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An Ex Vivo Analysis of Sertoli Cell Actin Dynamics Following Gonadotropic Hormone Withdrawal

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ABSTRACT: The receptors for the steroid hormone testosterone and the peptide hormone follicle-stimulating hormone are localized to the somatic Sertoli cell in the seminiferous epithelium. In the rat, prolonged gonadotrophic hormone withdrawal has been shown to result in substantial germ cell apoptosis. Previous studies have shown that, coincident with the loss of germ cells following hypophysectomy, the actin cytoskeleton of the Sertoli cell becomes disorganized and diffuse throughout the cell's cytoplasm. The molecular mechanisms that govern Sertoli cell actin filament dynamics in response to the loss of gonadotrophic hormones remain undefined. It was therefore hypothesized that hypophysectomy brings about a decrease in the amount of polymerized actin (F-actin) within the Sertoli cell and that this decrease is associated with changes in the expression of genes known to govern Sertoli actin dynamics. To this end, Sertoli cells were isolated from adult control and hypophysectomized rats. Sertoli cells from hypophysectomized rats were found to contain significantly less (72%) F-actin relative to untreated controls, although overall, β-actin protein and mRNA expression remained constant. The expression levels of genes known to directly influence the amount of F-actin in cells were then examined by Northern blot analysis. Cofilin and profilin I gene expression was unaffected by hypophysectomy, whereas the expression of profilin II and espin both decreased significantly (47% and 42%, respectively). Taken together, these results suggest that, following hypophysectomy, the actin cytoskeleton of the Sertoli cell shifts to a predominantly depolymerized state, perhaps in part because of decreases in profilin II and espin gene products.

Key words: Espin, profilin, cofilin.

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Within the mammalian seminiferous epithelium, the receptors for testosterone and follicle-stimulating hormone (FSH) are restricted to the somatic Sertoli cells (Sar et al, 1990; Rannikki et al, 1995), the cells to which germ cells maintain adhesion until the completion of spermatogenesis. Previous studies reported that the microfilament (actin) cytoskeleton of the Sertoli cell and the actin-based junctional complexes found at points of Sertoli–germ cell adhesion (ectoplasmic specializations; Muffly et al, 1993, 1994) become disorganized following hypophysectomy. The redistribution of the actin cytoskeleton, from a predominantly peripheral localization in normal Sertoli cells (Vogl et al, 1993) to a diffuse distribution throughout the cell (Muffly et al, 1993, 1994), was shown to occur in concert with the loss of adhesion between Sertoli cells and immature spermatids following hypophysectomy. Following the restoration of FSH and testosterone to hypophysectomized rats, actin was shown to relocalize to the periphery of the Sertoli cell before resumption of spermatogenesis (Muffly et al, 1994). These observations suggest that the peripheral distribution of actin filaments in Sertoli cells might be necessary for spermatid–Sertoli cell adhesion during spermatogenesis. As yet, the biochemical or molecular mechanisms by which actin redistributes in Sertoli cells in response to gonadotropin withdrawal or restoration is not known. From other experimental systems, it is known that under physiological ionic conditions, actin protein monomers (G-actin) spontaneously assemble into polymers (F-actin) in a polarized fashion and that in order to maintain a constant length, the rate of G-actin added to the ''growing'' or ''plus'' end of the polymer must equal the loss of G-actin from the ''minus'' end. Several proteins are known to directly influence actin polymer length by promoting either the addition of G-actin to, or loss from, the F-actin polymer. These include cofilin, which causes decreases in F-actin concentration (Nishida, 1985) by increasing the rate of G-actin loss from the minus end of the polymer, and the profilins, which stimulate the formation of F-actin by increasing the rate of G-actin addition to the plus end (Nishida, 1985; Honore et al, 1993). Additionally, the Sertoli cell–specific protein, espin, bundles actin filaments together at ectoplasmic specializations (Bartles et al, 1996; Chen et al, 1999; Vogl et al, 2000), potentially contributing to the formation of the characteristic hexagonal arrays of actin polymers seen at these junctional complexes. In vitro recombinant espin can cross-link actin

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filaments into partially ordered bundles (Chen et al, 1999). Because espin acts as an F-actin cross-linking protein, it might therefore be important for the initiation and maintenance of F-actin distribution at the ectoplasmic specialization, a junctional complex thought to be required for Sertoli–germ cell adhesion.

We hypothesize herein that the actin redistribution in Sertoli cells following hypophysectomy might result from alteration in the amount of F-actin within the cells and that changes in the expression of genes whose products govern actin filament dynamics, distribution, and stability in the Sertoli cell affect F-actin and its distribution. The results indicate that Sertoli cells isolated from hypophysectomized rats contain less F-actin than cells isolated from untreated controls, but that overall actin protein and mRNA expression remain constant. The expression levels of cofilin and profilin I were unaffected by hypophysectomy. In contrast, profilin II and espin mRNA expression significantly decreased following hypophysectomy. These results, taken together, suggest that after the loss of gonadotrophic hormones by hypophysectomy, actin redistributes in the Sertoli cell, perhaps as a consequence of a net decrease in the amount of polymerized F-actin, the latter, in turn, which could result from decreases in profilin II and espin gene products.

