Transcriptional and post-transcriptional control mechanisms coordinate the onset of spermatid differentiation with meiosis I in *Drosophila*

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Accepted 26 October 1997; published on WWW 8 December 1997

SUMMARY

The *aly*, *can*, *mia* and *sa* genes of *Drosophila* are essential in males both for the G2-meiosis I transition and for onset of spermatid differentiation. Function of all four genes is required for transcription in primary spermatocytes of a suite of spermatid differentiation genes. *aly* is also required for transcription of the cell cycle control genes *cyclin B* and *twine* in primary spermatocytes. In contrast *can*, *mia* and *sa* are required for accumulation of *twine* protein but not *twine* transcript. We propose that the *can*, *mia* and *sa* gene products act together or in a pathway to turn on transcription of spermatid differentiation genes, and that *aly* acts upstream of *can*, *mia* and *sa* to regulate spermatid differentiation. We also propose that control of translation or protein stability regulates entry into the first meiotic division. We suggest that a gene or genes transcribed under the control of *can*, *mia* and *sa* allow(s) accumulation of *twine* protein, thus coordinating meiotic division with onset of spermatid differentiation.

Key words: Meiosis, Cell-cycle, Transcription, Spermatogenesis

INTRODUCTION

Cell cycle progression is coordinated with developmental and cellular differentiation programs in multicellular organisms via regulation of core cell cycle components. For example, the timing of mitotic divisions in gastrulating *Drosophila* embryos is controlled by regulated transcription of the cell cycle phosphatase gene *string* under the control of pattern formation genes (Edgar and O’Farrell, 1990; Edgar et al., 1994). During differentiation of the *Drosophila* eye, activity of the gene *rux* ensures that cells are synchronised in G1 before the onset of terminal differentiation (Thomas et al., 1994). In *Drosophila* males, the progression into the meiotic divisions is coordinated with the onset of spermatid differentiation by at least four genes, *aly*, *can*, *mia* and *sa* (Lin et al., 1996). Our results indicate that during *Drosophila* spermatogenesis, transcriptional and post-transcriptional regulation of genes required for meiosis and differentiation may link the independent pathways of meiosis and spermatid differentiation.

*Transcription* in *Drosophila* occurs throughout larval and adult life, and all stages of differentiation are represented in a single adult testis (reviewed in Fuller, 1993). Gonial cells born at the apical tip by a stem cell mechanism undergo four rounds of synchronous mitotic divisions to produce cysts of 16 interconnected cells that then progress through pre-meiotic S phase. The resulting primary spermatocytes enter an extended G2 phase, lasting approximately 90 hours and characterised by a 25-fold increase in cell volume associated with robust gene expression (Lindsley and Tokuyasu, 1980). The two meiotic divisions are followed by spermatid differentiation, a series of dramatic morphological changes including flagellar elongation and nuclear shaping, which eventually results in the production of motile sperm. In *Drosophila*, most transcription is shut off upon entry into the meiotic divisions (Gould-Somero and Holland, 1974; Olivieri and Olivieri, 1965). Thus transcription of genes required post-meiotically occurs during the primary spermatocyte stage. The G2-M transition must be delayed until all transcripts required for post-meiotic events have accumulated. Translation of specific messages may be delayed until long after meiosis (Schäfer et al., 1995).

Although spermatid differentiation usually follows completion of meiosis, onset of spermatid differentiation is not dependent upon progression through the meiotic divisions. In testes mutant for the cell cycle regulator *twine*, spermatocytes skip many events characteristic of the meiotic divisions, but nonetheless undergo extensive spermatid differentiation, including flagellar elongation and nuclear shaping (Alphey et al., 1992; Courtot et al., 1992; White-Cooper et al., 1993). *Twine* and *string* are *Drosophila* homologues of the *S. pombe* mitotic activator *cdc25*, which encodes a dual specificity phosphatase that promotes the G2-M transition by removing inhibitory phosphates from the cdc2 protein kinase, thereby activating the cdk-cyclin complex (Dunphy and Kumagai,
Meiotic cell cycle progression and the onset of spermatid differentiation both require the action of four meiotic arrest genes, *aly*, *can*, *mia* and *sa* (Lin et al., 1996). In testes mutant for any one of these genes, mature primary spermatocytes accumulate, but meiotic and post-meiotic cells are not observed. The testes become filled with spermatocytes arrested at the G2-M transition with partially condensed chromatin. Based on comparison of this phenotype with that of *twine* mutant males, Lin et al. (1996) proposed that spermatogenesis follows a branched pathway, with *twine* acting in the branch leading to the meiotic divisions, an independent branch leading to spermatid differentiation, and *aly*, *can*, *mia* and *sa* acting at or upstream of the branch or coordinate control point.

