DEVELOPMENTAL BIOLOGY **204,** 567–576 (1998)
ARTICLE NO. DB989098

Biphasic Subcellular Localization of the DAZL-Related Protein Boule in *Drosophila* Spermatogenesis

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The *Drosophila boule* gene is expressed exclusively in the male germline and encodes an RNA binding protein closely related to the mammalian fertility factors encoded by the *DAZ* (Deleted in Azoospermia) and *DAZL* (DAZ-like) genes. Mutation of *boule* blocks both meiotic divisions. Differentiation nonetheless continues, resulting in tetraploid spermatids that fail to mature into sperm. We have found that Boule localizes premeiotically to a perinucleolar region and then translocates to the cytoplasm at the onset of meiosis. We show that deletion of the Y chromosome *ks-1* fertility locus eliminates Boule nuclear localization, although it does not perturb entry into meiosis. Based on these observations we propose that Boule acts in the cytoplasm to regulate the stability or translation of messenger RNA encoding an essential meiotic factor.

Key Words: **DAZ; meiosis; RNA binding protein; posttranscriptional regulation; Y loops.**

INTRODUCTION

Spermatogenesis, the progression from a single germline stem cell to a cohort of differentiated gametes, is a highly conserved process. In general, the pattern of development begins with a germline stem cell division, continues with mitotic expansion and reductional meiotic divisions, and concludes with cellular specialization to form mature sperm. The dramatic morphological changes accompanying spermatid differentiation are largely the product of the posttranscriptional regulation of RNA molecules synthesized prior to meiosis. In *Drosophila* spermatogenesis, for example, high levels of transcription in the spermatocyte stage are followed by a near complete cessation of transcription, with only a few RNAs transcribed postmeiotically (Olivieri and Olivieri, 1965; Lindsley and Tokuyasu, 1980).

Proteins required for the posttranscriptional regulation of male germ cell development have been identified in many species. For example, Prbp is an RNA binding protein believed to block precocious translation of the mouse protamine-1 gene during spermatogenesis (Lee *et al.*, 1996). In *Drosophila,* the *pelota* locus encodes a protein with sequence similarity to translation termination factors and is the ortholog of the yeast translation factor DOM34 (Eberhart and Wasserman, 1995; Davis and Engebrecht, 1998; Maines and Wasserman, 1998).

In this study, we focus our attention on *boule*, a *Drosophila* locus that, like *pelota*, is apparently required for the posttranscriptional regulation of gene expression in male germ cells. Loss-of-function mutations in *boule* have a meiotic arrest phenotype, resulting in azoospermia and male sterility (Castrillon *et al.*, 1993; Eberhart *et al.*, 1996). Male flies mutant for *boule* fail to enter the meiotic divisions, although their germline mitotic divisions are executed normally and primary spermatocytes are morphologically indistinguishable from wild type. As a result, *boule* mutants exhibit a cellular phenotype in which spermatid cysts contain 16 tetraploid cells rather than 64 haploid cells.

Although *boule* mutant germ cells do not execute meiosis, some spermatid differentiation does occur. Similar observations have been made in males mutant for *pelota* (Eberhart and Wasserman, 1995) and for *twine*, the homolog

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0012-1606/98 $25.00

of the cdc25 phosphatase (Alphey *et al.*, 1992; Courtot *et al.*, 1992; White-Cooper *et al.*, 1993). However, *boule* mutants exhibit only limited postmeiotic differentiation and, unlike *pelota* or *twine* mutants, do not progress to the sperm elongation and individualization stages.

The meiotic entry defect in *boule* males lies at the transition to metaphase of meiosis I (Eberhart *et al.*, 1996; Maines and Wasserman, 1998). Meiotic prophase appears wild-type in *boule* germ cells; chromosomes begin to condense and cyclin A enters nuclei, signaling the transition between the G_2 and M phases. However, chromosomes neither complete condensation nor congress at the metaphase plate. Furthermore, cyclin A persists in the nucleus, spindles do not form, and the nuclear envelope fails to break down. These defects place the requirement for Boule activity at, or prior to, the G_2/M transition.

Boule is closely related to the protein products of the vertebrate DAZL gene family (Reijo *et al.*, 1995, 1996; Cooke *et al.*, 1996; Shan *et al.*, 1996; Yen *et al.*, 1996; Seboun *et al.*, 1997; Houston *et al.*, 1998). This family includes the human Y chromosome *Deleted in Azoospermia* (*DAZ*) gene, as well as the DAZ-like autosomal genes identified in humans, mice, and *Xenopus*. All family members are found exclusively in the gonad, with the male testis being the predominant or, in some cases, exclusive site of expression.

There is strong genetic evidence that members of the DAZL family, like *boule*, are required for wild-type spermatogenesis. Y chromosome deletions spanning the human *DAZ* gene are associated with azoospermia or oligospermia (Reijo *et al.*, 1995, 1996; Vogt *et al.*, 1996; Mulhall *et al.*, 1997). At a cellular level, mutations in *DAZ* can result in a Sertoli cell-only phenotype (absence of germ cells), a maturation arrest phenotype (meiotic arrest), or an absence of detectable defects (Reijo *et al.*, 1995). Male and female mice null for the autosomal *dazl* locus are sterile; the observed absence of germ cells reveals a requirement for the gene in gamete differentiation (Ruggiu *et al.*, 1997).

