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# Haploid Expression of a Unique c-*abl* Transcript in the Mouse Male Germ Line

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**RNA from immature mouse testes was shown to lack a low-molecular-weight c-*abl* transcript previously noted to be the predominant species in adult testes. The developmental pattern of appearance of this c-*abl* variant was determined by analyzing RNA obtained from purified populations of testicular cells in different stages of spermatogenesis. The appearance of the c-*abl* testicular variant was coincident with the entry of the germ cells into their haploid state and suggested that the regulated expression of this proto-oncogene may be important in the normal differentiation of the male germ line.**

Cellular oncogenes (c-*onc*) have been identified because of their homology to retroviral oncogenes whose expression is known to drastically alter the phenotype and proliferation of the infected cell (4). This evidence, together with the remarkable degree of conservation of c-*onc* within eucaryotes, strongly suggests that protooncogenes exert a fundamental role in controlling normal cell growth and differentiation (4). For example, variation in expression of c-Ki-*ras* and c-*myc* has been demonstrated in regenerating rat liver (11, 14), and tissue-specific expression of c-*abl*, c-*fms*, and c-*fos* has been demonstrated in the mouse during normal embryogenesis (17, 18). Correlation of the appearance of c-*onc* transcripts with unique stages of differentiation within a particular cell lineage would aid in elucidating the role of c-*onc* in normal development.

The testis provides an ideal system for such an analysis, for it contains the complete spermatogenic cell lineage from spermatogonial stem cells to mature spermatozoa (see Table 1). Furthermore, it is technically possible not only to separate the germinal from the somatic component of the tissue but also to obtain enriched populations of germ cells at different developmental stages (16). Müller et al. (17) noted the presence of a unique c-*abl* transcript expressed at high levels in the mouse adult testis, in addition to the two higher-molecular-weight messengers characteristic of embryonic and adult somatic tissues (26). We sought to understand the possible role for this transcript in the differentiation of mammalian germ cells by examining its expression in developing testes and ovaries and, eventually, in purified populations of germ cells at different developmental stages.

In initial experiments, total and polyadenylated [poly(A)$^+$] RNA was isolated (1, 6) from testes of both mature (>40 days old) and immature (postnatal, days 7 to 9) Swiss Webster mice. As indicated in Table 1, testes at these two developmental stages contain many cell types in common: (i) somatic cells, including Sertoli, Leydig, and peritubular cells, as well as macrophages; and (ii) germ cells in premeiotic stages of spermatogenesis, including primitive type A spermatogonia and type A and type B spermatogonia (3). In addition to these cells, the mature testis also contains meiotic and postmeiotic germ cells (3). RNA from the two sources was analyzed in a series of Northern blot (15, 25) hybridization experiments by using a v-*abl*-specific probe from two different sources (10; ONCOR, Inc.) labeled with $^{32}$P by nick translation (27). Poly(A)$^+$ RNA isolated from brain and liver was used as somatic tissue controls. The results of a representative experiment are shown in Fig. 1.

In all the tissues examined, two poly(A)$^+$ transcripts of ~6.2 and ~8.0 kilobases (kb) were seen (Fig. 1b and c). Mature, but not immature, testes contained an additional transcript of ~4.7 kb (Fig. 1b, lanes 2, 4, and 5) which was the most abundant. This transcript was sufficiently abundant to be detectable in Northern blots of total RNA (Fig. 1b, lane 1).

In light of the recent report that transcription of the *Drosophila* homolog of the c-*abl* proto-oncogene occurs at a very high level in the growing oocyte (13), we also examined poly(A)$^+$ RNA isolated from mouse ovaries at different developmental stages. Postnatal day-11 ovaries contain a high level of growing oocytes, relative to somatic cells (21). In postnatal day-30 ovaries, fully grown oocytes, which can be fertilized, are found in well-developed follicles (21). As illustrated in Fig. 1b (lane 3) and 1c (lanes 1 and 2), only the two somatic transcripts were present in both day-11 and

