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A Molecular Analysis of *fushi tarazu*, a Gene in Drosophila melanogaster That Encodes a Product Affecting Embryonic Segment Number and Cell Fate

Amy J. Weiner, Matthew P. Scott* and Thomas C. Kaufman
Program in Genetics and in Molecular, Cellular, and Developmental Biology
Department of Biology
Indiana University
Bloomington, Indiana 47405

Summary

Mutations at the *fushi tarazu* locus in Drosophila melanogaster affect both segment number and the pattern of cuticular structures on alternating segments of embryos. The *ftz* gene has been cloned and characterized.

Two mutations, ftz^{w20} and ftz^{Rpl} are associated with lesions in a 3.2 kb fragment of DNA cloned in the Antennepedia Complex (ANT-C) chromosome "walk." The structure of DNA isolated from the ftz^{w20} and ftz^{Rpl} chromosomes indicates that the mutations are associated with a 4.9 kb insertion of DNA and a chromosomal rearrangement breakpoint, respectively.

The 3.2 kb genomic DNA fragment hybridizes to a 1.8 kb polyadenylated transcript which accumulates maximally at 2–4 hr of embryonic development. The ftz^{w20} and ftz^{Rpl} mutations have different phenotypic consequences for the developing embryo, although both mutations interrupt the 1.8 kb transcription unit. The genetic and molecular data indicate that the 1.8 kb transcript derives from the *ftz* locus. The gene products are synthesized and utilized several hours prior to the visibly detectable morphogenetic events which the gene apparently regulates.

Introduction

Embryos of many higher organisms are at least partially composed of repeated, morphologically identical units called metameres or segments. The origin and nature of the developmental information required for the formation of metameres, as well as the processes by which the developmental information within each segment functions to establish the future pattern of structures characteristic of each segment, are central issues in developmental biology.

In Drosophila, cell lineage (Weischaus and Gehring, 1976), transplantation (Illmensee, 1978), ablation (Underwood et al., 1980), and embryo manipulation studies (Chan and Gehring, 1971; Schubiger and Wood, 1977) indicate that the developmental fates of embryonic cells are determined at the cellular blastoderm stage (2.5–3.5 hr of development) and that the process of segmentation (6–10 hr of development) partitions certain preprogrammed groups of cells into developmentally autonomous units or compartments (Garcia-Bellido, 1973; see Lawrence, 1981 for review). At approximately 8–10 hr of development, the embryo is composed of a segmented head region, three thoracic segments, eight abdominal segments, and a caudal region (Turner and Mahowald, 1977). The thoracic and abdominal segments are approximately equal in width (Lohs-Schardin, et al., 1979) and can be distinguished from one another late in embryogenesis by the pattern of ventral cuticular structures, such as the setae or denticles, and by the presence of morphological markers such as Keilin's organs, ventral pits, and tracheal pits.

Mutations in at least three classes of genes—maternal-effect, homoeotic, and zygotic lethal genes—cause abnormalities in the establishment, organization, and utilization of positional information (Wolpert, 1969) within the developing embryo. Several maternal-effect genes have been described which are required for establishing and maintaining the anterior–posterior (Bull, 1966) and dorsal–ventral polarity of the embryo (Nusslein-Volhard, 1979). The homoeotic mutations affect the second class of genes. Homoeotic genes specify segmental identity but do not alter segment number (Lewis, 1963, 1978, 1981; see Ouweneel, 1976 for a review). The third class of genetic loci are represented by zygotic lethal mutations which either affect segment number and/or the pattern of segment-specific cuticular structures (Nusslein-Volhard and Wieschaus, 1980). A minimum of 20 zygotic lethal loci have been genetically characterized and shown to affect the pattern embryonic segmentation (Nusslein-Volhard and Wieschaus, 1980; Sander et al., 1980; Wakimoto et al., 1984). This paper describes the initial molecular characterization of one such locus, *fushi tarazu* (*ftz*).

We have physically mapped the *ftz* locus by characterizing genomic DNA isolated from mutant flies. The *ftz* locus encodes a 1.8 kb polyadenylated transcript that is maximally accumulated at the cellular blastoderm stage of development (2–4 hr of development). Since the period of RNA accumulation is coincident with the temperature-sensitive period of a temperature-sensitive allele of the *ftz* locus (Wakimoto et al., 1984), the data indicate that the *ftz* gene product is synthesized and utilized prior to the physical process of embryonic segmentation. The combined molecular and genetic data suggest that the *ftz* locus is among the first zygotic genes transcribed and is required for establishing the normal segmental cuticular pattern as well as the number of segment boundaries in Drosophila embryos.

Results

Mutations Associated with the *ftz* Locus

Cuticle preparations of homozygous ftz^{w20} embryos exhibit one-half the normal number of denticle belts as do embryos genotypically ftz^{w20}/Df when compared to wild-type embryos (Figures 1A and 1B). In contrast, ftz^{Rpl}/Df embryos typically have partial gaps and/or fusions in some denticle belts while others appear morphologically normal