Despite the difference in phenotypes among *DAZ*, *dazl*, and *boule*, the *DAZ* family members appear to have a high degree of functional homology. Expression of the *Xdazl* (*Xenopus DAZ*-like) gene in *Drosophila* via a cDNA construct rescues the *boule* meiotic entry phenotype (Houston *et al.*, 1998). Furthermore, *Xdazl* can function as an RNA binding protein *in vitro*. Thus, the *DAZ* family of RBPs is likely to fulfill similar roles in gamete development through conserved interactions with RNA.

In this work, we characterize Boule localization at different stages of spermatogenesis and the cell cycle. We find that Boule localization is biphasic in the germline, with nuclear localization in primary spermatocytes until near the end of the meiotic prophase and cytoplasmic localization from then onward. Furthermore, we find that Boule's nuclear, but not cytoplasmic, localization is dependent on the presence of a single fertility factor on the short arm of the Y chromosome. These findings allowed us to dissociate the nuclear localization of Boule from its essential role in meiosis and to formulate a model for Boule function in meiotic entry.

MATERIALS AND METHODS

Analysis of Squashed Testis Contents by Phase-Contrast Microscopy

For all analyses of testis contents, testes from 0- to 1-day-old males were dissected on a glass slide in a drop of testis Ringer's buffer (Ashburner, 1989). For phase-contrast microscopy, testis contents were released by making an incision at about one-third of the length from the apical end. The preparation was then gently squashed under the weight of a coverslip (Kemphues *et al.*, 1980).

Generation of Antiserum and Immunoblot Analysis

Polyclonal antiserum R383 was raised in a rabbit against bacterially expressed full-length Boule with an N-terminal $(His)_{10}$ tag (pET vector, Novagen). Dissected testes were homogenized in RIPAII buffer (50 mM Tris, pH 8.0, 250 mM NaCl, 0.1% SDS, 1% NP-40, and 0.5% deoxycholate), using 1 μl buffer for each pair of testes. After boiling for 5 min, the extracts were mixed with an equal volume of SDS–PAGE sample buffer. Extracts (10 testes equivalents per lane) were resolved on a 12% SDS–PAGE gel. Immunoblotting was performed as described (Gillespie and Wasserman, 1994) using a PVDF membrane (Millipore). The R383 antiserum was used at a 1:1000 dilution. Goat anti-rabbit conjugated to alkaline phosphatase (Calbiochem) was used as the secondary antibody at a 1:1000 dilution.

Immunofluorescence Staining and Microscopy

Fixation and indirect immunofluorescence staining of squashed testes followed the method of Gatti and colleagues (Cenci *et al.*, 1994). Boule staining was done using R383 at 1:1000. RB97D staining was done using a rabbit polyclonal antiserum at 1:100 (Heatwole and Haynes, 1996). Donkey anti-rabbit conjugated to Cy3 (Jackson Labs) was used at 1:200 or 1:500. Y loop staining was done using the X4-S5 mouse monoclonal (Glätzer, 1984) at 1:4. Donkey anti-mouse conjugated to Cy2 (Jackson Labs) was used at 1:50. To examine RNase sensitivity, the primary antibody incubation step included 20 μl of 50 μg/ml DNase-free RNase (Boehringer Mannheim) or 20 μl of PBS (mock treatment). Fluorescence micrographs were recorded on a cool charge-coupled (CCD) digital imaging camera (Hamamatsu) mounted on a Leica DMRXE microscope and imported into Adobe Photoshop.

Generation of X/Ø Males and Y Loop Segmental Aneuploids

X/Ø males were generated by crossing females carrying an attached X chromosome [genotype *C(1)RM, y[1], pn[1], v[1]/Ø* or *C(1)RM, y[1], v[1], bb$^-$/Ø*] to wild-type (Oregon R) males. Segmental deletions of the Y were generated by crosses with T(X;Y) lines. The T(X;Y) lines carry reciprocal translocations between the X and Y chromosomes, with the X breakpoint in the proximal heterochromatin and the Y breakpoints distributed along the length of the Y chromosome. By combining two X;Y translocations flanking an internal region, or a single translocation flanking a terminal region,

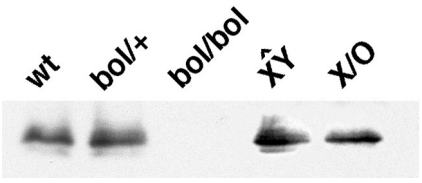

FIG. 1. Immunoblot analysis of Boule protein in testis extracts. Boule protein from the testes of wild-type (*wt*), *boule* heterozygous (*bol*/+), and *boule* mutant (*bol*/*bol*) attached XY and X/Ø males was analyzed by immunoblotting. The antigen detected has an apparent molecular weight of 32,000, slightly greater than the predicted molecular weight of 24,666. The attached XY stock was used to generate the X/Ø males and thus serves as a control for genetic background.

segmental deletions of the Y chromosome are generated (Goldstein *et al.*, 1982). To obtain males carrying terminal deletions (one element of the translocation), the T(X;Y) males were crossed to C(1)RM/Ø females. To obtain males carrying internal deletions required crossing males and females from different T(X;Y) lines flanking the region of interest. A given cross between the two T(X;Y) lines produced a deletion, and the reciprocal cross would produce a duplication for the region of interest.