Materials and Methods

Animal Treatment Groups

Hypophysectomized and untreated Sprague-Dawley rats (8–12 weeks old) were purchased from Charles River (Kingston, Mass), housed in a vivarium under a 14:10 hour light:dark cycle, and provided water and rat chow ad libitum. The hypophysectomized animals were maintained in the above conditions for 42 days before being euthanized. A third group of rats were administered luteinizing hormone (LH)–suppressive subdermal 2.5-cm testosterone and 0.1-cm 17β-estradiol (TE)–filled polydimethylsiloxane capsules (Silastic, Dow Corning, Midland, Md) for 56 days, and controls received empty capsules for 56 days (Stratton et al, 1973) before euthanizing. All protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

Sertoli, Germ, and Leydig Cell Isolation

Sertoli cells were isolated according to previously described methods (Anway et al, 2003), but omitting the 10-minute trypsin digestion. Briefly, 2 decapsulated testes from untreated rats, or 4 testes from either the hypohysectomized or TE-treated rats, were incubated in 0.5 mg/mL of collagenase in 1× Hanks solution (pH 7.4) at 34°C, shaken for 15 minutes to eliminate the interstitial cells, and then washed a total of 3 times. To separate the Sertoli and germ cells, the tubules were incubated in a mixture of enzymes (0.1% collagenase, 0.2% hyaluronidase, 0.04% DNase I, and 0.03% trypsin inhibitor in 1× Hanks [pH 7.4]) at 34°C with shaking for 40 minutes. The Sertoli cells were pelleted by centrifugation, washed in 1× Hanks, and repelleted a total of 3 times. The Sertoli cell–enriched pellets were then resuspended in 1× Hanks and subjected to hypotonic shock in a dilute Hanks solution (1:3.5, Hanks:water). Cells were collected by centrifugation, resuspended in 1× Hanks, and filtered through 53-μM nylon mesh. The cells were then washed and resuspended in F12/DMEM (Invitrogen, Carlsbad, Calif) (1:1) tissue culture media. Sertoli cell number and purity were estimated by hemacytometer and light microscopic analyses, as previously described (Anway et al, 2003). In each Sertoli cell preparation, an average of 7–8 million Sertoli cells per testis was obtained, with approximately 80% purity. Germ and myoid cells made up the contaminants.

Total testicular germ cells were isolated by incubating 2 decapsulated testes in 0.5 mg/mL collagenase in 1× Hanks solution (pH 7.4) at 34°C with shaking for 12 minutes and then washing a total of 3 times. Trypsin was then added to the tubules at 0.5 mg/mL in 1× Hanks solution at 34°C and shaken for 12 minutes. The tubules were then mechanically disrupted by repeated pipetting, followed by filtering through nylon mesh. The cells were next pelleted by centrifugation, washed in 1× Hanks, and repelleted a total of 3 times. The isolated germ cell preparations were approximately 90% pure, with Sertoli cells making up the principal contaminant.

Leydig cells were isolated from untreated rats by centrifugal elutriation and Percoll gradient centrifugation purification, as previously described (Klinefelter et al, 1987). The isolated Leydig cell preparations were approximately 90% pure, with immune and red blood cells making up the principal contaminants.

Fluorescence Microscopy

Sertoli cells freshly isolated from control or hypophysectomized rats were dried to microscope slides and fixed with 10% neutral buffered formalin. To label F-actin, the Sertoli cells were stained with a 1.5 μg/mL Texas Red–phalloidin conjugate (Sigma, St. Louis, Mo) solution in phosphate-buffered saline (PBS) and 1% DMSO for 1 hour at room temperature. The cells were then washed several times with PBS. Nuclei were stained with Vectashield Anti-Fade Mounting Medium containing 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, Calif). Images were obtained by a Nikon Microflex H-III automatic camera system with a 60× oil Zeiss PlanApo lens.

Sertoli Cell Actin Fractionation

Detergent-soluble G-actin monomeric proteins from freshly isolated Sertoli cells were separated from insoluble F-actin polymers according to Patterson et al (1999). Briefly, isolated Sertoli cells were gently lysed at 4°C in lysis buffer (1% Triton X-100, 20 mM HEPES-NaOH [pH 7.2], 100 mM NaCl, 1 mM sodium orthovanadate, and 0.5% protease inhibitor cocktail [Sigma]) by brief sonication. The lysate was centrifuged at 10 000 × *g* for 20 minutes at 4°C. Supernatants contained the detergent-soluble cytoplasmic proteins, and the pellets contained the insoluble polymers. The pellets were then dissolved in RIPA buffer (1% Triton X-100, 15 mM HEPES-NaOH [pH 7.5], 0.15 mM NaCl, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM sodium orthovanadate, 10 mM EDTA, and 0.5% protease inhibitor cocktail [Sigma]) by means of sonication.

