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Cis-acting regions sufficient for spermatocyte-specific transcriptional and spermatid-specific translational control of the *Drosophila melanogaster* gene *mst(3)gl-9*

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In *Drosophila* spermatogenesis transcription occurs only premeiotically while translation can be detected also in postmeiotic spermatids. To analyse the underlying processes *mst(3)gl-9*, a gene specifically expressed in the male germ cells of *Drosophila melanogaster*, was studied. The putative protein encoded by *mst(3)gl-9* is mostly composed of repetitive Cys-Gly-Pro motifs. The transcriptional and translational control of expression of *mst(3)gl-9* has been investigated by P-mediated transformation. Only 102 bp of 5′ upstream sequences and the first 201 bp of the gene are sufficient to maintain the gene specific characteristics of expression, namely premeiotic transcription and postmeiotic translation separated by 3 days of development.

Key words: Drosophila/P-element transformation/spermatogenesis/tissue specific transcription/translational control

Introduction

The complex process of spermatogenesis in *Drosophila melanogaster* is well characterized both at the cytological and genetic level (for review, see Lindsley and Tokuyasu, 1980). In short, an adult testis contains five to eight apical stem cells each of which divides in such a way that one daughter cell continues to function as a stem cell while the other becomes a primary gonial cell. The latter is surrounded by a pair of somatic cells, the so-called cyst cells, and undergoes four rounds of mitoses resulting in 16 primary spermatocytes per cyst. These spermatocytes increase their volume ~25-fold and during that phase genes are maximally expressed; typical for that stage is the unfolding of the Y chromosomal loop structures, the cytologically visible activity of the Y chromosomal fertility genes (Hess and Meyer, 1968). Before the cells enter the two meiotic divisions all detectable transcription, but not translation, ceases (Olivieri and Olivieri, 1965; Gould-Somero and Holland, 1974). Consequently, spermatids will mature normally even if they do not contain chromosomes (Lindsley and Grell, 1969). Thus, the complex postmeiotic morphogenetic events by which round and syncytial spermatids change to extremely long and individual motile sperm have to rely on stored transcripts; in other words, the complete program for spermiogenesis of the 64 spermatids in each cyst is entirely laid down in the 16 progenitor spermatocytes.

In order to gain more insight into the processes governing these key events for male fertility in *Drosophila* we have isolated a clone, named *mst(3)gl-9*, which encodes a transcript exclusively accumulated in the male germ line (Schäfer, 1986a,b). The present study is aimed at the structural analysis of this gene and, more importantly, at the identification of the sequences which guarantee that this gene is transcribed and translated at the correct stages of spermatogenesis.

Such an analysis is feasible in *Drosophila* due to the development of the system of P-mediated germ line transformation (Rubin and Spradling, 1982; Spradling and Rubin, 1982). In pursuing these experiments we made use of two different vectors. In Carnegie 20 (Rubin and Spradling, 1983) we used flanking sequences together with the entire gene whose transcript was made distinguishable from the endogenous gene by insertion of a small *lacZ* fragment. On the other hand, we marked the gene by fusion in phase to the *Escherichia coli* β-galactosidase structural gene employing a P-element vector specifically designed for this purpose (Molsberger *et al.*, 1988). The correct expression can then easily be visualized by histochemical staining of the fly's tissues for β-galactosidase activity, a method pioneered for *Drosophila* by Lis *et al.* (1983).

The results presented here show that 0.1 kb of 5′ upstream sequences together with the first 0.2 kb of the transcribed region of the gene are sufficient to guarantee that transcription of *mst(3)gl-9* is strictly male germ line dependent. In addition, we present evidence that the untranslated leader sequences of the mRNA contain all the signals for stabilizing the RNA and preventing premeiotic translation.

Results

Gene structure of mst(3)gl-9

To determine the gene structure of *mst(3)gl-9* the genomic 2.52 kb *Eco*RI fragment was sequenced and matched with

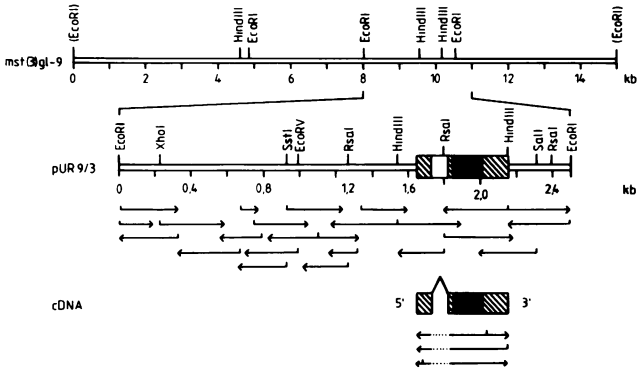

Fig. 1. Restriction map of *mst(3)gl-9*. A detailed map of the 2.5-kb *Eco*RI fragment shows the position and organization of the gene as well as the sequencing strategy. The gene is indicated by a box in which transcribed regions are striped, the translatable region is black and the intron is left blank. Arrows demonstrate direction and extent of the sequenced fragments. The same information is given for the cDNA clone.

