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Determinants of *Drosophila fushi tarazu* mRNA Instability

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The *fushi tarazu* gene is essential for the establishment of the *Drosophila* embryonic body plan. When first expressed, in early embryogenesis, *fushi tarazu* mRNA is uniformly distributed over most of the embryo. Subsequently, *fushi tarazu* mRNA expression rapidly evolves into a pattern of seven stripes that encircle the embryo. The instability of *fushi tarazu* mRNA is probably crucial for attaining this localized pattern of expression. mRNA stability in transgenic embryos was measured by a new method that does not use drugs or external interference. Experiments using hybrid genes that fuse *fushi tarazu* sequences to those of the stable ribosomal protein A1 mRNA provide evidence for at least two destabilizing elements in the *fushi tarazu* mRNA, one located within the 5′ one-third of the mRNA and the other near the 3′ end (termed FIE3 for *ftz* instability element 3′). The FIE3 lies within a 201-nucleotide sequence just upstream of the polyadenylation signal and can act autonomously to destabilize a heterologous mRNA. Further deletion constructs identified an essential 68-nucleotide element within the FIE3. Lack of homology between this element and other previously identified destabilization sequences suggests that FIE3 contains a novel RNA destabilization element.

mRNA stability plays a key role in regulating the expression of many transiently expressed genes, such as cytokines, oncogenes, and developmentally important genes (36). While much has been learned about the regulation of mRNA stability in cultured cells (4), few studies have been conducted with whole organisms or in a developmental context (36). This is due in part to the lack of a convenient method to assay mRNA stability in whole organisms. In this paper, we present a new method to measure mRNA stability in *Drosophila* embryos.

We have directed our attention to genes which establish the *Drosophila* embryonic body plan. Early zygotic mRNAs belonging to the gap, pair-rule, and segment polarity group of genes are expressed only transiently in the developing *Drosophila* embryo. As such, these mRNAs are likely to be unstable, and as indicated below, instability is probably crucial for achieving correct spatial and temporal pattern of expression. Here we focus on *fushi tarazu* (*ftz*), one of the best-characterized *Drosophila* pair-rule genes. The *ftz* mRNA is among the shortest-lived mRNAs (6- to 14-min half-life) among higher eukaryotes (4, 9) and shares the cytoplasm with a majority of mostly stable mRNAs.

ftz transcripts are first expressed at low levels at embryonic nuclear cycle 11 (about 1 h 40 min after egg laying at 25°C) in a broad band between 15 and 65% egg length (measured from the posterior pole) (5, 11, 13). During cellularization of the blastoderm (2 h 10 min to 2 h 50 min), *ftz* mRNA becomes transiently restricted to four broad bands and finally to seven narrow stripes that encircle the embryo (5, 13, 38, 45). Thus, it is during this very short time that the spatial pattern of *ftz* mRNA expression is established. Formation of stripes probably occurs by the cessation of transcription in interband regions coupled with rapid turnover of *ftz* mRNA. Because the striped pattern evolves very rapidly and because the FTZ protein activates its own transcription (positive feedback loop), it is important that *ftz* gene products be rapidly removed from interband regions. In fact, *ftz* mRNA and protein are both highly unstable (8, 9, 22). In support of this assertion, Kellerman et al. (22) have shown that certain dominant *ftz* alleles (ftz^{Ual}) carry mutations that lead to the stabilization of the *ftz* protein. These authors also report that the dominant mutant ftz^{Rpl} allele, which deletes all sequences 3′ from the homeobox, results in the stabilization of both RNA and protein. The truncated RNA may be more stable because it lacks *cis*-acting destabilizing sequence(s). Moreover, Welte et al. (39) have observed that heat treatment of early embryos mimics the phenotypes of certain *ftz* gain-of-function mutations. Their data strongly suggest that the phenotype is caused by the heat-induced stabilization of the *ftz* protein. The *ftz* mRNA stability has been shown to decrease as a function of time after fertilization. The estimated half-life varies from 14 min when *ftz* is first expressed to 6 min when the stripes have completely formed (9).

This study describes our initial characterization of *cis*-acting sequences that mediate the destabilization of *ftz* mRNA. To search for such sequences, we constructed hybrid genes that fuse *ftz* sequences to the stable ribosomal protein A1 (rpA1) mRNA and made deletions in the *ftz* portion of the hybrid genes. The stability of the corresponding mRNAs was measured in synchronized transgenic embryos in the absence of drugs or external interference. We found that the *ftz* mRNA contains at least two destabilizing sequences, one located in the 5′ one-third of the mRNA and another located in the 3′ untranslated region (UTR), near the polyadenylation signal (termed FIE3 for *ftz* instability element 3′).

