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Repression and Turnover Pattern *fushi tarazu* RNA in the Early Drosophila Embryo

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Summary

Embryonic expression of transcripts from the Drosophila gene *fushi tarazu* (*ftz*) progresses through a series of spatial patterns, culminating in a seven-banded pattern at the cellular blastoderm stage. We studied the generation of this pattern using inhibitors of RNA synthesis (α-amanitin) and protein synthesis (cycloheximide). Injections of α-amanitin revealed that *ftz* RNA turns over extremely rapidly in the embryo, and we think that this may be essential to effect rapid changes in *ftz* RNA patterns. Injections of cycloheximide added to the normal domains of *ftz* expression, creating novel expression patterns that were dependent on the time of injection. These novel patterns suggest that two superimposed systems of repression establish the normal, seven-banded pattern of *ftz* expression. One system sets up a banded pattern over the entire length of the embryo, and the other restricts actual expression to the middle portion of the embryo.

Introduction

Mutational analysis has identified a number of genes involved in establishing the metameric pattern in Drosophila (Nüsslein-Volhard and Wieschaus, 1980). Several of these genes have been cloned, and their products have been found to be expressed in reiterated bands that reflect the segmental organization of the embryo (Hafen et al., 1984; Kornberg et al., 1985; Fjose et al., 1985; Ingham et al., 1985; Kilchherr et al., 1986; Harding et al., 1986). In general, regions of the embryo that normally accumulate a certain gene product are altered or deleted when the gene coding for that product is mutant. Conversely, regions that normally lack a certain gene product develop abnormally when they express that product (Struhl, 1985). Thus, by their overlapping patterns of activity and inactivity, these genes may provide the spatial information for organized, metameric development in the embryo.

Embryos lacking a functional *fushi tarazu* (*ftz*) gene develop with only half the normal number of gnathal, thoracic, and abdominal segments (Wakimoto and Kaufman, 1981; Wakimoto et al., 1984). Transcripts of the *ftz* gene are first detected about 90 min after egg deposition (AED), when the dividing nuclei reach the egg cortex (nuclear cycle 10; Rabinowitz, 1941; Foe and Alberts, 1983). At this stage *ftz* transcripts are associated with nuclei throughout the embryo (Weir and Kornberg, 1985). Subsequently, during the syncytial blastoderm stages (nuclear cycles 11–13), expression of *ftz* RNA is restricted to a broad band between 15% and 65% egg length (Hafen et al., 1984; 0% egg length is at the posterior pole). During nuclear cycle 14, as the blastoderm cellularizes, gaps divide this broad band and form seven narrow bands of *ftz* RNA that encircle the embryo (Hafen et al., 1984). These seven bands appear to correspond to the regions that fail to develop in *ftz* mutant embryos.

Recent work has shown that the spatial patterns of *ftz* expression depend on both *cis*- and *trans*-acting elements. Studies using *ftz*/β-galactosidase hybrid genes have shown that 740 bp of DNA immediately 5′ to the *ftz* gene's open reading frame are sufficient to confer banded expression in transformed embryos, and that sequences further upstream are needed to suppress *ftz* expression in the anterior 35% of the embryo (Hiromi et al., 1985). Correct formation of the banded *ftz* pattern also depends upon a number of other genes, which are both maternally and zygotically expressed (Mohler and Wieschaus, 1985; Howard and Ingham, 1986; Carroll and Scott, 1986).

In this study, we employed metabolic inhibitors to analyze the mechanisms that pattern *ftz* RNA expression. The Drosophila embryo is suited for such a study because the spatial patterns of *ftz* expression are established before the nuclei are completely encapsulated within cell boundaries, at the end of the fourteenth nuclear cycle (Rickoll, 1976). It is therefore possible to deliver inhibitors simultaneously throughout the embryo (Zalokar and Erk, 1976; Foe and Alberts, 1983; Edgar and Schubiger, 1986), and to examine their global effects on the patterning of gene expression. We used in situ hybridization and Northern analysis to monitor expression of *ftz* RNA in embryos injected at different times with cycloheximide, which blocked protein synthesis, or α-amanitin, which blocked transcription. The results of these experiments suggest a model in which the seven-banded *ftz* RNA pattern forms through a combination of spatially differential repression of transcription and spatially uniform transcript degradation.

Results

Localization of *ftz* RNA Requires Concurrent Protein Synthesis

Injection of cycloheximide to an intracellular concentration of 20 μg/ml had the following effects on development: greater than 95% of protein synthesis was rapidly inhibited, as measured by incorporation of ^{35}S-methionine, the nuclear cycle was arrested in G2 of the cycle of injection (Zalokar and Erk, 1976; Edgar and Schubiger, 1986), and, provided arrest was induced later than nuclear cycle 9 or 10, high rates of transcription of many genes transcribed