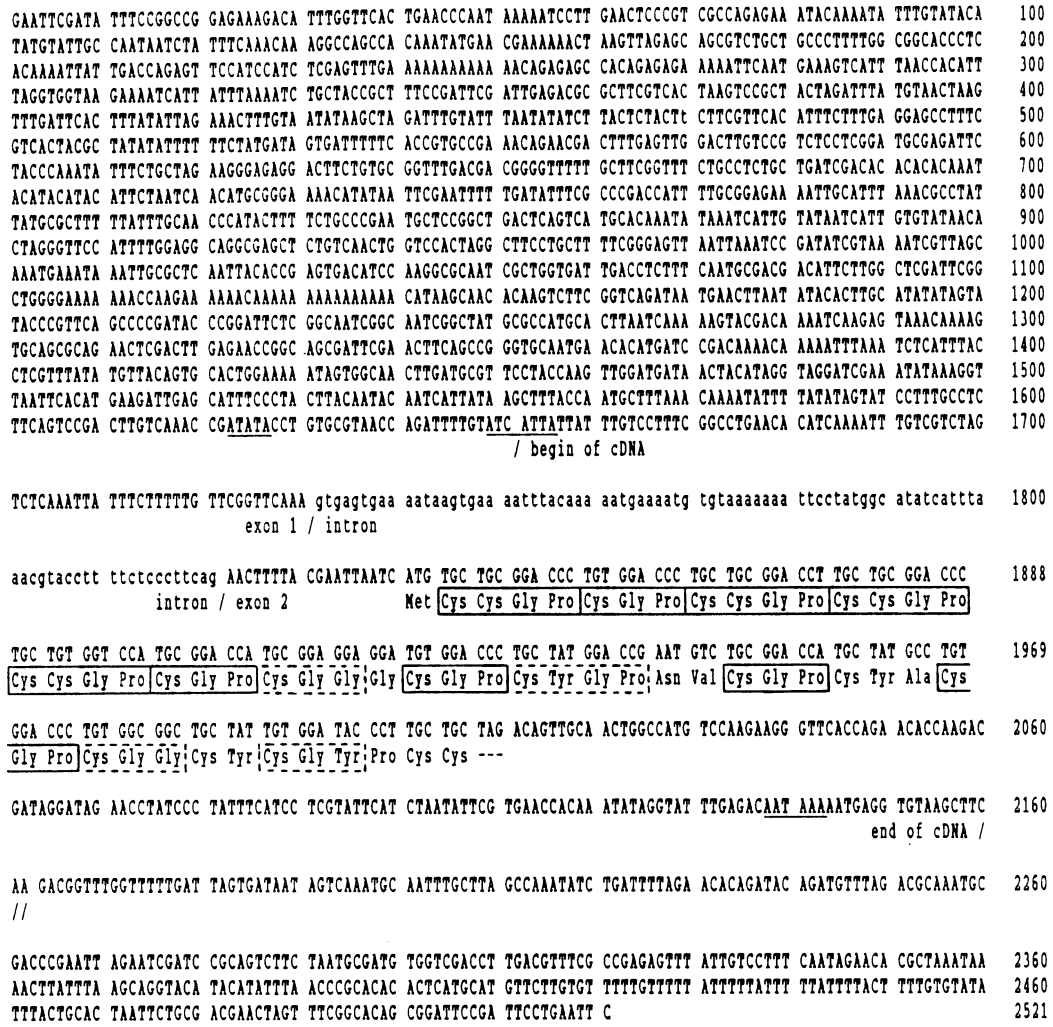

Fig. 2. Complete nucleotide sequence of the 2.5-kb *Eco*RI fragment with the deduced amino acid sequence starting from the first AUG in the cDNA sequence. Beginning and end of the cDNA sequence, as well as the exon/intron boundaries as defined by the sequence comparison, are indicated below the sequence. TATA-box, the transcription start site and a possible polyadenylation signal sequence are underlined. The repetitive Cys−Gly−Pro motifs are boxed with solid lines, variations of the motif are indicated by dotted lines.

the sequence of two different cDNA clones. The sequencing strategy is outlined in Figure 1, and the resulting sequence is shown in Figure 2. From the comparison it can be concluded that the gene is composed of two exons of 79 and 338 bp, respectively, separated by an intron of 91 bp. The cDNA sequence starts at a position where the genomic clone contains 6 out of 7 nt (ATCATTA) of a conserved sequence found at transcription start sites in insects (ATCA(G;T)T(C;T); Hultmark *et al.*, 1986). In addition, 5 out of 7 nt thought to be the consensus sequence for a TATA box (TATA(A;T)A(A;T); Breathnach and Cambon, 1981) can be seen at the appropriate distance of 30 bp from the cDNA start (ATATA). It should be pointed out that the TATA box is not embedded in a GC-rich region, as is normally the case. The cDNA positions the 3′ end of the gene at nucleotide 2160−2162, 22−24 nt behind the polyadenylation signal (AATAAA; Proudfoot and Brownlee, 1976). The resulting transcript has a calculated size of 417−419 nt, excluding the poly(A) tail. Within this transcript a small 165-nt long open reading frame is found, following the first ATG of the cDNA at nucleotide 1841 in the second exon. The deduced amino acid composition of the putative protein is highly unusual, since most of the protein is made of a short repeat of Cys−Gly−Pro or variations thereof. As a result, 38% of the amino acids are cysteine, 29% glycine and 20% proline.

