A small RNA in testis and brain: implications for male germ cell development

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Summary

BC1 RNA, a small non-coding RNA polymerase III transcript, is selectively targeted to dendritic domains of a subset of neurons in the rodent nervous system. It has been implicated in the regulation of local protein synthesis in postsynaptic microdomains. The gene encoding BC1 RNA has been suggested to be a master gene for repetitive ID elements that are found interspersed throughout rodent genomes. A prerequisite for the generation of repetitive elements through retroposition and subsequent transmission in the germline is expression of the master gene RNA in germ cells. To test this hypothesis, we have investigated expression of BC1 RNA in murine male germ cells. We report that BC1 RNA is expressed at substantial levels in a subset of male germ cells. Results from cell fractionation experiments, developmental analysis, and northern and in situ hybridization showed that the RNA was expressed in pre-meiotic spermatogonia, with particularly high amounts in syncytial ensembles of cells that are primed for synchronous spermatogenic differentiation. BC1 RNA continued to be expressed in spermatocytes, but expression levels decreased during further spermatogenic development, and low or negligible amounts of BC1 RNA were identified in round and elongating spermatids. The combined data indicate that BC1 RNA operates in groups of interconnected germ cells, including spermatogonia, where it may function in the mediation of translational control. At the same time, the identification of BC1 RNA in germ cells provides essential support for the hypothesis that repetitive ID elements in rodent genomes arose from the BC1 RNA gene through retroposition.

Key words: Neuronal, Testicular RNA, Repetitive elements, Retroposition, Spermatogonia, Spermatogenic development

Introduction

Dendritic BC1 RNA has been suggested to participate in the modulation of local translation-related processes in postsynaptic neuronal microdomains (reviewed by Tiedge et al., 1999; Brosius and Tiedge, 2001). BC1 RNA is a short non-translatable RNA polymerase III transcript that can be subdivided into three distinct molecular domains: a 5′ domain of 75 nucleotides, which is predicted to form a stable stem-loop secondary structure (Rozhdestvensky et al., 2001), a central A-rich region, and a unique 3′ part of 23 nucleotides. The 5′ BC1 domain has been shown to direct dendritic targeting of BC1 RNA and BC1-chimeric RNAs in neurons (Muslimov et al., 1997). The 5′ domain has also been reported to exhibit extensive sequence similarity with the family of repetitive ID elements (DeChiara and Brosius, 1987).

The original hypothesis of ID elements as markers for brain-specific gene expression (Sutcliffe et al., 1984) could not be substantiated (reviewed by Chikaraishi, 1986). Thus, subsequently it has been proposed that BC1 RNA is the actual functional entity (DeChiara and Brosius, 1987), and that its single gene is in fact a master gene from which ID elements were derived, either directly or through intermediates, by way of retroposition (Kim et al., 1994; Shen et al., 1997). In this mechanism, an RNA is first reverse transcribed into cDNA, which is then inserted into genomic DNA. A fundamental question that remained to be addressed in this scenario was whether BC1 RNA is in fact expressed in germ cells: only retroposition in such cells would result in germ-line transmission and amplification in the genome (Kim et al., 1994; Shen et al., 1997).

It was important, for this reason, to examine expression of BC1 RNA in germ cells. We report here that BC1 RNA is expressed at significant levels during early phases of male germ cell development. These results validate the retroposition model for the origin of repetitive ID elements. At the same time, they indicate that aspects of protein synthesis in male germ cells, such as RNA localization and regulation of local translation, may be subject to BC1-mediated control during spermatogenic development.

Materials and Methods

Animals, tissues and cells

Male Swiss Webster mice and Sprague-Dawley rats were used as sources for wild-type testis tissues. Unless otherwise noted, animals were 6-8 weeks old. For developmental studies, testes were obtained from mice at postnatal day 7 (p7), between postnatal days 7 and 8 (p7/8), and at postnatal day 17 (p17). For the purpose of this