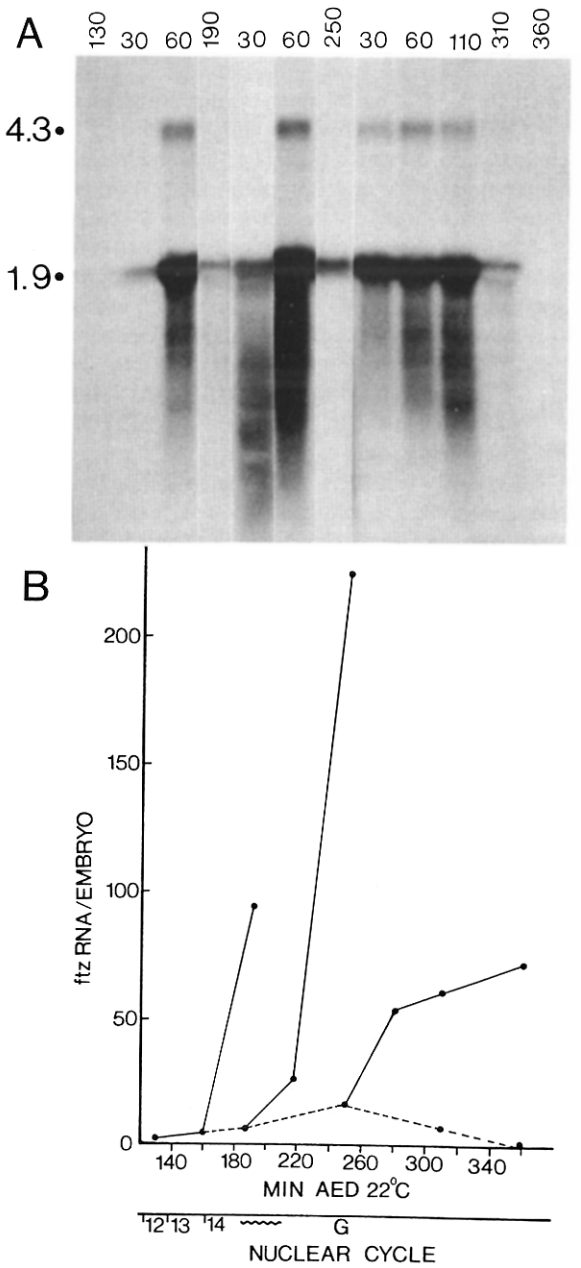

Figure 1. Accumulation of *ftz* RNA after Injection of Cycloheximide

(A) The accumulation of *ftz* RNA after injection of cycloheximide in cycles 12 (130 min AED), early 14 (190 min AED), and late 14 (250 min AED) was measured on this Northern blot, which had total nucleic acid from exactly 60 embryos in each lane. Vertical numbers above the gel (130, 190, 250, 310, 360) refer to min AED and show the amount of *ftz* RNA in uninjected embryos at those times. Horizontal numbers above the gel (30, 60, 110) indicate the incubation time (in min) after injection at 130, 190, and 250 min AED, respectively. RNA sizes are given to the left of the gel in kb.

(B) We show the accumulation of 1.9 kb *ftz* RNA in control embryos (dotted line) and after injection of cycloheximide in cycle 12, early cycle 14, or late cycle 14 (solid lines) as determined by densitometry of the Northern blot above. Amount of *ftz* RNA is indicated on the Y axis in arbitrary units. Developmental time in min AED or in nuclear cycle number (with slashes at the midpoints of mitoses) is indicated on the X axes. G indicates the onset of gastrulation. The wavy line represents the period during which *ftz* expression changes from uniform to the seven-banded pattern.

by RNA polymerases I, II, and III occurred (Edgar and Schubiger, 1986). Injections before the onset of cellularization, in early cycle 14, blocked cellularization, whereas later injections did not. Gastrulation was blocked by injections more than 10 min before the onset of gastrulation movements (240 min AED, 22°C).

Embryos were injected with cycloheximide at nuclear cycles 8, 10, 12, 13, and 14. One hour after injection, the embryos were fixed for in situ hybridization or their RNA was extracted for Northern analysis. Control embryos were processed and staged in parallel with experimental ones, but were fixed at the time the experimentals were injected. Our injection technique was designed to deliver the drug evenly throughout the embryo. The results of injections through the anterior and posterior poles were indistinguishable, and injections of buffer alone had no apparent affect on either morphological development or *ftz* RNA patterning.

Northern analysis and densitometry indicated that arrest by cycloheximide in cycle 12 (130 min AED), early cycle 14 (190 min AED), or late cycle 14 (250 min AED) resulted in the accumulation of manyfold more *ftz* RNA than was present in control embryos fixed at the time of injection (Figure 1). The *ftz* RNA that accumulated was of normal size (1.9 kb).

In situ hybridization showed that embryos arrested by cycloheximide in cycle 8 (the penultimate intravitelline division) or cycle 10 (the first blastoderm division) accumulated very little *ftz* RNA, and that embryos arrested in cycle 12 or later accumulated much more *ftz* RNA than controls (see Figure 1). Unlike controls, in which transcripts accumulated only between 15% and 65% egg length (Figure 2A), embryos injected in cycles 12, 13, and early 14 accumulated *ftz* RNA over their entire surface, except for a narrow anterior–ventral region (Figure 2D). The embryos arrested in early cycle 14 were incubated well past the time when normal, developing embryos established the banded *ftz* RNA pattern, indicating that the formation of bands required protein synthesis during cycle 14.

Injections of cycloheximide later in cycle 14 produced a continuum of patterns ranging from almost uniform to banded. As in younger arrested embryos, all of these patterns extended over the entire embryo, but had little or no *ftz* RNA in a narrow region on the anterior–ventral surface. Embryos that had *ftz* RNA distributed almost uniformly had faint bands whose intensity, relative to the adjacent interbands, was much less than in normal, banded embryos. Banded embryos had the seven normal *ftz* bands, two additional anterior bands, and a small patch of expression at the posterior end which probably included only the pole cells (Figure 2F). Intermediate patterns were also observed; these included portions of the nine-banded pattern (or ten-banded, if pole cell expression is considered a band), and various degrees of expression in the interbands between bands (Figure 2E).