Δ*kl-5* was generated by crossing T(X;Y) line V24 to C(1)RM/Ø. Δ*kl-5,3,2* was generated by crossing T(X;Y) line E16 to C(1)RM/Ø. Δ*kl-3,2* was generated by crossing T(X;Y) line V24 females to E16 males. Δ*kl-1, ks-1,2* was generated by crossing T(X;Y) line E16 males to wt females. Δ*kl-5,3,2,1* was generated by crossing T(X;Y) line F14 to C(1)RM/Ø. Δ*ks-1,2* was generated by crossing T(X;Y) line W19 to C(1)RM/Ø. Δ*ks-1* was generated by crossing T(X;Y) line V8 females to W19 males. Δ*ks-2* was generated by crossing T(X;Y) line V8 to C(1)RM/Ø.

RESULTS

Boule Protein Localization in Pre- and Postmeiotic Spermatocytes

We generated a polyclonal serum against bacterially expressed Boule protein that recognizes an antigen of appropriate size in wild-type, but not *boule* mutant testis extracts (Fig. 1). Using this serum, we examined Boule localization in flattened germ cell preparations (testis squashes) by immunocytochemistry. In wild-type testes, we first detected Boule at the beginning of the spermatocyte growth phase, following completion of the premeiotic S phase. Boule staining was diffuse at this stage (data not shown). However, as the primary spermatocytes matured, Boule became highly concentrated in nuclei (Fig. 2A, left). Within these nuclei, Boule protein staining was not uniform but was instead consistently restricted to a crescent-shaped region.

To define the nature of the subnuclear localization of Boule, we compared the Boule staining pattern to prominent nuclear structures. Comparison with chromatin localization, as assayed with the DNA-binding dye Hoechst, indicated that the Boule crescent was near, but not coincident with, one of the three readily detectable chromosome pairs (Fig. 2A, center). We identified the associated chromosomes as the XY pair, based on the distinct morphology of this chromosome set relative to the autosomes (Cenci *et al.*, 1994). In most cells, Boule staining did not overlap with that of the XY pair, but was very frequently found nearby, usually at the periphery of the nucleus.

Since the X and Y chromosomes contain the rRNA loci and are therefore closely associated with the nucleolus, we used phase-contrast microscopy to determine the location of Boule relative to this organelle. The spermatocyte nucleolus appears as a roughly spherical, phase-dense structure within the nucleus. As shown below (see Fig. 5A), the crescent-shaped region of Boule staining very often abutted and embraced the nucleolus, but was not coincident with it. The nuclear staining pattern was absent in the bol^1 mutant (Fig. 2E), confirming that this pattern is representative of Boule. The distinctive Boule crescent thus represents a perinucleolar pattern of protein localization.

Boule nuclear localization persisted until the end of the spermatocyte growth phase. At the time of nucleolar breakdown, prior to meiosis, Boule was in the cytoplasm rather than the nucleus (Fig. 2B). As a result, during meiosis Boule was excluded from the chromatin-containing region and was largely cytoplasmic (Fig. 2C). Boule remained cytoplasmic in postmeiotic spermatids, where it appeared to be uniformly distributed (Fig. 2D). Boule localization in spermatocytes is thus biphasic, being first nuclear and then cytoplasmic.

We also examined Boule localization in *twine* mutant spermatocytes, which fail to activate the cell cycle oscillator and hence do not undergo the meiotic divisions. Boule underwent a wild-type transition from the nucleus to the cytoplasm in the *twine* mutant (data not shown). We conclude that the translocation of Boule to the cytoplasm is not dependent on meiotic entry.

Boule Nuclear Localization Is RNA Dependent

Since *boule* encodes a putative RNA-binding protein, we wished to determine if either or both phases of Boule localization are RNA dependent. We therefore performed immunocytochemistry on testis squashes in the presence and absence of RNase. Mock-treated primary spermatocytes, shown in Fig. 3A, had the same pattern of localization as described above. In contrast, Boule nuclear localization was absent from primary spermatocytes in samples treated with RNase (Fig. 3B, arrows). However, the cytoplasmic localization of Boule in postmeiotic onion stage spermatids was unaffected by RNase treatment (Fig. 3B, arrowheads), indicating that nuclear but not cytoplasmic Boule localization is primarily dependent on RNA.

