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A conserved element in the leader mediates post-meiotic translation as well as cytoplasmic polyadenylation of a *Drosophila* spermatocyte mRNA

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We have previously shown that *Mst87F* (previously called *mst(3)gl-9*), a gene which is exclusively expressed in the male germ line of *Drosophila melanogaster*, is subject to negative translational control. While transcription of this gene takes place premeiotically, translation occurs only after the elongation of spermatids is complete. We report here the identification of a sequence element within the first 45 nucleotides of the leader which is crucial for this translational regulation. Sequence comparison with six other genes, which form a gene family with *Mst87F*, shows the conservation of a twelve nucleotide element within this leader segment. It is found in all genes at positions +28 to +39 of the leader. Deletion of this element or alteration of two nucleotides by *in vitro* mutagenesis both lead to the breakdown of the translational control mechanism. The poly(A) tail of the *Mst87F* mRNA becomes longer and heterogeneous in length when the mRNA is recruited for translation. We present evidence that the control for this additional polyadenylation also resides within the conserved element of the leader.

Key words: *Drosophila*/polyadenylation/sequence element/spermatogenesis/translational control

Introduction

The main focus in understanding regulatory mechanisms of eukaryotic gene expression in the last years has been the identification of promoters and upstream *cis* regulatory elements and the binding factors that activate or repress transcription (for review see Ptashne, 1988; Biggin and Tjian, 1989; Renkawitz, 1990). However, there is a growing realization that post-transcriptional regulation of mRNA can be just as important as the control of transcription. Such regulation could be mediated by mRNA stability, subcellular localization of the mRNA or translational control, which may be accompanied by structural modifications of the mRNA.

Although translational control is a mechanism which is used in a number of different biological systems, much less detailed information is available on this type of regulation. For example, it has long been known that in many species the embryo utilizes a large number of mRNAs that have been synthesized during oogenesis but stored and prevented from translation until after fertilization has occurred (see Davidson, 1986), but only recently has information begun to accumulate on how this regulation is achieved.

We have chosen spermatogenesis in *Drosophila melanogaster* to study the mechanism of translational control. Unlike mammalian spermatogenesis, transcription ceases in *Drosophila* well before the meiotic divisions (Olivieri and Olivieri, 1965; Hennig, 1967; Gould-Somero and Holland, 1974), yet drastic structural alterations take place postmeiotically during the maturation of spermatids. Therefore, the entire developmental program for spermiogenesis has to be transcribed in primary spermatocytes and executed in spermatids. As a consequence, all mRNAs coding for proteins needed in spermiogenesis have to be stored and prevented from premature translation. We have previously shown that *Mst87F* (new nomenclature for *mst(3)gl-9* as suggested by Lindsley and Zimm, 1990), a gene which is exclusively expressed in the male germ line in *Drosophila melanogaster* (Schäfer, 1986), is one of the genes subject to negative translational control (Kuhn *et al.*, 1988a). Its transcript is first detected in primary spermatocytes, but translation is prevented for at least three days until spermatids have fully elongated. We have continued the analysis of the expression of this gene by P-mediated germ line transformation (Rubin and Spradling, 1982) using fusion genes with varying portions of the leader sequences. We find that a region of 24 nucleotides is necessary for the observed translational control. Within these 24 nucleotides lies an element of twelve nucleotides which is conserved at an identical position in all seven cloned genes of a gene family to which *Mst87F* belongs. In this paper we demonstrate that this element is in fact a translational control element. This is the second identification of a short sequence element necessary for translational control and is the first example of a translational control element with a dual function, since it also mediates the cytoplasmic polyadenylation which occurs concomitantly with translation of the stored mRNA.

Results

A segment of the leader confers translational control

In earlier experiments, we showed that the presence of 102 bp of 5′ upstream sequences together with 201 bp of transcribed sequences is sufficient to maintain spermatocyte-specific transcription of *Mst87F* as well as repression of protein synthesis until late in spermiogenesis (Kuhn *et al.*, 1988a). To identify sequence elements responsible for this translational repression, we constructed fusion genes with progressive deletions of the *Mst87F* leader sequences linked to the β galactosidase gene (*lacZ*) of *E.coli* (Figure 1). Translation of the fusion protein was monitored by histochemical staining for β galactosidase activity. Each of these constructs contains 670 bp of 5′ upstream sequences to ensure a high level of transcription. Since the translation start (at position +189 corresponding to nucleotide 19 in the second exon) was deleted in all cases, translation of these fusion genes was directed by a synthetic start codon

