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Construction and Preliminary Characterization of a Series of Mouse and Rat Testis cDNA Libraries

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ABSTRACT: We have constructed a series of 23 cDNA libraries from mouse and rat testicular cells. These include libraries made from whole, intact adult testes; seminiferous tubule cells from adult testes; combined populations of primary spermatocytes from 18-day-old mouse testes; and isolated populations of primitive type A spermatogonia, type A spermatogonia, type B spermatogonia, preleptotene spermatocytes, leptotene plus zygotene spermatocytes, juvenile pachytene spermatocytes, adult pachytene spermatocytes, round spermatids, Sertoli cells from 6-, 8-, 17-, and 18–20-day-old mice, and peritubular cells from 18–20 day old mice, all recovered from outbred white Swiss (CD-1) mice. We also constructed libraries from whole adult testes from five other lines of mice: C57 Bl6/J, C3 HEB, BDF-1, Balb/c, and 129 Sv. Finally, there are two libraries made from populations of Sertoli cells and peritubular cells isolated from testes of 20-day-old Sprague-Dawley rats. Enzymatic dissociation, followed by gradient separation or plating/lysing techniques, was used to prepare populations of specific cell types in purities of 85–98%. cDNAs were synthesized from poly A+ mRNA primed with oligo dT and unidirectionally cloned into the lambda Uni-Zap XR expression vector from Stratagene. Primary titers ranged from 2.1×10^5 to 2.9×10^6 plaque-forming units, and insert sizes averaged 1.0–1.2 kb. These libraries have been amplified once and submitted to the American Type Culture Collection (ATCC) for distribution to interested investigators. ATCC accession numbers are provided.

Key words: Spermatogenesis, Sertoli cells, peritubular cells, gene expression.

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The mammalian testis is a complex organ comprised of many different cell types. These can be subdivided into germ cells, which participate in the process of spermatogenesis (McCarrey, 1993); somatic cells, including Leydig cells, which produce androgens (Ewing and Keeney, 1993); Sertoli cells, which foster the process of spermatogenesis (Jégou, 1993; Griswold, 1995); and peritubular cells, which surround the seminiferous epithelium and facilitate interactions among other cell types (Skinner, 1991). The spermatogenic cell lineage is well characterized and decends from primordial germ cells in the embryo and from prospermatogonia in the fetus and neonate. These stem cells give rise to premeiotic spermatogonia, meiotic spermatocytes, and postmeiotic spermatids, which undergo the final differentiative process of spermiogenesis, leading to formation of the mature male gametes (spermatozoa) (reviewed in McCarrey, 1993). Each testicular cell type is distinguished by unique morphological and functional characteristics that develop as a result of differential gene expression. Complimentary DNA (cDNA) libraries are constructed from messenger RNAs (mRNAs) isolated from a specific tissue or cell type and thus represent the unique collection of genes that are expressed in that particular tissue or cell type (McCarrey and Williams, 1994). We have constructed a series of cDNA libraries that have the potential to provide a useful resource for studies of the genes and/or their products that are expressed in various testicular tissues or cell types.

Methods

Source of Tissues and Purified Cell Types

Except where otherwise designated, outbred CD-1 white Swiss mice (Charles River) or rats (Sprague-Dawley) were used as the source of testicular tissues and cell types. All experiments were performed according to guidelines specified by the National Research Council (Institute of Laboratory Animal Resources, 1996) and approved by local institutional animal care and use committees. Following euthanasia, testes were dissected from male mice at the designated age and were decapsulated to remove the tunica albuginea. The remaining tissue was either used as such

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Table 1. *Summary of testis cDNA libraries*