Transcription of mst(3)gl-9

As has been previously shown (Schäfer, 1986a), the transcript of *mst(3)gl-9* is male germ line specific (Figure 3). No hybridization occurs to female RNA nor to RNA from sons or daughters of homozygous *tudor* mothers, which are devoid of germ cells (Boswell and Mahowald, 1985). To identify the developmental stage at which transcription of *mst(3)gl-9* starts, RNAs were prepared from the different developmental stages and probed with an *in vitro* transcript of the gene. As can be seen in Figure 3 the RNA is detected in third instar larvae whose testes contain only premeiotic male germ cells and becomes more prominent in pupae. Since primary spermatocytes have already developed in the testes of second instar larvae, this seems to indicate that either transcription starts later in the growth phase of spermatocytes or the transcript is accumulated from the beginning and the detection level is only sufficient to show it at that time. Fur-

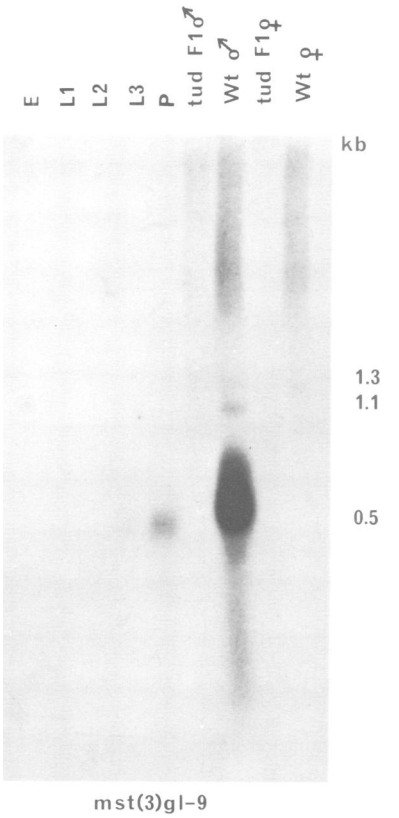

Fig. 3. Developmental Northern hybridized with *mst(3)gl-9*. 35 μg of total RNA from embryos (E), first (L1), second (L2) and third instar larvae (L3) as well as pupae (P) were separated on a 1.5% formaldehyde agarose gel. The same amounts were loaded from sons and daughters of homozygous *tudor* mothers (tud F1) as well as wild-type males and females (Wt). The lengths of the hybridizing transcripts are indicated.

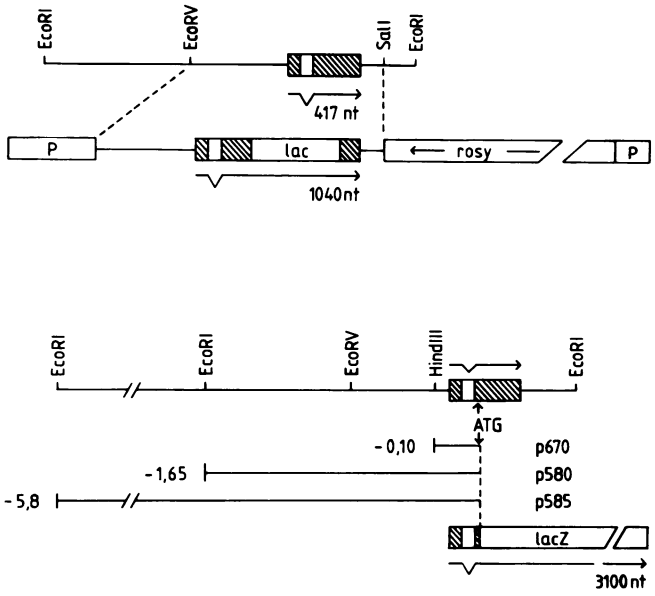

Fig. 4. Diagram of the various constructs used in the transformation experiments. p425 is based on Carnegie 20 and contains the whole gene enlarged by insertion of a 600-bp fragment of the *lacZ* gene (*lac*; top), *lacZ* fusions are constructed with pUCPlac (bottom). Transcribed regions of *mst(3)gl-9* are indicated by striped areas, the intron is left blank. The resulting transcripts from the various constructs are indicated by arrows and their calculated lengths are given in nucleotides (nt). For comparison the restriction map of *mst(3)gl-9* is given above the two types of constructs.