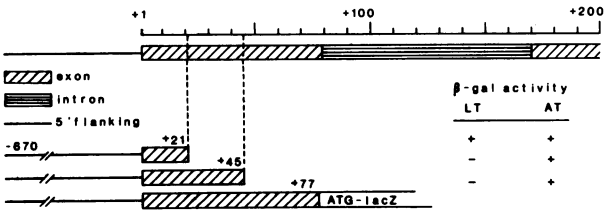

Fig. 1. *Mst87F1acZ* fusion genes. For comparison the region of the *Mst87F* gene relevant for the experiments is shown (top) with a scale given in nucleotides. The *Mst87F* parts of the various fusion genes are shown in the diagram (bottom). Only in the construct with +77 as the endpoint is the fusion to the ATG-*lacZ* sequences indicated. The small table in the right corner summarizes the histochemical data on the β galactosidase activity in the larval (LT) or adult testes (AT).

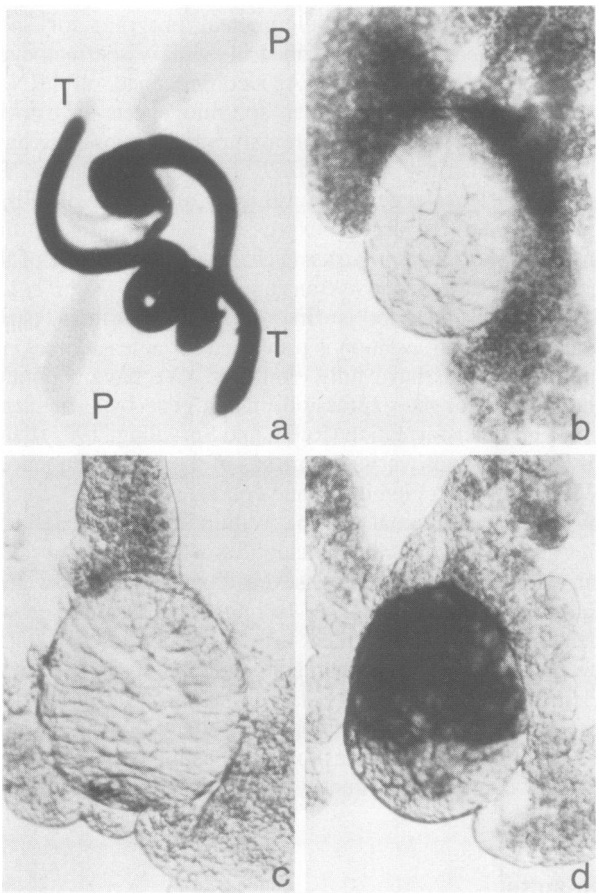

Fig. 2. Histochemical detection of the fusion proteins. Adult male reproductive organs (**a**) or larval testes anlagen (**b–d**) were dissected from animals transformed with the construct −670/+21 (a and d), −670/+77 (b) and −670/+45 (c) and assayed for β galactosidase activity. Abbreviations: testes (T), paragonia (P).

introduced in front of the *lacZ* gene (Kuhn *et al.*, 1988b). After transformation with these constructs, homozygous strains were tested for β galactosidase activity. Analysis of adult flies showed that staining remained restricted to the testis tubes irrespective of the fusion gene used for transformation (Figure 2a). Staining of larval testes anlagen, however, depended upon the nature of the fusion gene. If correct expression of the fusion gene occurs, the testes anlagen should be free of staining, since they only contain premeiotic stages of spermatogenesis. This was indeed the case if the intron and the beginning of the second exon were