* Present address: Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

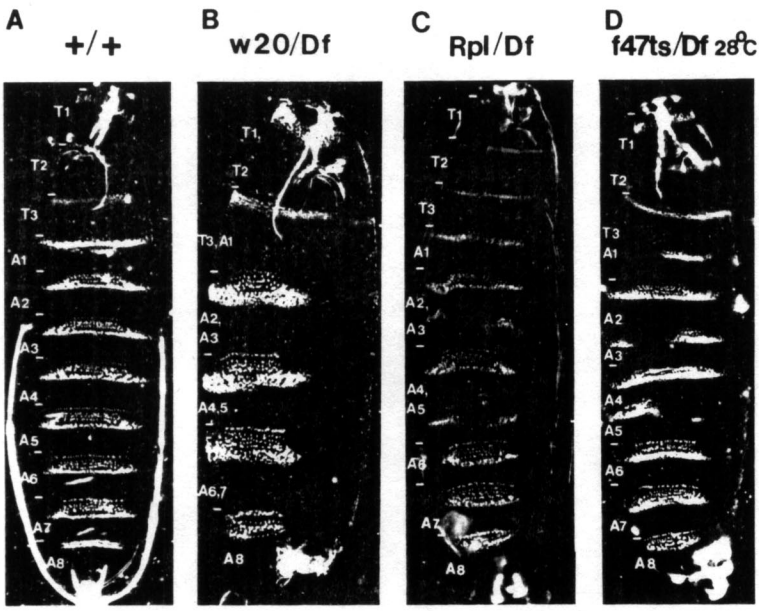

Figure 1. Cuticular Preparations of Wild-Type and Hemizygous *ftz* Embryos

Cuticular preparations of embryos (see Experimental Procedures) of the following genotypes are shown in Figure 1. (A) Wild-type Oregon R, P2 strain (138×). (B) ftz^{w20}/Df (138×). (C) ftz^{Rpl}/Df (138×). (D) ftz^{47ts}/Df raised at 28°C (138×). T1–T3 = first thoracic through third thoracic segments; A1–A8 = first through eighth abdominal segments.

(Figure 1C). The phenotype as well as the phenotypic variability observed in individual ftz^{Rpl} embryos, with respect to the number and morphology of affected segments, is similar to that of embryos that are hemizygous for a "leaky" temperature-sensitive allele of the *ftz* locus, ftz^{47ts} (compare Figures 1C and 1D). The genetic characterization of embryos hemizygous for the ftz^{w20} and ftz^{Rpl} mutations suggests that the ftz^{w20} allele eliminates gene function (Wakimoto et al., 1984) while the ftz^{Rp1} allele partially expresses the *ftz* gene function.

Heterozygous ftz^{Rpl} flies also exhibit a dominant postbithorax-like phenotype in which the metathoracic haltere is partially transformed into a mesothoracic wing. The mechanism by which the chromosomal rearrangement associated with the ftz^{Rpl} mutation causes a postbithorax-like phenotype is unclear.

Physical Localization of the *ftz* Locus

Lewis et al., (1980a, 1980b) genetically mapped the *ftz* locus in the Antennapedia Complex (ANT-C) between the *Deformed* (*Dfd*) and *Antennapedia* (*Antp*) loci. The ANT-C is cytologically located in the salivary gland polytene chromosome interval 84A4,5 to 84B1,2 and DNA from this region of the chromosome has been isolated and characterized by Scott et al. (1983). One method used to correlate the genetic map position of a particular locus with a physical map of cloned DNA involves the use of chromosomal rearrangements which are associated with mutations in the particular gene of interest. Chromosomal rearrangements can partially or completely inactivate a gene(s) within the vicinity of the breakpoint either by causing a direct disruption of the DNA sequence within a gene or through position effects (Spofford, 1976).

DNA breakpoints associated with *ftz* mutations were localized on the DNA map by detecting restriction site differences between mutant and non-mutant chromosomal DNA. Southern blots containing restriction digests of genomic DNA isolated from flies carrying one of seven mutant alleles of *ftz* (see Table 1), each heterozygous with a balancer chromosome, were probed with labeled DNA from the region of the ANT-C walk between the Dfd^{+R16} breakpoint and breakpoints associated with mutations at the *Antp* locus (Figure 2). Each Southern blot contained DNA extracted from the strain of flies bearing the chromosome on which each individual *ftz* mutation was induced as a control for irrelevant restriction site heterogeneity among chromosomes. For example, the ftz^{w20} and ftz^{47ts} mutations were induced on a ftz^{+} *red e* and ftz^{+} p^{p} chromosome (see Lindsley and Grell, 1968 or Lewis et al., 1980a, 1980b), respectively. The DNA from mutant flies as well as ftz^{+} *red e*/ftz^{+} *red e* and p^{p}/p^{p} are analyzed on the Southern blot shown in Figure 3. Anomalous restriction fragments were detected only on Southern blots hybridized to a probe from the +66 to +69.2 kb region of the ANT-C walk, which is designated the λA439 Hind III 3.2 kb fragment. Figure 3 shows that when a Southern blot containing Hind III (H), H plus Sal I (S), and Bam HI (B) digests of DNA extracted from ftz^{+} *red e*/ftz^{+} *red e* flies (lanes 1, 3, 11) is probed with the λA439 Hind III 3.2 kb DNA fragment the following bands are visible on the autoradiogram: an H 3.2 kb, an H plus S 1.7 kb/1.5 kb and a B 4.6 kb band. The sizes of the genomic DNA fragments observed in Figure 3 lanes 1, 3, and 11 are consistent with the fragment sizes predicted from the restriction map of the Canton S wild-type DNA in clone λA439 (see Figure 4). The presence of a 3.2 kb fragment in lanes containing S plus H digested DNA (Figure 3, lanes 3, 4, and 6) is due to the incomplete digestion of the genomic DNA by the Sal I enzyme. In contrast, genomic DNA isolated from the heterozygous mutant strain ftz^{w20}/