Serial reconstruction of nine sectioned embryos revealed that, in a manner characteristic of the normal *ftz* pattern, the two extra anterior bands followed a curvature toward the anterior–dorsal surface (Figure 3F). The extra

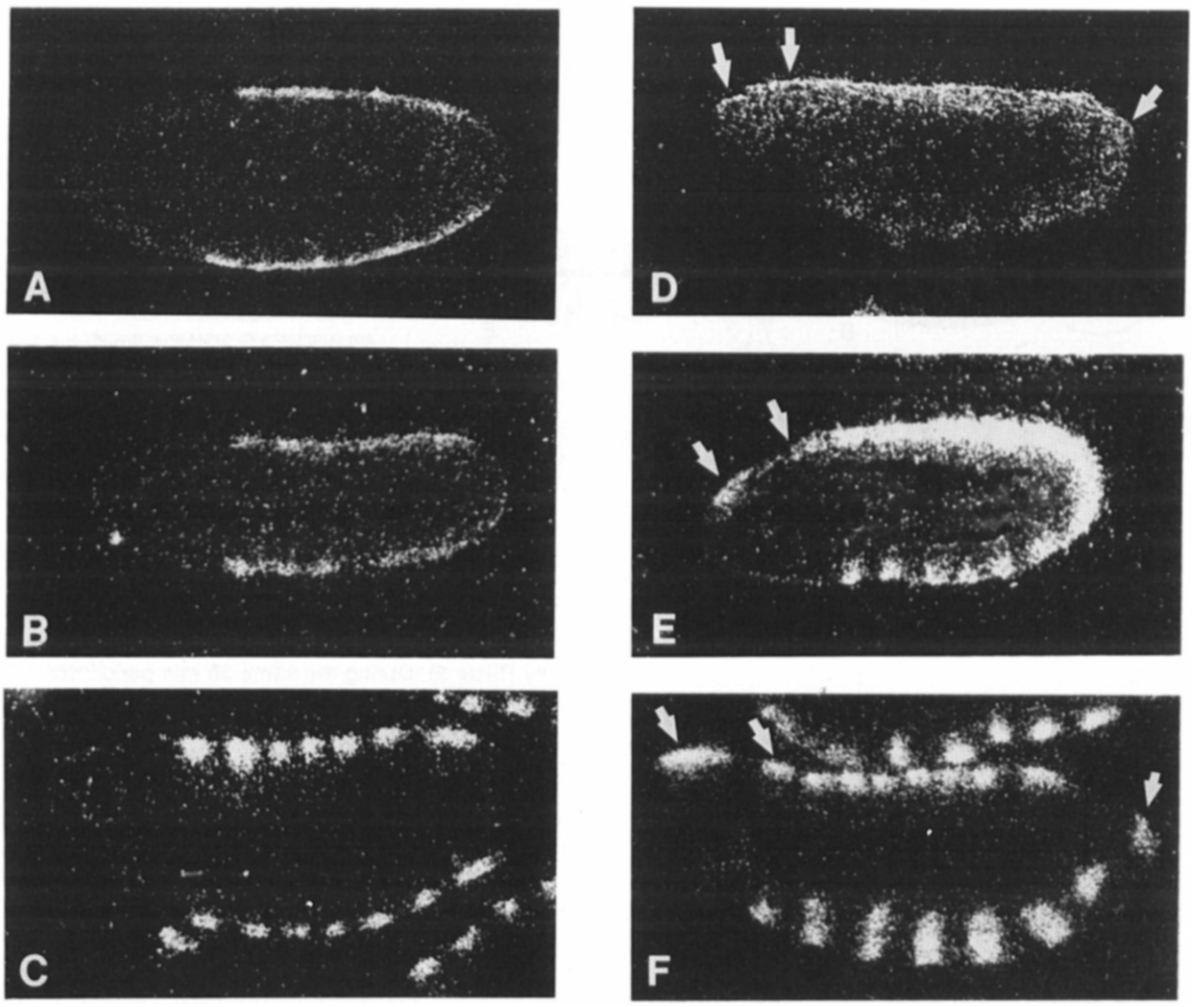

Figure 2. *ftz* Expression in Normal and Cycloheximide-Arrested Embryos

(A–C) show uninjected control embryos after in situ hybridization with *ftz* probe. Embryos were fixed in cycles 13 (A), early 14 (B), and late 14 (C). (D–F) show embryos fixed 1 hr after injection of cycloheximide during early cycle 14 (D), mid cycle 14 (E), and late cycle 14 (F). Note the extra regions of *ftz* RNA accumulation in cycloheximide-arrested embryos (arrows). Embryos injected in cycles 12 and 13 gave patterns similar to (D). Embryo F commenced gastrulation after injection. Sagittal sections of embryos are oriented with anterior to the left and dorsal up. The *ftz* probe used in (A) was labeled with ^{3}H, whereas (B–F) used ^{35}S-labeled probe.

bands also increased in width and spacing towards the anterior pole, as the normal bands do toward the posterior pole. Thus the extra bands appeared to be a regular extension of the normal *ftz* RNA pattern (also see Hiromi et al., 1985).

To better understand the temporal relationships of these patterns, we examined sections of embryos injected at four times in cycle 14. For analysis (Table 1), *ftz* RNA patterns in control and injected embryos were classified as uniform (Figures 3A and 3D), partially banded (Figures 3B and 3E), or fully banded (Figures 3C and 3F). Embryos injected in early cycle 14 (170 min AED) accumulated *ftz* RNA uniformly. Injections in mid cycle 14 (195 min AED), when most control embryos had recently formed bands (73%) and some were partially banded (26%), also resulted in uniform transcript accumulation in most embryos (82%). Injections later in cycle 14 (205 min and 240 min AED) produced progressively higher proportions of

Table 1. Frequencies of *ftz* RNA Patterns in Cycloheximide-Arrested and Control Embryos

Type	*ftz* Pattern (%)	Min AED, 22°C 170′	195′	205′	240′
Control	A	90	1	0	0
	B	10	26	2	1
	C	0	73	98	99
	(n)	(33)	(102)	(84)	(68)
CYH, 1 hr	D	100	82	26	5
	E	0	16	40	19
	F	0	2	34	76
	(n)	(79)	(60)	(121)	(150)

Control embryos were fixed at the time indicated, whereas experimental embryos (CYH, 1 hr) were injected at the time indicated and fixed 1 hr later. The *ftz* RNA patterns observed were classified into six groups as shown in Figures 2A–2F. Frequencies of these patterns are given in percent of the total number of embryos scored (parentheses). Each embryo was scored from a single section.