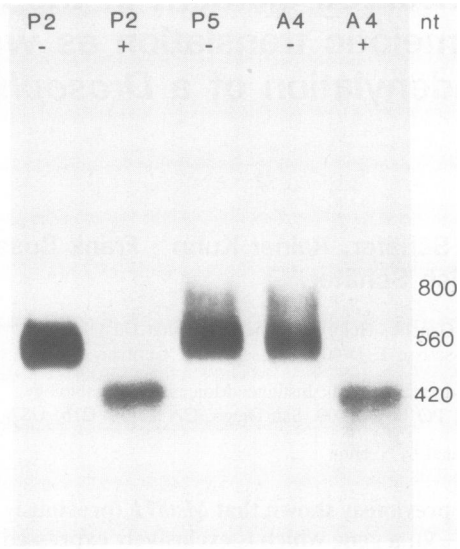

Fig. 3. Polyadenylation of the *Mst87F* mRNA. Total RNA from male Oregon-R pupae was isolated two (P2) or five (P5) days after pupation and from adult male Oregon-R flies four days (A4) after eclosion. Identical samples of P2 and A4 RNA were incubated with (+) or without (−) oligo (dT) and RNase H to show the extent of polyadenylation. Hybridization was done using an *in vitro* transcript containing only trailer sequences of the *Mst87F* gene. Sizes are given in nucleotides (nt). The sample with early pupal RNA (P2) was purposely overloaded to show the homogeneous size of the transcripts compared with late pupal RNA (P5).

deleted such that nearly all of the first exon was still contained in the fusion gene (Figure 2b, −670/+77). Therefore, neither sequences surrounding the translation start codon nor the intron play an important role in translational regulation. Deletion of half the first exon (−670/+45) did not affect translational regulation either (Figure 2c). However, when sequences between +45 and +21 were deleted in addition (−670/+21, Figure 1), the fusion gene was prematurely translated, leading to staining in half of the testes anlagen (Figure 2d). In the cytological preparations it could be observed that translation of the fusion genes was always restricted to that half of the testes anlagen which contains the further developed stages of spermatogenesis, i.e. primary spermatocytes. The stem cells and spermatogonia did not show translational activity of the fusion genes.

Translation is accompanied by poly(A) addition

In our earlier analysis of transcription it was already apparent that the size of the *Mst87F* transcripts was altered in adult stages compared with that in earlier stages of development (Kuhn *et al.*, 1988a). To determine whether we could correlate these structural differences with translational regulation, we analyzed the RNA from pupae and adults of various ages. While the *Mst87F* mRNA has a uniform length of 560 nucleotides (nt) in RNA populations from young pupae (P2, Figure 3), its size becomes larger and heterogeneous—up to 800 nt length—in older pupae (P5) and adults (A4). Incubation of these different RNA samples with oligo(dT) and RNase H selectively removes the poly(A) tail from these mRNAs and thereby eliminates the size differences observed in the native RNAs (P2 versus A4). Spermatogenesis in *Drosophila melanogaster* is correlated with development in such a way that the first germ cells undergo meiosis shortly after pupation and almost the entire

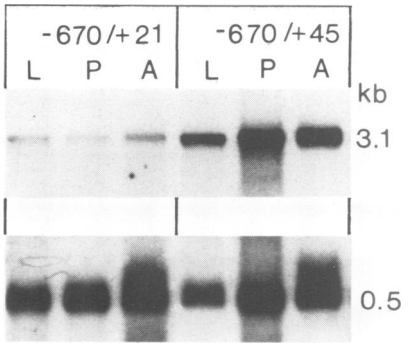

Fig. 4. Polyadenylation of the fusion transcripts. Northern transfers of total RNA from larval (L; 30 μg), early pupal (P; 20 μg), and adult (A; 5 μg) males harboring the fusion gene indicated above the lanes were hybridized with *lacZ* (*in vitro* transcript of the 600 nt *Hpa*I fragment) and *Mst87F* sequences to show the length heterogeneity both in the fusion transcripts (3.1 kb) and in the *Mst87F* mRNA (0.5 kb). The length heterogeneity of the fusion transcripts seems less pronounced due to the much larger size of these RNAs. Sizes are given in kb.

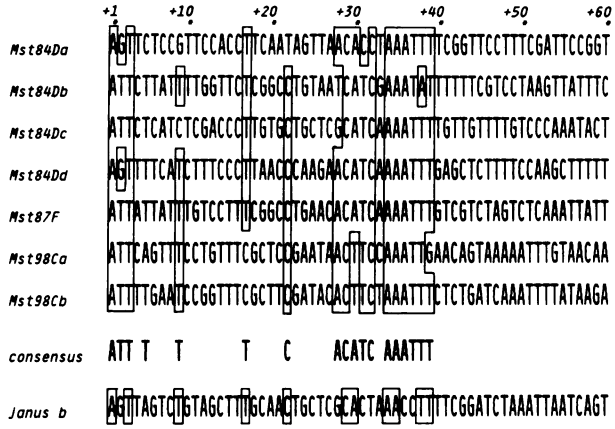

Fig. 5. Conserved sequence element within the leader. The first sixty nucleotides of the leader sequences from all seven cloned members of the gene family are compared. All nucleotides which are conserved in at least five of seven genes are boxed and are shown as consensus sequence below. For comparison the corresponding sequence of the 5′ untranslated region of the *janus B* gene (Yanicostas *et al.*, 1989) is shown (bottom); nucleotides which are identical to the consensus sequence are also boxed.