Table 1. Summary of Mutations at the *ftz* Locus in Drosophila melanogaster

Mutation	Reference	Mutagen[a]	Cytology	Molecular Anomaly
ftz^{w20}	Lewis et al., 1980a, 1980b; Wakimoto et al., 1984	E	Normal	DNA insertion into λA439 Hind III 3.2 kb fragment (see Figure 4)
$ftz^{X\text{-}ak5}$	Lewis et al., 1980a, 1980b	X	Normal	None
ftz^{Rpl}	I. Duncan, unpublished	X	T(2, 3)[b]	Translocation breakpoint disrupts the λA439 Hind III 3.2 kb fragment (see Figure 4).
ftz^{f47ts}	Wakimoto et al., 1984	E	Normal	None observed (restriction site heterogeneity in the parental strain)
$ftz^{E.fc.1fs}$	L. Cain, unpublished	E	Normal	None
$ftz^{E.fc.5}$	L. Cain, unpublished	E	Normal	None
$ftz^{E.fc.15}$	L. Cain, unpublished	E	Normal	None

[a] E = EMS and X = x-rays.
[b] The pattern of the salivary gland polytene bands in $ftz^{Rpl}/TM1$ chromosomes shows a 2, 3 reciprocal translocation between region 84AB and the second chromosome centromeric heterochromatin as well as an insertion of polytene chromosome material of unknown origin into 84AB (T. Kaufman, unpublished observations).

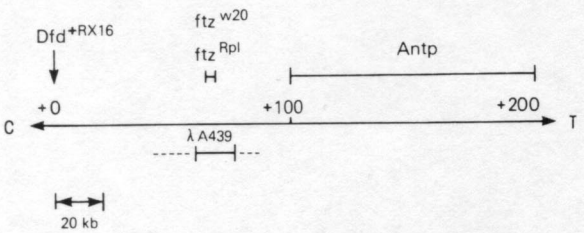

Figure 2. Location of Selected Mutations on the Physical Map of ANT-C DNA

The coordinates of DNA breakpoints associated with three loci, Dfd^{+RX16}, *ftz*, and *Antp*, located within the 300 kb of DNA cloned in the Antennapedia Complex (ANT-C) chromosome walk (Scott et al., 1983) are shown in Figure 2. λA439 was isolated from a λ recombinant DNA library containing DNA sequences from the Canton S strain of Drosophila melanogaster. The dashed lines represent the Drosophila DNA sequences extending ±20 kb from λA439 Drosophila insert DNA, which were examined for restriction site sequence heterogeneity in fly strains heterozygous for mutations at the *ftz* locus. Centromere (C), Telomere (T), $Deformed^{+RX16}$ (Dfd^{+RX16}), *fushi tarazu* (*ftz*), *Antennapedia* (*Antp*).

TM3 digested with the same enzymes and hybridized to the same probe showed unique bands on the autoradiogram in addition to the pattern of bands observed for ftz^{+} *red e*/ftz^{+} *red e* DNA. For example, an H 2.5 kb, 3.2 kb and 5.6 kb fragment hybridizes to the λA439 Hind III 3.2 kb probe in lane 2 that contains $ftz^{w20}/TM3$ DNA, while only an H 3.2 kb band is observed in DNA extracted from the parental, ftz^{+} *red e*/ftz^{+} *red e* genomic DNA (lane 1). The 3.2 kb band observed in lane 2 comes from the *TM3* balancer chromosomal DNA, while the two unique bands (2.5 kb and 5.6 kb) come from the DNA extracted from the ftz^{w20} chromosome. Furthermore, in contrast to the B 4.6 kb band observed in DNA extracted from ftz^{+} *red e*/ftz^{+} *red e* flies (lane 11), a new 9.5 kb band is observed in lane 12, which contains Bam HI digested DNA purified from $ftz^{w20}/TM3$ flies. These data suggest that the ftz^{w20} mutation is associated with a 4.9 kb insertion of DNA, which has at least one Hind III site and no Bam HI sites, into region +66 to +69.2 of the ANT-C cloned DNA.