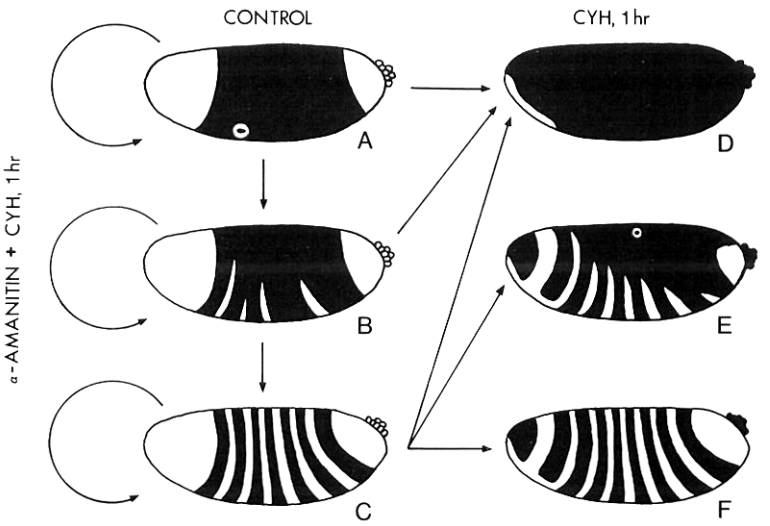

Figure 3. Schematic Representation of Normal and Drug-Induced *ftz* RNA Patterns

(A–C) show the normal progression of *ftz* RNA localization during cycle 14 as described by Hafen et al. (1984) and Weir and Kornberg (1985). (A–C) also represent *ftz* patterns 1 hr after coinjection of cycloheximide (CYH) and α-amanitin, which froze the *ftz* pattern as it was at the time of injection (Table 3). (D–F) show *ftz* patterns in embryos 1 hr after injection of cycloheximide alone. Arrows indicate the temporal relationships of these patterns, which are listed in terms of frequencies in Table 1. All embryos are oriented with anterior to the left and dorsal to the top. Patterns (D), (E), and (F) were derived from serial reconstructions of 11, 7, and 9 embryos, respectively. The two extra anterior *ftz* bands shown in embryo (F) have their midpoints at 76% and 92% EL.

fully banded embryos. All embryos that gastrulated during the hour after injection maintained the fully banded pattern.

The process that generates banded *ftz* expression is thus sensitive to cycloheximide, and the banded pattern remains sensitive until about 20 min after its genesis. In embryos with intermediate patterns, interbands were resolved ventrally more than dorsally, and anteriorly more than posteriorly (Figures 2E and 3E). These polarities mimicked the order of appearance of interbands during normal development (Figure 3B; Hafen et al., 1984; Weir and Kornberg, 1985), implying that the interbands established earliest were the first to become resistant to cycloheximide. About 10 min before gastrulation, the band/interband pattern in all regions of the embryo was resistant to cycloheximide.

ftz RNA Is Extremely Short-Lived

To measure the rate of turnover of *ftz* RNA in the embryo, transcription was blocked by injecting α-amanitin, and the amount of *ftz* RNA remaining after increasing periods of incubation was determined. α-amanitin was injected to an intracellular concentration of approximately 8 μg/ml, which is about 10-fold that necessary to block transcription by RNA polymerases I and II within 2 min (Edgar and Schubiger, 1986). At the end of an incubation period, the embryos were either fixed for in situ hybridization, or their RNA was isolated and fractionated for Northern analysis.

Northern analysis showed that the relative amount of *ftz* RNA per embryo decreased rapidly after transcription was blocked (Figure 4). Densitometry allowed us to calculate that *ftz* RNA decayed according to first order kinetics, with a half-life that dropped from about 14 min in early cycle 13 to about 7 min in cycle 14. In the same experiment, ribosomal protein 49 mRNA (O'Connell and Rosbash, 1984) was stable (Figure 4).

In situ hybridization was used to determine the spatial distribution of the *ftz* RNA degradation seen after injection of α-amanitin. When α-amanitin was injected just before or during resolution of the banded pattern (190 or 205 min AED), *ftz* RNA remained detectable for about 30 min after injection. During this 30 min period, *ftz* RNA patterns did not change, but faded away with apparent spatial uniformity (Table 2). During the same 30 min period, *ftz* RNA patterns in control embryos progressed to the seven-banded pattern. When α-amanitin was injected after bands formed (240 min AED), *ftz* RNA in the bands was rapidly lost. These observations suggest that rates of *ftz* RNA degradation are similar throughout the embryo.

The stability of other RNAs synthesized during cycle 14 was monitored in an analogous experiment (data not shown). Total RNA was labeled in vivo by injection of α-^{32}P-UTP in early cycle 14 (180 min AED). Thirty minutes later, α-amanitin was delivered to an intracellular concentration of 20 μg/ml by a second injection. The labeled RNA remaining after 10, 20, and 40 min was then analyzed by gel electrophoresis and autoradiography. Ribosomal RNA, small nuclear RNAs, 12 specific abundant poly(A)$^+$ RNAs, and the bulk of poly(A)$^+$ RNA synthesized in early cycle 14 were stable for at least the 40 min tested. Histone mRNAs, however, decayed rapidly after S phase (Edgar and Schubiger, 1986), as noted previously by Anderson and Lengyel (1980). Thus rapid RNA degradation is not a general property of the cycle 14 embryo, and degradation of *ftz* RNA must be subject to specific regulation.