process of spermiogenesis takes place during pupal stage. This means that the time point at which we observe elongation of the poly(A) tail correlates with the time point at which we have observed translation of the correctly regulated fusion genes.

If there is a functional correlation between translational control and additional poly(A) lengthening, then the fusion transcripts might be polyadenylated differently depending on their regulation. Indeed, the 3.1 kb fusion transcripts of −670/+45 show a clear heterogeneity in length in the adult RNA but not in larval and early pupal RNA, although the heterogeneity is not as pronounced as that of the 560 nt *Mst87F* mRNA due to the much larger size, while the corresponding transcripts from −670/+21 remain homogeneous in length even in the adult RNA (Figure 4). This indicates that the secondary poly(A) addition is only taking place on those fusion transcripts which are translationally controlled. When regulation is prevented, resulting in premeiotic translation, the length of the mRNA is not altered. The signal which directs the additional polyadenylation has to lie within the sequences present in these fusion constructs; more specifically it seems to be mediated by the same leader segment as the translational control.

Figure 4 also shows that the transcript accumulates from third instar larvae to adult as in the wildtype situation, irrespective of the fusion gene analyzed. In primer extension experiments (Davies *et al.*, 1986a), these fusion transcripts initiate at the correct transcription start point (data not shown). Taken together, these data rule out the possibility that the premeiotic β galactosidase activity seen in Figure 2d is caused indirectly by false regulation of transcription and not by direct interference with the translational process.

A 12 nucleotide sequence element in the leader confers translational control

In the course of our experiments we found that *Mst87F* is a member of a small gene family. We have cloned six additional genes and have shown that, like *Mst87F*, they are expressed exclusively in the male germ line and are all translationally regulated. They have extended sequence similarities within the translated portions and code for proteins that contain a high proportion of the repetitive cysteine−glycine−proline motif characteristic of the *Mst87F* protein. The leader and trailer sequences, however, are totally divergent (manuscripts in preparation). We compared the first 60 nt from the leader sequences of *Mst87F* with those of the other members of the gene family and found conserved nucleotides at identical positions among all seven genes. The most striking sequence conservation is a block of twelve nucleotides, of which only the sixth is variable (Figure 5). This sequence element is located at positions +28 to +39 in the leader of all seven genes. This is exactly within the region which when deleted leads to premature translation of the *Mst87F* mRNA. We therefore postulate that translational regulation of all members of this gene family in the male germ line is mediated by this sequence element which we have named TCE for translational control element.

To test this idea we first constructed a fusion gene, which lacks eleven nucleotides of the TCE but contains all other sequences from −600 to +51 (Figure 6), a gene fragment which is normally sufficient for correct regulation (Figures 1 and 2). This fusion gene is translated prematurely, leading to translational activity in the larval testes (Figure 7a). In order to identify those areas within the element which are important for regulatory function, we performed *in vitro* mutagenesis experiments. The gene fragments containing the altered sequences were fused to the *lacZ* gene and transformed into flies. In each case two nucleotides were altered simultaneously. In one case, nucleotides 5 and 7 of the TCE were altered (mut2) and in the other case nucleotides 9 and 10 were altered (mut4; Figure 6). As can be seen in Figure 7, either alteration leads to premature translation of the fusion gene. There is a clear difference in the effect of both constructions, however. While mutation of nucleotides 5 and 7 leads to premature translation in about half the testes anlagen (Figure 7c), a phenotype which is indistinguishable from the deletion of the complete element, mutation of nucleotides 9 and 10 only leads to very late premeiotic translation resulting in a very small area of translationally active cells in larval testes (Figure 7d). Since this was observed with at least three independent integra-