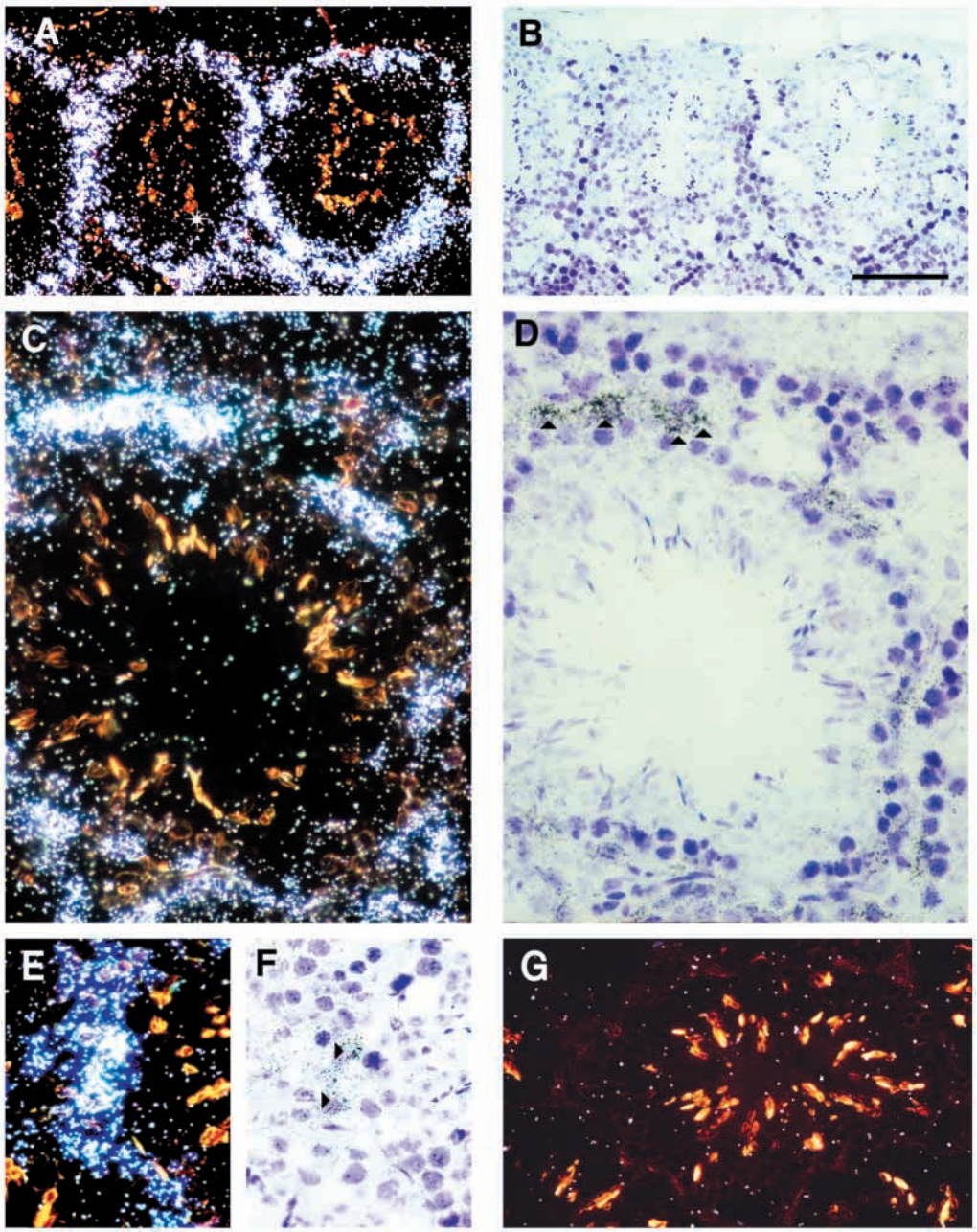

Fig. 1. Distribution of BC1 RNA in murine testis. Signal is represented as white autoradiographic silver grains in dark field photomicrographs. Labeling patterns in seminiferous tubules indicate an annular but patchy distribution of BC1 RNA along the tubular periphery (see low-power photomicrograph A with corresponding bright field B). Central adluminal regions are devoid of significant labeling (asterisk in A). Highest relative BC1 labeling appears concentrated in distinct patches overlying several neighboring cells (C-F; arrowheads in D,F) that are located adjacent to the basement membrane. Such cells often show the irregular nuclear outlines typical of enlarged A_{al} spermatogonia (Huckins, 1971). A sense-strand control is shown in G. Gold-colored silhouettes apparent in these and other dark field photomicrographs are produced by reflections from elongated spermatids in the adluminal regions of seminiferous tubules. (A,C,E,G) Dark field photomicrographs; (B,D,F) bright field photomicrographs. Bar, 100 μm (A,B); 50 μm (G); 30 μm (C-F).

analysis, the first 24 hours after birth are defined as postnatal day 1. Mutant mice of the W series genotype (Mintz and Russell, 1957) were used for work with germ-cell-deficient testes (Zakeri and Wolgemuth, 1987). Testes obtained from homozygous (W^V/W) mutant mice contain few if any germ cells although they exhibit a normal interstitium and seminiferous tubules lined by Sertoli cells (Coulumbre and Russell, 1954). Homozygous (W^V/W) mutant mice and wild-type (+/+) littermates were obtained through $W/+ \times W^V/+$ matings. All investigations involving animals were conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH Guide).

Separation of mouse testis cells was performed as described earlier (Wolgemuth et al., 1985). This preparation yields enriched populations of spermatogenic cells in meiotic prophase (mostly pachytene), of early (round) spermatids, and of cytoplasmic fragments of elongating spermatids and residual bodies (Ponzetto and Wolgemuth, 1985; Wolgemuth et al., 1985; Zakeri et al., 1988).