MATERIALS AND METHODS

Construction of transgenes. (i) f5r3 and r5f3. The *ftz* genomic clone λA439 was obtained from M. Scott (37). A 7.9-kb *Kpn*I-*Sal*I fragment was subcloned into the bacterial vector pGEM-3. This fragment contains the 5′ one-third of the *ftz* transcribed sequences and 6.1 kb of upstream sequences, which are necessary for correct expression of the *ftz* gene (17). A 1.3-kb *Sal*I-*Bam*HI genomic fragment of the ribosomal protein gene rpA1 (28), which includes the 3′ two-thirds of the rpA1 transcribed sequences, was then introduced into this vector, resulting in the f5r3 hybrid gene (see Fig. 2A). This gene encodes a 1.4-kb transcript, of which 1 kb is 5′ *ftz* transcribed sequence and 0.4 kb is 3′ rpA1 transcribed sequence. This transcript contains 114 bases of *ftz* 5′ UTR and 168 bases of rpA1 3′ UTR. The *Sal*I sites were in the protein coding regions of both genes, and the correct reading frame was preserved.

The reciprocal r5f3 construct (see Fig. 2A) was made by inserting the 4.0-kb *Sal*I-*Kpn*I fragment of λA439, which codes for the 3′ two-thirds of the *ftz* tran-

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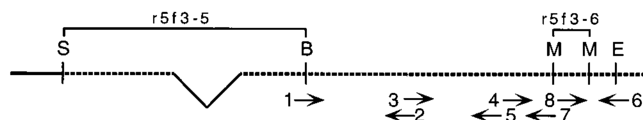

FIG. 1. r5f3 deletion constructs. Diagram of the 3′ two-thirds of *ftz*, showing restriction sites and the position of the intron (large notch). The PCR primers used to make the deletion constructs are shown by numbered arrows indicating the direction of polymerization. S, *Sal*I (this site is at the junction between rpA1 and *ftz* sequences in r5f3) (Fig. 2); *B*, *Bgl*II; M, *Msc*I; E, *Eco*RI (this site is located 10 nucleotides upstream from the polyadenylation signal). The restriction sites used to make the deletion constructs r5f3-5 and -6 are indicated at the top of the figure. Further details are given in Materials and Methods.

scribed sequences, into pGEM and adding the 1.1-kb *Bam*HI-*Sal*I fragment of rpA1 containing this gene's promoter and 5′ transcribed sequences. This hybrid gene encodes a 1.0-kb hybrid transcript, which includes 0.2 kb of 5′ rpA1 transcribed sequence and 0.8 kb of 3′ *ftz* transcribed sequence. This hybrid transcript includes 89 bases of rpA1 5′ UTR and 419 bases of *ftz* 3′ UTR. The protein coding region remains in frame.

The endogenous *ftz* and rpA1 transcripts are 1.8 and 0.6 kb, respectively [not including the poly(A) tail]. Thus, the hybrid transcripts can be easily resolved from the endogenous counterparts by agarose gel electrophoresis.

(ii) Deletion constructs. Deletion constructs were made either by replacing the 756-bp *Bgl*II-*Eco*RI fragment with PCR products which were subsets of this fragment or by restriction enzyme digestion (Fig. 1). The following PCR primers were used for synthesis of deletion constructs: primer 1, 5′**GCAGATCAAGATCTGGTTCC**3′; primer 2, 5′GTGAATTC**GCCGGACCTCAAGACAGATG**3′; primer 3, 5′GTAGATCT**CACAGGACCTCTACCATCTG**3′; primer 4, 5′CAAGATCT**GCAAAAGAGGCAGCGCAAAC**3′; primer 5, 5′CAGAATTC**CTGCCTTCTGCACTTGCGCAC**3′; primer 6, 5′**AAATAGAATTCTATGATAGG**3′; primer 7, 5′GCGAATTC**CAGCTAATCGATCGCTGAGA**3′; primer 8, 5′GCGAATTC**GGCCAAACACAAGCCCAAAACA**3′. Sequences complementary to the *ftz* gene are in boldface. The *Bgl*II (AGATCT) and *Eco*RI (GAATTC) restriction enzyme sites are underlined.

In the following constructs, the *Bgl*II-*Eco*RI fragment (Fig. 1) was replaced by PCR products synthesized with the listed primers: for r5f3-1, primers 1 and 2; for r5f3-2, primers 1 and 5; for r5f3-3, primers 4 and 6; for r5f3-4, primers 3 and 6; for r5f3-7, primers 1 and 7. Construct r5f3-8 was produced by inserting the PCR products of primers 6 and 8 into the unique *Eco*RI site of r5f3-2. The PCR products were repaired with Klenow enzyme and, in some cases, with T4 DNA polymerase or S1 nuclease. The PCR fragments were then gel purified and digested with *Bgl*II and/or *Eco*RI. The enzymes were removed by phenol extraction, and the PCR products were then ligated into the r5f3 hybrid construct which had been digested with *Bgl*II and/or *Eco*RI. A 150-fold molar excess of PCR product relative to vector was used in these ligations because the ligation efficiency of the PCR products was extremely low. Constructs r5f3-5 and r5f3-6 were generated by deleting the restriction fragments indicated in Fig. 1. The protein coding region remains in frame in all constructs. The constructs were sequenced to verify that no errors occurred during PCR amplification and to assure that the reading frame was maintained. All constructs contain the endogenous polyadenylation signal, which is located 10 nucleotides downstream of the *Eco*RI site.