Primers used to generate probes used in Northern blots

cDNA	Primer	Size, bp	Accession No.
Clusterin			
Sense	GCATGGAATGAGACAGAAGC	159	NM 012679
Antisense	GAGTACAGAACCCAGAGGAA		
β-actin			
Sense	CATCTATGAGGGTTACGCGCT	560	NM 031144
Antisense	GTGGACAGTGAGGCCAGGAT		
Profilin I			
Sense	GTGGAACGCCTACATCGACA	386	NM 022511
Antisense	GGTGAGAGGCCATTTCATA		
Profilin II			
Sense	CTACGTGGATAACCTGATGT	544	NM 030873
Antisense	CAGAGGGATACAAAGGAGACA		
Cofilin			
Sense	CAAGCTAACTGCTACGAGGA	468	X62908
Antisense	CAGACAAGCACAGAGTCACT		
Espin			
Sense	CGGGTGACAACTCGGAGTT	635	NM 019622
Antisense	CAAGAGGCCGAGACTCCTATT		

Western Blot Analyses

Proteins were isolated from soluble and insoluble Sertoli cell fractions isolated from control, hypophysectomized, and TE-implanted rats. Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, Ill) according to the manufacturer's specifications. Protein samples were added to an equal volume of 2× loading buffer (100 mM Tris, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol). For both G-actin and F-actin protein fractions, 5 μg of protein were reduced with 0.1% β-mercaptoethanol, boiled for 2 minutes, and loaded on 12% SDS–polyacrylamide gel electrophoresis gel as described by Laemmli et al (1970). Protein was transferred to Protran nitrocellulose (Schleicher & Schuell, Keene, NH) with a Trans-Blot SD Semi Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, Calif) according to the manufacturer's specifications.

Membranes were blocked for 1 hour with 5% nonfat dry milk in PBS (blocking solution) at room temperature, followed by incubation with an anti-mouse β-actin monoclonal antibody (1:1000; Sigma) for 1 hour in blocking solution at room temperature. Membranes were washed 3 times with PBS for 5 minutes and then incubated for 30 minutes at room temperature with secondary anti-mouse HRP-linked immunoglobulin (1:3000; Amersham Pharmacia, Piscataway, NJ) in PBS. Signal was detected with the SuperSignal WestPico Chemiluminescent kit (Pierce) according to manufacturer's specifications. The relative ratios of F-actin to G-actin in the samples were quantified by determining signal density with Scion Image 4.0.2 (Scion Corporation, Frederick, Md). The Western analysis experiment comparing F- to G-actin ratios in Sertoli cells isolated from TE-treated, hypophysectomized, and untreated control animals was performed 3 times with 3 different sets of samples.

Probe Generation

Primers used for the generation of complementary DNA (cDNA) probes employed in Northern blot analyses (next section) are listed in the Table. Clusterin, actin, profilin I, profilin II, cofilin, and espin cDNAs were cloned from a rat testicular cDNA library generated by performing first strand synthesis from total testis RNA. Briefly, testicular RNA (3 μg) isolated by the Trizol method (Invitrogen) from a control rat was reverse-transcribed in a 20-μL reaction at 46°C for 60 minutes with the use of 0.2 units of Superscript II (Invitrogen) and 50 ng of oligo-dT primer in single-strength first-strand synthesis buffer according to manufacturer's specifications. PCR was performed in a reaction volume of 50 μL containing 0.5 μL of the reverse transcription reaction single-strength buffer, 20 μM dNTPs, 1.5 mM $MgCl_2$, 400 nM forward primer, 400 nM reverse primer, and 0.5 units of AmpliTaqR DNA polymerase (Perkin Elmer, Boston, Mass). The PCR conditions were 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute and a final extension of 72°C for 2 minutes. PCR products were cloned into a p-GemT Easy vector (Promega, Madison, Wisc) according to manufacturer's specifications and sequenced to verify insert product.

Northern Blot Analyses

RNA was purified from isolated Sertoli, germ, and Leydig cells as well as from homogenized rat brain, prostate, and adrenal gland by the Trizol method (as in ''Probe Generation''). Total RNA (10 μg) from each cell and tissue type was fractionated in a 1% agarose–formaldehyde gel, transferred overnight to a nylon membrane (HybondTM-N, Amersham), and UV cross-linked (UV Stratagene 1800). cDNA fragments of clusterin, actin, profilin I, profilin II, cofilin, and espin were radiolabeled with (α-^{32}P) dATP and the Rad Prime DNA Labeling Kit (Invitrogen). Northern blots were hybridized overnight at 65°C with labeled cDNA probes in ExpressHyb hybridization solution (Clontech, Palo Alto, Calif). After hybridization, blots were washed in 2× SSC (sodium chloride/sodium citrate)/1.0% SDS for 30 minutes