Isolation and analysis of RNA

Total and poly(A)$^+$ RNA were isolated as described before (Zakeri et al., 1988; Chen et al., 1997a; Chen et al., 1997b). RNA was fractionated on agarose-formaldehyde gels, transferred to GeneScreen Plus membranes (New England Nuclear, Boston, MA), and immobilized by UV-illumination (Church and Gilbert, 1984). Integrity as well as equal loading and transfer of RNA was verified by ethidium bromide staining or by UV illumination of the membrane subsequent to transfer (Sambrook and Russell, 2001). Membranes were used for hybridization only if 28S and 18S rRNA bands were of equal intensities in all loaded lanes. Membranes were then hybridized to ^{32}P-endlabeled oligodeoxynucleotide probe HT005 (Chen et al., 1997b). This probe is complementary to the 60 3′-most nucleotides of BC1 RNA (DeChiara and Brosius, 1987). It was hybridized at 42°C in 1 M NaCl, 0.5 M Tris-HCl (pH 7.5), 5× Denhardt's reagent (Sambrook and Russell, 2001), 1% sodium dodecyl sulfate (SDS), 0.1 mg/ml yeast tRNA. The membranes were washed three times at 55°C in 0.5× SSC

(1× SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 0.1% SDS for 30 minutes.

In situ hybridization

Freshly removed testis tissues were quick-frozen in liquid nitrogen and embedded in TissueTek OCT embedding medium (Sakura Finetek, Torrance, CA). Tissue sections, prepared at 10 μm thickness on a Bright Microtome Cryostat (Hacker, Fairfield, NJ), were collected on gelatin and poly-L-lysine coated microscope slides (Tiedge, 1991).

Plasmid pMK1 (Tiedge et al., 1991) was used to generate RNA probes for the detection of BC1 RNA in tissue sections. ^{35}S-labelled probes were transcribed from linearized templates, using T3 (for sense strand) or T7 (for antisense strand) RNA polymerase. In situ hybridization experiments were performed as described previously (Tiedge, 1991; Tiedge et al., 1991). Prior to pre-hybridization, tissue sections were fixed with 4% formaldehyde (made freshly from paraformaldehyde) in 0.1 M sodium phosphate buffer pH 8.0.

Results

BC1 RNA is expressed in mouse testis

Male germ cell development proceeds in a peripheral-to-central fashion in seminiferous tubules in testis (reviewed by Willison and Ashworth, 1987). A_{single} (A_s) spermatogonia are the stem cells of spermatogenesis (reviewed by De Rooij and Grootegoed, 1998) that either remain as stem cells in mitotic cycles, or initiate, in several consecutive steps, differentiation into subsequent types of A and B spermatogonia that are committed to further spermatogenic development. The daughter cells of B spermatogonia (preleptotene spermatocytes) enter into a first meiotic cycle during which the cells are collectively referred to as primary spermatocytes. Secondary spermatocytes, the product of the first meiotic division, undergo a second meiotic division to produce spermatids. The subsequent process of spermiogenesis is a gradual development of round spermatocytes into spermatozoa, which are finally released into the lumina of seminiferous tubules (Willison and Ashworth, 1987; De Rooij and Grootegoed, 1998).

We used in situ hybridization in our initial approach to identify and localize BC1 RNA in male gonads. BC1 expression patterns were highly distinctive in mouse testis (Fig. 1). Labeling signal indicating the presence of BC1 RNA was seen concentrated in the basal (peripheral) layers of seminiferous tubules. Highest relative BC1 levels were detected in the outermost periphery. These layers are rich in

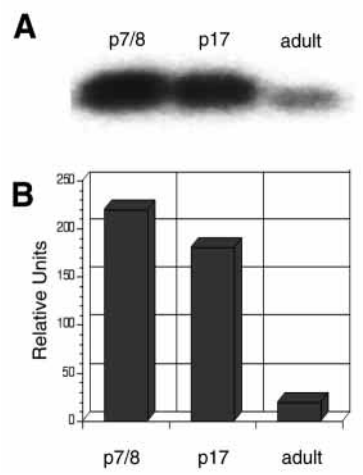

Fig. 2. Northern hybridization with total RNA from developing testes. (A) 10 μg were loaded per lane. No bands other than the ones shown were detected in this and the following northern hybridization experiments using BC1-specific probe HT005. Equal loading was verified as described in Materials and Methods. (B) Labeling intensities were quantified by phosphorimaging (Molecular Dynamics SF).