The results in Figure 3 show that the intensity of the H

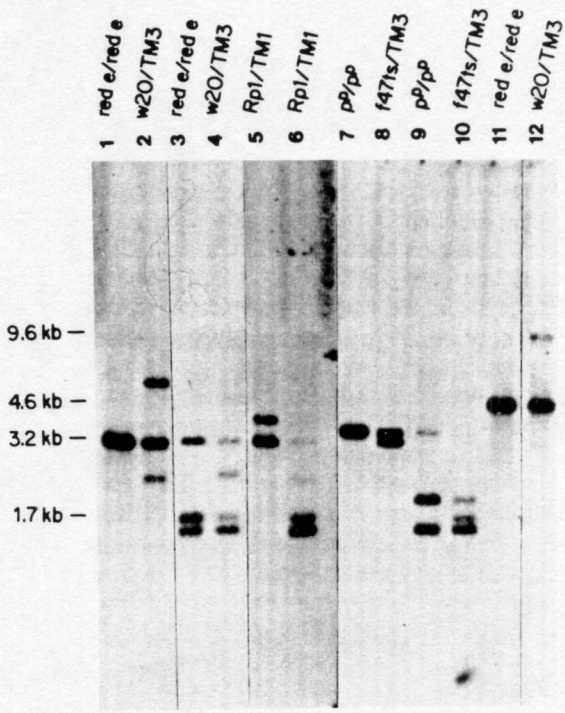

Figure 3. Genomic Southern Blot Analysis of DNA from *ftz* and ftz^{+} Flies

DNA was extracted from flies heterozygous for *ftz* mutations, and from some of the strains of flies from which the mutants were derived, digested with selected enzymes, electrophoresed on a 0.6% agarose gel in TA buffer (see Experimental Procedures), blotted according to the method of Southern (1975), hybridized to a nick-translated Hind III 3.2 kb probe (2×10^7 cpm), purified from the plasmid clone pDmA439 H 3.2, and exposed for three days. The λA439 Hind III 3.2 kb fragment is located at map coordinates +66 to +69.2 of the ANT-C walk. Lanes 1–12 contain the following DNA preparations digested with the designated restriction enzymes: (1) ftz^{+} *red e*/ftz^{+} *red e* plus Hind III; (2) $ftz^{w20}/TM3$ plus Hind III; (3) ftz^{+} *red e*/ftz^{+} *red e* plus Hind III plus Sal I; (4) $ftz^{w20}/TM3$ plus Hind III plus Sal I; (5) $ftz^{Rpl}/TM1$ plus Hind III; (6) $ftz^{Rpl}/TM1$ plus Hind III plus Sal I; (7) ftz^{+} p^{p}/ftz^{+} p^{p} plus Hind III; (8) $ftz^{f47ts}/TM3$ plus Hind III; (9) ftz^{+} p^{p}/ftz^{+} p^{p} plus Hind III plus Sal I; (10) $ftz^{f47ts}/TM3$ plus Hind III plus Sal I; (11) ftz^{+} *red e*/ftz^{+} *red e* plus Bam HI (12) $ftz^{w20}/TM3$ plus Bam HI. (See Lindsley and Grell, 1968, or Lewis et al., 1980a, 1980b, for a description of chromosomes.)

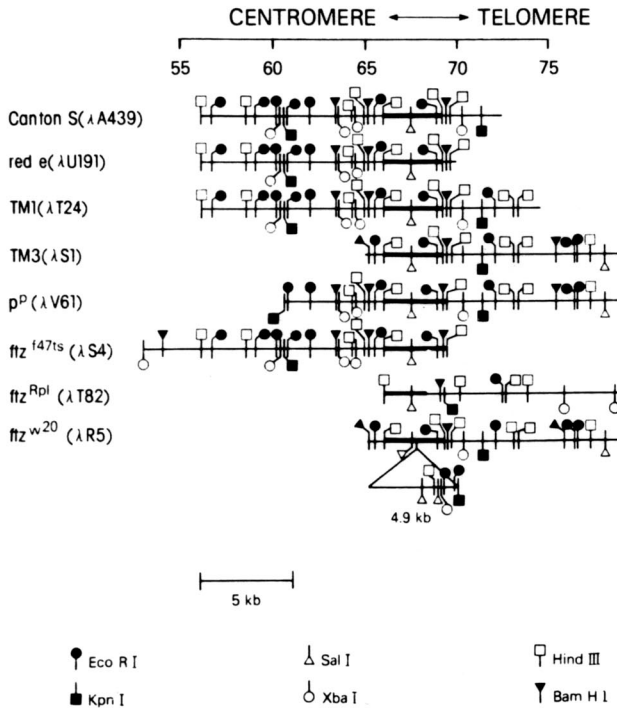

Figure 4. Restriction Maps of *ftz* DNA Isolated from Flies of Different Genotypes

Recombinant DNA libraries were constructed and screened with the λA439 Hind III 3.2 restriction fragment which contains DNA breakpoints associated with three *ftz* mutations. DNA was purified from phages recovered from plaques producing positive signals on Benton and Davis filters (see Experimental Procedures). Restriction maps were generated from single and double digests of phage DNA using the following enzymes: Eco RI, Hind III, Bam HI, Kpn I, Sal I, and Xba I. The thick black line represents the DNA sequence homologous to the λA439 Hind III 3.2 kb fragment. A description of the balancer and mutant chromosomes can be found in Lindsley and Grell (1968) or Lewis et al. (1980a, 1980b). The triangle in the ftz^{w20} (λR5) phage map represents the 4.9 kb DNA insertion found in the genomic DNA extracted from the ftz^{w20} chromosome but not in the DNA from the parental, *red e* (λU191) chromosome.

plus S 1.7 kb band is weaker than the 1.5 kb band in lane 4 which contains DNA isolated from ftz^{w20}/*TM3* flies. In contrast, the intensity of the H plus S 1.7 kb band is about equal to that of the 1.5 kb band in lane 3 which contains DNA purified from ftz^+ *red e*/ftz^+ *red e* flies. The data suggest that DNA from homozygous ftz^+ *red e* flies contains two copies of both the H plus S 1.7 kb and 1.5 kb fragments, while DNA from heterozygous mutant flies has two copies of the H plus S 1.5 kb fragment but only one copy of the H plus S 1.7 kb fragment. The copy of the H plus S 1.7 kb fragment seen in DNA from heterozygous mutant flies is derived frm the ftz^+ balancer chromsome (TM3). Therefore, the breakpoint associated with ftz^{w20} mutation was tentatively localized to the H plus S 1.7 kb fragment (map position +67.5 to +69.2).

Cytologically, the ftz^{Rpl} chromosome is a complicated rearrangement involving a reciprocal translocation between chromosomes two and three as well as an apparent insertion of chromosomal material of unknown origin adjacent to the translocation breakpoint located within polytene band interval 84AB (Kaufman, unpublished observations). A Southern blot containing Hind III digested DNA extracted from ftz^{Rpl}/*TM1* flies and hybridized to the λA439 Hind III 3.2 kb probe shows the expected H 3.2 b band derived from the balancer chromosome and a new 4.1 kb band apparently derived from the mutant chromosome (Figure 3, lane 5). The H 4.1 kb fragment is not observed in the Canton S wild-type strain in which the ftz^{Rpl} mutation was induced. From the data presented in Figures 4 and 5, the restriction maps of the DNA between map coordinates +66 and +69.2 of the *TM1* and Canton S chromosomes are identical. Therefore, the 4.1 kb band observed in lanes containing Hind III digested DNA from ftz^{Rpl}/*TM1* flies shown in Figure 3 is due to an alteration in the structure of the DNA derived from the ftz^{Rpl} chromosome and not to a difference between the *TM1* chromosomal DNA. Since the H plus S 1.7 kb fragment seen in Figure 3, lane 6, is approximately one-half the intensity of the H plus S 1.5 kb fragment, heterozygous ftz^{Rpl}/*TM3* flies appear to have only one copy of H plus S 1.7 kb fragment as was observed for ftz^{w20}/*TM3* flies. The data suggests that the ftz^{Rpl} breakpoint also resides in between map positions +67.5 and +69.2. (The weakly hybridizing bands in lane 6 are due to incomplete digestion of the DNA by the Sal I enzyme.)