Source tissue or cell type*	Purity of cell type	Primary titer	Percent recombinant†	Amplified titer	ATOC accession
Adult whole testis	—	4.50×10^7	>98	8.00×10^{11}	63413
Adult seminiferous tubule cells	—	1.77×10^8	>98	2.21×10^{11}	63414
6-Day primitive type A spermatogonia	≥85%	2.50×10^6	89.5	9.28×10^{11}	63415
8-Day type A spermatogonia	≥85%	3.85×10^6	96.5	2.94×10^{11}	63416
8-Day type B spermatogonia	≥85%	2.90×10^6	96.0	2.95×10^{11}	63417
18-Day preleptotene spermatocytes	≥85%	2.17×10^6	96.0	1.72×10^{11}	63418
18-Day leptotene + zygotene spermatocytes	≥85%	4.80×10^6	98.5	1.81×10^{11}	63419
18-Day pachytene spermatocytes	≥85%	2.53×10^5	96.0	1.60×10^{11}	63420
18-Day mixed primary spermatocytes‡	≥95%	1.52×10^6	90.5	1.10×10^{11}	63421
Adult pachytene spermatocytes	≥95%	3.73×10^7	98.0	3.65×10^{11}	63422
Adult round spermatids	≥95%	8.26×10^7	98.5	3.65×10^{11}	63423
6-Day Sertoli cells	≥85%	3.00×10^6	95.5	4.08×10^{11}	63424
8-Day Sertoli cells	≥90%	6.60×10^6	99.0	3.05×10^{11}	63425
17-day Sertoli cells	≥90%	8.40×10^6	96.0	1.26×10^{11}	63426
18–20-Day Sertoli cells	≥98%	2.70×10^6	98.5	7.20×10^{11}	63427
18–20-Day peritubular cells	≥98%	2.90×10^6	>98	9.60×10^{11}	63428
Adult testis C57 Bl6/J	—	6.10×10^6	>95	1.75×10^{12}	63429
Adult testis C3 HEB	—	1.35×10^6	>95	7.44×10^{11}	63430
Adult testis BDF-1	—	5.95×10^6	>95	1.36×10^{12}	63431
Adult testis Balb/c	—	1.10×10^7	>95	1.20×10^{12}	63432
Adult testis 129 Sv	—	1.40×10^7	>95	2.08×10^{12}	63433
20-Day rat Sertoli cells	≥98%	3.32×10^5	89.5	5.60×10^{11}	63434
20-Day rat peritubular cells	≥98%	2.80×10^6	99.0	7.20×10^{10}	63435

* Source tissue was obtained from outbred CD-1 white Swiss mice unless otherwise noted.

† Values for libraries pooled from multiple packaging reactions are expressed as greater than or equal to a minimum percentage.

‡ Produced by mixing equal plaque-forming unit (PFU)–containing portions of primary libraries made from purified preleptotene spermatocytes, leptotene + zygotene spermatocytes, and pachytene spermatocytes isolated from 18-day-old mice.

(whole testis) or was further processed to yield the desired testicular cell types, as described below.

Preparation of Germ Cell Populations

Enzymatic dissociation of testicular tissues was accomplished as described (Bellvé, 1993) in enriched Krebs-Ringer bicarbonate buffer (EKRB) [120.1 mM NaCl, 4.8 mM KCl, 25.2 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4.7H_2O$, 1.3 mM $CaCl_2$, supplemented with 11.1 mM glucose, 1 mM glutamine, 10 ml/L essential amino acids, 10 ml/L nonessential amino acids, 100 μg/ml streptomycin, and 100 U/ml penicillin (K^+ salt) (Gibco BRL, Gaithersburg, Maryland; Sigma, St. Louis, Missouri)]. An initial 15-minute treatment of decapsulated adult testes with collagenase (0.5 mg/ml) yielded seminiferous tubule sections from which interstitial cells in suspension were decanted away. These seminiferous tubules were further washed with EKRB buffer and were either used as such (seminiferous tubule cells) or were subjected to further dissociation with trypsin (see below). A similar collagenase treatment yielded seminiferous tubules from puberal (17–18 day) and prepuberal (6–8 day) testes. A subsequent 15-minute treatment with trypsin (0.5 mg/ml) followed by filtration through nylon mesh (80 μm for adult testes; 40–80 μm for prepuberal and puberal testes) yielded a fully dissociated cell suspension from which specific cell types were purified as described below.

Spermatogenic cell types (spermatogonia, spermatocytes, spermatids, and residual cytoplasmic bodies) and 6-day Sertoli cells were purified by the Sta Put gradient/unit gravity sedimentation method as described by other researchers (Romrell et al, 1976; Bellvé et al, 1977; McCarrey et al, 1992; Bellvé, 1993). Fractions containing the desired cell types were identified on the basis of cellular morphology under phase–contrast optics. The specific cell types recovered, ages of mice from which they were recovered, and purities of each recovered cell population are summarized in Table 1.

Preparation of Somatic Cell Populations

Sertoli cells from 17-day-old mice were isolated from seminiferous tubule cell suspensions by differential plating as described previously (O'Brien et al, 1989). Following enzymatic dissociation with collagenase and trypsin as described above, cell suspensions containing both Sertoli and spermatogenic cells were cultured in Primaria dishes (Falcon, Franklin Lakes, New Jersey) at 32°C in Eagle's Minimum Essential Medium (MEM) supplemented with 15 mM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin, 5 μg/ml insulin, and 200 ng/ml testosterone.

After 48 hours of culture to allow Sertoli cells to adhere to the tissue culture dishes, spermatogenic cells were eliminated by hypotonic lysis with 10 mM Tris–HCl (pH 7.4). Sertoli cells were cultured for 6 additional days and then lysed in the presence of guanidinium isothiocyanate and frozen at −20°C. Mouse Sertoli cells obtained by this method have characteristic morphologies and secretory products (transferrin, sulfated glycoprotein 1, and sulfated glycoprotein 2) and have purities greater than 90%, as monitored by alkaline phosphatase histochemistry (O'Brien et al, 1993).