(iii) rpA1 insertion constructs. The PCR product synthesized with primers 4 and 6 was inserted into the *Nae*I site in the 3′ UTR of the intact rpA1 gene to yield rp-*ftz* (Fig. 2D). The PCR product was digested with *Bgl*II and *Eco*RI, and the restriction site overhangs were filled with T4 polymerase. The fragments were then ligated into the blunt-ended *Nae*I site. As a control, a 97-bp fragment from the rpA1 gene (nucleotides 211 to 307) was inserted into the rpA1 *Nae*I site by the same method to yield rp-con (Fig. 2D). The FIE3 insert has a GC content of 63.5%, and the control insert has a content of 48.8%.

Transgenic flies. All hybrid gene constructs were introduced into the CaSpeR vector (27). A helper plasmid, phs-π (35), was coinjected at a concentration of 100 μg/ml in order to provide a transient source of transposase (32).

RNA analysis. Large numbers of adults homozygous for each transgene were allowed to lay eggs on fresh yeast paste at 25°C for 1 h. The embryos were raised at 25°C until they reached the desired age. They were then washed, quickly frozen in liquid nitrogen, and stored at −80°C. A small aliquot from some embryo collections was reserved and checked for synchrony at 2 to 3 h of development. Collections were discarded if more than 15% of the embryos were too old (retained) or too young (unfertilized). Polyadenylated RNA was prepared from each batch of embryos. We found it necessary to prepare polyadenylated RNA because *ftz* mRNA does not give a reliable signal on Northern (RNA) blots in the presence of a large excess of comigrating rRNA. The RNAs were fractionated by electrophoresis on 1 to 2% agarose gels, blotted, and hybridized with randomly primed probes. Estimates of RNA decay were obtained by quantification of the hybridized radioactivity with a Molecular Dynamics Phosphorimager. The rpA1 mRNA signals were used to correct for the amount of RNA loaded in each lane. For each construct, the Northern analysis was repeated at least four times with at least two independent RNA preparations.

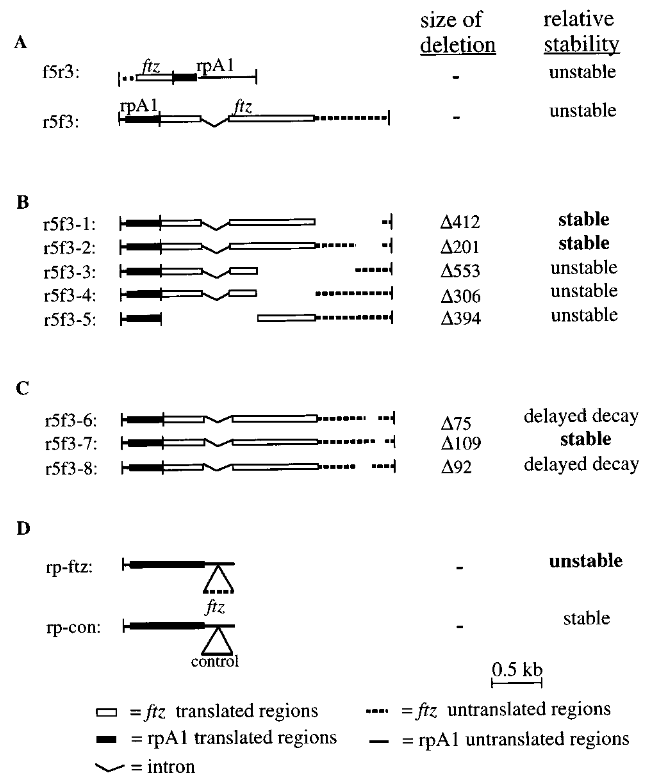

FIG. 2. Summary of hybrid constructs used to identify and delineate *fushi tarazu cis*-acting instability elements. (A) Reciprocal hybrid constructs. f5r3 fuses 5′ *ftz* sequences, including the promoter, to 3′ rpA1 sequences. r5f3 fuses 5′ rpA1 sequences, including the promoter, to 3′ *ftz* sequences. Each construct encodes a hybrid mRNA consisting of about one-third 5′ sequences from one gene fused in frame to the complementary two-thirds 3′ sequences from the other gene. (B) Overlapping deletions covering *ftz* sequences in r5f3. The number of nucleotides deleted in each construct is indicated to the right. (C) Overlapping deletions covering the 201-nucleotide destabilizing element defined by r5f3-2. The number of nucleotides deleted in each construct is indicated to the right (see Fig. 6A for further details). (D) Insertion of either the 201-nucleotide destabilizing element (*ftz*) or an unrelated sequence (control) into the 3′ UTR of rpA1. The relative stability of each mRNA in the *Drosophila* embryo is indicated in the far right column. The promoter regions and the insertion sequences in panel D are not drawn to scale.