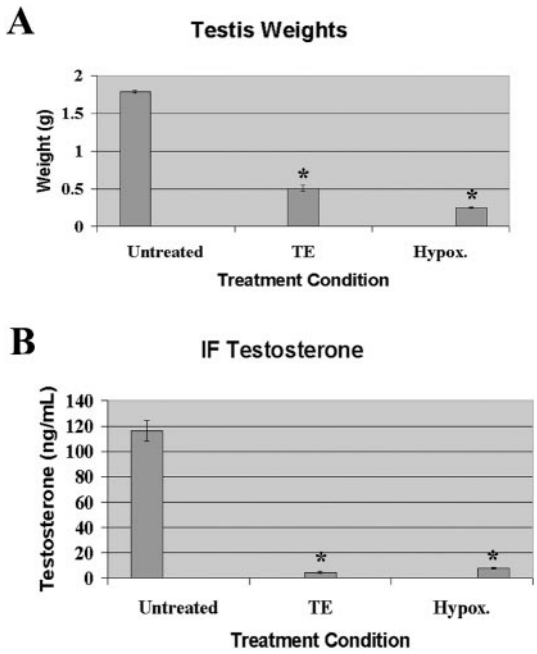

Figure 1. Effects of hypophysectomy (Hypox.) and testosterone 17β-estradiol treatment (TE) on testicular weight **(A)** and interstitial fluid (IF) testosterone concentration **(B)** compared with untreated controls. Shown are the means ± SEM (n = 3). In both cases, there was a significant decrease in the hypophysectomized and TE-treated animals compared with controls ($P < .0001$).

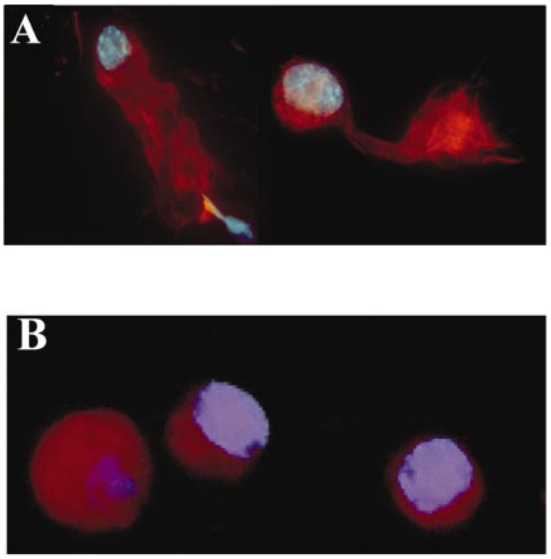

Figure 2. Freshly isolated Sertoli cells showing the distribution of actin (red) and DAPI-stained DNA (blue) from control **(A)** and 42-day hypophysectomized rats **(B)** (magnification 60×).

at 65°C; 1× SSC/0.5% SDS for 30 minutes at 65°C; and 0.1% SSC/0.1% SDS for 30 minutes at 65°C. After blots were washed, they were placed in a phosphor screen cassette for 8–12 hours. The signals were detected with a Typhoon 8600 and ImageQuant software (Amersham) and quantified densitometrically relative to clusterin with the use of Scion Image 4.0.2. The Northern blot analyses performed in this study employed 5 separate samples, with each sample containing pooled RNA from the isolated Sertoli cells of 2 rats.

Radioimmunoassays

Testicular interstitial fluid (IF) was collected according to previously described methods (Turner et al, 1984). All samples were stored at −80°C until assay for testosterone. IF testosterone concentrations were determined in duplicate for each sample by radioimmunoassay (RIA) according to a previously described method (Turner et al, 1984). Testosterone was assayed by RIA with testosterone antibody from ICN (Costa Mesa, Calif) and ^{3}H-testosterone from NEN (Boston, Mass). The sensitivity of the assay was 10 pg/tube.

Statistical Analyses

Data were expressed as means ± SEM. Statistical differences comparing testicular weight, IF testosterone concentration, and F- to G-actin protein content were determined by analysis of variance. Post hoc comparisons between treatment group means were made with Scheffe's least significant difference test. Statistical differences comparing gene expression between untreated and hypophysectomized Sertoli cells were analyzed by Student's *t* test. Means were considered significantly different at $P < .05$.

Results

The hypophysectomized rats used in these studies exhibited significant reductions in testicular weight (86%, Figure 1A) and intratesticular testosterone concentration (93%, $P < .0001$; Figure 1B) relative to controls, as did the TE-treated rats (Figure 1A and B). As shown previously (Awoniyi et al, 1990; Santulli et al, 1990), the reductions in testis weight in the 2 models result from germ cell loss in response to hormonal withdrawal.

Sertoli cells were isolated from control and hypophysectomized rats and examined immediately, without cell culture, for actin polymer morphology and protein levels. Cells were stained with the toxin phalloidin, which binds only to actin polymers (F-actin; Small et al, 1988). Figure 2A shows Sertoli cells isolated from untreated rats and stained for F-actin. The cells were characterized by extensive F-actin staining throughout the elongated cell cytoplasm. In contrast, in cells isolated from hypophysectomized rats, F-actin staining was perinuclear only (Figure 2B), and the cells exhibited a predominantly rounded-up morphology.

