic transformation observed in adults. The delayed activation of abd-A in PS6 of Ual2 homozygotes resembles the anterior expansion of Ubx expression seen after germ-band retraction of embryos in which ftz has been expressed indiscriminately under the control of a heat shock promoter (Ish-Horowicz and Gyrkovics 1988; Ish-Horowicz et al. 1989). In this case, however, anterior spread of Ubx expression is correlated with loss of the boundary between parasegments 5 and 6. In Ual2 embryos, the en stripe at the anterior margin of PS7 generally remains intact, suggesting that activation of abd-A in PS6 in Ual2 does not depend on loss of a parasegmental boundary. In a previous attempt to explain the Ual phenotype, a model was proposed in which both segmentation defects and homeotic transformations were considered to result from the same primary defect, the widening of ftz stripes (Duncan 1986). Although this model appears to be essentially correct with respect to segmentation defects, several inconsistencies indicate that homeotic transformations do not arise as suggested. These inconsistencies include exceptions (Duncan 1986, I. Duncan, unpubl.) to two important predictions of the model, that homeotic transformations should always remain intact in the Ual mutants, and that there should always be a strong correlation between segmentation defects and homeotic transformations. In addition, the model predicts early, rather than delayed, expression of abd-A in PS6 in Ual homozygotes and makes no provision for why homeotic transformations should occur almost exclusively in PS6.

If stripe widening is not the cause of homeotic transformations in Ual mutants, what is? Two striking differences in ftz protein expression are evident in Ual mutants: ftz protein accumulates to higher levels than in wild type, and it persists much longer in development. Because animals carrying extra doses of ftz+ show transformations of PS6 to PS8 but do not show marked protein persistence, whereas Ual mutant hemizygotes show only a low frequency of homeotic transformations but show strong protein persistence (D. Mattson and I. Duncan, unpubl.), it seems likely that the cause of the transformations is an excess of ftz protein, not abnormal persistence. One way that increased ftz protein could cause transformations of PS6 to PS8 is if the identities of these parasegments were distinguished by the relative amounts of ftz protein and some other protein present in a gradient in the embryo. Enhancement of homeotic transformations in Ual heterozygotes by alleles of the gap gene Krüppel (Kr) (Duncan 1986) suggests that ftz and Kr proteins may participate in such a mechanism. Kr is expressed at blastoderm in a broad central domain in which protein levels are high centrally and diminished toward the poles (Gaul et al. 1987; Gaul and Jackle 1989). PS6 is defined by the third ftz stripe, which lies in the region of peak Kr expression, whereas PS8 is defined by the fourth ftz stripe, which lies in a region of lower Kr expression. An overall increase in the amount of ftz protein, such as occurs in the Ual mutants, could transform PS6 toward PS8 by changing the ftz to Kr protein ratio in PS6 to one more like that normally present in PS8. Models of this type can accommodate, but do not explain, the delay in activation of abd-A in PS6 until after germ-band retraction. The model suggested here raises the possibility that the target of heat shock in causing transformations of PS6 to PS8 (Duncan 1986) is Kr, or some other gene whose product shows a graded distribution at blastoderm, rather than ftz, as suggested originally.

**Materials and methods**

**Sequence analysis**

Genomic Southern blots were carried out by standard methods (Maniatis et al. 1982). Genomic DNAs from each of the Ual mutants and their wild-type background alleles [Ual2 was induced on a pMcp chromosome, whereas Ual1 and Ual3 were induced on Canton-S] were restricted with EcoRI, Sau3A, KpnI, SalI and XbaI and Southern blots probed with pDm V61 H3.5 (Weiner et al. 1984), a 3.5-kb HindIII genomic clone that includes the ftz transcribed region. This would have detected rearrangement breakpoints throughout the ftz transcribed and regulatory regions. Genomic libraries from each of the mutants and their wild-type background alleles were constructed in the EMBL3 λ vector (Frischauf et al. 1983) and screened in duplicate with probes flanking the ftz transcription unit to identify clones bearing the complete ftz-coding region. A 2.6-kb PstI–HindIII fragment from each allele was then subcloned into the pEMBL18+ and pEMBL19+ vectors (Dente et al. 1985), and dideoxy DNA sequencing was carried out using the Sequenase system (U.S. Biochemical Corp.) and synthetic primers complementary to sites spaced throughout the
gene. The entire PstI-HindIII subclone was sequenced from one strand, and the region containing the \textit{Dal} mutations was sequenced from both strands. Homology searches of the NBRF protein data base were conducted with the ProfileSearch and WordSearch programs of the University of Wisconsin Genetics Computer Group software package [Devereux et al. 1984].