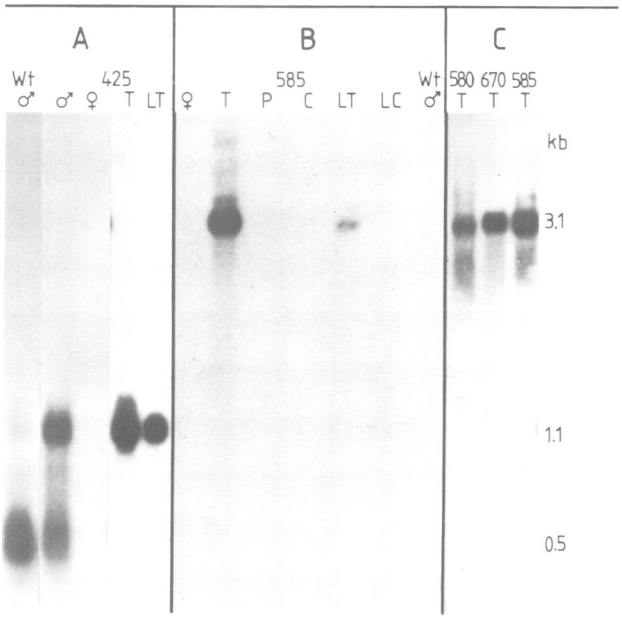

Fig. 5. Northern hybridizations to total RNAs from transformed lines. RNAs were isolated from flies carrying p425 (**A**), p585 (**B**) and p580, p670 or p585 (**C**). RNAs were isolated from males or females (symbols), from paragonia (P), testes—adult (T) or larval (LT)—as well as the carcasses (C and LC, respectively). As a control RNA from wild-type Oregon R males was also hybridized (Wt). RNA from 50 pairs of organs were used or 35 μg in the case of whole flies and carcasses. Hybridization was performed with the 600-bp *HpaI lacZ* fragment to show the transcripts generated from the constructs. The filter containing the first three samples in panel A was in addition hybridized with *mst(3)gl-9* sequences to show the endogenous transcript. The sizes of the transcripts are indicated.

ther increase of the signal supports the idea that the message is stable, i.e. is accumulated all along, and still stable in post-meiotic stages where transcription does not occur. The hybridizations with *mst(3)gl-9* to RNA always lead to a very broad signal at around 500-nt length. This is due to cross-hybridization with transcripts from related genes of similar but not identical size. The signals at 1.1- and 1.3-kb lengths are also the result of cross-hybridization with other messages (see Discussion). It should be noted that none of the RNAs of developmental stages were prepared from sexed material, partly accounting for the very faint signals compared to adult male RNA.

Transformations

To find out which sequences are necessary for correct stage and sex specific expression we performed P-mediated germ line transformations with constructs containing decreasing lengths of 5′ upstream sequences. To distinguish the transcript of the introduced gene from that of the endogenous gene, two different ways were chosen. In a construct using Carnegie 20 as a vector the gene was marked by insertion of a small *lacZ* fragment downstream of the translated portion of *mst(3)gl-9* (Figure 4). The construct contains 670 nt of the 5′ sequences, together with the modified gene and 132 nt of the 3′ sequences (p425). After chromosomal integration an RNA of 1040 nt plus poly(A) tail is transcribed from this plasmid.

In constructs using pUCPlac as vector we marked the gene by fusion in phase to the *E.coli* β-galactosidase structural gene. These plasmids contain 5.8-kb (p585), 1.6-kb (p580)