Cycloheximide Stabilizes *ftz* RNA

Cycloheximide and α-amanitin were coinjected during nuclear cycle 13, and at five different times in cycle 14 (140, 170, 190, 205, 210, and 240 min AED). In situ hybridizations to sections of these embryos showed that the spatial patterns of *ftz* transcripts did not change following injection, and that the *ftz* RNA present at the time of injection was largely stable for more than 1 hr (Table 3; Figures 3A–3C). This stabilization is probably not peculiar to *ftz* RNA; cycloheximide stabilizes mRNAs in many systems, including Drosophila (Lindquist et al., 1982).

Discussion

Expression of the *ftz* gene during early Drosophila development provides a molecular marker for the processes

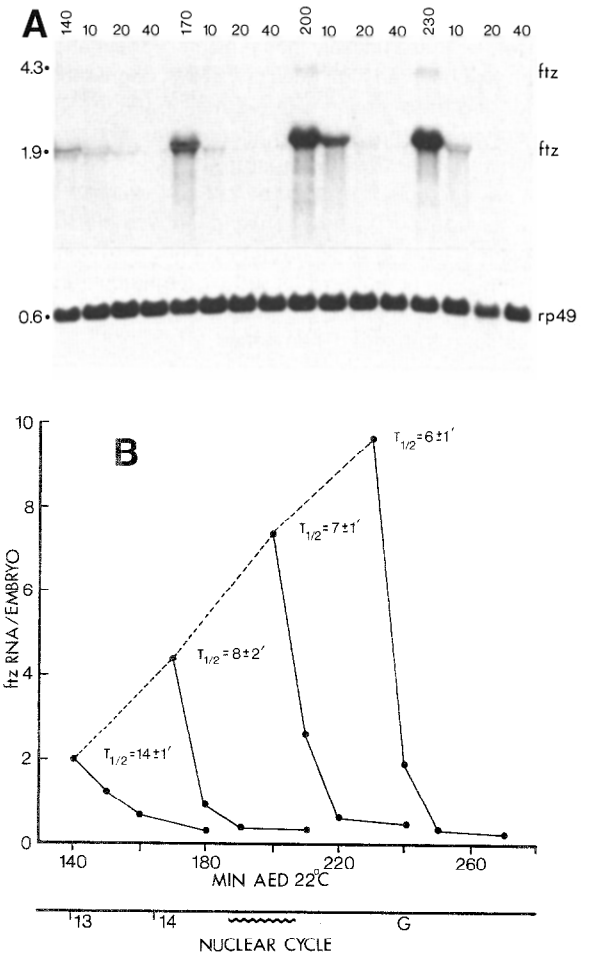

Figure 4. Accumulation and Turnover of *ftz* RNA in the Embryo

(A) The rates of turnover of *ftz* RNA at different times during development were determined by blocking transcription with α-amanitin and assaying the amount of *ftz* RNA that remained after increasing intervals. RNA was assayed on this Northern blot, which had total nucleic acid from exactly 60 embryos in each lane. Vertical numbers above the gel (140, 170, 200, 230) indicate times of α-amanitin injection in min AED and show the amount of RNA in the embryos before injection. Horizontal numbers above the gel (10, 20 40) indicate the incubation time after injection of α-amanitin at 140, 170, 200, and 230 min AED, respectively. RNA sizes are indicated to the left in kb, and the probe that hybridized to these RNAs is indicated to the right. As a control, we hybridized the same blot with probe for ribosomal protein 49 mRNA (rp49), which was evidently stable after injection of α-amanitin.

(B) We show the accumulation (dotted line) and the decay after α-amanitin injection (solid lines) of the 1.9 kb *ftz* mRNA at four times in nuclear cycles 13 and 14, as determined by densitometry of the Northern blot shown in (A). $T_{½}$ times are given in min to the right of each decay curve, and are means calculated from two T_0 points on each curve (0 min and 10 min after injection). Levels of *ftz* RNA are given in arbitrary units, which are normalized to control values to be approximately the same as the units in Figure 1. Other details are as described in the legend to Figure 1.

Table 2. Frequencies of *ftz* RNA Patterns in α-Amanitin Injected and Control Embryos

		Min AED, 22°C					
α-Amanitin Injected:		N.I.	190′	N.I.	205′	205′	N.I.
Fixed:		190′	205′	205′	210′	215′	215′
ftz Pattern (%)	A	53	54	0	0	0	0
	B	41	37	52	59	52	7
	C	6	9	48	41	48	93
	(n)	(66)	(65)	(152)	(67)	(91)	(45)

α-amanitin was injected at the times indicated (190 or 205 min AED), and development was stopped by fixation at the intervals indicated. Control embryos were not injected (N.I.). The *ftz* RNA patterns observed were classified into three groups as shown in Figures 2A–2C. Frequencies of these patterns are given in percent of the total number of embryos scored (parentheses). Each embryo was scored by a single section. Note that the relative frequencies of uniform (A), partially banded (B), and fully banded (C) *ftz* patterns remained approximately constant after injection of α-amanitin, but that uninjected control embryos (N.I.) progressed toward higher proportions of fully banded patterns with time.