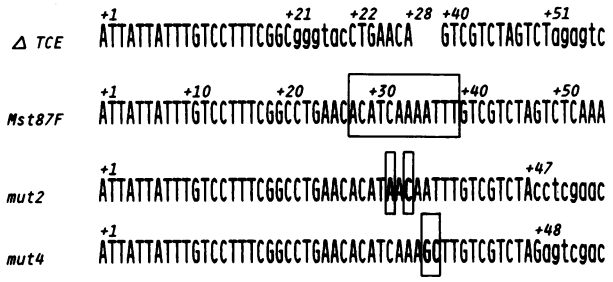

Fig. 6. *In vitro* mutagenesis. The nucleotide sequences of the transcribed *Mst87F* portion in the various fusions are shown. *Mst87F* nucleotides are shown as capital letters and their terminal nucleotide is numbered corresponding to the gene sequence. Sequences from the construction are typed in lower case letters. As a reference the wildtype leader sequence is shown between the two types of constructs. Since small deletions have occurred during the ligations, the endpoints in the two mutations are different. The mutagenized nucleotides in mut2 and mut4 and the TCE in the wildtype leader are boxed.

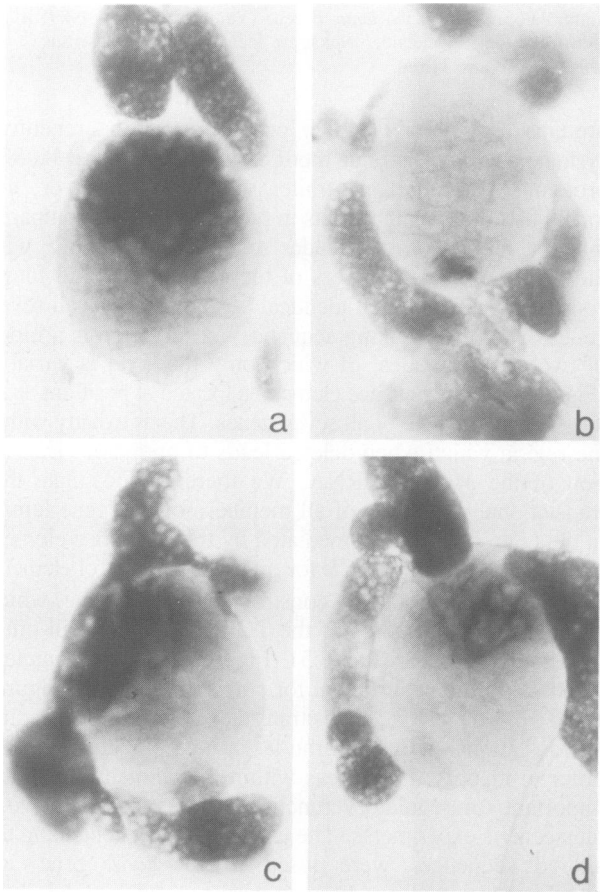

Fig. 7. Premeiotic translation of the 'mutagenized' fusion genes. Testes anlagen of transformed third instar larvae were assayed for β galactosidase activity. The integrated fusion genes contained no TCE (**a**), mut2 TCE (**c**) or mut4 TCE (**d**). As a control, testes anlagen from third instar larvae of w^{1118} were also incubated (**b**). All organs were incubated for 5 h to show the differences in staining more clearly.

tion lines for each construct, this difference cannot be due to influences from the surrounding genome, but must be due to the mutations present in the fusion gene.

The analysis of the fusion transcripts from the constructs with modified leader sequences also shows a striking

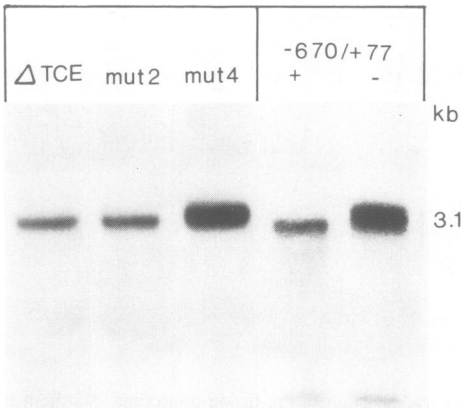

Fig. 8. Polyadenylation of fusion genes with altered leader sequences. Total RNA was isolated from adult males carrying the fusion genes indicated above the lanes and probed with a *lacZ in vitro* transcript. Two identical samples from −670/+77 RNA were incubated with (+) and without (−) RNase H and oligo (dT). The identical separation in the various RNA samples is apparent from the weak background hybridization signals. From all these experiments several exposures were compared to ensure that the homogeneous and heterogeneous hybridization signals are due to the nature of the fusion transcripts and not due to differences in abundance. Similar results were obtained for the other fusion transcripts.