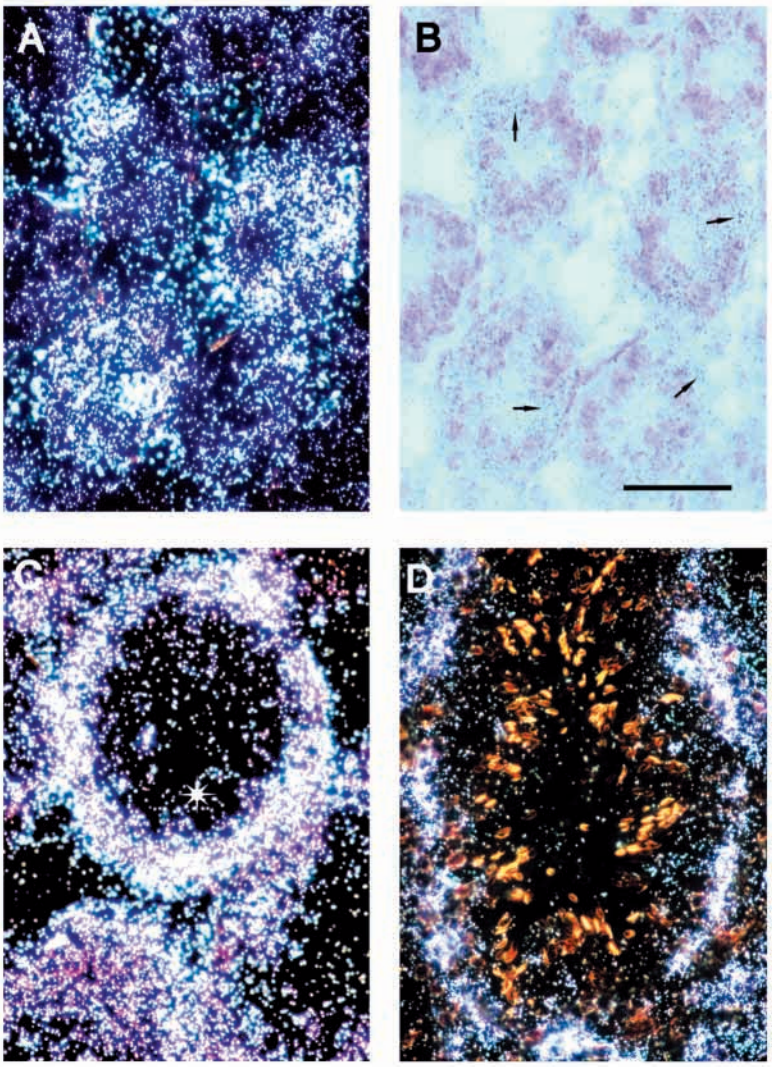

Fig. 3. BC1 expression in developing seminiferous tubules. (A,B) At postnatal day 7 (p7), BC1 labeling in developing tubules is robust but heterogeneous. Arrows indicate hotspots of BC1 expression in several tubules. Note that more heavily counterstained cells typically exhibit little or no specific BC1 labeling. (C) At p17, only cells in peripheral-most layers show strong labeling; asterisk indicates the position of cells in adluminal layers (presumably spermatocytes) that exhibit little specific labeling. (D) The adult expression pattern is shown for comparison. Exposure time for D was three times longer than for A-C. (A,C,D) Dark field photomicrographs; (B) bright field photomicrograph. Bar: 40 μm (A,B); 50 μm (C,D).

spermatogonia but also contain other cell types such as non-spermatogenic Sertoli cells and, during epithelial stages VI to VIII (Fig. 1A,B), significant numbers of preleptotene spermatocytes. BC1 expression levels were seen decreasing in a peripheral-to-central gradient in seminiferous tubules. Labeling in layers adjacent to basal layers (rich in spermatocytes) was thus weaker than in basal layers. Little if any signal was detectable in central adluminal regions that are rich in elongating spermatids (Fig. 1A-D). Equivalent results were obtained with rat testis (data not shown). No specific BC1 labeling signal was detectable in either rat or mouse epididymis (not shown).

The distribution of the labeling signal in seminiferous tubules is consistent with the notion that BC1 RNA is expressed at highest relative levels in spermatogonia and early spermatocytes, but disappears from germ cells as these progress through subsequent spermatogenic development. At times, particularly during epithelial stages VI and VII, remarkably strong labeling was observed concentrated in distinct patches, typically overlying clusters of several neighboring cells along the extreme tubular periphery (Fig. 1C-F). Location, light nuclear counterstain, and a relatively large size would provisionally identify such cells as A_{al} spermatogonia (aligned type A spermatogonia). These are undifferentiated spermatogonia (proliferating predominantly through stages VII to XII of the spermatogenic cycle) that are aligned in a chain-like fashion and are connected by intercellular cytoplasmic bridges (reviewed by De Rooij, 1998; De Rooij and Grootegoed, 1998). Such syncytial arrangements are considered important for the synchronization of subsequent spermatogonial differentiation (De Rooij, 1998). While the intensity of the labeling signal over such cellular clusters typically gives them a striking appearance, it should be emphasized that these cells constitute a clear minority among all labeled cells. Thus, the bulk of BC1-expressing cells is likely to consist of other types of spermatogonia and/or spermatocytes (e.g. preleptotene, see above), even though relative expression levels per cell may not be quite as high.

These data are potentially directly relevant to the function of BC1 RNA in germ cells. However, the results described above required substantiation because light microscopic in situ hybridization cannot provide the resolution necessary to ascribe the labeling signal unambiguously to a particular cell type. Thus, in the above experiments, no definitive distinction could be made between spermatogonia and spermatocytes on one hand, or between spermatogenic and non-spermatogenic cell types (e.g. Sertoli cells) on the other. It was therefore necessary to ascertain the presence of BC1 RNA in spermatogonia, spermatocytes, and/or other testicular cells by using additional experimental approaches.