Hind III digested genomic DNA extracted from ftz^{f47ts}/*TM3* flies and from ftz^+ p^p/ftz^+ p^p flies (the parental strain) have an identical 3.5 kb band which is detected by hybridization to the λA439 Hind III 3.2 kb probe, indicating that the structural anomaly in this region of the molecular map is not specific to the ftz^{f47ts} chromosome. The Hind III 3.2 kb band in the lane containing Hind III digested DNA from ftz^{f47ts}/*TM3* flies is from the *TM3* chromosome (Figure 3, lanes 7 and 8). The Hind III 3.2 kb (or Hind III 3.5 kb) DNA fragments from six chromosomes were subcloned into the plasmid vector pUC8. The restriction maps, shown in Figure 5, indicate that the ftz^{f47ts} and p^p chromosomal DNA have identical restriction maps which differ from all the other chromosomal DNA restriction maps with respect to the absence of the Hind III restriction site located at map position +69.2 (Figures 4 and 5).

Recombinant Clones Bearing Mutant DNA

Recombinant genomic DNA libraries were constructed in phage λ from DNA isolated from ftz^{w20}/*TM3*, ftz^{Rpl}/*TM1*, ftz^{f47ts}/*TM3*, *red e*/*red e*, and p^p/p^p flies in order to deter-

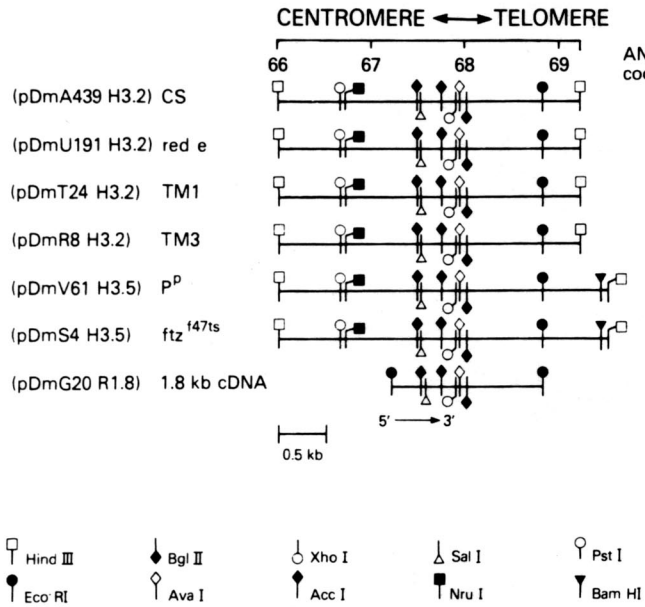

Figure 5. Restriction Maps of pUC8 Subcloned DNA Containing DNA Sequences Homologous to the λA439 Hind III 3.2 kb DNA Fragment

Phage DNAs described in Figure 5 were digested with Hind III. Fragments homologous to the λA439 Hind III 3.2 kb fragment were subcloned into the pUC8 Hind III site to generate the following plasmids: pDmA439 H3.2, pDmU191 H3.2, pDmT24 H3.2, pDmR8 H3.2, pDmV61 H3.5, and pDmS4 H3.5. Phage λG20 was isolated from a 0–5 hr embryonic cDNA library (gift from Goldschmidt-Clermont, Saint, and Hogness) screened with the λA439 H3.2 kb DNA probe. The λG20 Eco RI 1.8 kb insert DNA was subcloned into the pUC8 Eco RI site to generate pDmG20 R1.8. Restriction maps were constructed using the following enzymes: Hind III, Eco RI, Bam HI, Bgl II, Ava I, Xho I, Acc I, Nru I, and Pst I. The direction of transcription of the *ftz* gene was determined using single-stranded M13 clones (see Experimental Procedures).

mine more precisely the molecular structure of the *ftz* locus in mutant and wild-type flies. Each library was screened with the λA349 Hind III 3.2 kb probe. Restriction maps of DNA purified from each recombinant phage recovered from these screens are shown in Figure 4. The maps of the DNA from three ftz^+ strains (Canton S, *red e,* and p^p) agree in their regions of overlap with the exception of discrepancy in the size of the Hind III 3.2 kb (or 3.5 kb) fragments mentioned above. The restriction map of the ftz^{Rpl} chromosomal DNA (λT82) confirms that a breakpoint associated with the ftz^{Rpl} mutation occurs within the H plus S 1.7 kb DNA fragment. In agreement with the Southern blot data shown in Figure 3, the ftz^{Rpl} chromosomal rearrangement generates a new H 4.1 kb fragment and a new H plus S 2.5 kb DNA fragment, both of which are homologous to the wild-type Hind III 3.2 kb DNA fragment. The ftz^{Rpl} rearrangement breakpoint was more precisely mapped within the Canton S Bgl II plus Eco RI 0.8 kb DNA fragment of pdmA439 H3.2 (data not shown). Since all of the restriction sites that lie to the right of the Sal I site in DNA extracted from the ftz^{Rpl} chromosome (λT82) fail to correlate with the restriction sites observed in wild-type, Canton S chromosomal DNA (λA439), the structural changes observed in the ftz^{Rpl} chromosomal DNA are not due to a small insertion or deletion of DNA in this region of the chromosome. The precise origin of the new DNA, which is introduced into the DNA within polytene chromosome bands 84AB, is under investigation.