difference between the two mutations in the TCE. The fusion transcripts of Δ TCE and mut2 have a very homogeneous length in adult males (Figure 8), as expected since these transcripts are no longer translationally controlled and the elongation of the existing poly(A) tail is characteristic of a translationally controlled mRNA. The fusion transcripts from the construct mut4, on the other hand, show a heterogeneous length resulting in a significant broadening of the hybridization signal towards higher molecular weights as already documented for −670/+45 and −670/+77 (Figures 4 and 8). The extent of size variation is indistinguishable between the different fusion constructs (compare mut4 and −670/+77 in Figure 8). This length heterogeneity is again based on a secondary elongation of the poly(A) tail as shown by the size reduction after oligo(dT) and RNase H treatment (Figure 8). Furthermore, the hybridization signal of the fusion transcripts devoid of a poly(A) tail has a similar width to those obtained from Δ TCE and mut2. This RNase H experiment therefore demonstrates that it is indeed possible to document length variations in the size range of 3.1 kb. The observed size differences between mut2 and mut4 fusion transcripts support the conclusion drawn earlier, that modification of nucleotides 9 and 10 results in a much weaker effect on the translational control mechanism than modification of nucleotides 5 and 7.

Discussion

This study deals with the identification of a small element in the leader of the *Mst87F* mRNA which confers translational control on this mRNA in *Drosophila* spermatogenesis. Moreover, the element mediates the secondary elongation of the poly(A) tail that is concomitant with translation of stored mRNAs. In all cases where a fusion transcript is translationally controlled, the poly(A) tail becomes elongated at the time when translation is permitted. When translational regulation is prevented, this secondary polyadenylation is

no longer observed. Both of these regulatory processes are abolished if the complete TCE is deleted or if nucleotides 5 and 7 of the TCE are mutated.

Alteration of the poly(A) tail length has been observed in a number of developmental systems where translational control occurs, yet its functional importance for translational control is unclear (for review see Jackson and Standart, 1990; Munroe and Jacobson, 1990). In many cases the poly(A) tail increases drastically in length at maturation or fertilization of oocytes when translation of stored mRNAs begins (Rosenthal *et al.*, 1983; Vassalli *et al.*, 1989; McGrew *et al.*, 1989). In contrast, a number of translationally controlled mRNAs in mouse spermatogenesis show a drastic reduction in poly(A) tail length upon recruitment for translation (Kleene *et al.*, 1984, Kleene, 1989). The sequences necessary for this change in poly(A) tail length can reside either in the leader sequences as for the 35 nt long leader of the ribosomal protein S19 in *Xenopus* (Mariottini and Amaldi, 1990) or in the trailer sequences, as is the case for three different mRNAs in *Xenopus* that share a U-rich sequence 5′ to the polyadenylation signal (McGrew *et al.*, 1989).

The mRNA of *Mst87F* initially has a poly(A) tail of 140 nt which is twice as long as the average in *Drosophila* (Izquierdo and Bishop, 1979). Upon translational recruitment it is extended even further to up to 380 nt (which is almost as long as the mRNA itself with 417 nt). The element responsible for this cytoplasmic polyadenylation is indistinguishable from the TCE located in the leader of the mRNA. It is not dependent on sequences in the vicinity of the polyadenylation signal, since cytoplasmic polyadenylation is observed on all fusion transcripts containing a functional TCE and the trailer sequences of the *Drosophila* heat shock protein gene 70 (for construction of the vector, see Kuhn *et al.*, 1988b), which shares no sequence similarity with the *Mst87F* trailer other than the polyadenylation signal (AATAAA; Proudfoot and Brownlee, 1976) itself.

Translational regulation should be common to many genes whose products are essential for spermiogenesis in *Drosophila*. We propose that an element similar or identical to the TCE is present in other translationally controlled RNAs in *Drosophila* spermiogenesis. This element might exist on all these transcripts or only on a subset which is translated shortly before the end of spermiogenesis like *Mst87F*. The gene for the testis-specific β2 tubulin is both transcribed and translated in primary spermatocytes (Michiels *et al.*, 1989) and is not translationally regulated. No significant similarity to the conserved sequence element can be found in the sequence of its leader (Michiels *et al.*, 1987). Similarly, the α2 tubulin mRNA, which is expressed in the chordotonal organs and in the testes (Bo and Wensink, 1989), is not translationally regulated and hence does not share sequence similarity to the TCE (Theurkauf *et al.*, 1986).