Table 3. Frequencies of *ftz* RNA Patterns in Embryos Coinjected with Cycloheximide and α-Amanitin and in Control Embryos

		Min AED, 22°C			
α-Amanitin/CYH Injected:		N.I.	190′	N.I.	210′
Fixed:		190′	250′	210′	270′
ftz Pattern (%)	A	10	20	0	0
	B	64	53	2	5
	C	26	27	98	95
	(n)	(59)	(60)	(48)	(61)

α-amanitin and cycloheximide (CYH) were injected together at the times indicated (190 or 210 min AED), and development was stopped by fixation at the times indicated. Control embryos were not injected (N.I.). The *ftz* RNA patterns were classified and scored as described in Tables 1 and 2. Note that the relative frequencies of the different *ftz* patterns did not change after injection of α-amanitin and CYH, but progressed toward higher proportions of fully banded patterns in uninjected control embryos. *ftz* RNA appeared to be slightly less abundant in the injected embryos.

that subdivide the embryo into metameric units. Expression of *ftz* RNA initially spans the entire embryo and becomes progressively eliminated, first from the polar regions and then from the interbands, resulting in a seven-banded pattern at the cellular blastoderm stage (Hafen et al., 1984; Weir and Kornberg, 1985). In this study, we describe the consequences of blocking RNA or protein synthesis at different times during this progression. Our results suggest a model for how the seven-banded *ftz* RNA pattern is established.

Cycloheximide, which arrested protein synthesis, had the following effects on the patterning of *ftz* RNA: one, cycloheximide blocked the formation of interbands, and allowed reexpression of *ftz* RNA in the interbands up to 20 min after they formed; two, cycloheximide permitted *ftz* expression in the polar regions of the embryo, where it was normally absent after cycle 10. This polar expression was uniform in embryos exhibiting uniform expression in the normal *ftz* domain, but was banded in older embryos that had the seven normal *ftz* bands. Although these effects could be secondary consequences of the metabolic shock of translation arrest, their temporal and spatial specificity suggests that the patterning of *ftz* expression may require continuous protein synthesis either to replenish unstable proteins or, at certain times, to synthesize new ones.

Since cycloheximide added to the normal spatial domains of *ftz* expression (Figure 2), it is likely that the drug

disrupted systems that normally prevent *ftz* expression in certain regions of the embryo. We propose that the effects of cycloheximide distinguish two different systems of repression which, superimposed, generate the seven-banded *ftz* pattern. One system, *polar* repression, eliminates *ftz* expression in the anterior 35% and posterior 15% of the embryo. A second system, *periodic* repression, eliminates *ftz* expression in a phased manner, forming the interbands.

These two systems differed both with respect to their times of activation and their times of cycloheximide sensitivity. *Polar* repression was evident by the twelfth nuclear cycle, and could be abolished by cycloheximide until just after gastrulation in late cycle fourteen, which was the latest time we tested. *Periodic* repression was first evident in mid cycle fourteen, and became resistant to cycloheximide before gastrulation, at a time when polar repression was still sensitive to cycloheximide. Consequently, injections of cycloheximide in late cycle fourteen selectively abolished polar repression, and caused the expression of two extra anterior bands of *ftz* RNA. This result demonstrates the separability of polar and periodic repression. It also suggests (as noted previously by Hiromi et al., 1985) that the information for periodic *ftz* expression spans the entire embryo, but is not normally evident in the polar regions, where polar repression is usually dominant.

That a system of repression eliminates *ftz* expression in the anterior polar region has been suggested previously by Hiromi et al. (1985). Embryos transformed with hybrid genes consisting of 6.1 kb of 5′ *ftz* controlling sequences and the coding sequence for E. coli β-galactosidase expressed the bacterial enzyme in seven *ftz*-like bands. However, embryos transformed with similar hybrid genes lacking 3.7 kb from the most 5′ portion of the *ftz* controlling region expressed β-galactosidase in two additional anterior bands. These extra bands appear to coincide with those caused by cycloheximide (Edgar and Weir, unpublished observation). The existence of DNA sequences required for polar, but not periodic, repression supports our hypothesis that there are, mechanistically, at least two separate repression systems. *ftz* expression patterns in embryos mutant for other segmentation genes are also consistent with our hypothesis: certain gap and pair-rule mutations (*hunchback*, *knirps*, *runt*, and *hairy*) cause expansion of *ftz* expression into the interband regions but not into the polar regions (Carroll and Scott, 1986; Howard and Ingham, 1986).

ftz expression in the pole cells was observed in our experiments with cycloheximide, but not with the *ftz*/β-galactosidase deletion constructs discussed above (Hiromi et al., 1985). This suggests that a third system, which is cycloheximide-sensitive but does not require the same *ftz* DNA sequences as somatic polar repression, may prevent *ftz* expression in the pole cells. Interestingly, Zalokar (1976) found that total transcription was undetectable in the pole cells during cycle 14, suggesting that repression there may be quite general.

At the molecular level, localized repression of *ftz* expression might occur by transcriptional regulation, differential RNA degradation, or directed RNA transport. We cannot definitively exclude a role for differential RNA degradation or for RNA transport, but several observations render these unlikely mechanisms. For instance, although we observed high rates of *ftz* RNA degradation after blocking transcription with α-amanitin (Figure 4), our data suggested that this degradation was not spatially differential (Table 2). Furthermore, in experiments that might have detected transport of *ftz* RNA, we saw no evidence for transport; when transcription was blocked by α-amanitin (Table 2) or when transcription and degradation were simultaneously blocked by α-amanitin and cycloheximide (Table 3), the progression of *ftz* RNA patterns from uniform to banded was halted.

We suggest that both polar and periodic repression occur at the level of transcription, and that the new patterns of *ftz* expression caused by cycloheximide are due to derepression of transcription, as has been observed with cycloheximide in other systems (Wall et al., 1986). Polar repression probably occurs at the transcriptional level because the *ftz* DNA sequences required for it are not within the transcribed portion of the gene (Hiromi et al., 1985). This makes a mechanism that involves RNA degradation or transport improbable. Periodic repression probably occurs via transcriptional control because *ftz* RNA does not accumulate in the interbands of embryos injected with cycloheximide in late cycle 14, even though cycloheximide seems to stabilize *ftz* RNA at this time (Figure 1). Thus at least during the later, cycloheximide-resistant phase of periodic repression, *ftz* transcription probably does not occur in the interbands.