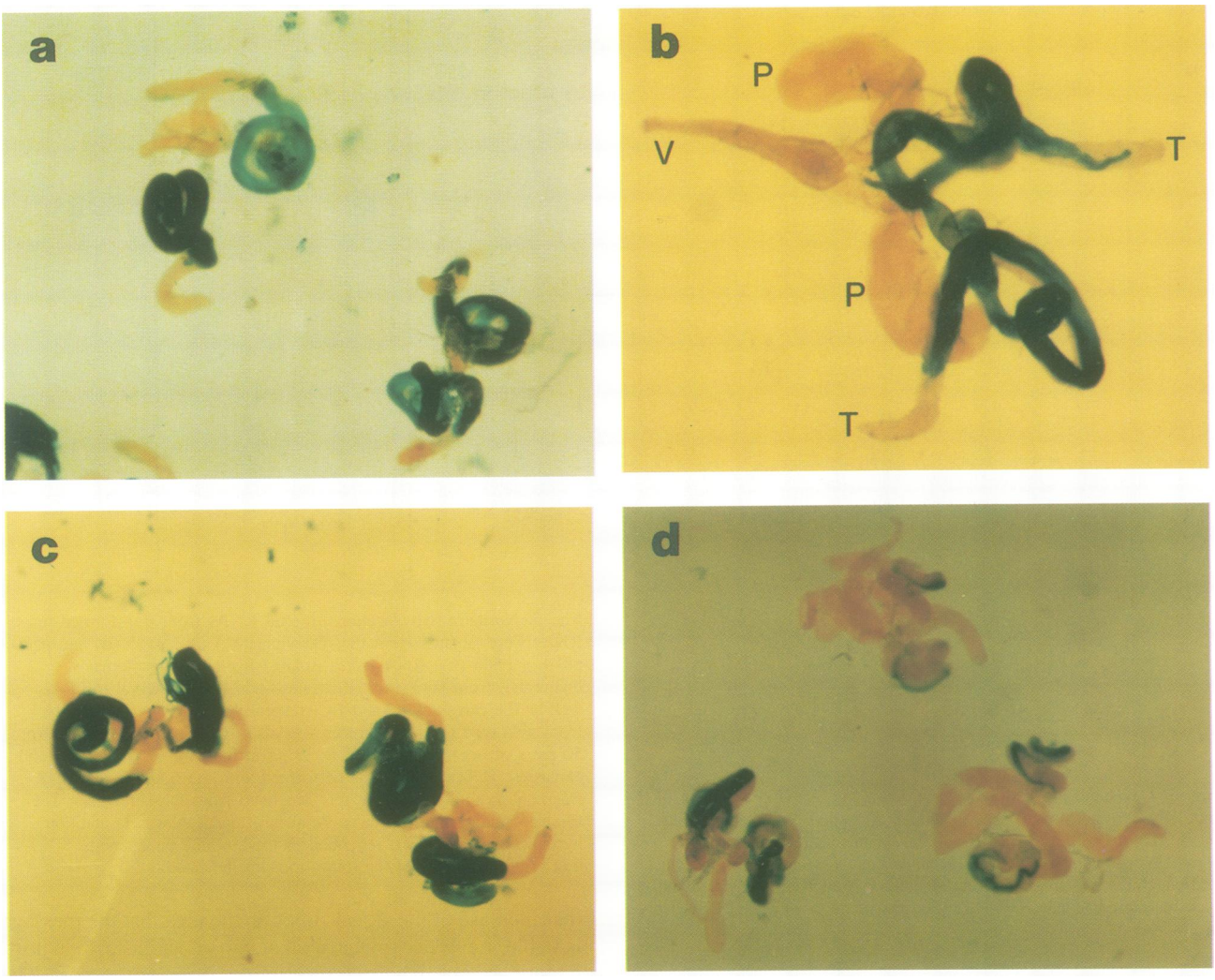

Fig. 6. Demonstration of β-galactosidase activity in the testes of transformed flies. The testes were dissected from males transformed with p585 (**a**), p580 (**b**), p670 (**c**) or from X/0 males carrying p585 (**d**). The parts of the male reproductive system are marked with T (testes), P (paragonium) and V (vas deferens).

and 0.1-kb (p670) 5′ upstream sequences together with 0.2 kb of the gene, i.e. exon 1, the intron and 31 bp of exon 2 including the first three amino acids of the putative protein. Transcription of the fusion gene results in an RNA of at least 3100 nt in length (Figure 4). After injection of appropriate embryos transformed individuals were selected and stocks homozygous for individual insertions were established.

Maintenance of transcriptional specificities

Figure 5 documents the hybridization patterns obtained with an *in vitro* RNA transcript of the *HpaI lacZ* fragment to RNAs isolated from transformed flies. In Figure 5A hybridization to a transcript of 1.1-kb length occurs only in RNA from males transformed with p425, i.e. the sex specific transcription is maintained, moreover the same signal can be observed when RNA from larval or adult testes is used. Figure 5B shows hybridization to 3.1-kb long transcripts only in testis RNA—adult as well as larval—from males transformed with p585. This demonstrates that tissue and stage specific transcription is maintained as well. The same result was obtained for RNAs isolated from flies transformed with p580 or p670 (Figure 5C). The experiments prove, therefore, that only 102 bp in front of the gene are sufficient to maintain the gene specific transcription characteristics.

Detection of the fusion protein

Transformed flies were dissected and tested for β-galactosidase activity. Visible staining appeared after 5 min and was considered sufficient after 1−2 h, since longer periods did not alter the pattern. As shown in Figure 6, only the testes of all transformants were stained. Outside the testes only the described endogenous activities (Glaser *et al.*, 1986) could be detected. No difference in the staining pattern was ever observed comparing transformants carrying different constructs (Figure 6a,b,c).

It should be especially emphasized that the tip of the testis containing the early stages of sperm development (i.e. stem cells, spermatogonia and spermatocytes) always remained completely unstained. In addition, in favourable cases staining of spermatid bundles can be distinguished (see Figure 6b). This suggests that translation of the fusion gene starts later in sperm development.