By comparing the restriction maps of DNA purified from the ftz^{w20} (λR5) and the ftz^+ *red e* (λU191) chromosomes (Figure 3), it can be seen that the structure of the ftz^{w20} genomic DNA differs from that of wild-type DNA by the insertion of 4.9 kb of DNA into the H plus S 1.7 kb DNA fragment located at DNA map position +67.5 to +68.7. The inserted 4.9 kb DNA sequence has one internal Hind III site that splits the λA439 Hind III 3.2 kb fragment into two new fragments of 2.6 kb and 5.6 kb, which is consistent with the genomic Southern results shown in Figure 3. Since the new 3.2 kb fragment generated by the insertion of 4.9 kb of DNA (see map of clone λR5, Figure 3) hybridizes very weakly to the λA439 Hind III 3.2 kb probe (data not shown), the insertion breakpoint is probably very close to the λA439 Sal I site. The ftz^{w20} 4.9 kb inserted DNA element has a restriction site pattern which is different from that of previously reported Drosophila repetitive DNA elements. The inserted DNA is moderately repeated in the genome (data not shown) and does not hybridize to any other DNA within the coordinates +0 to +220 of the cloned ANT-C DNA.

Transcriptional Analysis of the *ftz* Locus

Results from developmental genetic experiments indicate that the ftz^+ gene product is required prior to, but not after, 6 hr of embryogenesis (Wakimoto et al., 1984). In an effort to find an early embryonic transcript derived from the *ftz* locus, a library of cDNA clones prepared from 1–5 hr embryonic RNA was screened with the λA439 Hind III 3.2 kb DNA fragment within which the ftz^{w20} and ftz^{Rpl} mutations map. A phage containing a 1.8 kb insert homologous to the probe was isolated. The recovery of a cDNA clone is consistent with the conclusion that the *ftz* gene is transcribed during early embryogenesis, a period of Drosophila development during which a rapid increase in the transcription of the zygotic genome occurs (Lamb and Laird, 1976; Zalokar, 1976; Anderson and Lengyel, 1979).

The 1.8 kb cDNA insert was purified and subcloned. A comparison of the restriction map for the 1.8 kb cDNA insert and the homologous region of genomic DNA (see Figure 4), indicates that if any introns exist, they are not

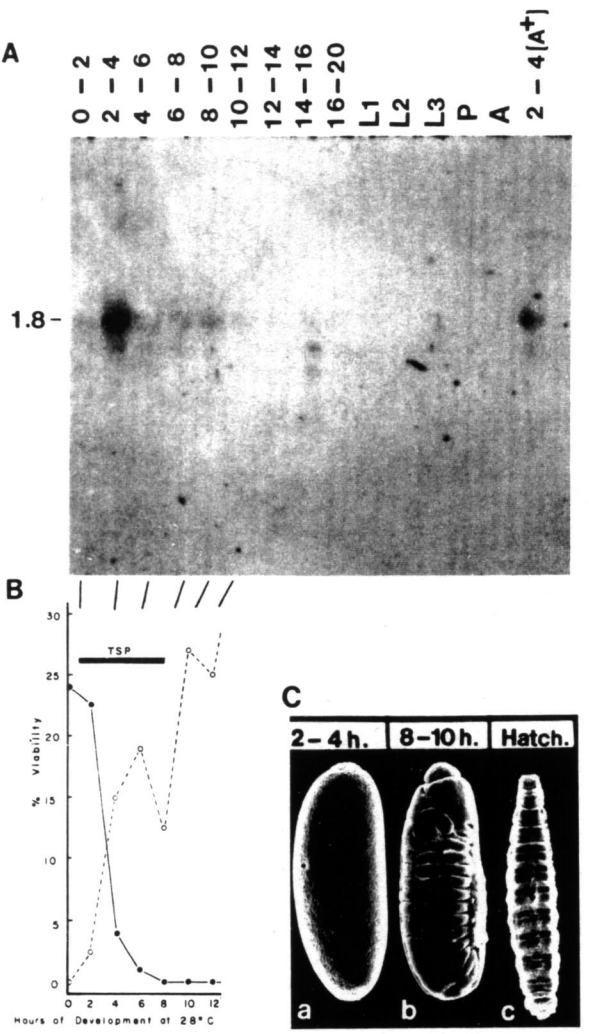

Figure 6. Analysis of the Putative *ftz* Transcript

(A) RNA prepared from animals from several stages of Drosophila development were electrophoresed on a 1.2%, 2.2 M formaldehyde-agarose gel, blotted onto nitrocellulose, and hybridized to a ^{32}P-labeled single-stranded M13Mp8 A439 Xho I plus Sal I 0.4 kb probe (see Experimental Procedures and the restriction map of pDmA439 H3.2 in Figure 5). The hybridization probe contained 10^7 cpm, and the blot was exposed for 14 days. The lanes contain 10 μg of total RNA from embryos of the age in hours indicated above each lane of the figure, first instar larvae (L1), second instar larvae (L2), third instar larvae (L3), pupae (P), and adults (A). Approximately 1 to 2 μg of oligo(dT) purified 2–4 hr polyadenylated embryonic RNA (2–4A$^+$) was loaded into the extreme right-hand lane. The transcript size was determined by comparing the relative mobility of the band in the 2–4 hr lane to that of the nematode myosin and ribosomal RNAs: 6.125, 3.5, and 1.7 kb, respectively. (B) The graph depicts the results of a temperature-shift study of embryos expressing a temperature-sensitive allele of *ftz*, *ftz*^{f47ts} (taken from Wakimoto et al., 1984). Mutant/*Df* embryos, viable at 18°C, die when shifted up to the restrictive temperature (28°C) before 2–4 hr; while mutant/*Df* embryos raised at 28°C will survive if shifted down to 18°C before 2–4 hr of embryonic development. O = shift up (18°C ↔ 28°C) and ● = shift down (28°C → 18°C). (C) a, b, and c show scanning electron micrographs of wild-type, Oregon R, 0–2 hr (52×), 8–10 hr (116×), and hatching stage, ~20 hr (124×) embryos which developed at 25°C. (Photos courtesy of R. F. Turner.)

large enough to alter the pattern of the restriction sites mapped in these clones.