Expression of BC1 RNA in testis is developmentally regulated

In developing prepubertal testis, different spermatogenic cell types make their initial appearances in sequential order. In murine testis, meiosis does not usually begin until after postnatal day 8, prior to which date most seminiferous tubules contain spermatogonia (and Sertoli cells) but not subsequent germ cell types (Nebel et al., 1961; Bellvé et al., 1977). However, it should be cautioned that because of strain differences, the precise timing may be variable (Kluin et al., 1982). By postnatal day 17, seminiferous tubules contain (in addition to spermatogonia) spermatocytes at various phases of meiotic development. Spermatids typically first appear after postnatal day 17. We took advantage of this well-defined temporal progression to establish the regulation of BC1 expression in developing testis, and to identify BC1-expressing cell types at various developmental stages.

We examined mouse testes from animals between postnatal days 7 and 8 (p7/8; spermatogenic cell types represented: mostly spermatogonia), from animals at postnatal day 17 (p17; spermatogenic cell types represented: mostly spermatogonia and spermatocytes), and from adult animals (all spermatogenic cell types represented). Northern hybridization with a probe specific for BC1 RNA revealed that relative BC1 expression levels were highest at p7/8, and showed a substantial decrease between then and adulthood (Fig. 2). The strong BC1 hybridization signal obtained with testes from p7/8 animals indicates that developing spermatogonia express significant amounts of BC1 RNA, although we cannot rule out contributions from early spermatocytes that may have been present at this time. The sharp decrease in BC1 signal intensities between postnatal day 17 and adult age is most probably due to dilution of BC1 expression in testis as a result of increasing development of non-expressing cell types. However, a simultaneous decrease of BC1 RNA levels in expressing cell types is also possible.

These data were confirmed and extended by in situ hybridization (Fig. 3). The earliest time point analyzed was postnatal day 7 (p7) (i.e. slightly earlier than in the above northern experiments). A robust BC1 hybridization signal was seen in seminiferous tubules of this developmental stage (Fig. 3A,B). Care was taken to ascertain cell types present in the tubules screened. We detected Sertoli cells and spermatogonia, but not subsequent types of spermatogenic cells, at this developmental stage. The results therefore indicate that BC1-expressing spermatogenic cells at this developmental stage are spermatogonia. The BC1 labeling pattern in p7 seminiferous tubules was heterogeneous as a significant percentage of cells in any given tubule remained unlabeled. These unlabeled cells are presumably non-spermatogenic Sertoli cells that do not express BC1 RNA (see below). Nonetheless, both northern hybridization and in situ hybridization confirmed that, compared with adult testis, average signal intensities were substantially higher at the end of the first postnatal week, thus suggesting rather high BC1 copy numbers per cell in expressing spermatogonia. At p17, labeling remained strong, but the more centrally located cells (most likely spermatocytes) exhibited a much lower BC1 hybridization signal (Fig. 3C). In adult testis, overall BC1 labeling signals were lower than at postnatal day 17: signal intensities remained low in adluminal tubular regions, but they also decreased in peripheral regions (Fig. 3D; please note that autoradiographic exposure time was three times longer for Fig. 3D than for Fig. 3A-C).

In summary, the results obtained with developing testes suggest that BC1 RNA is initially expressed in spermatogonia at rather robust levels. In subsequently differentiating cell types, levels of BC1 RNA appear to decrease gradually until little or no labeling remains detectable in elongating spermatids. In addition, expression levels in peripherally located BC1-positive cell types were observed to decrease during the late phase of postnatal

development, indicating that BC1 expression in testis is not only cell-type specific, but also developmentally regulated.

BC1-expressing cells in testis are identified as spermatogonia and spermatocytes

The morphological and developmental data point to spermatogonia and spermatocytes as the main BC1-expressing cell types in testis. However, for lack of resolution, they do not allow us to discriminate these cell types from non-germinal ones. To establish whether BC1 RNA is in fact expressed in germ cells and/or in non-spermatogenic cells in testis, we used germ-cell deficient testes obtained from homozygous progeny (W^V/W) of W series mutant mice (Zakeri and Wolgemuth, 1987). Gonads from such homozygous animals are virtually devoid of germ cells (Coulumbre and Russell, 1954; Mintz and Russell, 1957). We analyzed W^V/W mutant testes for the presence of BC1 RNA by both northern and in situ hybridization.

Northern hybridization revealed a robust signal in testis from wildtype (+/+) mice (Fig. 4E). In germ-line deficient W^V/W animals, in contrast, a hybridization signal was barely detectable. These results were confirmed by in situ hybridization. With germ cells absent, non-germinal cells are located peripherally in mutant seminiferous tubules. Low-level labeling was observed over such cells, but labeling intensities here were not significantly higher than over central lumina that are devoid of cells (Fig. 4A-D). We conclude that little or no BC1 RNA is expressed in non-germinal cells of W^V/W mutant testis, and that BC1 expression in wildtype testis is therefore attributable to germ cells.