Sertoli cells were isolated from 8-day-old mice by similar procedures, except that insulin and testosterone were omitted from the medium, and culture intervals were reduced. Seminiferous cell suspensions were cultured overnight, and then spermatogenic cells were removed by gentle pipetting followed by hypotonic lysis. The Sertoli cells were cultured for 1 additional day and then lysed for RNA isolation. Unlike Sertoli cells from 17-day-old mice, Sertoli cells from 8-day-old mice were actively dividing throughout this culture period.

Sertoli cells were isolated from testes of 18- to 20-day-old mice and 20-day-old rats by sequential enzymatic digestion (Dorrington et al, 1975) by a modified procedure described by Tung et al (1984). Decapsulated testis fragments were digested first with trypsin (1.5 mg/ml; Gibco) to remove the interstitial cells and then with collagenase (1 mg/ml type I; Sigma) and hyaluronidase (1 mg/ml; Sigma). Sertoli cells were collected immediately for RNA preparation. Peritubular cells were obtained from the collagenase digestion supernatant as described by Skinner et al (1988) after tubule segments had sedimented and collected immediately for RNA preparation.

Preparation of RNA

Total RNA was isolated from each testicular tissue or purified cell population by high-speed homogenization in the presence of guanidinium isothiocyanate followed by ultracentrifugation of the homogenate through a cushion of 5.7 M CsCl at 35,000 rpm for 18 hours at 18°C as described (Chirgwin et al, 1979). Pelleted RNA was resuspended in H_2O treated with diethyl pyrocarbonate, quantified by UV spectrophotometry, and processed by mRNA fractionation on commercially available oligo dT columns (Stratagene, La Jolla, California) according to the manufacturer's instructions.

Preparation of cDNA

Two to five micrograms of poly A+ mRNA were used as template for cDNA synthesis using the directional cDNA library construction kit from Stratagene according to the manufacturer's instructions with minor modifications. Modifications included the use of Chromaspin-400 size selection columns from Clontech (Palo Alto, California) to selectively recover double-stranded cDNA molecules of >400 bp and drop dialysis of the size-selected cDNA against autoclaved H_2O at room temperature for 2–4 hours prior to ligation to the lambda vector arms.

Unidirectional Ligation of cDNA into the Expression Vector

Double-stranded cDNA molecules prepared as described above had an *Eco*RI hemi-restriction site at their 5′ ends (relative to the orientation of the original template mRNA) and an *Xho*I hemirestriction site at their 3′ ends. These molecules were combined with precut lambda Uni-Zap XR vector arms (Stratagene) in an insert:vector ratio of approximately 1–3:1 to produce recombinant bacteriophage DNA. This involved mixing 30–50% of the synthesized cDNA with 1 μg of digested vector in a total reaction volume of 5 μl. Unidirectional ligation was accomplished at a site within the endogenous lambda β-galactosidase gene such that the integrated cDNA was in the same 5′–3′ orientation as the endogenous *β-gal* gene. As a result, transcription initiated from the *β-gal* promoter will produce a chimeric *β-gal/* cDNA mRNA that can then be translated to produce a fusion β-galactosidase/cDNA–encoded protein.

Propagation and Preliminary Characterization of the Primary Library

Recombinant lambda DNAs were packaged into infectious phage particles by mixing this DNA with Gigapack III Gold packaging extract (Stratagene) according to the manufacturer's instructions. Aliquots of each primary library were used to infect XL1 Blue MRF′ *Escherichia coli* bacteria in the presence of 2.5×10^{-3} M isopropylthiogalactoside and approximately 4 mg/ml X-gal to facilitate blue–clear color selection to distinguish nonrecombinant and recombinant phages, respectively. This mixture was added to top agarose, plated on NZY agar (Gibco-BRL), and incubated overnight at 37°C. The total number of plaques was then counted and the proportion of recombinant clones (clear plaques) calculated. Primary titers for each library represent the total number of plaque-forming units (PFU) produced from individual or pooled packaging reactions as indicated in Table 1.