To determine the developmental profile and size of the putative *ftz* transcript, RNA from several stages of Drosophila development was electrophoresed on 1.2% 2.2 M formaldehyde-agarose gels (see Experimental Procedures) and hybridized to a single-stranded M13 mp8 probe containing the Xho I plus Sal I 0.3 kb DNA sequence represented in the pDm G20R1.8 cDNA subclone. The results shown in Figure 6A show that a 1.8 kb transcript is maximally accumulated at 2–4 hr of embryogenesis (cellular blastoderm stage embryos, see Figure 6C,a), and that a very low level of the 1.8 kb transcript is observed up to 10–12 hr of embryogenesis (segmentation stage embryos, see Figure 6C,b). The fact that the putative *ftz* transcript is approximately the same size as the cDNA clone, which is homologous to the λA439 Hind III 3.2 kb probe, suggests that most of the transcribed region of the *ftz* gene is contained within the λ439 Hind III 3.2 kb DNA fragment. The 1.8 kb transcript is present in oligo dT purified 2–4 hr poly (A)$^+$ RNA. The correlation between the period of maximal accumulation of the 1.8 kb transcript and the temperature-sensitive period of *ftz*^{f47ts}/*Df* flies (Walkimoto et al., 1984; see Figure 6B) strongly supports the notion that the 1.8 kb RNA is transcribed from the *ftz* locus.

Discussion

Confidence in the accurate localization of the *ftz* gene on the physical DNA map is based on the following facts. First, two mutations, *ftz*^{w20} and *ftz*^{Rpl}, both map within the same region of genomic DNA from which a two to four hour transcript is synthesized. Second, the putative *ftz* transcript exhibits the developmental accumulation profile predicted by developmental genetic studies and is consistent with the biology of animals affected by mutations in the *ftz* gene. Third, although there is a small degree of restriction site heterogeneity at the limits of the +66 to +69.2 region, no restriction site heterogeneity has been detected within the transcribed region of the DNA isolated from approximately 18 chromosomes, except for those found in the DNA purified from the *ftz*^{w20} and *ftz*^{Rpl} chromosomes.

Molecular Lesions at the *ftz* Locus

The molecular structure of the *ftz* locus in *ftz*^{w20} flies suggests that the insertion of 4.9 kb of repetitive DNA into the transcribed region of the *ftz* gene, or some aberration associated with the insertion event, may have caused an inactivation of the gene. The *ftz*^{Rpl} rearrangement breakpoint, which also lies within the transcribed region of the *ftz* locus, maps within a minimum of 0.3 kb 3′ to the site of the 4.9 kb DNA insertion associated with the *ftz*^{w20} mutation. Therefore, although at least part of the 3′ region of the *ftz* transcription unit is displaced by the *ftz*^{Rpl} rearrangement, the *ftz*^{Rpl} lesion apparently does not eliminate the synthesis of a partially functional gene product

since ftz^{Rpl}/Df embryos frequently exhibit only partial gaps or fusions in one or more segments. Therefore, either quantitative or qualitative changes in the *ftz* gene product from homozygous or hemizygous ftz^{Rpl} embryos causes a condition in the embryos that causes lethality.

Transcription of the *ftz* Locus

The physical process of segmentation and the development of cuticular structures occurs between 6 to 16 hr of embryonic development. The data presented here suggest that the *ftz* transcript and the functional gene product are required for the establishment of conditions necessary for later processes such as segmentation and cellular differentiation, but are apparently not necessary for the maintenance of these conditions. If the *ftz* locus encodes a protein, as suggested by the presence of an open reading frame in sequenced regions of the *ftz* transcription unit (Scott and Weiner, 1984), the protein is probably only necessary at the blastoderm stage, judging from the temperature-sensitive period of the homozygous or hemizygous ftz^{1471s} embryos.

Role of *ftz* Locus in Development

Embryos that have no apparent *ftz* activity (ftz^- embryos) exhibit alterations in segment number as well as changes in the pattern of cuticular structures within each segment. In ftz^- embryos each double-width segment has only one set of denticle belts, with extra rows of setae (Figure 1B) and one set of tracheal pits (Wakimoto et al., 1984). The denticles observed in ftz^-/ftz^- embryos have a morphology characteristic of the more anterior of the "fused" pair of segments rather than that of a hybrid of the denticle pattern of both segments, as is seen in some other zygotic segmentation mutants such as *paired* (Nusslein-Volhard and Wieschaus, 1980). Thus the *ftz* locus affects the number of rows of denticles within each belt, the location of the denticle belts within each double segment, and the segmental identity of each denticle belt. Whether the absence of denticle belts in the more posterior region of a double-width segment and the increase in rows of denticle belts near the anterior boundary of each segment is due to a transformation of posterior to anterior structures or to a deletion and duplication of structures is unclear. Despite the fact that the *ftz* locus maps within the ANT-C, the *ftz* locus is not a conventional homoeotic mutation either with respect to the type of pattern alterations caused by mutations at this locus, the period of developmental expression of the gene, or the structure of the *ftz* gene relative to the *Ubx* gene in the Bithorax complex (BX-C) (Bender et al., 1983b) or the *Antp* gene in the ANT-C (Garber et al., 1983; Scott et al., 1983). Interestingly, the *ftz* 1.8 kb cDNA contains sequences that are homologous to the 3′ exon of the *Antp* and *Ubx* transcription units, respectively (Scott and Weiner, 1984). The data suggest that there may be a functional or ancestral relationship between the *ftz* gene and the *Antp* genes which are physically located about 30 kb from each other in the ANT-C.