These results, together with the developmental data and the fact that the highest relative BC1 expression levels were observed in the extreme periphery of wildtype seminiferous tubules, support the notion that spermatogonia and spermatocytes are the most prominent BC1-expressing cell types in testis. To test this hypothesis, and to evaluate the respective relative contributions to BC1 expression by different types of germ cells, we analyzed enriched populations of testicular cells at specific stages of spermatogenesis. Methods for separation of such cells have previously been described and are routinely used by us (Ponzetto and Wolgemuth, 1985; Wolgemuth et al., 1986; Zakeri et al., 1988). Using this approach, cells were separated into enriched populations of spermatocytes in the prophase stage of meiosis (mainly pachytene), of early (i.e. round) spermatids, and of cytoplasmic fragments of elongating spermatids and residual bodies. RNA isolated from these fractions was then analyzed by northern hybridization.

BC1 RNA was detectable at significant levels in total RNA and in poly(A)$^+$ RNA from entire testis, but not in poly(A)$^-$ RNA (Fig. 4F). BC1 RNA is known to contain a central A-rich region, and previously it has been identified in the poly(A)$^+$ RNA fraction from rat neural tissues (DeChiara and Brosius, 1987). Total RNA from spermatogenic cells in meiotic prophase (predominantly pachytene spermatocytes) showed a significantly lower BC1 RNA hybridization signal than total RNA from entire testis (Fig. 4F). Little or no BC1 signal was observed with total RNA from early spermatids and total RNA from elongating spermatids/residual bodies. In these latter cases, the hybridization signal was barely above background (Fig. 4F), confirming that spermatids contain little if any BC1 RNA.

In summary, the combined results of these and previous experiments indicate that BC1 expression in seminiferous tubules is to a significant part attributable to spermatogonia, with additional contributions from spermatocytes. The identification of spermatogonia as a prominent BC1-expressing cell type in testis also supports our original notion (see above) that the clustered, most strongly labeled cells observed at the extreme periphery of seminiferous tubules are in fact syncytial ensembles of spermatogonia. These results are of direct relevance for the functional role of BC1 RNA in male germ cells, as will be discussed below.

Discussion

Dendritic BC1 RNA is a neuronal small RNA that is not normally expressed in non-neuronal somatic cell types (reviewed by Brosius and Tiedge, 2001). The identification of BC1 RNA in male germ cells, as reported in this paper, is of dual significance: (1) BC1 expression in the germ line is a prerequisite for the propagation of ID repetitive elements in rodent genomes; and (2) high levels of BC1 RNA in early spermatogenic development indicate a functional role in such cells. We will discuss the implications of these two conclusions in turn.

Germ-line retroposition

By showing that BC1 RNA is expressed in spermatogenic germ cells, we established what has until now been a missing link between BC1 RNA and repetitive ID elements. ID elements, which show a high degree of sequence similarity to the 5′ domain of BC1 RNA, form a subtype of short interspersed repetitive elements (SINEs) that are distributed at high but divergent copy numbers throughout rodent genomes (Sapienza and St-Jacques, 1986; Kass et al., 1996). Our results thus provide prerequisite support for the theory that ID elements can be generated by germ-line retroposition of BC1 RNA (DeChiara and Brosius, 1987; Deininger et al., 1992; Kim et al., 1994; Shen et al., 1997; Aleman et al., 2000). Following self-priming through association of the 3′ terminal series of U-residues with the central A-rich region (Kim et al., 1994; Shen et al., 1997), BC1 RNA becomes a substrate for cellular or viral reverse transcriptase (Brosius and Tiedge, 1996). Reverse transcription of the BC1 5′ domain and subsequent integration into germ-line DNA can thus account for the generation of high numbers of ID elements in the genome. The fact that BC1 RNA is expressed in male germ cells, as reported here, indicates that germ-line retroposition and transmission of the 5′ domain are potentially ongoing processes in modern rodents, thus continuing to contribute to genomic diversification (Brosius, 1999). Furthermore, this process is likely to be efficient given the rather robust steady-state levels of BC1 RNA observed in spermatogonia. Although germ-line retroposition will often prove neutral or even deleterious, depending on the site of genomic integration, insertion of 5′ BC1 domains into target gene sites corresponding to untranslated mRNA regions may in certain cases add functionality to such transcripts. ‘Retroposed functionality’ may encompass altered RNA stability, translatability or localization competence, hypotheses that we plan to address in future research.

Cell-type specific expression of BC1 RNA in the course of spermatogenic development

Spatiotemporal BC1 expression patterns in seminiferous tubules are highly distinctive. Results with germ-cell deficient animals indicate that BC1 RNA is not expressed in Sertoli or other non-spermatogenic cells in testis. Early onset of BC1 expression in p7 testes documents substantial amounts of the RNA in spermatogonia. Developmental analysis, cell separation experiments and in situ hybridization further identify BC1 RNA in spermatocytes, albeit at lower average levels. In round and elongating spermatids, the RNA is not detectable at significant levels. Taken together, these results therefore suggest that BC1 RNA is expressed at high levels in spermatogonia (as well as in cells directly derived from them, i.e. preleptotene spermatocytes). By the time spermatogenic development has reached the pachytene spermatocyte stage, BC1 expression levels have significantly decreased. BC1 RNA disappears from male germ cells by the time development has proceeded to the early spermatid stage.