Because phalloidin, by binding to and stabilizing F-actin, can sometimes artificially shift the ratio of polymerized to monomeric actin in the direction of the polymerized form, we also quantified F-actin in Sertoli cells by Western blot analyses. Isolated Sertoli cells from untreated, hypohysectomized, and TE-treated rats were frac-

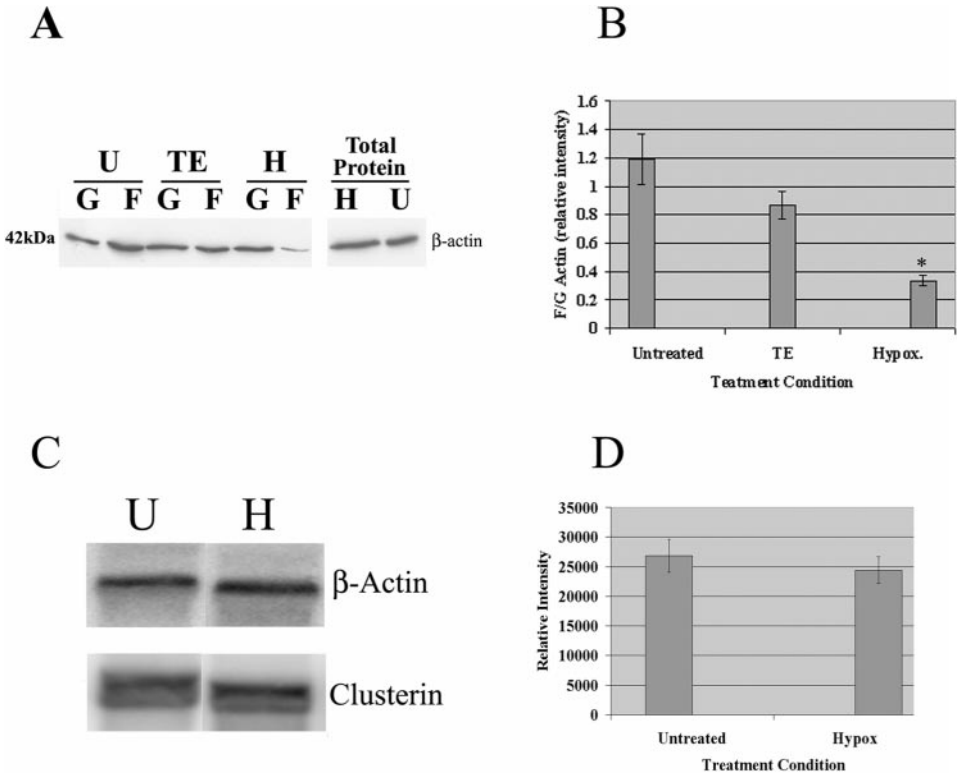

Figure 3. **(A)** Western blot analysis showing the quantity of detergent-soluble G-actin (G) relative to F-actin (F) in Sertoli cells isolated from adult untreated (U), 56-day TE-treated (TE), and 42-day hypophysectomized (H) rats. Also, total β-actin protein from whole Sertoli cells isolated from untreated and hypophysectomized animals. **(B)** Relative decrease in F:G actin ratios after hypophysectomy and TE treatment. Shown are the means ± SEM (n = 3); the asterisk (*) denotes statistical significance relative to untreated controls ($P < .005$). **(C)** Northern blot analysis of β-actin gene expression in Sertoli cells isolated from untreated and hypophysectomized rats. **(D)** Quantification of β-actin gene expression relative to clusterin. Shown are the means ± SEM (n = 5).

tionated so as to separate F-actin polymers from detergent-soluble monomeric G-actin. Figure 3A shows that, following hypophysectomy, freshly isolated Sertoli cells contained significantly less F-actin than Sertoli cells isolated from either untreated controls or animals treated with TE. The ratio of F- to G-actin in Sertoli cells from hypophysectomized animals was 72% less than that of untreated controls ($P < .005$; Figure 3B). The ratio of F- to G-actin in Sertoli cells from TE-treated rats also was decreased relative to controls, although far less so (about 35%, not significant; Figure 3B). Given that there was no decrease in overall actin protein expression in Sertoli cells after hypophysectomy (Figure 3B), it was not surprising to find by Northern blot analysis that β-actin mRNA levels were unchanged relative to the Sertoli cell–specific transcript clusterin (Figure 3C through D). Taken together, these results indicated that after hypophysectomy, Sertoli cells possess significantly decreased F-actin relative to untreated and TE-treated control cells, but not as a consequence of reduced expression of the β-actin mRNA or protein.

We next considered the possibility that the decrease in F-actin following hypophysectomy might be brought about by changes in the expression of genes known to directly influence actin dynamics, stability, and distribution. The profilins are a family of actin binding proteins that facilitate the formation of F-actin (Schluter et al, 1997). The testicular expression of one of the family members, profilin III, is known to be restricted to germ cells (Braun et al, 2002); thus, it was not examined. Figure 4A shows a Northern blot of profilin I and profilin II expression in several cell and tissue types. Profilin I was expressed in each of adrenal, brain, and prostate tissue and germ, Leydig, and Sertoli cells. Profilin II, however, was expressed only in the brain and testis, and in the latter, only in Sertoli cells (Figure 4A). After hypophysectomy, profilin II expression in Sertoli cells was reduced significantly from its levels in cells from the testes of untreated rats (47%, $P < .024$; Figure 4B and C). In contrast, expression levels of the profilin I transcript, also present in Sertoli cells, were unchanged following hypophysectomy (Figure 4D and 4E).