RESULTS

Measurement of mRNA stability in early embryos. We have developed a new method for the in vivo analysis of mRNA stability in early *Drosophila* embryos in the absence of external interference or drugs. It takes advantage of the fact that most genes are not transcribed during the first 3 h of embryogenesis (3, 25). In the absence of transcription, the stability of preexisting (maternal) mRNAs can be directly determined from changes of steady-state levels as a function of time.

Hybrid constructs which contain sequences from the *ftz* and from the rpA1 genes were generated (Fig. 2). rpA1 was chosen because the mRNA is stable in early embryos (1) and can thus serve as a reporter for potential destabilizing *ftz* mRNA sequences. Moreover, rpA1 acts as a strong promoter in ovaries and is silent in early embryos. All but one of the constructs (f5r3) reported in this work are driven by the rpA1 promoter.

Hybrid constructs were introduced into flies by P element-mediated transformation. For each construct, the transformant

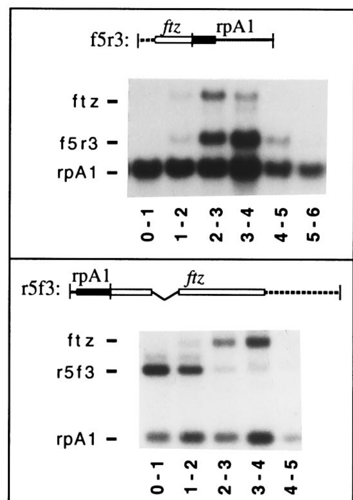

FIG. 3. First-generation constructs for identification of *fushi tarazu* mRNA-destabilizing sequences. Constructs carrying either the 5′ one-third (f5r3) or the 3′ two-thirds (r5f3) of *ftz* mRNA were transformed into the *Drosophila* germ line. Polyadenylated RNAs (about 100 ng) from synchronized transgenic embryos were analyzed on Northern blots hybridized with rpA1 and *ftz* probes. The migration of each mRNA species is indicated on the left. Embryo age ranges (in hours) are indicated below the autoradiograms.

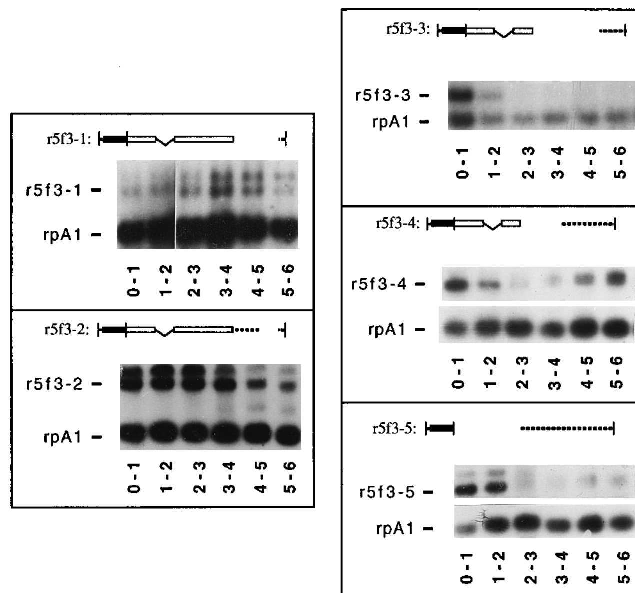

FIG. 4. Second-generation constructs for mapping the 3′ *fushi tarazu* instability element. A series of overlapping deletions covering the 3′ *ftz* mRNA sequences of the r5f3 construct were made and transformed into the flies. The stability of the resulting mRNAs in embryos was analyzed as described in the legend to Fig. 3.

strains which expressed the highest levels of hybrid mRNA were chosen for analysis of mRNA stability. To measure mRNA stability, poly(A)-containing RNAs were prepared from synchronous embryos and analyzed on RNA blots with a mixture of rpA1 and *ftz* probes. Isolation of poly(A)-containing RNA was necessary because rRNAs interfere with detection on Northern blots of RNAs (such as *ftz* mRNA) in the size range of 1.8 to 2.0 kb. The stable rpA1 mRNA served as a quantification standard to correct for differences in the amount of RNA analyzed in each lane.

Multiple destabilizing sequences exist in the *ftz* mRNA. To determine the approximate location of *ftz* destabilizing sequences, two hybrid genes were initially constructed (Fig. 2A). One, f5r3, encodes the 5′ one-third of the *ftz* mRNA fused in frame to the 3′ two-thirds of the rpA1 mRNA. The other, r5f3, is exactly complementary to the first and encodes the 5′ one-third of the rpA1 mRNA fused in frame to the 3′ two-thirds of the *ftz* mRNA. Analysis of the corresponding mRNAs is shown in Fig. 3. f5r3 is the only construct in this work to use the *ftz* promoter. The pattern of expression of this hybrid mRNA is similar to that of *ftz* mRNA. Significantly, the f5r3 mRNA is rapidly degraded after 3 to 4 h of development, indicating that the mRNA is unstable. These results indicate that the 5′ one-third of the *ftz* mRNA contains a destabilizing element.