To discover the molecular mode of action of the meiotic arrest genes in the coordinate control of meiosis and spermiogenesis, we analysed the expression state of core cell cycle regulators in mutant versus wild-type testes. Here we show that *aly*, *can*, *mia* and *sa* act via two different mechanisms to regulate expression of *twine* protein. Wild-type function of *aly* is required for expression of *twine* mRNA, and also several other messages in primary spermatocytes. In contrast, wild-type function of *can*, *mia* and *sa* is not required for expression of *twine* mRNA, but is needed for accumulation of *twine* protein. All four genes are also required for expression of a number of transcripts needed for post-meiotic differentiation. We propose that *aly*, *can*, *mia* and *sa* act in a pathway to regulate a program of gene expression in primary spermatocytes required for post-meiotic spermatid differentiation. Expression of a gene or genes in this program allows accumulation of *twine* protein and progression from the extended G2 phase into the first meiotic division. Thus the regulatory circuitry of spermatogenesis in *Drosophila* appears to incorporate a fail-safe mechanism to ensure that transcripts required for post-meiotic differentiation are expressed before allowing entry into the first meiotic division.

**MATERIALS AND METHODS**

**Drosophila stocks**

*Drosophila* stocks were maintained on standard cornmeal molasses food at either room temperature (22°C) or at 25°C. Unless otherwise noted *aly*, *can*, *mia* and *sa* (Lin et al., 1996) and *twine*HBS (Alphey et al., 1992; Courtot et al., 1992) alleles were used homozygous for all experiments. An isogenic red *e* stock that is the background chromosome for both strong loss of function alleles *aly* and *can* was used as a wild-type control, except for the northern analysis in which Oregon R was used.

**Western blotting**

40 testes of each genotype were dissected from newly eclosed adult males in EB− buffer (10 mM Tris, pH 7.5, 80 mM sodium β-glycerophosphate, 20 mM EGTA, 15 mM MgCl₂, 2 mM Na₂VO₃, 1 mM benzamidine, 0.2 mM PMSF, 1 μg/ml leupepsin, pepstatin A and aprotinin), and transferred to a 20 μl drop of EB+ (EB with 0.1% NP-40). The apical tip of each testis was cut off and the contents of the testis were emptied into the drop by lifting the shear out of the buffer by the distal end. 20 μl of SDS-PAGE sample buffer was added, the samples were transferred to an Eppendorf tube and immediately boiled for 10 minutes. Samples were stored at −20°C, boiled again for 5 minutes, and spun in a microfuge for 10 minutes before loading. Embryos were frozen, homogenised with an Eppendorf pestle in EB+, solubilised with an equal volume of sample buffer, boiled and centrifuged as for testes. 2 μl (approximately two testes) of each sample was run on a 10% SDS-polyacrylamide gel and stained with Coomassie Blue to assess protein loading; 18 μl (18 testes) of each sample was run on a duplicate gel for Western blotting.

Western blots were blocked with 5% non-fat dried milk and then probed sequentially, starting with a monoclonal anti-actin antibody (N350, Amersham) diluted 1:100 (v:v) in PBS, 0.1% Tween-20, 5% milk, followed by HRP-conjugated sheep anti-mouse secondary antibody diluted 1:1000 (Amersham). Cdc2 protein was detected with an anti-PSTAIR antibody (06-181, Upstate Biotechnology Incorporated) diluted 1:2500. Cyclin A (Rb270) and cyclin B (Rb271) antibodies (both provided by D. Glover) were diluted 1:1000 (Whitfield, 1990). Rabbit antibodies to cdc2, cyclin A and cyclin B were followed by HRP-conjugated donkey anti-rabbit secondary antibody diluted 1:1000 (Amersham). Detection was by ECL (Amersham).

**Immunofluorescence**

Immunofluorescence staining of squashed testes was carried out as previously described (White-Cooper et al., 1993). The anti-cyclin B antibody was diluted 1:500 (v:v). DNA was visualised by using propidium iodide in the mounting medium at 1 μg/ml.

**In situ hybridisation**

In situ hybridisation to whole testes was carried out as previously described (Tautz and Pfeifle, 1989) with the following exceptions. Testes were dissected from newly eclosed males in TB1 (Kemphues et al., 1980). Fixation was for 20 minutes in paraformaldehyde. All steps involving methanol or heptane were omitted. Proteinase K (50 μg/ml) treatment was for 5-7 minutes. The hybridisation buffer (HB) was adjusted to pH 4.5 with citric acid to stabilise the RNA probe; pre-hybridisation, hybridisation and washes in HB were carried out at 65°C. Pre-hybridisation and all subsequent steps were carried out in cell culture inserts (Falcon) in 24-well tissue culture dishes. Hybridisation was carried out in a total volume of 300 μl, and all washes were with 1 ml of buffer. After hybridisation the testes were washed for at least 3 hours in six changes of HB at 65°C. Subsequent washes were as described by Tautz and Pfeifle (1989). Alkaline phosphatase-conjugated sheep anti-digoxigenin antibody (Boehringer Mannheim) was preadsorbed against embryos and used at a final concentration of 1:2000 (v:v) overnight at 4°C.