Since *ftz* RNA initially accumulates to high levels with a uniform distribution (Figures 2 and 4), generation of the banded distribution must involve rapid losses of *ftz* RNA from the interbands. The rapid degradation of *ftz* RNA we observed after blocking transcription with α-amanitin could effect these losses. After a shutdown of *ftz* transcription in the interbands, RNA degradation at the rate we observed ($T_{1/2}$ = 6–8 min) would remove about 90% of the *ftz* RNA in the interbands within 20 min. This time frame corresponds well with the 15–30 min actually observed for the transition from uniform to seven-banded expression in precisely staged populations of embryos (see control data in Tables 1, 2, and 3).

Considering our data, the combination of spatially regulated repression of transcription and rapid, uniform RNA degradation provides the simplest, sufficient explanation for how the seven-banded pattern of *ftz* RNA is generated: transcription ceases in the polar regions and interbands and earlier, uniformly distributed transcripts are degraded. Other genes involved in segmentation in the Drosophila embryo, such as *hairy* (Ingham et al., 1985), *evenskipped* (Harding et al., 1986), and *paired* (Kilchherr et al., 1986) are similar to *ftz* in that their transcripts initially accumulate in broad regions of the embryo and later become restricted to more tightly focused regions. We suggest that their patterns of expression may also be regulated by combinations of repression and RNA turnover.

Experimental Procedures

Egg Collections and Staging of Embryos

Eggs were collected from three to ten day old wild-type Drosophila melanogaster ("Sevelen" strain) raised in bottles. Ten minute collections

of synchronously developing embryos were obtained after precollections of 1 hr on fresh food and 30, 10, and 10 min on agar plates supplemented with baker's yeast and acetic acid. Afterwards, experimental embryos were collected at 10 min intervals. Development was at 21–22.5°C. Min AED listed in the text and tables were standardized to 22°C. Embryos were individually staged as described by Edgar et al. (1986) and Edgar and Schubiger (1986).

Microinjections

Microinjections were carried out according to Edgar and Schubiger (1986). Intracellular concentrations of α-amanitin and cycloheximide (Sigma) were estimated according to Foe and Alberts (1983), assuming a dilution factor of 50× and even diffusion throughout the egg. Protein synthesis was measured by incorporation of injected ^{35}S-methionine (New England Nuclear) according to Zalokar (1976). ^{32}P-UTP (3000 Ci/mmol, 10 mCi/ml; New England Nuclear) for labeling RNA in vivo was concentrated to 50 mCi/ml for injection.

In Situ Hybridization

After incubation, the embryos were rinsed from the parafilm injection surface with heptane and pipetted into a two-phase mixture of heptane and 4% filtered paraformaldehyde in phosphate-buffered saline (PBS). The embryos were shaken for more than 30 min and then transferred to a two-phase mixture of heptane and 90% methanol, 50 mM EGTA for 10 min with shaking. They were then rehydrated in PBS, transferred to a drop of PBS containing 0.03% Triton X-100 on parafilm, and manually devitellinized using a tungsten needle. The PBS/Triton X-100 was then aspirated off with a drawn out Pasteur pipette, and the embryos were covered with a small drop of O.C.T. embedding medium (Miles). After arranging the embryos in this drop using a tungsten needle, the drop and its parafilm support were frozen at −70°C. The frozen drop was then affixed to a cryostat chuck using O.C.T. Frozen sections were cut at −12°C and collected on slides coated with polylysine hydrobromide (0.5 mg/ml, 150,000–300,000 MW fraction; Sigma). In situ hybridization was as described by Kornberg et al. (1985), except that sections were acetylated (0.1 M triethanolamine, 0.5% acetic anhydride, 5 min), and 20 mM DTT was included in the hybridization solution. Nick-translated DNA probes were prepared as follows: 3 hr reactions (20 μl, 16°C) were carried out with 0.4 μg of predigested DNA, 40 μM dCTP, dGTP, and dTTP, ^{35}S-dATP (35 μCi, 650 Ci/mmol Amersham) and 7U DNA polymerase I (Boehringer-Mannheim, nuclease-free). Incubation was terminated by adding 25 μl 10 mM EDTA, 2 mg/ml denatured salmon sperm DNA, and heating to 65°C for 10 min. Our *ftz* probe was pDmV61H35, a 3.5 kb genomic clone (Laughon and Scott, 1984). Autoradiography was for 11 days at 4°C.

RNA Isolation, Gels, and Northern Blots

At the completion of an incubation period, embryos were quickly frozen by placing the injection slide on dry ice at −70°C. After thawing, they were rinsed from the injection surface with heptane, transferred to an Eppendorf tube, and total nucleic acid or poly(A)$^+$ RNA was isolated as described by Edgar and Schubiger (1986).

Nucleic acid samples were run on 1% agarose gels containing 6.5% formaldehyde (Maniatis et al., 1982). Labeled RNA gels were dried on a Hoefer gel drier and exposed using Kodak XAR-5 film for 14 days. Northern gels used total nucleic acid from exactly 60 embryos per lane, and were transferred to Hybond nylon membrane (Amersham) and hybridized according to Stroeher et al. (1986). Single-stranded RNA probes were made using SP6 polymerase (Promega) and α-^{32}P-UTP (New England Nuclear) from the plasmid p65FZ.1, which contains the 2.0 kb PstI, EcoRI fragment from p523B (Kuroiwa et al., 1984; the complete *ftz* genomic transcription unit), or the plasmid pGRP49, which contains the rp49 transcription unit (O'Connell and Rosbash, 1984). Exposure times of the gels shown were 4 hr (*ftz*, Figure 1), 30 min (*ftz*, Figure 4), and 15 min (rp49, Figure 4).