\textbf{Antibody labeling}

\textbf{Fixation} All embryos were fixed with a modification of the two-phase heptane/fixative procedure [Mitchison and Sedat 1983]. Embryos were dechorionated in 50% bleach in water, rinsed in PBS (10 mM phosphate, 150 mM NaCl, 0.05% Triton-X 100), and fixed for 30 min at room temperature with vigorous shaking in 3.2 ml of PMG [50 mMPIPES (pH 6.9), 1 mM MgSO$_4$, 2 mM EGTA], 0.8 ml of 37% formaldehyde, and 4 ml of heptane. The aqueous phase was replaced with 4 ml of methanol, and the tube was shaken vigorously for 30 sec to remove vitelline membranes. Embryos were then washed four times in methanol and stored at 5°C in absolute methanol.

\textbf{Histochemical staining} Embryos were treated for 15 min in 3% H$_2$O$_2$ in methanol to block endogenous peroxidase, rehydrated in PBS-Tx, and blocked for 2 hr in 5% powdered milk in PBS-Tx. Primary antibodies were added in block and incubated for 1.5 hr. Embryos were washed three times at 10 min per wash in PBS-Tx and incubated with biotinylated secondary antibodies (Vector Laboratories) for 1 hr in block at a dilution of 1:200. After a third wash procedure, the color was developed with 0.25-0.5 mg/ml diaminobenzidine [Amersham] in 50 mM Tris (pH 7.2), 0.012% H$_2$O$_2$, with or without 0.4% NiCl$_2$ or CuCl$_2$. For double-labeling, embryos were stripped of the initial primary and secondary antibodies by a 5-min treatment in 2 M glycine-HCl (pH 2.5) and then reblocked for an additional hour. Embryos were then stained for the secondary antigen as above.

All embryos were dehydrated from PBS (without Triton X) into 95% ethanol, followed by 100% ethanol, and were mounted in Permount (Fisher) containing 30% methyl salicylate. Embryos were photographed under differential interference contrast or bright-field optics with Polaroid type 55, Kodak Ektachrome 400, or Kodak EKTAR 125 films, using a Zeiss Axiomat photomicroscope or a Photomicroscope III.

The primary antibodies used were as follows: Monoclonal mouse anti-\textit{ftz} and monoclonal mouse anti-\textit{abd-A} antibodies were generated in this laboratory [see below]. The \textit{ftz} antibody was used as ascites at a concentration of 1:500, and the \textit{abd-A} antibody was used as hybridoma culture supernatant at a concentration of 1:10. Polyclonal rabbit anti-\textit{eve} was kindly provided by M. Frasch and was used at a concentration of 1:5000. Monoclonal mouse anti-\textit{en} is the mAb 4D9 antibody of Patel et al. [1989b]. The mAb 4D9 cell line was obtained from the American Type Culture Collection, and the hybridoma culture supernatant was used at a concentration of 1:4.

\textbf{Western blots} Half-hour egg collections were made for each genotype and aged until embryos were 2.75 to 3.25 hr old. Embryos were then dechorionated in 50% bleach in water and washed with PBS-Tx. To determine the relative synchrony of each genotype, a portion of each collection was observed under the microscope and scored for the percentage of embryos at blastoderm. After dechorionation, embryos were homogenized in SDS lysis buffer [2.5% SDS, 60 mM Tris, 0.005% bromophenol blue, 10% glycerol], boiled for 5 min, and stored at -20°C. The total protein content of each lysate was then determined by Lowry analysis, and equivalent protein amounts (~580 \mu g total protein per lane) were run on a 10% polyacrylamide gel and electroblotted onto nitrocellulose.

\textit{ftz} protein was detected by use of a rabbit anti-\textit{ftz} polyclonal antibody (kindly provided by H. Krause). To control for the amounts of protein loaded, a rabbit polyclonal antiserum against \textit{Drosophila} cytoplasmic myosin [Kiehart and Feghali 1986], provided by Dr. Kathryn Miller, was used. Both antibodies were visualized with \textit{125I}-labeled protein A [ICN] and quantitated by gamma counting.