TABLE 1. Cell types and stages of mouse spermatogenesis in immature and mature testes

| Stages of spermatogenesis | Cell types |
|---|---|
| Postnatal (7 to 9 days) and adult | |
| Somatic | Leydig cells, Sertoli cells, peritubular cells, macrophages |
| Premeiotic | Primitive type A spermatogonia, type A spermatogonia, type B spermatogonia |
| Adult | |
| Meiotic | Spermatocytes in prophase of meiosis (leptotene, zygotene, pachytene, diplotene), secondary spermatocytes |
| Postmeiotic | Early (round) spermatids (stages 1 to 8),[a] elongating spermatids, elongated spermatids, sperm, residual bodies |

[a] Staging is according to Oakberg (20).

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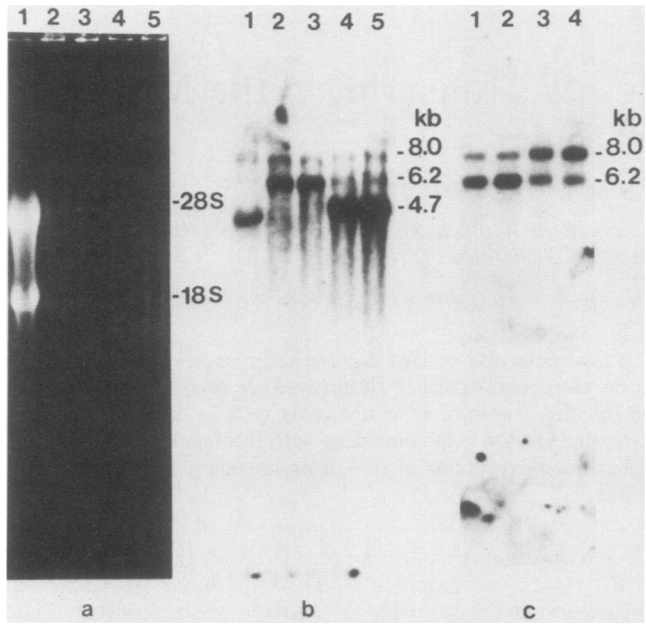


FIG. 1. (a) Ethidium bromide staining of total RNA isolated from mouse (Swiss Webster) mature testes (lane 1; 30 μg) and poly(A)$^+$ RNAs isolated from mouse immature testes (lane 2; 5 μg), mouse day-11 ovaries (lane 3; 5 μg), and mouse mature testes (lane 4, 5 μg; lane 5, 10 μg). Total RNA was isolated according to the method of Cathala et al. (6) and enriched for poly(A)$^+$ RNA through one cycle of oligodeoxythymidylate cellulose chromatography (1). RNAs were subjected to electrophoresis in denaturing formaldehyde-0.8% agarose gel (15). (b) Northern blot (15) of the RNA gel shown in panel a. The nitrocellulose filter was hybridized to the probe P abl.1 (ONCOR, Inc.) specific for v-*abl* and then labeled with $^{32}$P by nick translation (27). Prehybridization and hybridization were carried out according to the procedure of Wahl et al. (25). Exposure time was 3 days. Size (in kb) was determined by using 28S (5.0 kb) and 18S (2.0 kb) rRNA bands as internal markers. It should be noted that enough residual rRNA was present in the poly(A)$^+$-selected samples to allow visualization in the ethidium bromide-stained gel (panel a, lanes 2 to 5). (c) Northern blot of poly(A)$^+$ RNAs isolated from mouse day-11 ovaries (lane 1; 4 μg), mouse day-30 ovaries (lane 2; 4 μg), mouse brain (lane 3; 10 μg), and mouse liver (lane 4; 10 μg). RNAs were isolated and processed as described above.

day-30 ovaries, at levels comparable to those observed in somatic tissues and immature testes.

The qualitative and quantitative differences in expression of the 4.7-kb transcript between mature and immature testis cells suggested that its presence was correlated with meiotic stages of spermatogenesis, since, as noted above, only the mature testes contained germ cells in these later stages. To determine the developmental stage during which the 4.7-kb transcript appears, cells from adult testes were purified by velocity sedimentation at unit gravity into discrete populations by using a Celsep apparatus. Details on the method of cell separation, criteria for analysis of cell types, and average purities of populations of cells are described elsewhere (D. J. Wolgemuth, E. Gizang-Ginsberg, E. Engelmyer, B. J. Gavin, and C. Ponzetto, Gamete Res., in press).