The Y chromosome is thought to carry major regulatory

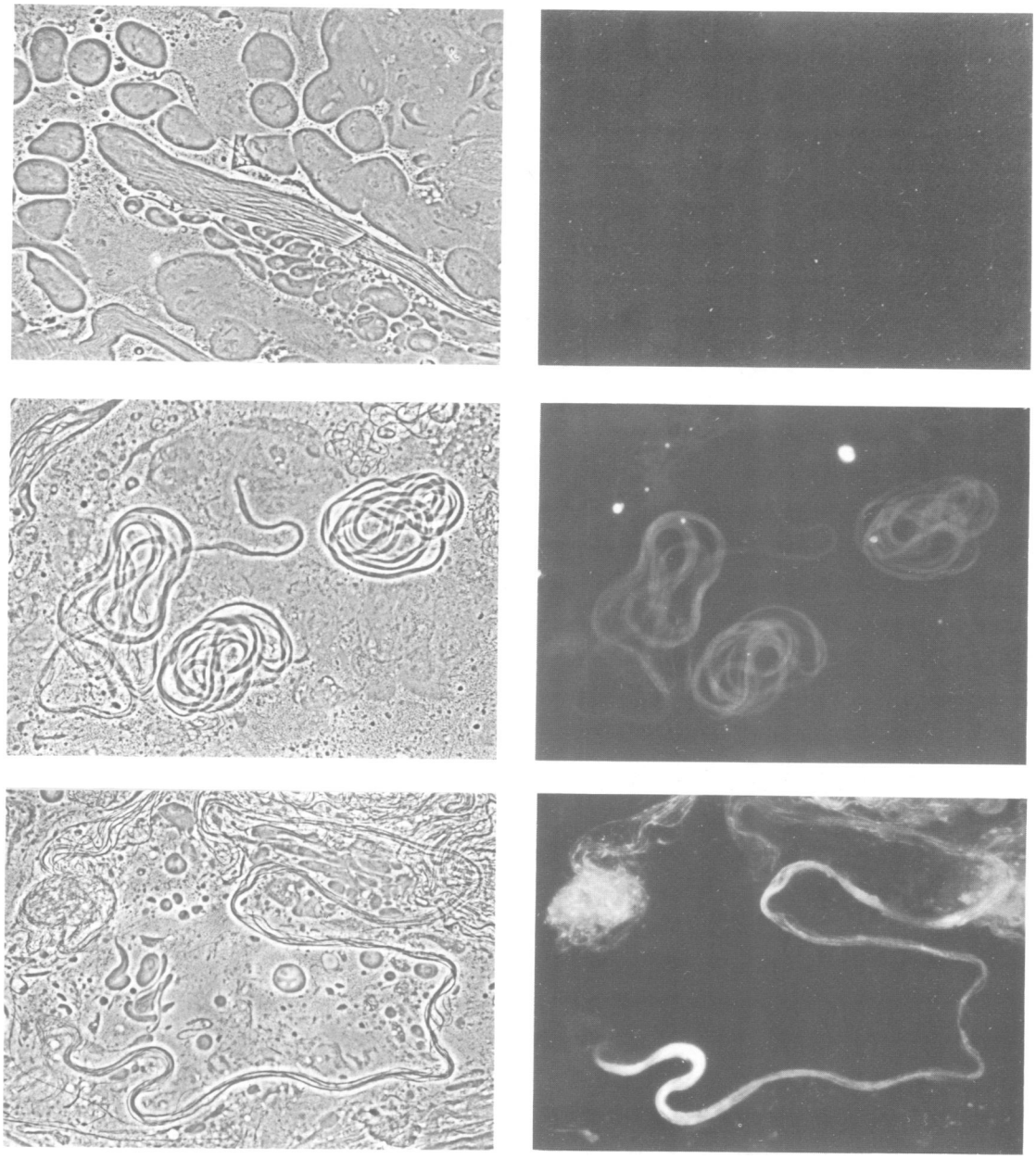

Fig. 7. Indirect immunofluorescence in squash preparations of testis material from flies transformed with p585. The various stages of sperm development were photographed under phase contrast (left panel) or epifluorescent illumination (right panel). Spermatocytes and elongating spermatids are completely devoid of fluorescence (top), while fully elongated spermatids and sperm show fluorescence (middle and bottom).

genes for spermatogenesis (Hess and Meyer, 1968). It had already been shown that the absence of this chromosome had no influence on the accumulation of the *mst(3)gl-9* RNA (Schäfer, 1986b). It was tested whether the same was true for the production of the fusion protein. Figure 6d documents that staining still occurred exclusively in the testes of X/0 males. Again, the staining of X/0 transformants was identical irrespective of the construct used. The intensity of the staining, however, was found to be much more variable than in the case of X/Y transformants. This can be attributed to the retarded development and the ongoing degeneration of later stages of sperm development in the testes of X/0 flies (Kiefer, 1966).

To further specify the stage of sperm development in which the fusion protein begins to be synthesized, indirect immunofluorescence with β-galactosidase antibodies was performed on squash preparations of testes (Figure 7). In accordance with the observed staining pattern, fluorescence occurred only at very late stages of sperm development after elongation of spermatids has been completed. The observed fluorescence was always extended over the entire length of the sperm.

These data clearly show that translational control must act on the fusion constructs. We have proved that transcription occurs premeiotically (Figures 3 and 5) as had to be expected, since it was shown that there is no nuclear RNA synthesis after the onset of meiosis in *D.melanogaster* (Olivieri and Olivieri, 1965; Gould-Somero and Holland, 1974). The synthesis of protein, on the other hand, starts only after meiosis separated by at least three days of storage of the RNA following the time scale of spermatogenesis (Lindsley and Tokuyasu, 1980). This must mean that nucleotides −102 to +201 still contain all the sequence information necessary for the observed transcriptional and translational control.