For all hybridisation experiments wild-type testes were mixed with each of the different mutant testes, to provide a positive control in each well. The colour reaction was allowed to develop until the wild-type testes was directly comparable to the corresponding wild-type testes in each well were sufficiently stained, so the staining in the mutant testes was directly comparable to the corresponding wild-type samples. Testes were dehydrated in an ethanol series, mounted in GMM and viewed by DIC optics (Tautz and Pfeifle, 1989). Mutant testes were easily distinguished from wild type by their strikingly different morphologies.

**RNA probe synthesis**

RNA probes were made by using the Genius 4 kit from Boehringer Mannheim, according to the manufacturer’s instructions. Probes were hydrolysed to give an average probe length of 100 nt. Each probe reaction was resuspended in 200 μl dH₂O. Immediately before use probes were diluted 1:200 (v:v) in HB and heated to 80°C for 5 minutes. Antisense RNA probes were generated from the following cDNAs: *cyclin A* (Lehner and O’Farrell 1989), *cyclin B* (Lehner and O’Farrell, 1990), *twine* (Alphey et al., 1992), *polo* 9a2 (Llamazares et al., 1991), *pelota* (Eberhart and Wasserman, 1995), *roughex* (Thomas et al., 1994), *boule* (Eberhart et al., 1996), *fzo* (Hales and Fuller, 1997), *Mst87F S’* gene-specific fragment (Kuhn et al., 1988),
Construction of twine-lacZ reporter

A genomic 3.7kb BamHI fragment containing the twine gene was previously cloned into the P element transformation vector pW8 (Alphey et al., 1992). A 661 bp BgIII fragment was excised from this construct and replaced with a 3091 bp BamHI fragment from pMC1871, which encodes β-galactosidase. This construct was injected into flies as described (Alphey et al., 1992). An insertion on the second chromosome was isolated. In both wild-type and mutant backgrounds the insertion was maintained homozygous. These flies express a twine-lacZ fusion, which includes the NH2-terminal 224 amino acids of twine.

β-galactosidase activity assay

Testes were dissected from newly eclosed males in TB1 (Kemphues et al., 1980) and fixed in 1% glutaraldehyde in PBS for 15 minutes. Testes were washed 3x 10 minutes in X-gal buffer (10 mM NaPO₄, pH 6.8, 150 mM NaCl, 1 mM MgCl₂) and then stained overnight at 37°C in X-gal buffer containing 5 mM K₄Fe(CN)₆, 5 mM K₃Fe(CN)₆, 0.01% X-gal. Testes were mounted in 80% glycerol and observed with DIC optics.

Northern analysis

Total RNA was isolated from adult male flies according to the method of Chomczynski and Sacchi (1987). Approximately 35 μg of total adult male RNA was separated on a 1.5% agarose gel containing formaldehyde, blotted onto Hybond nylon membrane (Amersham) in 20× SSPE and pre-hybridised in 500 mM sodium phosphate (pH 6.5), 7% SDS at 65°C. Hybridisation was carried out in 50% formamide, 5× SSPE, 50 mM Na-phosphate, pH 6.5 at 65°C by using 32P-labelled, antisense, in vitro-transcribed probes. Antisense RNA probes were generated from cloned cDNAs for gonadal 1500 bp male-specific cDNA (Schulz and Butler, 1989), janusB 400 bp gene-specific fragment of cDNA (Yanicostas et al., 1989), Mst78D (S. Viswanathan and M. T. Fuller, personal communication) and don juan (Santel et al., 1997).

RESULTS

**aly** is required for cyclin B and twine expression in primary spermatocytes

The level of cyclin B protein was reduced significantly in *aly* mutant testes compared to wild type (Fig. 1). Although low levels were still detected by western blotting of testis extracts, the three different *aly* alleles tested all caused a similar marked reduction in cyclin B protein level when compared to wild-type testis extracts. Cyclin B protein appeared to be expressed at normal levels in *can, mia* and *sa* mutant embryos, indicating that these genes might influence cell cycle progression through a biochemically distinct pathway from *aly*. *aly* is not required for the expression of all cell cycle genes, since both cyclin A and cdc2 proteins were expressed at high levels in *aly* testes. Equivalence of loading was determined by an actin loading control (Fig. 1) and by Coomassie Blue staining of a duplicate gel (not shown).