Densitometer measurements were done in the following manner. Autoradiographic exposures of 15 min, 30 min, 1 hr, 2 hr, 4 hr, and 12 hr for each blot were scanned using a Quick Scan R and D densitometer. The values obtained were then graphed to determine the linear range of the film for each band. Values within the linear range of the film were then normalized to a constant exposure time and used to construct the graphs shown in Figures 1 and 4.

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References

Anderson, K. V., and Lengyel, J. A. (1980). Changing rates of histone mRNA synthesis and turnover in Drosophila embryos. Cell *21*, 717–727.

Carroll, S. B., and Scott, M. P. (1986). Zygotically active genes that affect the spatial expression of the *fushi tarazu* segmentation gene during early Drosophila embryogenesis. Cell *45*, 113–126.

Edgar, B. A., and Schubiger, G. (1986). Parameters controlling transcriptional activation during early Drosophila development. Cell *44*, 871–877.

Edgar, B. A., Kiehle, C. P., and Schubiger, G. (1986). Cell cycle control by the nucleo-cytoplasmic ratio in early Drosophila development. Cell *44*, 365–372.

Fjose, A., McGinnis, W. J., and Gehring, W. J. (1985). Isolation of a homeo-box containing gene from the *engrailed* region of Drosophila and the spatial distribution of transcripts. Nature *313*, 284–289.

Foe, V. E., and Alberts, B. M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. J. Cell Sci. *61*, 31–70.

Hafen, E., Kuroiwa, A., and Gehring, W. J. (1984). Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during Drosophila embryonic development. Cell *37*, 833–841.

Harding, K., Rushlow, C., Doyle, H. J., Hoey, T., and Levine, M. (1986). Cross-regulatory interactions among pair-rule genes in Drosophila. Science *233*, 953–959.

Hiromi, Y., Kuroiwa, A., and Gehring, W. J. (1985). Control elements of the Drosophila segmentation gene *fushi tarazu*. Cell *43*, 603–613.

Howard, K., and Ingham, P. (1986). Regulatory interactions between the segmentation genes *fushi tarazu*, *hairy*, and *engrailed* in the Drosophila blastoderm. Cell *44*, 949–957.

Ingham, P. W., Howard, K. R., and Ish-Horowicz, D. (1985). Transcription pattern of the Drosophila segmentation gene *hairy*. Nature *318*, 439–446.

Kilchherr, K., Baumgartner, S., Bopp, D., Frei, E., and Noll, M. (1986). Isolation of the *paired* gene of Drosophila and its spatial expression during early embryogenesis. Nature *321*, 493–499.

Kornberg, T., Siden, I., O'Farrell, P., and Simon, M. (1985). The *engrailed* locus of Drosophila: in situ localization of transcripts reveals compartment-specific expression. Cell *40*, 45–63.

Kuroiwa, A., Hafen, E., and Gehring, W. J. (1984). Cloning and transcriptional analysis of the segmentation gene *fushi tarazu* of Drosophila. Cell *37*, 825–831.

Laughon, A., and Scott, M. P. (1984). Sequence of a Drosophila segmentation gene: protein structure homology with DNA-binding proteins. Nature *310*, 25–31.

Lindquist, S., DiDomenico, B., Bugaisky, G., Kurtz, S., Petko, L., and Sonoda, S. (1982). Regulation of the heat-shock response in Drosophila and Yeast. In Heat Shock from Bacteria to Man, M. Schlesinger, M. Ashburner, and A. Tissières, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 167–175.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning:

A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Mohler, J., and Wieschaus, E. F. (1985). Bicaudal mutations of Drosophila melanogaster: alteration of blastoderm cell fate. Cold Spring Harbor Symp. Quant. Biol. *50*, 105–111.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. Nature *287*, 795–801.

O'Connell, P., and Rosbash, M. (1984). Sequence, structure, and codon preference of the Drosophila ribosomal protein 49 gene. Nucl. Acids Res. *12*, 5495–5513.

Rabinowitz, M. (1941). Studies on the cytology and early embryology of the egg of Drosophila melanogaster. J. Morph. *69*, 1–49.

Rickoll, W. L. (1976). Cytoplasmic continuity between embryonic cells and the primitive yolk sac during early gastrulation in Drosophila melanogaster. Dev. Biol. *49*, 304–310.

Stroeher, V. L., Jorgensen, E. M., and Garber, R. L. (1986). Multiple transcripts from the *Antennapedia* gene of Drosophila. Mol. Cell Biol., in press.

Struhl, G. (1985). Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the Drosophila segmentation gene *ftz*. Nature *318*, 677–680.

Wakimoto, B. T., and Kaufman, T. C. (1981). Analysis of larval segmentation in lethal genotypes associated with the *Antennapedia* gene complex in Drosophila melanogaster. Dev. Biol. *81*, 51–64.

Wakimoto, B. T., Turner, F. R., and Kaufman, T. C. (1984). Defects in embryogenesis in mutants associated with the *Antennapedia* gene complex of Drosophila melanogaster. Dev. Biol. *102*, 147–172.

Wall, R., Briskin, M., Carter, C., Govan, H., Taylor, A., and Kincade, P. (1986). A labile inhibitor blocks immunoglobulin κ-light-chain-gene transcription in a pre-B leukemic cell line. Proc. Natl. Acad. Sci. USA *83*, 295–298.

Weir, M. P., and Kornberg, T. (1985). Patterns of *engrailed* and *fushi tarazu* transcripts reveal novel intermediate stages in Drosophila segmentation. Nature *318*, 433–439.

Zalokar, M. (1976). Autoradiographic study of protein and RNA formation during early development of Drosophila eggs. Dev. Biol. *49*, 425–437.

Zalokar, M., and Erk, I. (1976). Division and migration of nuclei during early embryogenesis of Drosophila melanogaster. J. Mic. Biol. Cell *25*, 97–106.