The reciprocal r5f3 mRNA is transcribed maternally from the rpA1 promoter and accumulates to high levels in the unfertilized egg (Fig. 3, 0- to 1-h time point). The abundance of the hybrid mRNA decreases rapidly over the first 3 h of embryogenesis. The radioactive signals were quantified with a Phosphorimager, and after correction for the amount of RNA loaded, the hybrid mRNA was found to have a half-life of about 50 min. Thus, the r5f3 mRNA is unstable compared with the essentially stable rpA1 mRNA, indicating that the 3′ two-thirds of the *ftz* mRNA also contains destabilizing sequences. In conclusion, the *ftz* mRNA seems to contain at least two instability elements. Each element can act independently to destabilize a hybrid mRNA.

The 3′ *ftz* instability element (FIE3) is contained within a 201-nucleotide region of the *ftz* 3′ UTR. To further localize the *ftz* instability element(s) in the 3′ two-thirds of the mRNA, we produced a series of five overlapping deletions of the r5f3 hybrid gene (Fig. 2B). Transgenic fly lines were established for each of the constructs, and embryos from each strain were used to analyze the stability of the corresponding hybrid mRNAs. The results are shown in Fig. 4.

The r5f3-3, r5f3-4, and r5f3-5 mRNAs rapidly decay in early embryos, indicating that they contain an instability element (Fig. 4). By 2 to 3 h of development, only trace amounts of these mRNAs are detectable. The reappearance of these mRNAs at 4 to 5 h of development can be attributed to the onset of zygotic transcription from the rpA1 promoter. The mRNAs from older embryos tend to produce broader bands than those from young embryos, perhaps resulting from greater heterogeneity in the length of the poly(A) tails.

In contrast to the mRNAs discussed above, the r5f3-1 and r5f3-2 hybrid constructs give rise to substantially stabilized transcripts (Fig. 4). Their abundance does not diminish appreciably during the first 3 h of development. This indicates that the sequences required for destabilization are absent from the corresponding mRNAs. Thus, the 201 nucleotides which are deleted from both r5f3-1 and r5f3-2 are necessary for the destabilization of the hybrid transcript. This destabilizing sequence (FIE3) is located in the 3′ UTR of the *ftz* mRNA near the polyadenylation signal.

Despite its initial stability, the abundance of the r5f3-2 mRNA appears to drop to a lower plateau after 3 h of embryogenesis (Fig. 4). This may mean that the 3′ portion of the message contains an additional destabilizing element that is active from 3 to 4 h onward, when *ftz* mRNA degradation is most active. The higher stability of the r5f3-1 RNA during this same time would place such a putative element in the proximal portion of the 3′ UTR.

We observed that hybrid genes with altered sequences near the polyadenylation signal consistently give rise to two transcripts (Fig. 4, r5f3-1 and r5f3-2; see also Fig. 6, r5f3-7; these

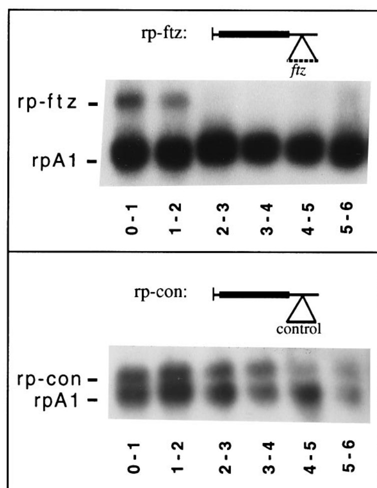

FIG. 5. Third-generation constructs show that the 3′ instability element is sufficient to destabilize a normally stable message. A 201-nucleotide instability element defined by r5f3-2 (Fig. 4) was inserted in the 3′ UTR of the rpA1 gene (rp-*ftz*). As a control, an unrelated sequence was inserted at the same position (rp-con). The stability of the resulting mRNAs in embryos was analyzed as described in the legend to Fig. 3.