On the other hand, it has been reported that sequence similarities to the *Mst87F* gene exist within the leader sequences of the translationally controlled *janus B* gene, which is also exclusively transcribed in *Drosophila* spermatocytes (Yanicostas *et al.*, 1989). Indeed, we find six nucleotides which match the TCE consensus sequence and which are located at an identical position in the *janus B* leader (Figure 5). The fact that the nucleotides at positions +9, +17 and +22 are also conserved in *janus B* may indicate that sequences outside the TCE are also of relevance for the observed translational regulation.

Although transcription in mammalian spermatogenesis continues after the meiotic division (Geremia *et al.*, 1978), this does not imply that translational control has no impact on the process of sperm differentiation. It is well established that the protamine mRNAs are transcribed in round (early) spermatids but translated only in elongated (late) spermatids during mouse spermiogenesis (Kleene *et al.*, 1984). The region involved in this translational control has recently been identified for the mouse protamine gene *mP1* (Braun *et al.*, 1989). In contrast to *Mst87F* regulation, sequences upstream of the translational start site are of no importance, rather sequences downstream of the stop codon are necessary and sufficient for translational control. The 156 nucleotides of the 3′ untranslated region of the gene contain a conserved sequence element shared among mouse, human and bovine *mP1* genes which might be responsible for this kind of regulation. It is remarkable in this respect that the leader of *janus B* totally overlaps with the 3′ untranslated region of the *janus A* gene, a gene which is transcribed both in oocytes as well as in spermatocytes (Yanicostas *et al.*, 1989). It may indicate that translational control in *Drosophila* spermatogenesis can in some cases be mediated by 3′ untranslated sequences.

The best studied cases of translational control are the ferritin genes of rat and man. Here, the first 150 nt of the leader contain a conserved sequence element which forms a loop structure and which confers translational control on the ferritin genes in response to the cellular level of iron (Aziz and Munro, 1987; Hentze *et al.*, 1987). It has been demonstrated that these sequences represent a binding site for a protein present in the cytoplasm (Leibold and Munro, 1988) and this so-called iron-responsive element binding protein has already been isolated and characterized (Rouault *et al.*, 1989; for a review see Klausner and Harford, 1989). In analogy, we postulate that the TCE (perhaps in cooperation with further upstream nucleotides) also represents a binding site for a translational repressor. This translational repressor could be synthesized in primary spermatocytes and could then bind to all those mRNAs containing the TCE or similar elements. The fact that the TCE was found at identical position in all genes seems to suggest that the action of the postulated repressor may somehow be position dependent. After synthesis of this repressor ceases, translation would be permitted by lowering the concentration of the repressor and gradual dissociation of the bound protein from these binding sites. Deletion of the TCE would prevent the binding of this repressor and consequently, translation would start as soon as transcription occurred. Modification of nucleotides 5 and 7 might destroy the binding site for the repressor, whereas modification of nucleotides 9 and 10 might only weaken the binding such that a low level of translation could occur premeiotically. It is tempting to postulate that the same repressor molecule mediates both translational control and secondary polyadenylation, since these phenomena are tightly linked. When translational control is still operating to a certain extent—as in the case of mut4—then changes in poly(A) tail length can be observed. Our working hypothesis is that a bound repressor molecule will, upon release of the control, move along the mRNA molecule and elicit a secondary addition of A residues. This could explain why an RNA of different size with unrelated sequences (3 kb *lacZ*) and a different 3′ end (trailer from the *Drosophila hsp70* gene) behaves like the genuine *Mst87F* mRNA. Alternatively, the bound repressor could sequester the mRNA thereby allowing

translation and secondary polyadenylation only late in spermiogenesis.