Since spermatocytes derive from spermatogonia, the origin of BC1 RNA in the former may be explained by 'carry-over' from the latter. Alternatively, BC1 RNA may still be transcribed in spermatocytes; if so, transcription would have to be assumed to proceed at gradually reduced levels (and/or be offset by an increasingly higher rate of degradation), particularly in pachytene spermatocytes. At any rate, BC1 transcription ceases, and pre-existing BC1 RNA is eliminated, by the end of spermatocyte development at the latest. BC1 RNA levels remain negligible during spermiogenesis (the developmental interval spanning differentiation from early spermatids to spermatozoa), even though RNA synthesis is known to continue about halfway through this period (Kierszenbaum and Tres, 1978). BC1 expression is thus a hallmark of early but not late stages of spermatogenic development.

Functional relevance of BC1 expression in spermatogonia and spermatocytes

The characteristic and specific expression of BC1 RNA in male germ cells, in particular in spermatogonia and early spermatocytes, indicates a functional role in spermatogenic development. In neurons, BC1 RNA has been suggested to

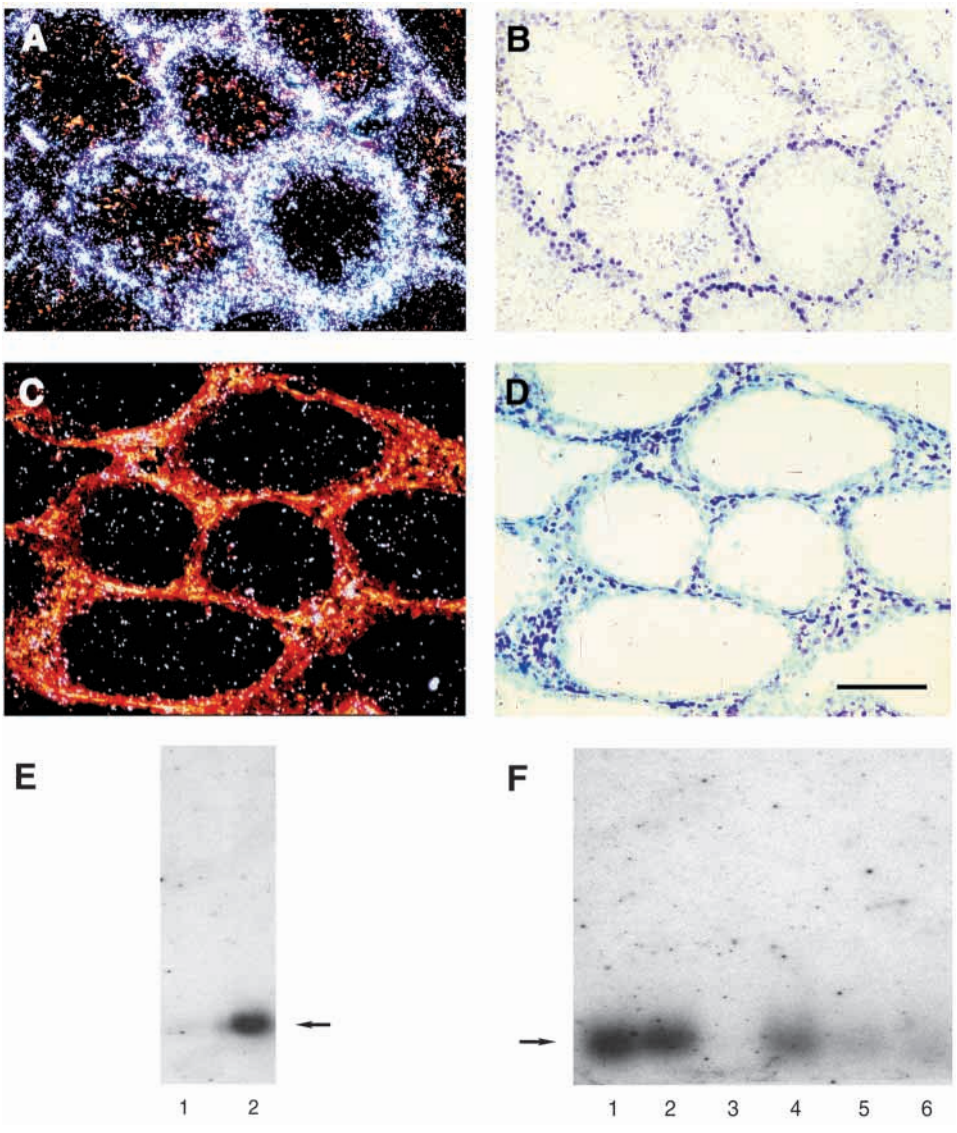

Fig. 4. Cell-type-specific expression of BC1 RNA in seminiferous tubules. In situ hybridization was performed with testes from homozygous (W^V/W) germ-line deficient mutant mice (C,D), and with testes from wild-type (+/+) littermates (A,B). Little specific signal is observed in seminiferous tubules of germ-line-deficient animals. Note that most of the brightness associated with cells in C is not caused by silver grains but by reflections from cellular structures. (A,C) Dark field photomicrographs; (B,D) bright field photomicrographs. Bar, 100 μm. (E) Northern hybridization with 10 μg total RNA isolated from germ-line-deficient adult testes (lane 1) or wild-type adult testes (lane 2). BC1 RNA is not detected in germ-line-deficient testes. Equal loading was verified as described in Materials and Methods. (F) Northern hybridization with RNA isolated from enriched populations of spermatogenic cells. Lane 1, total RNA from entire adult testis (total RNA loaded in this and other lanes: 15 μg); lane 2, poly(A)$^+$ RNA from entire testis (5 μg); lane 3, poly(A)$^-$ RNA from entire testis (25 μg); lane 4, total RNA from cells in the prophase of meiosis (mainly pachytene); lane 5, total RNA from early spermatids; lane 6, total RNA from the fraction containing cytoplasmic fragments of elongating spermatids and residual bodies. For the experiment shown in F, a filter used for a previous publication [(Zakeri et al., 1988) see figure 5, third panel)] was reprobed with BC1-specific probe HT005. Arrows in E and F indicate the position of the BC1 RNA band on the two blots.

participate in the regulation of translation-related processes in postsynaptic microdomains (reviewed by Brosius and Tiedge, 2001). Translation regulation also plays an important role in the modulation of gene expression in male germ cell development. A large number of mRNAs have been reported to be subject to translational control in murine testis (reviewed by Schäfer et al., 1995). In seminiferous tubules, overall protein synthetic activities are highest in basal layers, decreasing in a peripheral-to-central gradient (Monesi, 1965; Dadoune et al., 1981) in a fashion similar to the pattern of BC1 expression.