\textbf{Isolation of monoclonal antibodies} The monoclonal antibodies mAb DMabd-A.1 and mAb DM Fritz.1 were generated by using the following general protocol: BALB/c mice [2-6 months old] were immunized with \textit{ftz} and \textit{abd-A} proteins produced by the T7 expression system of Studier and Moffatt [1986] [\textit{abd-A} construct kindly provided by Dr. Shige Sakonju, and \textit{ftz} construct kindly provided by Dr. Allen Laughon]. Mice received an initial injection of 100 \mu g of protein in PBS emulsified 1:1 with Freund's complete adjuvant. The mice were rested for 2 weeks and were then given three boosts of 100 \mu g of protein in Freund's incomplete adjuvant at 1 week intervals. Five days after the final boost, serum samples were tested for staining on fixed embryos. Mice giving good serum responses were boosted with 50 \mu g of protein without adjuvant on three successive days, rested for 1 day, and fused on the next day. Spleen cells were fused in the presence of 50% PEG to SP2/0 myeloma cells, using standard protocols. Hybridoma supernatants were screened 1-2 weeks later on fixed embryos, and positive preclones were cloned by limiting dilution.

Both antibodies isolated in this study are subtype IgG 2a Kappa [isotyped using the ScreenType mouse immunoglobulin isotyping kit, BMB]. Although no epitope mapping has been undertaken, the \textit{ftz} antibody must recognize an epitope within the amino-terminal 3% of the protein, as it stains the \textit{ftzab} protein.

\textbf{In situ hybridization} In situ hybridization to intact embryos was performed by using the Genius Nonradioactive DNA Labeling and Detection Kit [Boehringer Mannheim]. A 2.2-kb \textit{PstI-\textit{EcoRI}} DNA fragment was labeled according to kit instructions. Embryos were fixed in paraformaldehyde and hybridized and stained according to the method of Tautz and Pfeifle [1989]. Following color development [4-6 hr], embryos were washed thoroughly in PBS + 0.1% Tween + 20 mM EDTA, dehydrated stepwise into ethanol, and mounted in Permount.

\textbf{Embryo injections} Embryos were injected with cycloheximide [Sigma] at 1 mg/ml in 5 mM KCl, and 0.1 mM \textit{NaPO$_4$} (pH 6.5) as described by Weir et al. [1988]. To ascertain that the dose of inhibitor used was not limiting, one injection series was carried out at 10 mg/ml cycloheximide. To determine whether \textit{ftz} protein degradation is energy dependent, cycloheximide was injected (1 mg/ml) along with 2-deoxy-D-glucose at 1 M and sodium azide at 0.5 M.

Embryos were prepared and injected by standard methods and were then incubated at 25°C 100% relative humidity for appropriate times, washed off the injection slide in a stream of heptane, and transferred to acid-washed glass screw-cap tubes con-
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...taining fixative and heptane, as described above, and shaken vigorously for 30 min at room temperature. After fixation, embryos were rinsed in PBS, and vitelline membranes were removed manually. Embryos were then transferred to PBSTx, dehydrated into methanol, and stored at 5°C. Antibody staining was moved manually. Embryos were then transferred to PBSTx, dehydrated into methanol, and stored at 5°C. Antibody staining was as described above.

Quantitation of embryo staining intensities

An underlying assumption in our analysis is that the staining intensity of a stripe is proportional to the amount of ftz protein present. Because of variation in staining intensities from day to day, only embryos that were injected and fixed on the same night and stained together with the same reagents for the same length of time were compared. Stained embryos were photographed on a Zeiss Axiomat photomicroscope, using 4 x 5-inch Polaroid type 55 film. Only one embryo for each data point was photographed. The embryo chosen represented an average staining intensity of the 5–10 embryos that comprised each time point. Negatives were scanned for absolute optical density by use of a Molecular Dynamics 300A Computing Densitometer. For each negative, optical densities were measured for the anterior two stripes, the unlabeled anterior pole, and a region outside the embryo. To correct for background staining, the average optical density for the first two stripes was then subtracted from the optical density value for the anterior pole of the embryo. To normalize these values for slight differences in photographic exposure, each was then divided by the optical density of a region of the negative outside of the embryo. In fact, the optical densities of the anterior pole and of regions outside of the embryo hardly varied, so that qualitatively very similar results were obtained by simply plotting uncorrected stripe optical density values.

Croses

All flies were raised on the medium of Lewis (1960) at 25°C and 75% relative humidity. All Ual2 embryos described were from homozygous parents produced by crossing Ual2 P P cu Ubx* e/TM1 males to In(3L)P, Me Ual2 P P/TM3, Sb Ubx*1986.17 set females. (Ubx*1986.17 is an EMS-induced Ubx allele recovered by I. Duncan.) Although Ual2 homozygotes have low viability, all classes of progeny except homozygous Ual2 escapers die in this cross. Because ftz*Rpl is a recessive lethal, all Rpl embryos were collected from heterozygous parents. To avoid the production of gross aneuploids from adjacent segregations, parents were made heterozygous for the Rpl translocation and of both translocations lie in the same arm.

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