Two additional proteins important in the regulation of actin dynamics in Sertoli cells are cofilin and espin. Cofilin is one of the most abundantly transcribed genes in the Sertoli cell (unpublished data). It facilitates the con-

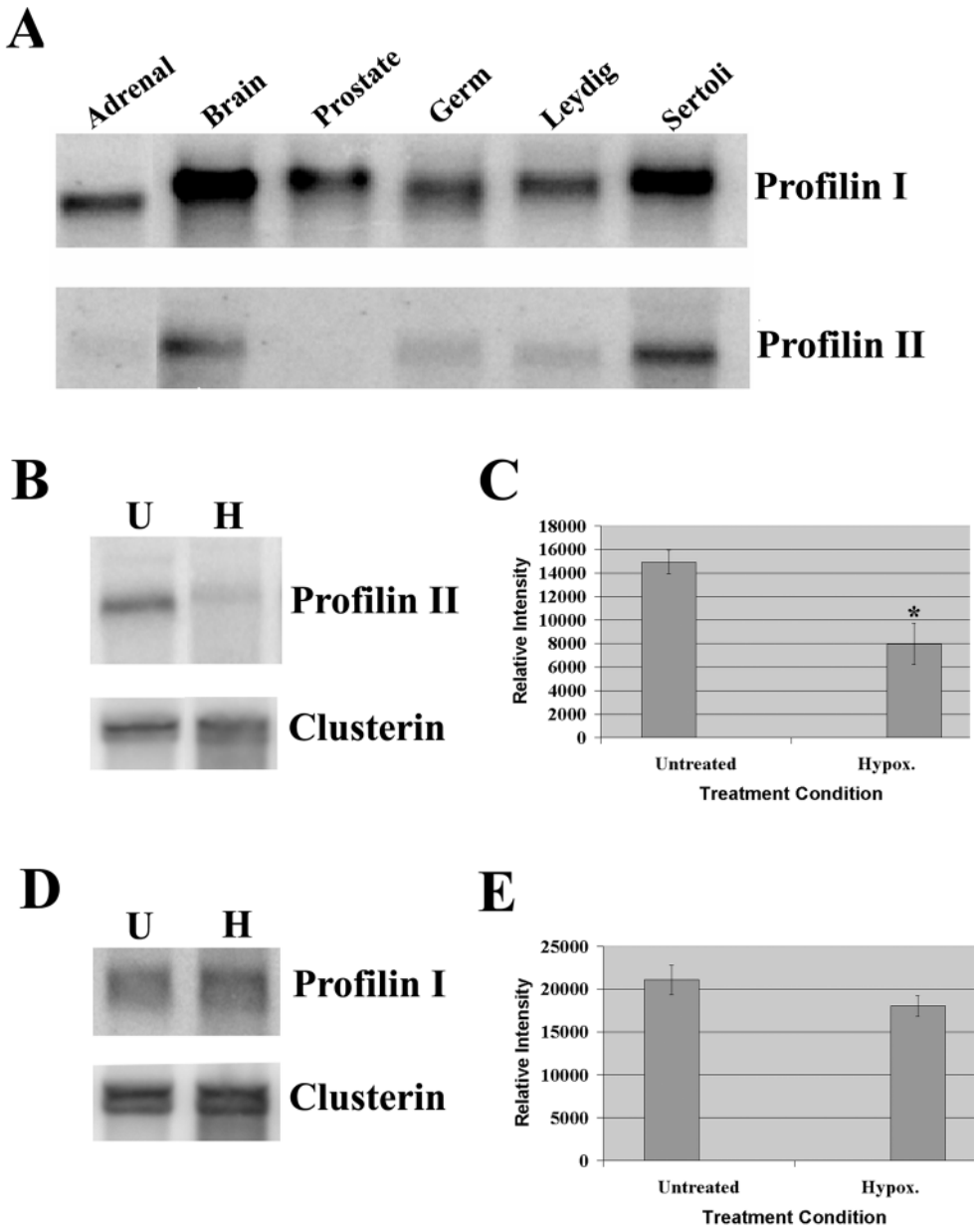

Figure 4. **(A)** Northern blot demonstrating profilin I and profilin II gene expression in adrenal, brain, and prostate tissues as well as in isolated Sertoli, Leydig, and germ cells. **(B)** Northern blot analysis of profilin II gene expression in Sertoli cells isolated from untreated (U) and hypophysectomized (H) rats. **(C)** Quantification of profilin II gene expression relative to clusterin. Shown are the means ± SEM (n = 5); the asterisk (*) denotes statistical significance ($P < .024$). **(D)** Northern blot analysis of profilin I gene expression in Sertoli cells isolated from untreated and hypophysectomized rats. **(E)** Quantification of profilin II gene expression relative to clusterin. Shown are the means ± SEM (n = 5).

version of F-actin to G-actin and thus causes loss of overall actin polymer length (Bamburg et al, 1999). As seen in Figure 5A and B, however, Northern blot analysis revealed no significant difference in cofilin expression in response to hypophysectomy. Espin is a 110-kd protein with a large spliceoform that is unique to the Sertoli cell and, in particular, to the actin-rich ectoplasmic specializations that are present at sites of germ–Sertoli cell adhesion (Bartles et al, 1996; Chen et al, 1999). Espin message in Sertoli cells isolated from hypophysectomized rats exhibited a significant decrease (42%, $P < .0001$) relative to the message levels seen in untreated controls (Figure 6A and B).