Discussion

This work deals with the identification of *cis*-acting regions sufficient for the correct transcription and translation during spermatogenesis in *D.melanogaster*. We show that 102 bp of 5′ upstream sequences plus 201 bp of the gene are sufficient for the selective transcription of the *mst(3)gl-9−lacZ* fusion gene in premeiotic male germ cells. The fact that a fusion protein is synthesized at all allows us to conclude that the first AUG in *mst(3)gl-9* is the actual translation start of the gene. Furthermore, there is no indication that the post-transcriptional control mechanisms acting on the fusion product would not reflect the *in vivo* situation for the *mst(3)gl-9* protein. Thus, the sequences sufficient for the correct regulation of *mst(3)gl-9* lie extremely close to and/or within the gene. A similar situation has been described only for two other genes in *Drosophila*. For the salivary glue protein gene *Sgs-3* 130 bp of 5′ sequences together with 1100 bp of the gene will guarantee tissue- and stage-specific accumulation of the *Sgs-3* RNA. RNA levels, however, will substantially increase if additional sequences further upstream are present (Raghavan *et al.*, 1986). The minimal sequence requirement for correct expression of the *ninaE* opsin gene is a segment from −120 to +67 bp and, again, sequences further upstream will enhance transcription (Mismer and Rubin, 1987). Our experiments do not indicate such requirements for *mst(3)gl-9* since RNA accumulation among the different constructs seems at least similar. Whether this reflects the *in vivo* situation or whether this is caused by features of the fusion construct is an open question for the time being, especially since only one integration site per construct was extensively studied. Studies on related genes make it likely that additional sequences are not necessary. We know that *mst(3)gl-9* is a member of a small gene family of which four additional members are clustered within 4.0 kb at one locus (unpublished data). Two of these genes are so close together that at least one member of the gene family has to rely on considerably less than 200 bp of upstream sequences for its transcriptional control (in preparation). Since most of the members of this gene family code for RNAs of sizes similar to that of *mst(3)gl-9* the observed hybridization to 0.5-kb RNAs is at least due to five different transcripts. Therefore, we cannot compare the abundance of the fusion transcript with that of the unaltered gene.

The histochemical staining gave evidence for and the immunofluorescence pattern gave proof of the postmeiotic translation of the fusion protein. Since it is well established that transcription ceases before meoisis during spermatogenesis in *Drosophila* (for review, see Baker and Lindsley, 1982) post-transcriptional processes which have to be postulated for *Drosophila* spermatogenesis also have to be executed on the fusion transcript. These include at least three steps. First, the primary transcript has to be properly spliced to generate an active and antigenic fusion protein. Second, a very stable RNA has to be guaranteed since the post-elongation period starts ~3 days after the onset of meiotic divisions (Lindsley and Tokuyasu, 1980). Third, translation has to be prevented or, what we find very unlikely, the protein would have to be immediately degraded during the first days of spermiogenesis to give the observed protein pattern. Hybridizations to Northern transfers with RNAs from transformed flies always gave a single hybridization signal which suggests that the RNA is stored as spliced mRNA and not as primary transcript. Therefore, we favour the idea that the latter two mechanisms only rely on the final mRNA although we cannot yet exclude a supportive function of the intron.

If our hypothesis is correct both stability of the RNA and translational control are mediated by 110 nt representing the entire untranslated leader and the 10 nt following the AUG start codon. There are examples where structural features for mRNA stability reside in the 5′ end of this mRNA, although in the majority of the cases they are found at the 3′ end (for review, see Raghow, 1987). The stabilizing ability of the 5′ end is so effective that even the 3.1-kb *mst(3)gl-9−lacZ* transcript is not degraded; this is especially noteworthy since an *hsp70−lacZ* mRNA was found to be less stable than the endogenous *hsp70* mRNA (Simon and Lis, 1987).

In those cases where translational control has been assigned to specific parts of the transcript it was always the untranslated leader as in the adenovirus major late transcript (e.g. Logan and Shenk, 1984), the *GCN4* mRNA of yeast (e.g. Mueller and Hinnebusch, 1986), or the transcripts of the *Drosophila* heat shock genes *hsp22* (Klemenz *et al.*, 1985) and *hsp70* (McGarry and Lindquist, 1985). In the case of the *hsp22* mRNA it was even possible to localize the sequences for translational control to the extreme 5′ end, namely to the first 26 nt which are quite conserved among heat shock RNAs (Hultmark *et al.*, 1986). Since four genes have already been isolated which are closely related to *mst(3)gl-9* it may be possible to identify sequence similarities in the untranslated leader of the various members of the gene family, too. Further deletions and site-directed mutagenesis in concert with P-mediated germ line transformation should then identify unequivocally the functional regions. Such experiments are currently under way.

So far, we have no data on the function of this testis-specific protein with the unusual repetitive motif of Cys−Gly−Pro. Searching the protein sequence library (NBRF, release 10.0 from August 1986) for sequence similarities was without success. On the other hand, in Northern experiments we can detect a transcript in mouse RNA which hybridizes to *mst(3)gl-9*. It has a size of 1.5 kb and is present exclusively in RNA isolated from testis material (unpublished). It may, therefore, be legitimate to relate our data to what is known in the mammalian system.

Dense fibres are located on the outside of the axoneme in the mid-piece of the mammalian sperm. They contain proteins rich in cysteine and proline (e.g. 20 and 8%, respectively, in bull sperm; Baccetti *et al.*, 1973). These fibres are believed to have significant passive elastic properties and may function to stiffen or provide elastic recoil for the sperm tail (for review, see Guraya, 1987). Since the mid-piece of the mammalian sperm is functionally equivalent to the *Drosophila* sperm tail we speculate that *mst(3)gl-9* and the other members of the gene family may code for proteins with similar function in *Drosophila*. The first detection of *mst(3)gl-9−lacZ* fusion protein in the post-elongation period of spermatogenesis is in good agreement with this interpretation. At that time the accessory and central microtubules of the axoneme undergo drastic changes and develop into complex structures with electron dense cores. Coarse fibres and the accessory tubules form the so-called satellites on the outside of the axoneme. They initially appear as depositions of amorphous material, but later develop into highly ordered

components of the sperm tail (for review, see Lindsley and Tokuyasu, 1980). Our working hypothesis is that the CGP-rich proteins encoded by this gene family are involved in this process and, therefore, help maintain the elastic properties of the unusually long *D.melanogaster* sperm (~1.8 mm). We are currently employing immunological methods to prove or disprove this theory.