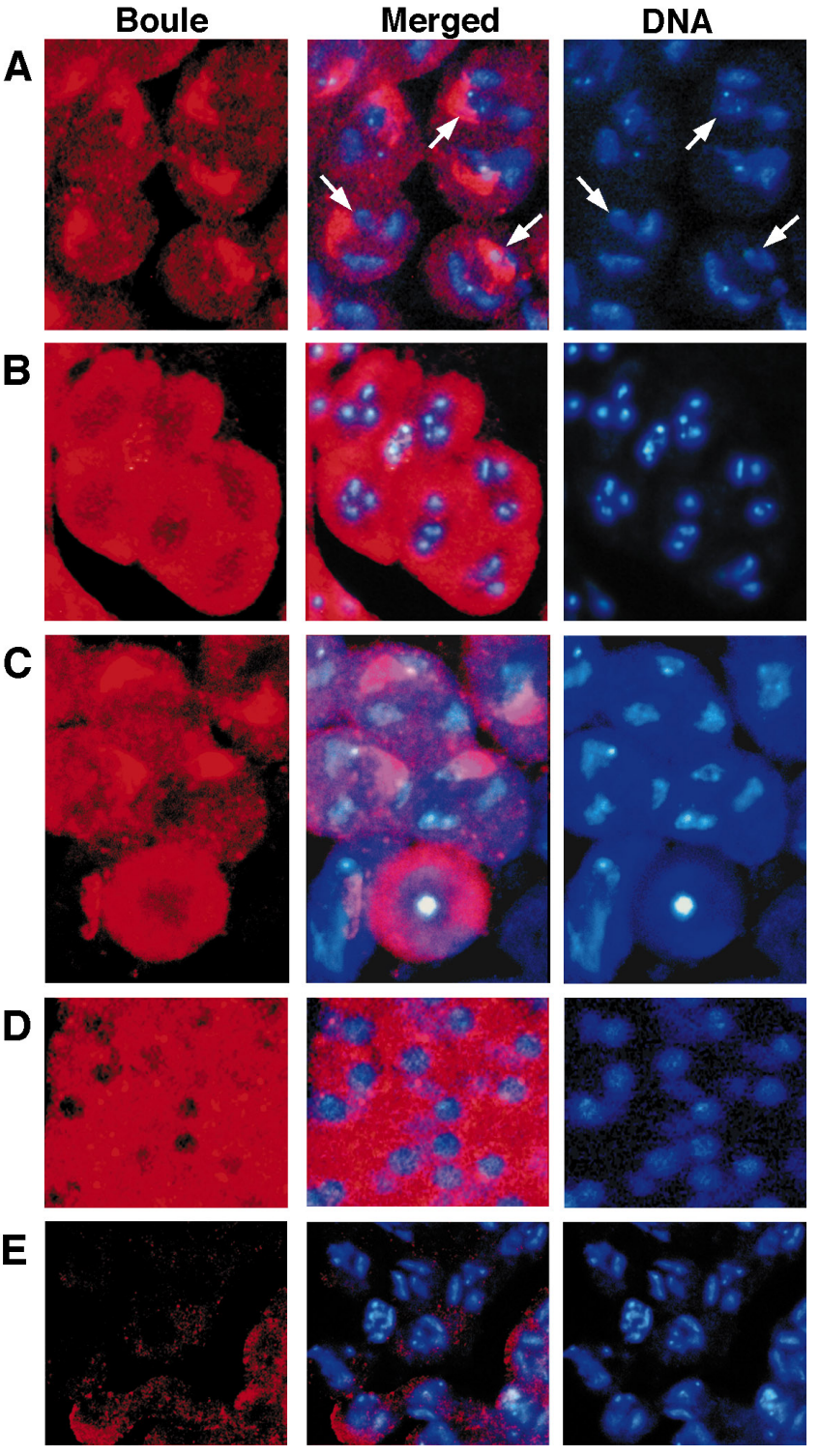

FIG. 2. Boule protein localization in wild-type and mutant testis squashes. For each row, Boule staining is shown on the left in red, DNA staining is shown on the right in blue, and a merged image is shown in the center. (A) Late primary spermatocytes. In these premeiotic cells, Boule localizes to a crescent-shaped region within the nucleus. Comparison with the DNA staining pattern reveals that the Boule crescent is adjacent to the XY pair, the smallest and most diffuse of the three significant chromatin masses (arrows). (B) Mature spermatocytes. The end of the primary spermatocyte stage and progression to prophase of meiosis I are distinguishable by the breakdown of the large nucleolus seen by phase-contrast microscopy (not shown) and the beginning of chromosome condensation, as seen by Hoechst staining. At this stage, Boule is found in the cytoplasm of the cells and the subnuclear crescent is absent. (C) Meiotic cell. The cell at the bottom of this panel is in metaphase of meiosis I, as apparent from the congression of the chromosomes at the metaphase plate. Boule staining in this cell is exclusively cytoplasmic, indicating that the translocation of Boule from the nucleus to the cytoplasm has already occurred. The remaining cells are late primary spermatocytes in which Boule is still nuclear. (D) Onion-stage spermatids. In these postmeiotic cells Boule is cytoplasmic. (E) Late primary spermatocytes from a *bol*^{1} mutant testis. No staining above background levels is seen.