In addition to primary titers and proportion of recombinant PFU, a small sample (10–50 clones) from each library was characterized for average insert size. This was accomplished by using the polymerase chain reaction (PCR) to amplify insert cDNA fragments from individually selected plaques. In each case, individual plaques were picked into 50 μl of SM buffer (0.1 M NaCl, 0.05 M Tris.HCl [pH 7.5], 10 mM $MgSO_4$), incubated for ≥1 hour, and extensively processed by vortex to release phage particles from the agar plug. Four microliters of this solution was then added to a PCR reaction mix according to the manufacturer's instructions (Perkin-Elmer, Foster City, California) along with 10 picomoles/μl each of T3 (5′-AATTAACCCTCACTAAAGGG-3′) and T7 (5′-GTAATACGACTCACTATAGGGC-3′) PCR primers that hybridize to vector sites that flank the cDNA integration site and 2 μl of Taq Gold polymerase (Perkin-Elmer). This mixture was subjected to an initial denaturation step at 94°C for 12 minutes, followed by an annealing step at 55°C for 5 minutes and 35 cycles of PCR (72°C for 90 seconds, 94°C for 45 seconds, and 55°C for 45 seconds) and a final extension/annealing step at 72°C for 10 minutes. Twelve microliters of product from each 50 μl PCR reaction were electrophoresed through 1% agarose, stained with ethidium bromide, and compared with molecular markers to determine the size of each product. cDNA insert sizes were calculated as the size of each PCR product minus 100 bp to account for vector sequence represented in each product.

Amplification and Storage of cDNA Libraries

At least 200,000 PFU from each primary library were used to produce amplified libraries. These PFU were mixed with XL1 Blue MRF′ *E. coli* and plated as described above on four 150-mm Petri dishes at a density of 50,000 PFU per dish. Plates were incubated at 37°C for 6.5 hours to propagate phages. Phage particles were recovered by overlaying each plate with 8 ml SM buffer and gently rocking the plates at 4°C overnight to elute the phage. This phage-containing solution was then recovered, and each plate was washed with an additional 2 ml SM buffer. The combined 40 ml of solution containing eluted phage represented the amplified version of each library. After titering, each amplified library was prepared for storage by adding DMSO to a final concentration of 7% and freezing aliquots at −80°C.

Results

A total of 23 testis-derived libraries were constructed. The source tissue or cell type, purity of the source cell type (where applicable), primary library titer, percent recombinant phage in each library, amplified library titer, and the ATCC accession number for each are listed in Table 1. On the basis of the preliminary characterization performed, each library showed an average insert size of approximately 1.0–1.2 kb (data not shown).

Discussion

The cDNA libraries described here represent potentially useful research resources for investigators interested in studying genes and/or their encoded products expressed in the mammalian testis. As constructed, these libraries can be screened by hybridization with nucleic acid probes or by affinity with an antibody probe or any other means based on detection of either the specific DNA sequence of the cloned cDNA or the mRNA or protein products encoded by that cDNA.

Because the cDNAs were ligated unidirectionally into the lambda vector in the same 5′–3′ orientation as the endogenous lambda *β-gal* gene into which they were inserted, the normal coding DNA strand can be transcribed from all of the cDNAs as part of a chimeric mRNA produced by transcription initiated at the *β-gal* promoter following infection of host bacterial cells. However, only one out of three of the clones carrying any particular cDNA can be expected to produce a fusion protein with the same amino acid sequence as that normally encoded by the mRNA from which the cDNA was derived. The other two out of three clones will, on average, produce chimeric mRNAs in an incorrect reading frame to encode the correct amino acid sequence.

The primary titers of all of these libraries are sufficiently large to be expected to include representation of even relatively rare transcripts expressed in the source tissue or cell type, since libraries with primary titers of $>10^6$ should include even those mRNAs that are present as rarely as one molecule per cell (Sambrook et al, 1989). Amplification of 200,000 PFU from each primary library can be expected to include representation in the amplified library of the vast majority of the cDNAs present in the primary library.

Because each of these libraries is derived from template mRNA isolated from a different testicular tissue, cell type, combination of cell types, line of mice, or species (mouse or rat), the representation of cDNAs and, hence, expressed genes, will vary in each case. This variation will include both qualitative differences (genes that are expressed in one testicular tissue or cell type but not at all in another) and quantitative differences (genes that are expressed at relatively higher or lower levels in different testicular tissues or cell types). Quantitative differences resulting from different proportions of particular transcripts in the total mRNA pool recovered from each source tissue or cell type will be reflected by variation in abundance of each corresponding cDNA in each library. Amplification of each primary library one time is not expected to have significantly skewed this relative representation of specific cDNAs. Thus, the detection of any particular cDNA in a particular library will be indicative of expression of the corresponding gene in that source tissue or cell type, and the relative abundance of positive clones of a particular cDNA in different libraries will be indicative of the relative expression level of the corresponding gene in each source tissue or cell type.

To facilitate access by interested investigators, these libraries have been submitted to the ATCC, and the corresponding accession numbers are provided in Table 1. That accessibility, along with the background information provided in this report, should facilitate the use of these libraries in a large variety of studies.

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