Materials and methods

Nucleic acids

Standard protocols were taken from Maniatis *et al.* (1982). RNA isolation and radiolabelling procedures were peformed as described previously (Schäfer, 1986a).

Sequence analysis

The sequence of the chromosomal *mst(3)gl-9* gene was determined by dideoxy sequencing techniques (Sanger *et al.*, 1977) using a cloned gene from the wild-type strain Canton S (Schäfer, 1986a). Overlapping restriction fragments were subcloned into M13 mp8/9 (Messing and Vieira, 1982) or plasmid vector pTZ18U (Mead *et al.*, 1986) and sequenced. In those cases where suitable restriction fragments were not available, sequencing was done using exonuclease III deletion clones (Henikoff, 1984). The sequence of both strands was determined.

mst(3)gl-9 cDNAs were isolated from a cDNA library of adult males from the strain Oregon R constructed by L.Kauvar (Poole *et al.*, 1985). Approximately 2×10^5 phages were screened with a nick-translated *Hin*dIII fragment (nt 1550–2159; Figures 1 and 2) under standard conditions. Phage inserts were subcloned in the vector pTZ18U and the nucleotide sequences determined as described.

Transformation plasmids

Carnegie 20 construct. The transformation plasmid p425 was constructed by modifying the *mst(3)gl-9* gene contained on the 2.5-kb *Eco*RI fragment (Figure 1) within plasmid pUR9/3. pUR9/3 was linearized at the unique *Bal*I site within the *mst(3)gl-9* untranslated region 14 bp downstream of the stop codon and blunt-end-ligated to the 625-bp *Hpa*I fragment from the *E.coli lacZ* gene of plasmid pMC1871 (Casadaban *et al.*, 1983). The resulting recombinant that had the *mst(3)gl-9* gene and the *lacZ* fragment in the same transcriptional orientation was identified by restriction digestion. The *Eco*RV–*Sal*I subfragment which contains 670-bp upstream sequences, the enlarged *mst(3)gl-9* gene and 132 bp of downstream sequences was then subcloned into the *Hpa*I–*Sal*I digested polylinker of Carnegie 20 (Rubin and Spradling, 1983).

mst(3)gl-9–lacZ gene fusions. To simplify the construction of *mst(3)gl-9–lacZ* gene fusions, plasmid p465 was constructed containing sequences from −5.8 kb to +0.2 kb including the ATG codon. Plasmid pUR9/3 was digested with *Ava*II and a 0.9-kb *Ava*II fragment purified by agarose gel electrophoresis. The fragment was made 'blunt end' by filling in the recessed 3′ termini with Klenow fragment and ligated into *Hin*cII digested and phosphatase treated pUC9 (Vieira and Messing, 1982). A recombinant plasmid containing the *Sma*I, *Bam*HI and *Eco*RI polylinker sites adjacent to the ATG codon was digested wtih *Hin*dIII and recircularized, resulting in the deletion of a 0.6-kb *Hin*dIII fragment. Finally, a genomic 5.9-kb *Hin*dIII fragment (Figure 1) was inserted into the unique *Hin*dIII site. The three *mst(3)gl-9–lacZ* transformation plasmids were made by subcloning the respective restriction fragments from plasmid p465 in the P-element vectors pUCPlac, which contain a polylinker in all three reading frames in front of a truncated *E.coli lacZ* gene (Molsberger *et al.*, 1988).

P-element-mediated transformation

Germ line transformation was done as described by Rubin and Spradling (1982); the techniques for handling and injection of the embryos followed the protocol of Technau and Campos-Ortega (1985). All DNAs were CsCl purified and extensively dialysed against 0.1 mM $NaPO_4$, pH 6.8, 5 mM KCl. ry^{506} embryos were injected with a mixture of 400 μg/ml p425 DNA and 100 μg/ml of helper plasmid p25.7wc (Karess and Rubin, 1984). In the case of the pUCPlac constructs the DNA (500 μg/ml) was mixed with 100 μg/ml phsπ DNA (Steller and Pirotta, 1986) and injected into Oregon R embryos. G_1 offspring were mated on instant food containing G418 (1 mg/ml). Resistant flies were crossed to balancer stocks (*SM5;TM3*) and homozygous lines with a single chromosomal insertion were established. The identity of the various constructs was verified after integration by Southern analysis.

Whole animal β-galactosidase assay

Staining for β-galactosidase activity was done as described in Glaser *et al.* (1986). The staining reaction was stopped after 1–2 h. Organs were then transferred to glycerol (100%) and photographed using an Olympus SZH photomicroscope.

Indirect immunofluorescence

To detect *mst(3)gl-9–lacZ* fusion proteins indirect immunofluorescence was performed on squash preparations of testes as described in Glätzer (1984).

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These sequence data will appear in the EMBL/GenBank/DDB7 Nucleotide Sequence Databases under the accession number Y00831.