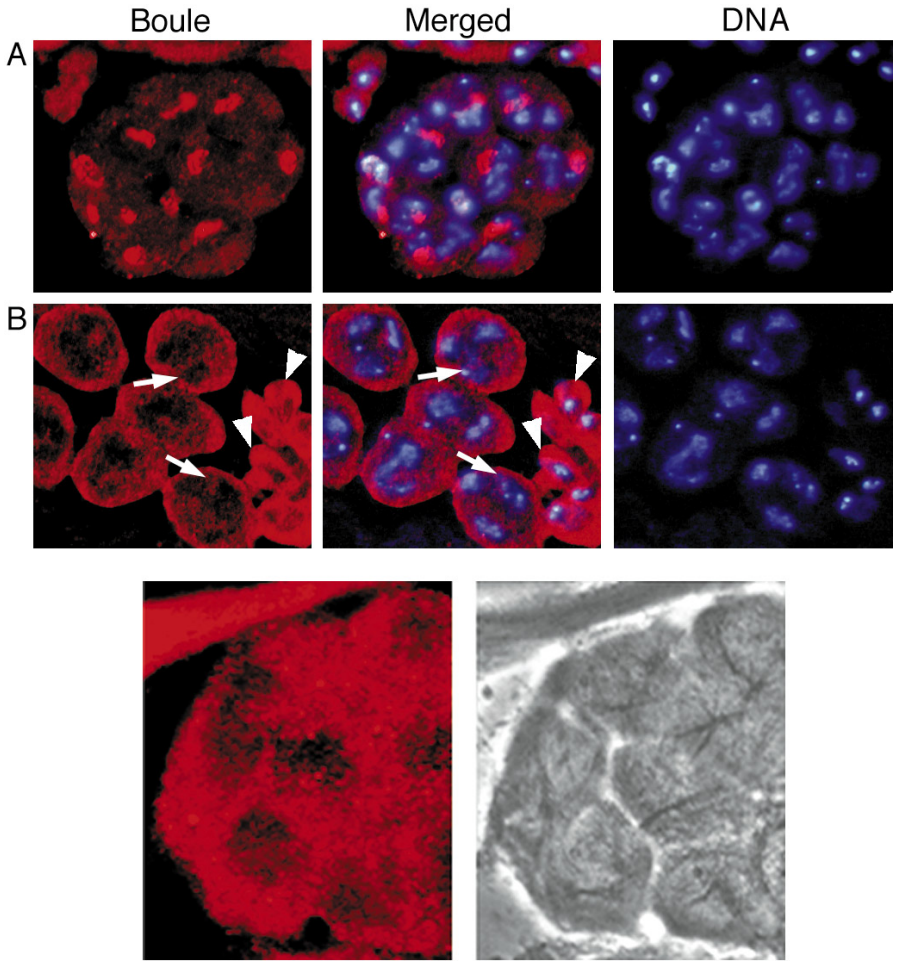

FIG. 3. RNase sensitivity of Boule staining in primary spermatocytes. In both rows, Boule staining is shown on the left in red, DNA staining is shown on the right in blue, and a merged image is shown in the center. (A) Mock-treated sample. Staining patterns in premeiotic and postmeiotic cells are comparable to wild-type samples shown in Fig. 1. (B) RNase-treated sample. The distinctive crescent of Boule staining is absent in primary spermatocyte nuclei (arrows). In postmeiotic onion-stage cells (arrowheads), however, Boule staining is unaffected and cytoplasmic.

FIG. 4. Boule staining in X/Ø primary spermatocytes. (Left) Boule staining, shown in red, is cytoplasmic and the distinctive perinucleolar crescent is absent. (Right) Phase image. The cells display crystalline inclusions characteristic of X/Ø spermatocytes.

Nuclear Boule Localization Is Not Required for Meiotic Entry

In the course of dissecting the mechanism for Boule nuclear localization, we discovered a means of uncoupling that localization from Boule's function in meiosis. Whereas in wild-type spermatocytes Boule was exclusively nuclear, in spermatocytes of X/Ø males, which specifically lack the Y chromosome, Boule was present at wild-type levels but wholly cytoplasmic (Figs. 1 and 4). Although X/Ø males are sterile, with an arrest of spermatogenesis at the late spermatid stage, premeiotic stages of spermatogenesis appear wild-type and meiotic entry is unaffected (Bridges, 1916; Kiefer, 1966; Meyer, 1968). Thus, the formation of the Boule subnuclear crescent is not essential to Boule's function in meiosis.

The Boule Nuclear Localization Determinant Maps to the Short Arm of the Y Chromosome

The results presented above strongly suggest that the cytoplasmic Boule protein in wild-type late spermatocytes is sufficient to mediate entry into meiosis. We were curious nonetheless as to the possible function of nuclear Boule at earlier stages. Our results indicated that this nuclear localization was dependent on RNA and on the presence of the Y chromosome. The Y chromosome