The wild-type function of *aly* is required for the transcription or accumulation of both cyclin B and twine mRNAs. In wild-type testes, cyclin B transcripts were detected by in situ hybridisation at low levels in the mitotic cells at the apical tip (Fig. 2A, arrow), but were not detected at the position where

**Fig. 2.** Cyclin B and twine mRNAs were reduced in *aly* testes. RNA in situ hybridisation to wild type (A,C,E) and *aly* (B,D,F) testes. (A,B) cyclin B message in wild type (A) and *aly* (B) testes. Arrows, cyclin B mRNA in the mitotic cells at the apical tip of the testis; large arrowheads, mature primary spermatocytes; small arrowhead, post-meiotic cysts. (C,D) twine mRNA was detected in wild type (C) but not *aly* (D) testes. (E,F) cyclin A mRNA was present in both wild type (E) and *aly* (F) testes.
similar to that of cyclin B, except that twine mRNA was not detected in the mitotic cells at the apical tip of the testis (Fig. 2C), as has been shown previously (Alphey et al., 1992; Courtot et al., 1992). In aly mutant testes twine mRNA was not detected by in situ hybridisation (Fig. 2D). can, mia and sa mutant testes expressed both twine and cyclin B mRNA at normal levels (not shown).

The lack of cyclin B and twine mRNAs in aly mutant spermatocytes was not due to a general defect in transcription, as cyclin A (Fig. 2F) and other messages were abundant in the mutant spermatocytes. In wild-type testes cyclin A mRNA was detected at low levels in both mitotic and S-phase cells at the apical tip of the testis. Cyclin A showed high levels of expression throughout the primary spermatocyte stage, with the message disappearing during meiosis (Fig. 2E). In aly, can, mia and sa mutant testes cyclin A mRNA was expressed in mitotic and S-phase cells and spermatocytes as in wild type. However cyclin A transcript levels remained high in the arrested mature primary spermatocytes, only disappearing at the base of the testes where the cells finally degenerate (Fig. 2F, data not shown for can, mia and sa), suggesting that the wild-type function of aly, can, mia and sa is required directly or indirectly for the normal shut down of transcription and/or turnover of cyclin A message at meiosis.

**Accumulation of twine and cyclin B proteins occurs just before the first meiotic division in wild type**

The timing of entry into the meiotic divisions in wild type may be controlled by post-transcriptionally regulated accumulation of cyclin B and twine proteins. Although cyclin B mRNA was expressed at high levels in early spermatocytes (Fig. 2A), the accumulation of cyclin B protein was delayed in wild-type testes until the late primary spermatocyte stages. Immunofluorescence with an anti-cyclin B antibody showed high levels of cyclin B protein in the mitotic region at the apical tip of the testis (Fig. 3A, arrow). Only low levels of cyclin B protein were detected during the primary spermatocyte growth phase (Fig. 3A, small arrowhead). Cyclin B protein began to accumulate in the cytoplasm of late primary spermatocytes as chromosome condensation was initiated just before the entry into the first meiotic division (Fig. 3B,C, small arrowheads), and was present at high levels in pro-metaphase I cells (Fig. 3B,C, large arrowheads). Cyclin B protein was degraded at the metaphase to anaphase transition of meiosis I, and re-accumulated in preparation for the second meiotic division (not shown) (Gonczy et al., 1994). In aly mutant testes cyclin B protein was detected in the mitotic cells at the apical tip, but did not accumulate in the mutant spermatocytes (not shown).

Accumulation of twine protein, as assayed by a twine-lacZ reporter fusion protein (Fig. 4A), also appeared to be delayed until just before the entry into the first meiotic division, days after the transcript was first detected. A reporter construct expressing a twine-lacZ fusion protein was derived from a 3.7 kb genomic fragment carrying the entire twine gene (Alphey et al., 1992) (Fig. 4A). The reporter construct was transcribed in wild type in the same pattern as endogenous twine (Fig. 4B). However β-galactosidase activity of the reporter protein was not detected until just before the onset of the first meiotic division. In testes from otherwise wild-type males carrying the reporter construct, most primary spermatocyte cysts had no detectable β-galactosidase activity, even up to the mature primary spermatocyte stage (Fig. 4D, arrow). β-galactosidase activity from the reporter was first detected at low levels in primary spermatocytes cysts about to enter the first meiotic division (Fig. 4C, arrow). Activity of the reporter protein persisted through the meiotic divisions (Fig. 4D, large arrowhead) and into early post-meiotic stages (Fig. 4D, small arrowhead), but was absent from later stages of spermatid elongation. Neither β-galactosidase activity nor the fusion construct mRNA was detected when the reporter construct was present in an aly mutant background (data not shown), indicating that the reporter construct contains regulatory sequences that confer dependence on aly for transcription.