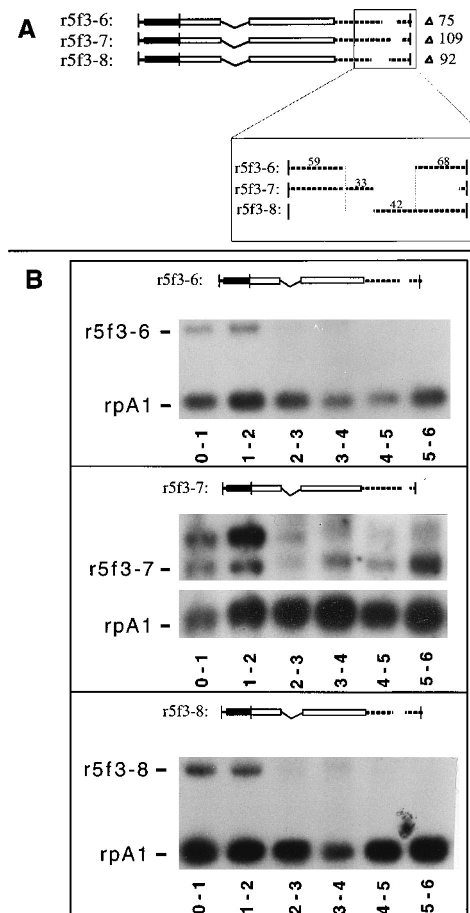

FIG. 6. Fourth-generation constructs define an essential 68-nucleotide element. (A) Schematic diagram of the deletions covering the 201-nucleotide instability element defined by r5f3-2 (Fig. 4). (B) The constructs illustrated in panel A were transformed into flies, and the stability of the resulting mRNAs was analyzed as described in the legend to Fig. 3. The r5f3-7 mRNA is stable while degradation of the remaining two hybrid mRNAs is delayed in development.

three deletions end 10 nucleotides upstream from the polyadenylation signal). In all cases, the smaller of the two mRNAs is of the expected size. The larger mRNA is approximately 150 bases longer and probably corresponds to read-through transcription and polyadenylation at a cryptic downstream site. A putative polyadenylation signal is present in the *ftz* gene approximately 150 bp downstream of the normal polyadenylation signal. In support of this interpretation, we found that an oligonucleotide complementary to the downstream sequence hybridizes exclusively to the larger mRNA (results not shown).

The FIE3 is sufficient to destabilize a normally stable mRNA. In order to determine whether the FIE3 is sufficient to destabilize an otherwise stable mRNA, the 201-bp destabilizing region defined by the r5f3-2 deletion was inserted into the 3′ UTR of the intact rpA1 gene (Fig. 2D, rp-*ftz*). Transgenic flies were obtained, and the stability of the resulting rp-*ftz* mRNA was measured as done previously. As shown in Fig. 5, the rp-*ftz* mRNA is unstable, indicating that the *ftz* 3′ instability element is sufficient to destabilize a heterologous mRNA. The half-life of the mRNA was estimated to be about 58 min, which is similar to the 50-min half-life estimated for the r5f3 hybrid mRNA (Fig. 3).

As a control, an unrelated sequence was inserted into the same site of the rpA1 mRNA (Fig. 2D, rp-con). Figure 5 shows that this mRNA is stable, indicating that the destabilization of the rpA1 mRNA is sequence specific and is not due to the disruption of the rpA1 3′ UTR.

Further deletions identify functional elements within FIE3. A further series of constructs consisting of three overlapping deletions within the 201-nucleotide FIE3 was generated (Fig. 2C and 6A). The stability of the resulting mRNAs was analyzed as before. Two of the hybrid mRNAs from this series, r5f3-6 and r5f3-8, were found to be unstable. Thus, the 134 nucleotides which were deleted from one or both of these hybrid mRNAs (Fig. 6A) are probably not necessary for *ftz* mRNA degradation.

The r5f3-7 mRNA is stabilized, indicating that the 109 nucleotides which were deleted from this mRNA contain an essential component of the FIE3. Of these 109 nucleotides, 42 overlap with sequences which are deleted from the unstable r5f3-6 mRNA and therefore cannot contain essential destabilizing sequences (Fig. 6A). This indicates that the 68 nucleotides just upstream of the *ftz* polyadenylation signal may contain sequences necessary for *ftz* mRNA destabilization. Two r5f3-7 transcripts were detected (Fig. 6B). As discussed above, it might be due to the weakening of the normal polyadenylation signal caused by deletion of nearby sequences.

The decay of the r5f3-6 and r5f3-8 mRNAs follows a different temporal pattern of decay from that of the other unstable hybrid mRNAs. In several repeat experiments, these transcripts were essentially stable for the first 2 h of embryogenesis and only afterwards were they rapidly degraded (Fig. 6B). (Note that the apparent initial stability of r5f3-5 mRNA shown in Fig. 4 is actually due to the underloading of RNA in the 0- to 1-h lane, as indicated by the weaker rpA1 signal.) It appears that the 134 nucleotides upstream of the 68-nucleotide element contain sequences that act in conjunction with this essential element to determine the timing of *ftz* mRNA degradation.

DISCUSSION

Using a new approach to measure mRNA stability in *Drosophila* embryos, we defined a 201-nucleotide instability ele-

ment (FIE3) in the 3′ UTR of the *fushi tarazu* (*ftz*) mRNA. This element is necessary for the degradation of a hybrid *ftz* mRNA and sufficient to destabilize a heterologous mRNA. Further deletion analysis identified a 68-nucleotide sequence that is essential for FIE3 function. At least one additional destabilizing element may be present in the 5′ one-third of the *ftz* mRNA.