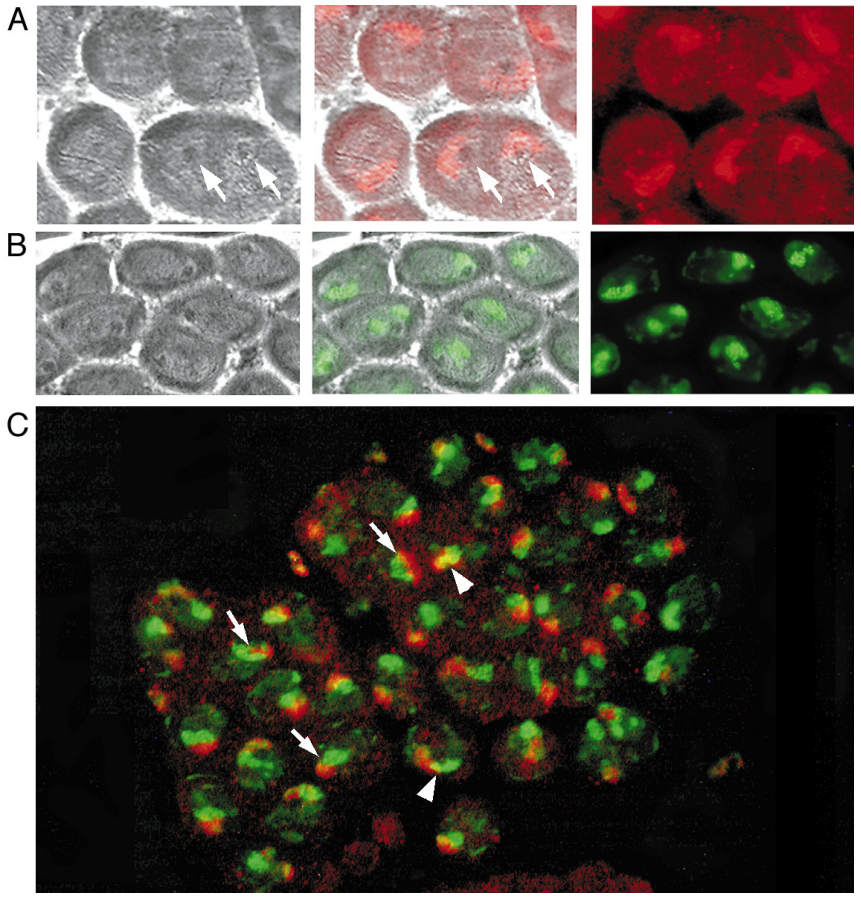

FIG. 5. Boule and Y loop antigen staining in wild-type primary spermatocytes. Boule staining is shown in red; Y loop antigen staining is shown in green. In the first two rows, the image on the left was acquired by phase-contrast microscopy, while that on the right was generated by indirect immunofluorescence. A merged image is shown in the center. (A) Boule localization. Nucleoli are evident as prominent, phase-dense structures within the nuclei (arrows). In most cells the crescent-like pattern of Boule staining is adjacent to, and partially encircles, the nucleolus. (B) Y loop antigen localization. The Y loop antigens, like Boule, frequently abut the nucleolus. (C) A merged image of spermatocytes stained for both Boule and the Y loop antigens. In some of the cells, there is a close association between the two patterns (arrows) and occasional overlap, shown in yellow (arrowheads).

encodes six fertility loci, each spanning four or more megabases of DNA, that are heavily transcribed in the primary spermatocyte (Gatti and Pimpinelli, 1983; Bonaccorsi *et al.*, 1988). Indeed, several of the resulting lampbrush chromosome structures, or Y loops, are visible by phase microscopy at the nuclear periphery of unstained primary spermatocytes (Cenci *et al.*, 1994). We hypothesized, therefore, that Boule might bind to one or more Y loop structures.

To compare Y loop localization with that of Boule, we immunostained testis squashes with an antibody that recognizes Y loop associated antigens in *Drosophila* chromatin (Glätzer, 1984). This antiserum recognizes a subset of the Y loops, predominantly the antigen associated with the *kl-5* and to a lesser extent the *ks-1* fertility factors (see below). Like Boule, the Y loop antigens occupied a crescent-like region that was often adjacent to the nucleolus (compare Figs. 5A and B). In flattened spermatocytes double stained with anti-Boule and anti-Y loop sera (Fig. 5C), we found that Boule and the Y loop antigens localized to neighboring regions (arrows) that occasionally overlapped (arrowheads). The similarity and close spatial association of the localization patterns suggested that Boule associates with Y loops.

To delineate the region of the Y required for Boule nuclear localization, we carried out a deletion analysis. Four of the Y chromosome fertility factors lie on the long arm (*kl-1, kl-2, kl-3, kl-5*) and two on the short arm (*ks-1* and *ks-2*), with numbering on each arm ordered from proximal to distal. We analyzed eight distinct sets of Y chromosome synthetic deficiencies for alterations in Boule staining (Figs. 6 and 7). Only the three deficiencies affecting *ks-1* disrupted the nuclear localiza-