The model implies that transcription of the genes subjected to translational control can only be permitted during the time interval in which the repressor is present. In other words, during the extensive growth phase of the primary spermatocytes there has to be a time-span in which transcription of the translational repressor gene is allowed but that of the translationally controlled genes is prevented. For this negative transcriptional regulation one would expect to find sequence elements in the upstream sequences of the gene. Since we have already shown that 102 bp of upstream sequence are sufficient to mediate wildtype *Mst87F* expression (Kuhn *et al.*, 1988a), such elements would have to be located very close to the transcription start site. Our current experiments are aimed at identifying such elements within this small 5′ region of the gene as well as the *trans*-acting factors involved in transcriptional and translational regulation of *Mst87F* expression.

Materials and methods

RNA isolation

RNAs from the different development stages were isolated according to the method of Chirgwin *et al.* (1979) or following the protocol described by Chomczynski and Sacchi (1987).

RNase H experiments

(Sippel *et al.*, 1974). 30 to 40 μg of total RNA were incubated with 8 μg oligo (dT) (15mer, Boehringer-Mannheim) and 6 U RNase H (Boehringer-Mannheim) in a total volume of 40 μl buffer containing 20 mM Tris−HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT for 60 min at 37°C (Restifo and Guild, 1986). The reaction mixture was then cooled on ice, phenol extracted and analyzed on a 1.5% formaldehyde−agarose gel.

Northern analysis

If not indicated otherwise, 35 μg of total RNA were used in each sample. RNAs were separated in formaldehyde−agarose gels as described in Davies *et al.* (1986b) including ethidium bromide in the samples (Fourney *et al.*, 1988). The transfer was done to Hybond nylon membrane (Amersham) using standard protocols. The membrane was prehybridized in 500 mM Na phosphate pH 6.5, 7% SDS and hybridized in 50% formamide, 5 × SSPE and 50 mM Na phosphate pH 6.5 at 65°C using an *in vitro* transcript.

Gene fusions

Clone p444, containing a 913 bp *Ava*II fragment of *Mst87F* with 712 bp 5′ flanking sequences and 201 bp transcribed sequences in pUC9 (Vieira and Messing, 1982) was used to generate deletions with Bal31 after linearization with *Eco*RI. The resulting fragments were digested with *Eco*RV, generating a constant endpoint at −670 and cloned into the *Sma*I site of pTZ18U (Mead *et al.*, 1986). The extent of deletion was determined by sequencing. The *Mst87F* fragment with endpoint at +45 was cut out of the clone with *Kpn*I and *Xba*I and ligated into the P-element vector pW-ATG-lac1 (Kuhn *et al.*, 1988b). Clones with endpoints at +77 and +21 were linearized with *Eco*RI, made blunt ended with the Klenow fragment of DNA polymerase I, *Xba*I linkers were added and the fragments inserted into *Xba*I digested pW-ATG-lac1.

To generate a fusion gene lacking only the TCE, the gene fusion with endpoint +21 was linearized with *Sst*II and Bal31-treated to destroy the *Kpn*I and *Xba*I sites in the multicloning site. After the ends were trimmed with Klenow enzyme, the clone was ligated. This fusion gene now contains about 600 nt of upstream sequences from *Mst87F*. A double-stranded oligonucleotide containing nucleotides +22 to +28 and +40 to +49 flanked by a *Kpn*I (5′) and an *Xba*I (3′) site was ligated into the *Kpn*I and *Xba*I site located between the *Mst87F* fragment and the *lacZ* gene.

In vitro mutagenesis

An *Mst87F* fragment spanning from −102 to +201 was cloned into phage m13mp9amE. 22 nt oligonucleotides containing the mutated consensus sequence were annealed with the single stranded insert and extended by the use of the Klenow fragment. *In vitro* mutagenized double-stranded molecules were selected by the method of Kramer *et al.* (1984).

P-mediated germ line transformations

(Rubin and Spradling, 1982). Injections were done into a white strain from *Drosophila melanogaster* (*w*−*2*; Sweden and later w^{1118}; Geneva). As a helper plasmid either phsπ or pUChsπΔ2−3 was used. Transformation details were as described in Kuhn *et al.* (1988a). Southern hybridizations showed that all transformant lines contained a single P-element insertion of the correct construction (data not shown).

X-gal staining

Organs from third instar larvae or adult flies were dissected in Ringer's solution and then tested for β galactosidase expression as described in Glaser *et al.* (1986). Staining was for 5 h at room temperature and was always monitored after an overnight incubation. Organs were transferred to glycerol (Figure 2) or phosphate buffer (Figure 7) and photographed using a Zeiss photomicroscope.

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