Discussion

We have shown in this study that Sertoli cells isolated from hypophysectomized rats exhibit decreased amounts of F-actin. In contrast, however, loss of actin polymer was

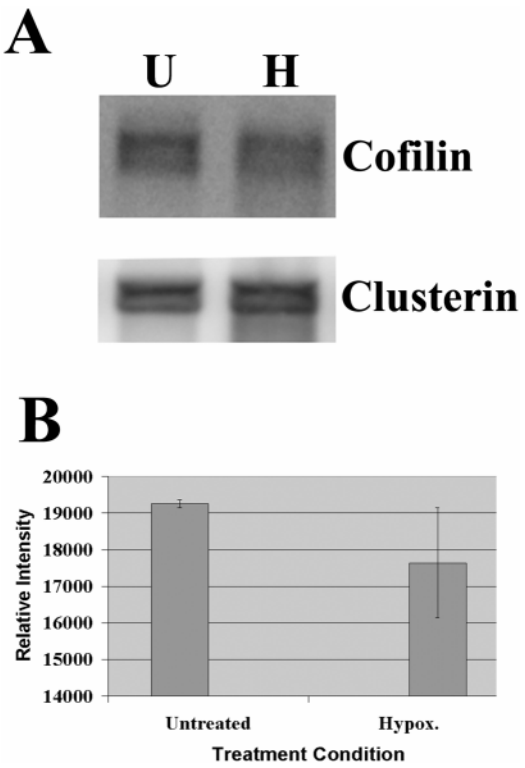

Figure 5. **(A)** Northern blot analysis of cofilin gene expression in Sertoli cells isolated from untreated (U) and hypophysectomized (H) rats. **(B)** Quantification of cofilin gene expression relative to clusterin. Shown are the means ± SEM (n = 5).

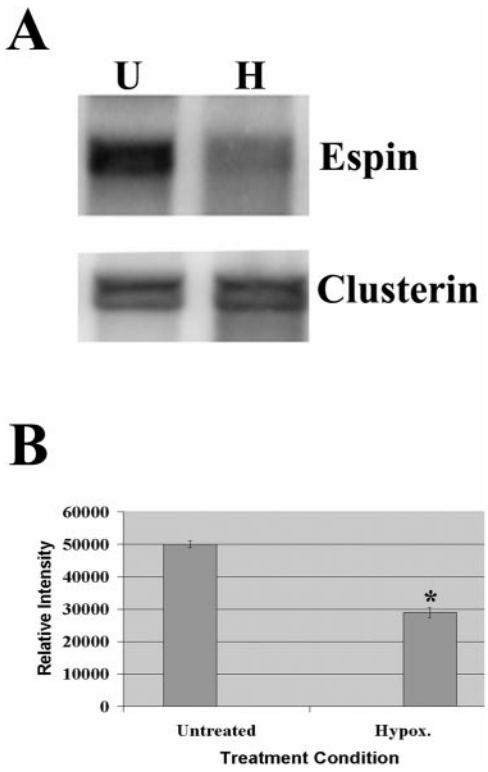

Figure 6. **(A)** Northern blot analysis of espin gene expression in Sertoli cells isolated from untreated (U) and hypophysectomized (H) rats. **(B)** Quantification of espin gene expression relative to clusterin. Shown are the means ± SEM (n = 5); the asterisk (*) denotes statistical significance ($P < .0001$).

not seen in TE-treated animals in which intratesticular testosterone concentrations were reduced to the same low levels that characterized hypophysectomized rats. This analysis agrees with previous morphological studies which similarly have suggested that the actin cytoskeleton and actin-rich junctional complexes in the Sertoli cell were affected by hypophysectomy (Muffly et al, 1993, 1994) but not by the suppression of intratesticular testosterone alone (O'Donnell et al, 2000; Show et al, 2003).

The decrease in the amount of F-actin in Sertoli cells following hypophysectomy could be due either to the reduced expression of the β-actin gene or protein (or both) or to a change in the steady-state actin dynamics that govern F-actin concentration within Sertoli cells. As demonstrated, hypophysectomy did not affect the overall expression of the β-actin gene or protein in Sertoli cells. This suggests that the available pool of monomeric G-actin available for the formation of F-actin polymers remained unchanged despite hypophysectomy. Actin polymerization spontaneously occurs under physiologic conditions when G-actin monomers form an F-actin polymer. G-actin is constantly being added to the plus end of the F-actin polymer while it is also being lost from the minus end in a process known as ''treadmilling'' (Brenner and Korn, 1983). If the rate of G-actin addition to the plus end equals the rate of G-actin loss from the minus end, the F-actin polymer would maintain a constant length. The so-called ''critical concentration'' is the concentration of the cellular G-actin monomer pool required to maintain a constant F-actin polymer length, with the overall addition of G-actin to one end equaling the overall loss of G-actin from the other (Wegner and Engel, 1975; Pollard, 1986).