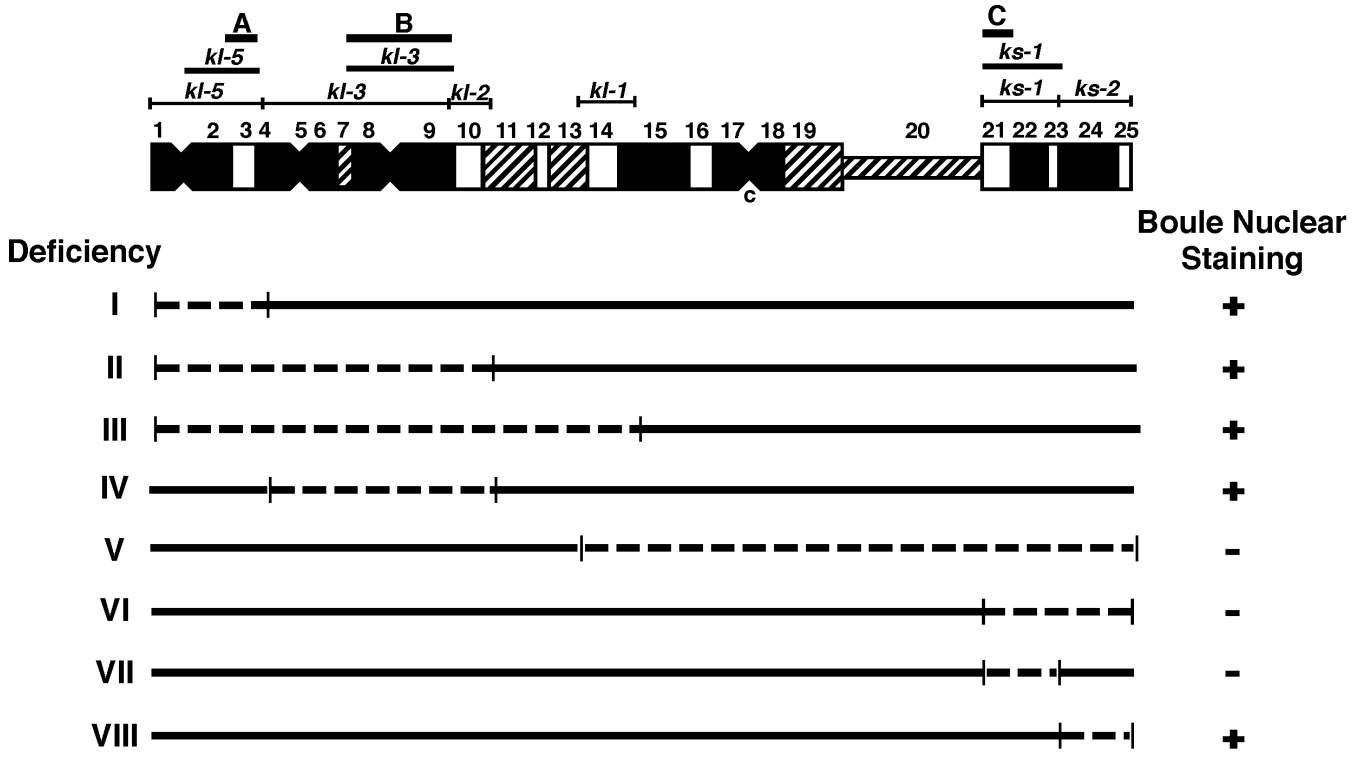

FIG. 6. Schematic of the Y chromosome and segmental deletions. The shaded diagram is a schematic representation of the Hoechst staining pattern of the *Drosophila melanogaster* Y chromosome (figure adapted from Gatti and Pimpinelli, 1983; Bonaccorsi *et al.*, 1988). The thin and thick lines immediately above the chromosome indicate the maximum and minimum sizes, respectively, of the *ks* and *kl* fertility factors. Above these lines are three bars indicating the loop-forming regions that give rise to lampbrush loops A, B, and C. Eight segmental deficiencies are diagrammed below the cytogenetic map, with dashed lines indicating the deficient regions. The factors removed by each deficiency are as follows: I, *kl-5;* II, *kl-5,3,2;* III, *kl-5,3,2,1;* IV, *kl-3,2;* V, *kl-1, ks-1,2;* VI, *ks-1,2;* VII, *ks-1;* and VIII, *ks-2.* To the right of each deficiency, a plus or minus designates the presence or absence of Boule nuclear localization on immunostaining.

tion of Boule. In all three such cases Boule was predominantly cytoplasmic in primary spermatocytes. Staining in later spermatids was not affected by any of the deficiencies.

These results indicate that Boule nuclear localization depends on the *ks-1* region of the Y. However, since the *ks-1*-dependent localization of the RNA binding protein RB97D (Heatwole and Haynes, 1996) is wild-type in a *boule* mutant (data not shown), the Y loop structure derived from the *ks-1* region is not substantially altered in the absence of Boule.

DISCUSSION

Biphasic Localization of Boule

Boule localization in wild-type males is biphasic. In premeiotic spermatocytes, Boule occupies a discrete subnuclear compartment. At meiosis, Boule translocates to the cytoplasm, where it remains during early spermatid differentiation. The movement of Boule, an RNA binding protein, thus parallels the movement of the messenger RNA population from its site of synthesis to its site of action.

Boule translocates from the nucleus to the cytoplasm just prior to the time at which a defect in spermatogenesis is first apparent in a *boule* mutant (Eberhart *et al.*, 1996). Mature primary spermatocytes in *boule* males appear wild-type, but fail to initiate the meiotic divisions. In theory, the requirement for Boule in meiotic entry could reflect an influence on RNA processing or storage in the nucleus, on RNA stabilization or translation in the cytoplasm, or on the translocation of RNA between these compartments. Our data favor a role in the cytoplasm. Nuclear Boule staining is abolished in X/Ø spermatocytes, yet these cells initiate the meiotic divisions in an apparently wild-type manner. Furthermore, although there are minor defects in the execution of meiosis in X/Ø testes, these defects map to fertility loci other than *ks-1*, the region we have found to be critical for Boule nuclear localization.