**can, mia and sa are required for accumulation of the twine-lacZ fusion protein in late primary spermatocytes**

can, mia and sa affect progression through the cell cycle by a different mechanism than aly. Both cyclin B and twine mRNAs were expressed in a normal pattern and at normal levels in can, mia and sa testes, in contrast to aly testes. In can, mia and sa
mutant testes, cyclin B protein was expressed at normal levels (Fig. 1), and immunofluorescence experiments revealed that the timing of accumulation of cyclin B protein was normal (data not shown). However the wild-type function of can, mia and sa was required for expression of the twine-lacZ reporter fusion protein. Although transcripts encoded by the twine-lacZ reporter fusion construct were detected in the same pattern as the endogenous twine message when the reporter was crossed into a can, mia or sa mutant background (Fig. 4E-G), no β-galactosidase activity was detected (Fig. 4H-J). No in situ hybridisation staining was detected by the lacZ probe in testes not carrying the transgene.

The meiotic arrest genes control transcription of several genes required for post-meiotic spermatid differentiation

aly, can, mia and sa are required for the transcription in primary spermatocytes of several genes involved in post-meiotic spermatid differentiation. The fuzzy onions (fzo) gene product is required for mitochondrial fusion in early haploid spermatids (Hales and Fuller, 1997). fzo transcription initiates in early primary spermatocytes and the mRNA is present throughout the growing stages in wild type (Fig. 5A). fzo mRNA was greatly reduced in aly, can, mia and sa testes, despite the presence of primary spermatocytes in the mutant tissue (Fig. 5B-E). Message levels in mutant testes ranged from undetectable (Fig. 5E) to low levels (Fig. 5C) under conditions in which the in situ hybridisation signal in wild type was strong (Fig. 5A), indicating that transcription may be reduced to a low basal level, but not entirely turned off. The variability in signal was detected within each genotype, with the average level of staining comparable between the different mutants. Similarly severe reductions in message level were observed for Mst87F (Fig. 5G-J), a gene normally transcribed in primary spermatocytes but not translated until mid- to late-spermatid stages, days after the completion of meiosis (Kuhn et al., 1988). Several other genes also showed dramatic reductions in transcript levels in meiotic arrest mutant testes when assayed by in situ hybridisation (Table 1). Reduced transcript levels in aly, can, mia and sa spermatocytes were not due to a general defect in transcription as a number of genes were transcribed at normal levels in mutant spermatocytes. For example, mRNA for pelota, a gene required for entry into meiosis but not for spermatid differentiation (Eberhart and Wasserman, 1995), was expressed at levels comparable to wild type in aly, can, mia and sa mutant testes (Fig. 5K-O; see also Table 1).

The drastic reduction in level of certain transcripts in mutant testes was confirmed by Northern blot analysis (Fig. 6). Total RNA from wild-type and mutant adult males was probed sequentially for expression of gonadal (gdl) (Schulz and Butler, 1989), janB (Yanicostas et al., 1989), Mst87F and Mst75C (Schäfer, 1986). gdl is expressed as two differentially terminated variants in the testis (Schulz et al., 1990). Both forms were dramatically reduced in can, mia and sa mutant testes, and barely detectable in aly (Fig. 6). Likewise levels of both janB and Mst87F transcripts were reduced drastically in can, mia and sa, and barely detectable in aly mutant testes. This reduction is seen for all of the members of the Mst75C gene family detected in this experiment (see Materials and methods). The northern blot was probed with Mst75C, a gene expressed in the male ejaculatory bulb, as a loading control (J. Weinert and M. A. Schäfer, unpublished). Expression of Mst75C is not affected in any of the meiotic arrest mutants.

Comparison of the effects of aly, can, mia and sa mutations on transcript levels suggests that genes normally transcribed in primary spermatocytes can be grouped into three classes (Table 1). The transcription of the first (general) class of genes is independent of aly, can, mia and sa function. The second

![Fig. 4](image-url) Post-transcriptional control of twine depends on can, mia and sa. (A) Construction of a twine-lacZ reporter fusion construct from a 3.7 kb genomic fragment capable of rescuing the twine mutant phenotype. (B) In situ hybridisation with a lacZ probe in a whole testis from a wild-type male carrying the reporter construct. The twine-lacZ reporter is transcribed in wild-type testes in the same pattern as endogenous twine. (C,D) High magnification views of whole mount testes stained for β-galactosidase activity from wild-type males carrying the twine-lacZ reporter. (C, arrow) Cyst of 16 mature primary spermatocytes showing perinuclear localisation of β-galactosidase activity. (D) Arrow, cyst of mature primary spermatocytes with no detectable β-galactosidase activity; large arrowhead, cyst in anaphase I showing accumulation of β-galactosidase activity; small arrowhead, cyst of early spermatids at the onion stage show persitance of β-galactosidase activity in the nucleus. (E-G) In situ hybridisation to mutant testes with a lacZ probe. The twine-lacZ transgene is transcribed in the same pattern as endogenous twine. (H-J) β-galactosidase assays showed no β-galactosidase activity in can, mia or sa mutant testes. (E,H) w; P[w+, twine-lacZ]; can. (F,I) w; P[w+, twine-lacZ]; mia. (G,J) w; P[w+, twine-lacZ]; sa.
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Table 1. Transcripts normally expressed in primary spermatocytes fall into three classes

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Transcript expression in wild-type and mutant testes was determined by RNA in situ hybridisation or Northern analysis. +, Transcript was detected at near wild-type levels; -, transcript was severely reduced or absent; (ND) not determined.