Experimental Procedures

Recombinant DNA Libraries

All libraries were constructed by ligating Sau 3A partially digested adult fly DNA to Bam HI cut EMBL-4 (Murray and Lehrach, 1983). Preparation of adult fly DNA, EMBL-4 vector DNA, and ligation reactions are described in Scott et al., 1983.

Restriction Mapping

Single and double restriction enzymes digests of phage DNA or plasmid DNA analyzed on 0.6% or 1.0% gels, respectively, containing 40 mM Tris-HCl (pH 7.8), 20 mM Na Acetate, and 2.0 mM EDTA were used to construct all recombinant DNA maps. Restriction digests were performed in 1X TA buffer (33 rnM Tris-Acetate [pH 7.9], 66 mM potassium acetate, 10 mM magnesium acetate, 100 μg/ml BSA, and 0.5 mM DTT). TA buffer was supplemented with 0.1 M NaCl for digests containing Sal I. All enzymes were purchased from either New England Biolabs or BRL. Hind III digested phage λ DNA and Hae III digested εX174 DNA served as size markers (New England Biolabs).

Construction of Subclones

Hind III DNA fragments were purified on 0.7% low-melting-point agarose gels in 40 mM Tris-HCl (pH 7.8), 20 mM Na Acetate, and 2 mM EDTA buffer. Gel pieces were melted in 2–3 volumes of 50 mM Tris, 1 mM EDTA (pH 8.0), at 70°C for 10–20 min. Liquified agarose was extracted two times with phenol. The DNA was ethanol precipitated two times. Ethanol precipitated were vacuum dried, resuspended in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (TE), extracted with water-saturated n-butanol, and ethanol precipitated. Ethanol precipitates were resuspended in TE, then two parts insert DNA were ligated to one part Hind III digested pUC8DNA (Messing and Vieira, 1982), and used to transform JM 83 cells. Transformed cells containing recombinant plasmids were selected on agar plates containing 25 mg/ml Xgal (5-bromo-4-chloro-3-indolyl-B-D-galactopyranoside), 50 μg/ml ampicillin, and 2X YT media (Miller, 1972). Plasmid DNA was prepared according to the method of Marko et al. (1982).

Hybridization Probes

Restriction digest fragments of phage or plasmid DNA were purified from low melting point agarose gels and nick translated (see Maniatis, 1982). Unincorporated radioactivity was removed by spermine precipitation (Hoopes and McClure, 1982). Nick-translated probes were typically 10^7 cpm/μg.

Single-stranded M13 probes were prepared according to Hu and Messing, 1982. Briefly, 0.1 μg of pHm235 primer DNA was boiled for 1 min, quick cooled and mixed with 1 μg of single-stranded M13 template DNA in a 15 μl volume containing 60 mM NaCl, 10 mM Tris-HCl (pH 7.4), 7 mM $MgCl_2$, 1 mM DTT and 100 μg/ml BSA. The mixture was heated for 15 min at 65°C, cooled and incubated with a 5 μl volume containing 40 μM of each dXTP (-dATP), 10 μCi α^{32}P dATP (2,000–3,000 Ci/mmole, Amersham) plus 0.9 U of New England Biolabs E. coli DNA polymerase Klenow fragment for 90 min at 15°C. The reaction was terminated and the labeled product was purified by spermin precipitation. Probes had a specific activity of approximately 10^7 cpm/μg DNA templates.

RNA Blots

The preparation of Drosophila RNAs, Northern blot procedures and hybridization conditions and size markers are described in Scott et al., 1983.

Determination of the Direction of Transcription

The direction of transcription of the 1.8 transcript was determined by using single-stranded M13mp8 probes containing the sense and antisense strands of the Xho I plus Sal I 0.4 kb DNA fragment contained in the plasmid clones pDmA439 H3.2 and pDmG20 R1.8. Since the polarity of M13 phage DNA is known (Messing and Vieira, 1982) and the poly-linker Sal I site is 5′ to the Hind III site in the (+) strand of M13mp8, the orientation of the Drosophila Xho I plus Sal I 0.4 kb fragment in the M13mp8 RF was determined to be 5′ Xho I to 3′ Sal I in one clone and vice versa in another. Only the single-stranded M13mp8 DNA containing the insert DNA fragment orientated in a 5′ Xho I to 3′ Sal I direction hybridizes to any transcripts (data not shown). Therefore, the 5′ to 3′ direction of transcription is from

the Sal I site toward the Xho I site. Since the Sal I site is proximal to the Xho I site in the genome (Figure 4), the direction of transcription in the genome is proximal to distal (i. e., from the centromere toward the telomere).

Fly Culture and Cuticle Preparations

Flies were raised on standard cornmeal, molasses, agar media supplemented with bakers' yeast at 25°C. Flies from which DNA for recombinant libraries was isolated were starved overnight with a wetted paper towel to prevent desiccation.

Cuticular preparations were made from late embryos according to the method of Van Der Meer (1977) and cuticular structures were classified according to the morphological criteria of Lohs-Schardin et al. (1979).

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