Y Chromosome Transcripts May Serve as Storage Sites for Boule Protein

If nuclear localization is not a prerequisite for Boule's function in meiotic entry, what purpose, if any, is served by

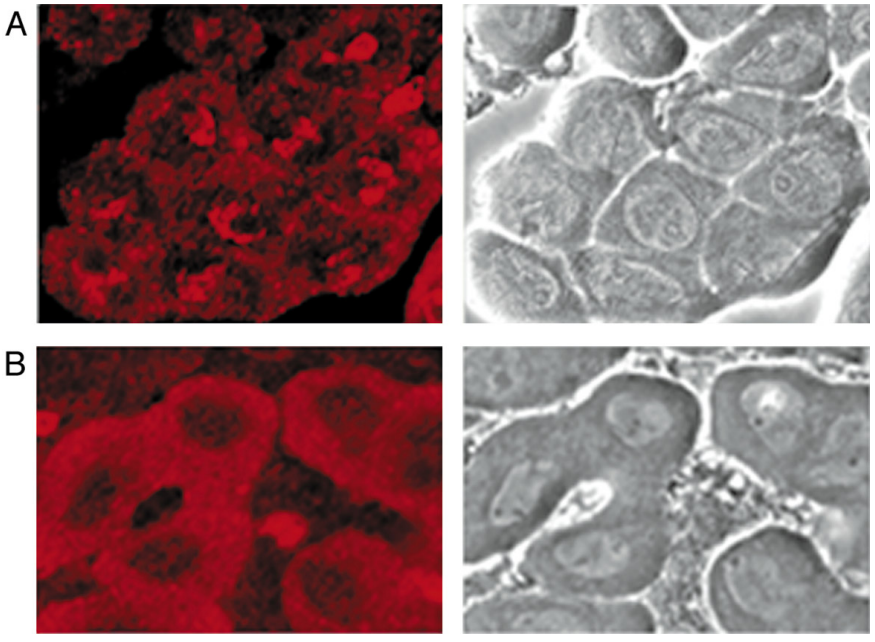

FIG. 7. Boule staining in Y chromosome segmental deficiency males. Images acquired by indirect immunofluorescence (left) and phase-contrast microscopy (right) are shown, with Boule staining shown in red. (A) Midgrowth phase spermatocytes from Δ*kl-5,3,2,1* males. As in wild-type spermatocytes, Boule appears in a perinucleolar crescent. (B) Late growth phase spermatocytes from Δ*ks-1* males. Boule staining is cytoplasmic. The distinctive perinucleolar pattern is absent from this and from all earlier stages.

the premeiotic accumulation of Boule in a perinucleolar compartment? One possibility is that nuclear localization is essential for the postmeiotic function of Boule. Although the tetraploid spermatids in *boule* mutants carry out some aspects of spermiogenesis, they do so to a much lesser extent than comparable cells in a *twine* mutant. However, our results argue that this function of Boule, like its role in meiotic entry, is independent of nuclear localization. In particular, Boule is cytoplasmic in male germ cells lacking just the *ks-1* region, yet spermatid differentiation in such cells is nearly wild-type.

An alternative role for Boule nuclear localization is to store Boule prior to its function in meiosis and spermatid differentiation. According to this model, the Y chromosome loops would serve to bind up Boule, consistent with the proposal that these loops serve a general role as stockpiles for RNA binding proteins (Hennig *et al.*, 1989; Hackstein and Hochstenbach, 1995). The *ks* and *kl* regions each consist mainly of satellite and other repetitive DNA sequences. The breakdown of the lampbrush Y chromosome loops, which occurs at the end of the spermatocyte growth phase (Cenci *et al.*, 1994), could serve in part to release Boule and other factors in a synchronous fashion.

There is evidence that other RNA binding proteins essential for male fertility are compartmentalized in a manner similar to that seen for Boule. *Drosophila* RB97D, an RRM protein required for male fertility (Heatwole and Haynes, 1996; Castrillon *et al.*, 1993), binds specifically to the Y chromosome *ks-1* loop in spermatocytes in an RNase-sensitive manner (Heatwole and Haynes, 1996). Furthermore, localization of the mammalian Boule homolog DAZ shifts from the nucleus to the cytoplasm during spermatogenesis (Reijo *et al.*, manuscript in preparation).

Boule Likely Affects the Stability or Translation of the Message Encoding an Essential Meiotic Factor

The results reported here suggest that Boule acts in the cytoplasm to effect meiotic entry. Given that Boule contains conserved RNA binding motifs, Boule likely interacts with mRNA molecules to influence their stability or translation. Boule may cooperate in this process with the product of the *pelota* gene, which is also required for meiotic entry. Since a *pelota* cDNA expression construct rescues the growth defects in yeast null for the *pelota* ortholog *dom34* (Eberhart and Wasserman, 1995; Davis and Engebrecht, 1998), and since *dom34* has been implicated in translational regulation (Davis and Engebrecht, 1998), *pelota* likely influences gene expression posttranscriptionally.

The meiotic arrest phenotype seen in *boule* mutant males presumably reflects the fact that the earliest acting target of this locus is an essential meiotic regulator. In mammals, processes other than meiosis appear to be the critical targets for the closely related *DAZ* and *dazl* gene products. What has likely been conserved, therefore, among the DAZ/Boule family is a general mechanism for translational regulation in gametogenesis.

ACKNOWLEDGMENTS

We thank William Mattox for the anti-Y loop serum, Susan Haynes for the anti-RB97D serum, and Maurizio Gatti for the T(X;Y) lines. We also thank David Page for communication of unpublished results, Stephen DiNardo for helpful discussions, and Alisha Tizenor for assistance with figure preparation. This work was funded by support from the Perot Family Foundation and an NIH MSTP training grant (to M.H.C), by fellowship support from NIH (to J.Z.M.), and by a grant from the Council for Tobacco Research (to S.A.W.).

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Received for publication August 15, 1998
Revised September 25, 1998
Accepted September 25, 1998