One population was enriched for spermatocytes in the pachytene stage of meiotic prophase (a minimum average of 73% purity for the particular experiment illustrated in Fig. 2). Pachytene spermatocytes have been shown to be the most transcriptionally active spermatogenic cells, and its has been proposed that RNAs, important for postmeiotic germ cell differentiation, could be synthesized during this stage and stored as long-lived mRNAs to be translated during spermiogenesis (for a review, see reference 2). On the other hand, very recent molecular evidence (e.g., references 7, 8, and 12), combined with genetic evidence on transmission distortion in the T-complex (9), suggests that postmeiotic gene expression may occur. Since the genetic consequences of haploid gene expression would be profound (2, 9), we were particularly interested in examining c-*abl* transcripts in postmeiotic cells. To this end, a second population of cells was obtained, consisting of predominantly early spermatids (minimum average purity, 81%). Finally, we also obtained a third population of cells, containing residual bodies and cytoplasmic fragments of late spermatids (minimum average purity, 76%). This population was of developmental interest since although these nucleus-deficient structures are known to contain RNA, neither the integrity nor the function of these RNAs has been defined (2). It should be noted that the major contaminants of this residual body and cytoplasmic fragment population are sperm and elongated spermatids. Since sperm contain virtually no RNA and elongated spermatids only occasionally contain adherent cytoplasmic fragments, the actual purity of cell type in this population was greater than 96%.

In all experiments, poly(A)$^+$ RNA isolated from spermatocytes in the meiotic prophase was shown to contain predominantly the ~6.2- and ~8.0-kb transcripts (an example is shown in Fig. 2b, lane 2). In contrast, the 4.7-kb transcript was overwhelmingly abundant in poly(A)$^+$ RNA from early spermatids (Fig. 2b, lane 3). It is interesting that high levels of the 4.7-kb transcript were still present in poly(A)$^+$ RNA from residual bodies and cytoplasmic fragments (Fig. 2b, lane 4). The trace amount of the 4.7-kb transcript seen in the meiotic prophase population is most likely attributed to the ~10% contamination of this fraction with binucleated early spermatids (Wolgemuth et al., in press). Parallel experiments, carried out using corresponding RNAs excluded from the oligodeoxythymidylate column, consistently showed no hybridization of the v-*abl* probe to the poly(A)$^-$ RNAs from all sources (Fig. 2a, b, and c, lanes 5, 7, and 8).

The 4.7-kb c-*abl* mRNA probably corresponds to the testis-specific 3.7-kb transcript previously described by Müller et al. (17), and the ~6.2- and ~8.0-kb transcripts probably correspond to the 5.5- and 6.5-kb species seen in mouse tissues (26). The sizes of the transcripts reported herein were calculated by using the residual rRNA present in the poly(A)$^+$ samples as internal markers (Fig. 1a). The testicular c-*abl* transcript clearly migrated slightly ahead of the 28S RNA (~5.0 kb). In addition, one of the filters was rehybridized with an α-tubulin cDNA probe (24). Two transcripts (~1.7 and ~2.1 kb) were visualized (Fig. 2c). These sizes are consistent with the sizes of α-tubulin transcripts recently reported by Distel et al. (7) and Stacey and Evans (23) and also observed in our laboratory (E. Gizang-Ginsberg and D. J. Wolgemuth, Abstr. DNA Congr., 1985, p. 88).