General transcripts do not require the function of aly, can, mia or sa for their expression. Some transcripts required for meiosis depend on aly for their expression. A set of genes involved in spermatid differentiation depend on aly, can, mia and sa. None of the transcripts tested depend on twine for their expression.

(meiotic) class of genes requires the normal function of aly, but not can, mia or sa. Expression of the third (spermiogenic) class of genes requires the wild-type activity of all four of the meiotic arrest genes.

DISCUSSION

The aly, can, mia and sa genes of Drosophila are required for meiotic cell-cycle progression and spermatid differentiation. We have shown that the genes act to control the onset of the meiotic divisions by regulating expression of cell cycle proteins. In addition, the meiotic arrest genes control the transcription of a number of genes involved in spermatid differentiation. Based on these data we propose a model for the coordinated regulation of meiosis and spermatid differentiation in wild-type Drosophila testes.

Regulation of meiotic cell cycle progression in males

Control of the timing of cell cycle progression by regulated production or activation of core cell cycle components is a common theme in development, although the exact mechanism by which the control is exerted can vary. Transcriptional control of stg sets the timing of the G2-mitosis transition in early Drosophila embryos. Transcription of stg under the control of patterning genes occurs 25 minutes before, and triggers the onset of, mitosis in the mitotic domains of the gastrulating embryo (Edgar and O’Farrell, 1990; Edgar et al., 1994). In contrast, translational control of the expression of cyclin B appears to regulate mitotic cell cycle progression in the embryonic germ cells of Drosophila. A maternally provided, alternatively spliced, variant of cyclin B mRNA containing an additional element in the 3’ UTR is localised to the pole plasm and incorporated into the presumptive germ cells upon cellularisation (Dalby and Glover, 1992).

![Fig. 5. Transcripts for several genes involved in spermatid differentiation are greatly reduced in aly, can, mia and sa mutant testes. In situ hybridisations to whole mount wild-type (A,F,K) and mutant testes. (A) fzo mRNA was detected in wild-type primary spermatocytes and meiotic cells, but was severely reduced or absent from aly (B), can1 (C), mia (D) and sa (E) mutant testes. Part of a stained wild-type testis included in the hybridisation as a positive control is visible in (B). (F) Mst87F was transcribed in wild-type primary spermatocytes and persisted through meiosis and spermatid elongation, but was severely reduced or absent from aly (G), can1 (H), mia (I) and sa (J) mutant testes. pelota mRNA was expressed in wild type (K), aly (L), can1 (M), mia (N) and sa (O) testes at comparable levels. In wild-type testes pelota mRNA normally disappears during meiosis and is undetectable in post-meiotic stages; however pelota mRNA persists in the arrested cells of all of the mutants.](image-url)
Translation of the message remains repressed until the pole cells have migrated into the embryonic gonad, at which time cyclin B mRNA is translated and mitosis resumes (Dalby and Glover, 1993). The onset of the meiotic divisions in mouse spermatogenesis may be controlled by regulated expression of cell cycle genes. In mouse testes cdc25C expression is restricted to germ cells in late pachytene, diplotene and round spermatids (Wu and Wolgemuth, 1995). Additionally cyclin A1 is expressed in late pachytene spermatocytes and disappears soon after meiosis (Sweeney et al., 1996). Cdc2 kinase activity in mouse testes is also regulated post-translationally: the HSP70-2 protein is required for cdc2/cyclin B complex formation in pachytene spermatocytes. HSP70-2 mutant males are sterile due to failure of spermatocytes to progress through meiosis I (Zhu et al., 1997).

The timing of entry into the first meiotic division in males is tightly regulated in many organisms so the primary spermatocyte stage lasts for a defined period: 22 days in man, 11 days in mouse (Handel, 1987) and approximately 3.5 days in Drosophila melanogaster (Lindsley and Tokuyasu, 1980). Our data suggest that the timing of the G2/M transition of meiosis I in Drosophila males may be regulated post-transcriptionally. In wild-type spermatocytes, accumulation of cyclin B protein and a twine-β-galactosidase fusion protein is delayed until just prior to onset of the first meiotic division, days after the appearance of their respective mRNAs. The level of cyclin B protein could be regulated either by translational control or by changes in protein stability. The same may be stated for the twine-lacZ fusion protein, and by inference twine protein, since the reporter fusion contains 224 amino acids from the amino terminus of twine. Accumulation of cdc25 activity is not the sole limiting step in the initiation of the first meiotic division in spermatocytes (Sigrist et al., 1995). Expression of the cdc25 homologue stg under control of a heat shock promoter in twine mutant testes partially rescued the twine phenotype but did not cause premature entry into the meiotic divisions, perhaps due to a lack of cyclin B protein or another as yet unidentified factor in younger primary spermatocytes.