Since general transcription ceases about midway through spermiogenesis, protein synthesis in the late phase of spermiogenesis can be achieved only by translation from stored mRNAs that have been transcribed previously (Schäfer et al., 1995). However, in view of our results that BC1 RNA is virtually undetectable in spermatids, a direct role in translation regulation during late spermiogenesis must appear unlikely. Conversely, high BC1 expression levels in spermatogonia and early spermatocytes clearly point to a more prominent role of the RNA in these cell types. It is notable in this context that at times strikingly high BC1 expression levels were observed in clusters of neighboring spermatogonia. Such paired (A_{pr}) or aligned (A_{al}) A spermatogonia are undifferentiated spermatogonia, derived from single A (A_s) spermatogonia through several rounds of mitosis (reviewed by De Rooij, 1998; De Rooij and Grootegoed, 1998). Forming pairs/chains, A_{pr}/A_{al} spermatogonia of the same clonal origin remain connected through intercellular cytoplasmic bridges, allowing for the intercellular transport of cytoplasmic components. It is assumed that such interconnectivity between A_{al} spermatogonia is required for the exquisite synchronization of subsequent differentiation into B spermatogonia and spermatocytes (De Rooij, 1998). However, the mechanisms underlying synchronous spermatogonial differentiation, and the possible role of cytoplasmic interconnectivity in such mechanisms remain unclear.

In neurons, BC1 RNA is specifically transported to dendritic microdomains (Muslimov et al., 1997) where it has been suggested to operate in the modulation of local protein synthesis (Brosius and Tiedge, 2001). We suggest that the synchronization of spermatogonial differentiation is a translation-controlled process that requires intercellular molecular communication between spermatogonia. We further propose that BC1 RNA may be a mediator of such coordinated intercellular modulation at the translational level, in an activity that may necessitate movement of this RNA (and possibly other RNAs and/or proteins) through spermatogonial cytoplasmic bridges. Since BC1 RNA remains detectable, albeit at gradually decreasing levels, in spermatocytes up to the pachytene stage, it is certainly possible that the RNA continues to be operational in early spermatocytes. Our suggestions are conjectural at this time but establish a testable hypothesis that can be addressed in future research. We find it noteworthy at this point that more than 100 years ago, Wilhelm His was one of the first to comment on similarities between developing male germ cells and neurons (His, 1890), a prescient observation that would be extended to the molecular level by the identification of equivalent RNA transport in both cell types.

Intercellular transport of RNAs and proteins between spermatogenic cells has previously been suggested to subserve an important role in male germ cell development (Willison and Ashworth, 1987). Movement of RNA has also been reported to occur between post-meiotic spermatids, where it is assumed to serve a different purpose, namely, to facilitate the sharing of gene products among genotypically different haploid cells (Morales et al., 1998). It should also be noted that other small RNAs (*lin-4* and *let-7*, no apparent relationship with BC1 RNA) have been implicated in the regulation, through translational modulation, of developmental timing in *C. elegans* (Lee et al., 1993; Pasquinelli et al., 2000; Reinhart et al., 2000). There is thus increasing evidence for roles of, and interplay between, small RNAs, RNA transport and translation regulation in various developing eukaryotic systems.

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