Avoiding the use of drugs was an important consideration in deciding on an experimental plan since transcription inhibitors can lead to artifactual results (33). The method used here can be used to study the stability of any *Drosophila* mRNA, with the limitation that measurements must be done when the rpA1 promoter is inactive, during the first 3 h of embryogenesis. The method is most reliable for those mRNAs normally present in the early embryo because the same mRNA could be regulated differently at other developmental times or in other cell types. For example, the r5f3 mRNA is unstable in the early embryo (Fig. 3) but is stable in the ovary (29).

The *ftz* mRNA may contain multiple destabilizing elements. Both the 5′ and the 3′ portions of the *ftz* mRNA that were originally tested appear to contain destabilizing elements. The identification of a destabilizing element in the 3′ UTR of the *ftz* mRNA is in line with the presumed stabilization of the mRNA encoded by the ftz^{Rpl} allele, which deletes part of the *ftz* coding sequence and the entire 3′ UTR (22). There are several precedents for mRNAs with more than one instability element. These include the proto-oncogenes c-*fos*, c-*myc*, and beta interferon and the *Saccharomyces cerevisiae* genes *STE3* and *HIS3* (15, 16, 33, 40, 42). Dual destabilizing elements could interact to increase degradation efficiency. Alternatively, a second element could act as a redundant degradation signal to insure rapid turnover of *ftz* mRNA. As argued in the Introduction, rapid removal of *ftz* mRNA from interband regions is probably crucial for normal embryonic development.

The f5r3 hybrid construct is driven by the *ftz* promoter and is expressed at the same time as the endogenous *ftz* gene. This makes quantification of mRNA half-life difficult because mRNA degradation cannot be easily separated from mRNA synthesis. However, we could deduce that the f5r3 mRNA is unstable because the transcript is degraded soon after the *ftz* promoter is turned off. By contrast, mRNAs lacking destabilizing sequences are relatively stable in these embryos (e.g., Fig. 4, left panels). In principle, the instability of the f5r3 mRNA could be due to the removal of a dominant 5′-stabilizing sequence from the rpA1 mRNA rather than to the presence of an *ftz* instability sequence in the hybrid mRNA. We consider this possibility to be unlikely because a similar hybrid RNA consisting of 5′ tubulin sequences fused to the same 3′ rpA1 sequences appears to be stable in the early *Drosophila* embryo (reference 26 and unpublished observations).

The stability of the r5f3 mRNA (50-min half-life) is intermediate between that of rpA1 (which is essentially stable) and *ftz*. This may be because *ftz* mRNA stability was measured soon after fertilization and because the degrading activity is low in very early embryos. This hypothesis is supported by the observation that the half-life of the *ftz* mRNA gradually decreases from 14 min in blastoderm embryos to 6 min at cellularization (9). Recall that r5f3 mRNA stability was measured prior to the time when *ftz* is normally expressed. That the r5f3 mRNA is stable in the ovary and is destabilized upon egg activation (30) suggests that the *ftz* degradation system is activated at fertilization and is consistent with the hypothesis that the *ftz* degrading activity increases as embryogenesis progresses. This increased activity could be due to the synthesis and accumulation of a nuclease, of an essential cofactor, or of a modifying enzyme (e.g., a kinase). Another possible explanation for the longer half-life of the hybrid mRNAs is that the extremely rapid turnover of the intact *ftz* mRNA is the result of the interaction between the 5′ and 3′ instability elements. The two explanations, which are not mutually exclusive, can be tested experimentally.

Inhibition of protein synthesis stabilizes the *ftz* mRNA (9), suggesting that the degrading activity depends on the synthesis of an unstable protein or on the transit of ribosomes along the message. Yet efficient translation of the r5f3 mRNA itself is probably not necessary for degradation, because the rpA1 mRNA is poorly translated in early embryos (1, 12, 21) because of sequences in its 5′ UTR (26). Most hybrid mRNAs analyzed in this work contain the same rpA1 5′ UTR sequences and yet display widely different stabilities. Thus translational efficiency of the hybrid mRNAs seems not to correlate with their stability.

Similarity of the FIE3 sequences to related genes. We compared the essential 68-nucleotide sequence within the FIE3 to the *ftz* gene from *Drosophila hydei* (20). The *ftz* 3′ UTRs from *Drosophila melanogaster* and *D. hydei* have a 25-nucleotide region with 84% identity (Fig. 7A). This region is located 336 nucleotides downstream of the *D. hydei* translation stop codon and 16 nucleotides upstream of the poly(A) signal. The same region in *D. melanogaster* is located 360 nucleotides downstream of the translation stop codon and 44 nucleotides upstream of the poly(A) signal. Interestingly, an RNA secondary structure folding program (18, 19, 46) predicts that this region of high identity will form a stem-loop structure in the *ftz* mRNAs of both *D. melanogaster* (Fig. 7B) and *D. hydei* (not shown). *D. melanogaster* and *D. hydei* diverged an estimated 60 million years ago (34). This conservation at the level of primary sequence and possibly secondary structure, and at the level of position within the 3′ UTR, suggests that this region may be functionally important. Further experiments are needed to test this hypothesis.