The meiotic arrest genes act through different biochemical mechanisms to control the G2-M transition of meiosis I

Although mutations in aly, can, mia and sa appear to cause arrest at the same point in the G2-M transition of meiosis I (Lin et al., 1996), the genes apparently control cell cycle progression by different biochemical mechanisms. aly, but not can, mia or sa, is required for the transcription of cyclin B and twine. The wild-type function of can, mia and sa instead appears to be required either to allow translation of twine message or to stabilize twine protein in mature primary spermatocytes. In either case aly, can, mia or sa mutations presumably cause cell cycle arrest at the same point in the G2-M transition, due to lack of active cdc2/cyclin B kinase complex. Cdc2 protein resolved into two distinct isoforms in western blots (Fig. 1). The slower migrating form, which is
enriched compared to the faster migrating form in twine mutant
testes, has been identified as a hyperphosphorylated, inactive
form (Sigrist et al., 1995). The slower migrating form of cdc2
also appeared to be enriched compared to the faster migrating
form in aly, can and sa. Production of twine protein, but not
cyclin B, is dependent on can, mia and sa. Thus, although both
cyclin B and twine protein accumulation are regulated post-
transcriptionally in wild-type testes, the genetic control of their
expression is different.

A model for coordinate control of spermatid
differentiation and meiotic cell cycle progression

We propose that can, mia, and sa act together or in a pathway
to activate a tissue and stage-specific transcription program in
primary spermatocytes (Fig. 7), and that failure to initiate this
program results in a global block in spermatid differentiation
due to the lack of an array of gene products. The wild-type
functions of can, mia and sa appear to be required for
transcription in primary spermatocytes of a set of genes
encoding products involved in post-meiotic spermatid
differentiation. Transcription of these genes is initiated early in
the primary spermatocyte stage, several days before the arrest
point of the meiotic arrest mutants. Therefore the lack of
transcription of this set of genes is likely to be a cause of the
arrest rather than merely a downstream consequence.

Of the eight genes identified so far that depend on can, mia
and sa for transcription, some information about the function
or time of action of the gene products is available for six. The product of the fzo gene is required for mitochondrial fusion,
a post-meiotic event. Although fzo is transcribed in primary
spermatocytes, the protein was not detected by
immunofluorescence staining of testes until late in meiosis II
(Hales and Fuller, 1997). Expression of Mst87F, of four
related genes at 84D and two related genes at 98C is regulated
translationally. Although mRNAs are transcribed in primary
spermatocytes, the proteins do not accumulate until days after
the meiotic divisions. All of these genes encode proteins that
are components of a structure in the sperm tail (Schafer et al.,
1993). Similarly the translation of janB and df mRNAs is
delayed until several days after the completion of meiosis
(Santel et al., 1997; Yanicosatas and Lepesant, 1990). While
the function of JanB is unknown, df is thought to serve a dual
function; it is found in the sperm tail, but sequence
comparisons suggest a possible role as a chromatin component
(Santel et al., 1997). The role of the other genes whose
transcription depend on aly, can, mia and sa is not yet known.
gdl mRNA is translated premeiotically, but no function has
been proposed for either of the proteins encoded in the
bicistronic large transcript. Mst78D is a male-specific, germ-
line-dependent transcript encoding a novel protein of
unknown function (S. Viswanathan and M. T. Fuller,
unpublished). In contrast, all the genes identified thus far, that
do not depend on the function of can, mia and sa for
transcription, are required either for pre-meiotic events or for
the meiotic divisions.

We propose that aly acts upstream of can, mia and sa (Fig.
7), possibly to control expression or activation of components
of the transcription machinery that drives expression of the
spermatid differentiation genes. Wild-type function of aly is
required for accumulation of at least three different mRNAs in
primary spermatocytes that are not dependent on can, mia and
sa, suggesting that aly is able to act independently of can, mia
and sa. However aly mutations cause the same phenotype, and
fail to express the same set of spermatid differentiation genes,
as can, mia and sa mutations. This strongly suggests that aly
might affect spermatid differentiation through an effect on
expression or activity of either can, mia or sa.

The block in meiotic cell cycle progression in can, mia and
sa mutant testes could be due to a cross-regulatory mechanism
that serves to coordinate meiosis and the spermatid
differentiation program. We propose that a gene or genes
encoded in primary spermatocytes under the control of can,
mia and sa encode(s) product(s) required either directly or
indirectly to relieve the translational repression of twine
message or to stabilise the twine protein (Fig. 7, gene X). Such
a cross-regulatory mechanism between the pathways leading
to spermatid differentiation and meiosis could serve in wild
type to ensure that spermatocytes do not enter meiotic division
until the proposed transcription program for post-meiotic
spermatid differentiation genes has been successfully initiated.
A late cross-regulatory mechanism may also explain why
mutations that block spermatid differentiation but not meiotic
cell cycle progression have not yet been isolated.