Since equal amounts of RNA were loaded in each lane in the Northern analysis, the results (Fig. 2b) show that the 4.7-kb c-*abl* transcript is present at the highest level in residual bodies and cytoplasmic fragments (lane 4). In contrast, at this stage of spermatogenesis, the major α-tubulin messenger is reduced (Fig. 2c, lane 4). This may suggest a higher stability of the c-*abl* versus the α-tubulin mRNA.

c-*abl* is present in the mouse genome as a single genetic locus (10). The various transcripts could therefore result from the utilization of different promoters (5, 19), one of

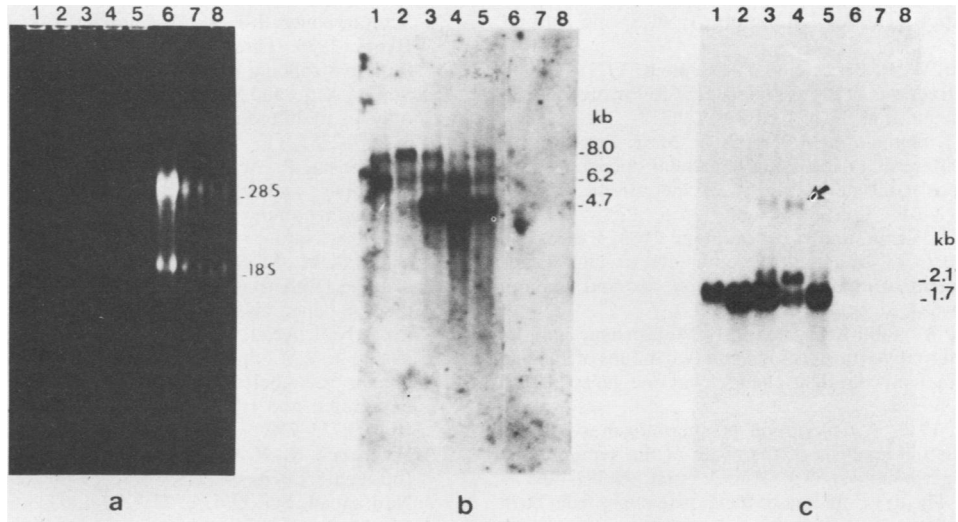


FIG. 2. (a) Ethidium bromide staining of poly(A)$^+$ (5 μg per lane; lanes 1 to 5) and poly(A)$^-$ (10 to 20 μg per lane; lanes 6 to 8) fractions of RNAs isolated from immature (days 7 to 9) (lane 1) and mature (lane 5) testes and from purified populations of testicular cells as described below. Lanes: 2, pachytene spermatocytes (in meiotic prophase); 3, early spermatids (postmeiotic); 4, residual bodies and cytoplasmic fragments (enucleated cell fragments from elongated spermatids); 6, 7, and 8, poly(A)$^-$ RNA from pachytene spermatocytes (~20 μg), early spermatids (~10 μg), and residual bodies (~10 μg), respectively. Cells in different stages of spermatogenesis, isolated from mature testes, were separated by sedimentation at unit gravity through a 2-to-4% gradient of bovine serum albumin using a Celsep apparatus (Wolgemuth et al., in press). To obtain sufficient total RNA from purified cells to allow poly(A)$^+$ selection, total RNAs isolated from six different cell separations were combined. (b) Northern blot of the RNA gel shown in panel a. The probe used and the hybridization conditions were as described for Fig. 1. (c) After removal of the counts the filter shown in panel b was rehybridized with a $^{32}$P-labeled nick-translated *Pst*I fragment isolated from plasmid pT1, containing chicken brain α-tubulin cDNA (24). Arrow indicates residual counts due to the 4.7-kb band visualized by the previous hybridization with the v-*abl*-specific probe.

which might be male-germ-line specific and developmentally regulated as well. Conversely, the presence of the novel 4.7-kb transcript could be due to differential polyadenylation (14) or stage-specific differential splicing of the preexisting larger messengers (19, 22). Characterization studies of the three mRNA species, currently in progress, will clarify the origin of the multiple RNA forms.

Irrespective of the exact mechanism by which the haploid-specific messenger is generated, the regulated transcription of c-*abl* during spermatogenesis strongly suggests an important function for its gene product in the development of male germ cells. This hypothesis is being tested in experiments to determine whether the 4.7-kb transcript gives rise to a protein product and to ascertain whether the presence of the testicular c-*abl* variant is characteristic of other species.

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