A six-base WUUGUA (where W is A or U) sequence is present in the *ftz* genes of both *D. melanogaster* and *D. hydei*. It occurs twice in the 3′ UTR of the *D. melanogaster* gene, one of which is within the 68-base destabilizing region (Fig. 7A). The same sequence appears near the 3′ end of many other unstable early zygotic mRNAs, including the pair-rule genes *even-skipped*, *odd-skipped*, and *runt* as well as the gap gene *hunchback*. It does not appear in the stable rpA1 mRNA. These data seem to suggest that this sequence is important for instability of pattern-determining mRNAs. However, the sequence is not present in the pair-rule genes *paired* or *hairy* and is found near the 3′ end of the stable tubulin gene. Therefore, if the WUU GUA sequence is involved in instability, it may be context sensitive and perhaps act in conjunction with other destabilizing elements within the FIE3. This is the case for the AU-rich element of the c-*fos* mRNA, whose function depends on the presence in *cis* of a second U-rich element (6, 44).

There are many examples of other genes with destabilizing regions in their 3′ UTRs (reviewed in the Introduction). In *D. melanogaster*, there is some evidence that the pair-rule gene *even-skipped* has a destabilization signal in its 3′ UTR (7). However, there is no homology between the 68-bp FIE3 and the *eve* 3′ UTR. No multiple AU-rich sequence motifs of the type present in proto-oncogene and cytokine mRNAs (4) were identified in the FIE3. Thus, these experiments identify a novel *Drosophila* destabilization element.

Model for the FIE3-mediated degradation. The following statements may be made about the studies of *ftz* mRNA degradation to date. (i) In the present study, two potential functional elements were identified within the FIE3: a 68-nucleotide core element and an auxiliary element (the 33-nucleotide element that is deleted by both r5f3-6 and r5f3-8 [Fig. 6A]).

FIG. 7. Comparison between the nucleotide sequences of *D. melanogaster* and *D. hydei ftz* mRNAs and predicted secondary structure. (A) Nucleotide sequence comparison. The 3′-terminal nucleotide sequences of the *D. melanogaster* (Dm) and *D. hydei* (Dh) *ftz* mRNAs are shown. The putative 68-nucleotide core instability element lies between the downward arrows. Bars, boldface letters, and asterisks indicate identical nucleotides within a 25-nucleotide high-similarity region. Dots represent gaps inserted to maximize matches. Horizontal brackets (oblique in panel B) above the sequences indicate the WUUGUA motif (see Discussion). The polyadenylation sequence is underlined. (B) Predicted secondary structure of the 68-nucleotide putative instability element shown in panel A. Note that the region of highest similarity forms the most stable predicted hairpin structure.

The 68-nucleotide element that is absent in the stable r5f3-7 mRNA, but is present in the unstable r5f3-6 and r5f3-8 mRNAs (Fig. 6A), is essential for degradation. Degradation of transcripts lacking the auxiliary element is biphasic: such transcripts are stable in early embryogenesis and are then rapidly degraded later in embryogenesis (Fig. 6B). (ii) The activity that degrades *ftz* mRNA in the embryo appears to increase as development progresses. Edgar et al. (9) estimate that the *ftz* mRNA half-life is 14 min at 2 h 30 min after egg laying, 8 min at 3 h 10 min, 7 min at 3 h 30 min, and 6 min at 4 h 00 min (in these experiments, embryos were incubated at 22°C and development occurs considerably more slowly than at 25°C). (iii) Selective mRNA degradation is likely to involve the interaction of protein factors with specific mRNA sequences. Evidence for such interactions has been obtained for a variety of mRNAs, such as those encoding cytokines, c-*myc*, c-*fos*, *Drosophila oskar* and *bicoid*, histones, erythropoietin, and the transferrin receptor (2, 10, 14, 23, 24, 31, 41, 44). For *ftz*, we have recently observed that injection of FIE3 RNA into early embryos resulted in delayed stripe formation and in segmentation defects (43). The results are consistent with the hypothesis that the injected FIE3 RNA competes with embryonic factors that specifically recognize the FIE3 and are required for *ftz* mRNA degradation.

On the basis of these observations, we propose a model to explain how the FIE3 sequence elements interact to promote *ftz* mRNA degradation. The competition experiments mentioned above suggest the involvement of a protein factor(s) in FIE3-mediated RNA degradation. In early embryogenesis, when the amount or activity of this putative factor(s) is low, the auxiliary element may be needed to promote cooperative binding and degradation. Later in embryogenesis, when the cofactor(s) amount or activity increases, the auxiliary element may be dispensable. The auxiliary element may be part of an independent degradation system or may act synergistically with the core 68-nucleotide element to promote mRNA degradation. It is also possible that the auxiliary element is not required and that the deletions in r5f3-6 and r5f3-8 simply change the folding of the FIE3 to make the critical 68-nucleotide region less accessible, thus increasing the activity threshold required for efficient degradation. Ultimately, the characterization of the *ftz* instability elements is essential to investigate the importance of mRNA stability to *Drosophila* embryonic development.

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