The signal that activates the G2/M transition in male meiosis
could be accumulation of the product of the proposed cross-
regulatory gene to a threshold sufficient to allow expression of
twine protein. Alternatively, timing of the G2/M transition for
meiosis I could be set via a less direct mechanism, involving
the proposed cross-regulatory gene, but not set directly by its
level. For example accumulation of twine protein may require
an extrinsic signal received or transduced by a gene or genes
controlled by the can, mia and sa transcription program. The
degenerative spermatocyte (des) gene, encoding a novel protein
that may be membrane associated, is a possible candidate for a
component of such a signalling pathway (Endo et al., 1996).
Mutations in des, like aly, can, mia and sa, cause a block in
both meiotic cell cycle progression and the onset of spermatid
differentiation. des mutations are also semi-lethal, suggesting a
role for this gene outside the testis (Endo et al., 1996). Pole cell
transplantation experiments also implicate extracellular signals
in the regulation of meiotic progression and spermatid
differentiation. Male (XY) germ cells transplanted into a female
(XX) host initiate spermatogenesis in the host ovary. However
the transplanted cells arrest as primary spermatocytes and fail
to undergo the meiotic divisions or initiate spermatid
differentiation (Steinmann-Zwicky et al., 1989).

Part of the program of spermatid differentiation regulated by
can, mia and sa could act to destabilize or turn off transcription of
certain messages expressed in primary spermatocytes but
not needed or deleterious after meiosis. In wild-type testes,
cyclin A mRNA is present in primary spermatocytes but not
detectable in post-meiotic cells. Loss of cyclin A mRNA could
be an important mechanism to prevent DNA replication during
meiosis II or in haploid spermatids. In wild-type testes cyclin
A protein is degraded at metaphase I and is not resynthesised
for the second meiotic division (Gonczy et al., 1994; Sigrist et
al., 1995). In males mutant for aly, can, mia or sa, cyclin A
message and cyclin A protein (Lin et al., 1996) persist in the
arrested primary spermatocytes, suggesting that the wild-type
function of the meiotic arrest mutants and/or the transcription
program they control is required directly or indirectly for
disappearance of cyclin A message midway through
Coordination of meiosis with differentiation

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spermatogenesis. A similar effect on message stability was seen for all of the other pre-meiotic genes tested.

Regulatory mechanisms that control and coordinate meiosis and differentiation may be evolutionarily conserved

Yeast meiosis bears striking similarities to Drosophila spermatogenesis. In both cases S phase is followed by an extended G2 phase, characterised by high levels of transcription of genes required for meiosis and subsequent differentiation into spores or sperm. Many yeast mutants, including certain alleles of cdc2 in S. pombe, are analogous to twine in that the mutant cells fail to complete one or both of the meiotic divisions, but still differentiate into spores (Grallert and Sipiczki, 1990; Nakaseko et al., 1984). However meiosis and differentiation are coordinated since mutations in some genes, mei4 in S. pombe or NDT80 in S. cerevisiae, like the meiotic arrest mutants of Drosophila, block both the meiotic division cycle and subsequent differentiation (Egel and Egel-Mitani, 1974; Xu et al., 1995). The failure to accumulate both cell cycle and spermiogenesis mRNAs in aly mutants suggests that there may be parallels in the genetic control of animal spermatogenesis and yeast sporulation. In S. pombe the expression of both cdc13 (cyclin B) and cdc25 (twine) mRNAs is dependent on the function of the mei4 transcription factor (Iino et al., 1995). In S. cerevisiae the expression of some B-type cyclins and some sporulation genes before the first meiotic division is dependent on the function of the NDT80 gene (S. Chu and I. Herskowitz, personal communication).

We thank David Glover for anti-cyclin A and anti-cyclin B antibodies and Christian Lehner, David Glover, Steve Wasserman, Bob Schulz, Larry Zipurski, Karen Hales, Sridhar Viswanathan, Ansgar Santel and C. Yanicostas for cDNAs for in situ hybridisation. We are also grateful to members of the Fuller laboratory and Mary Ann Handel, Scott Hawley and Ina Herskowitz for their critical reading of the manuscript. This work was supported by NIH grant #HD32936 to M.T.F. H.W.-C. was supported by the Stanford Medical School Dean's fund and the Walter and Idin Berry #HD32936 to M.T.F. H.W.-C. was supported by the Stanford Medical School Dean's fund and the Walter and Idin Berry Fellowship Fund. M.A.S acknowledges the support of Deutsche Forschungsgemeinschaft grant # Scha 515/1-2.

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