If the amount of F-actin contained within a cell is reduced, this suggests that the critical concentration has been raised; more G-actin is required to maintain the previous polymer length than is currently available in the soluble cytoplasmic pool. To lower the critical concentration and return F-actin to normal concentration, the cell would have to either increase the concentration of the soluble pool of G-actin available for treadmilling or express actin binding proteins that enhance the rate of G-actin addition to F-actin, decrease the rate of its loss, or both. Dynamic changes in actin polymer length are commonly observed in motile cells in which turnover of actin polymers provides mechanical energy for motility (Carlier and Pantaloni, 1997; Carlier et al, 1997). The finely tuned mechanism of controlling the F- to G-actin ratio (ie, the critical concentration) in these motile cells lies in the spe-

cific actin binding proteins expressed by these cells that govern the amount of G-actin addition to and loss from F-actin at any given time.

In the case of the Sertoli cells isolated from hypophysectomized rats in this study, a decrease in the amount of F-actin suggests that the critical concentration for steady-state actin polymer length has been elevated. Potentially, this could be because of a decrease in the amount of G-actin available for treadmilling or a change in the availability of actin binding proteins that the Sertoli cell uses to control F- to G-actin ratios. As previously noted, Sertoli β-actin protein and mRNA remained constant after hypophysectomy, which suggested that the reason for the decrease in F-actin was the altered expression of proteins that govern actin dynamics in Sertoli cells.

We therefore examined the expression of actin binding proteins in the Sertoli cell that are known to influence steady-state actin dynamics by directly affecting the cell's critical concentration (Webber, 1999). The profilins are a family of actin binding proteins that enhance the addition of G-actin to the plus end of the F-actin polymer (Schluter et al, 1997; Didry et al, 1998). Thus, the presence of the profilins causes a decrease in the critical concentration of G-actin required for steady-state actin dynamics by promoting polymer growth. The Sertoli cell expresses profilin I and profilin II, the former expressed in every testicular cell type examined, the latter with its expression restricted to the Sertoli cell. Following 42 days of hypophysectomy, Sertoli cells expressed significantly less profilin II message relative to untreated controls, whereas profilin I transcript levels appeared to be unaffected by hypophysectomy. Profilin I and profilin II are biochemically similar in that they both bind actin with the same relative affinities, though they differ in that profilin II does not bind the membrane-associated phospholipid phosphatidyl inositol (4,5)-bisphosphate (PtdIns [4,5] P2) with as great an affinity as does profilin I (Lambrechts et al, 1995, 1997). Additionally, profilin II binds poly(L)-proline tracts with much greater affinity than profilin I (Lambrechts et al, 1997). Moreover, several proteins associated with cell signaling have been shown to interact physically with profilin II and not with profilin I. These include Rho-associated coil-coiled–forming protein kinase (ROCK 2), membrane-associated protein HEM 2, dynamin I, and several members of the synapsin family (Witke et al, 1998). Proteins that interact with profilin II in Sertoli cells have yet to be examined, although it is possible that the withdrawal of gonadotropic hormones from the Sertoli cell not only affects the expression of profilin II but also the behavior of upstream signaling proteins that are substrates for the FSH or androgen receptors, that interact specifically with profilin II, or that are affected by a combination of factors. A complete understanding of the mechanism of profilin II interaction with downstream components of Sertoli cell signaling pathways would provide a clearer picture as to how Sertoli actin dynamics are regulated during the course of spermatogenesis.

Cofilin is an actin binding protein that increases the rate of actin turnover by facilitating the loss of G-actin from the minus end of the F-actin polymer. Thus, the expression of cofilin serves to promote the depolymerization of F-actin and the elevation of the critical concentration. In this study, expression of the cofilin gene was unaffected in Sertoli cells after 42 days of hypophysectomy. Therefore, if the rate at which cofilin-mediated G-actin leaves actin filaments remains constant while the rate of profilin II-mediated G-actin addition is inhibited, then it is possible that the critical concentration would increase such that overall F-actin concentration would decrease in a manner similar to that observed in freshly isolated Sertoli cells after hypophysectomy. This model does not take into account the potential overlapping function of profilin I, which could compensate for the decrease in profilin II expression. However, although a profilin II knockout mouse has not yet been generated, the profilin I knockout possesses several defects unable to be compensated for by overlapping expression of profilin II (Witke et al, 2001). This, along with the fact that both profilins can interact with unique proteins, membrane-associated phospholipids, and structural motifs (Lambrechts et al, 1995, 1997; Witke et al, 1998), suggests that the profilin family members have distinct functional roles in cellular biology.

Although it is not a protein that directly influences actin dynamics, espin is an actin bundling protein whose long 110-kd splice variant is unique to the Sertoli ectoplasmic specialization (Bartles et al, 1996; Chen et al, 1999). It has been observed that the characteristic bundles of actin polymers at the site of espin immunolocalization (Vogl et al, 2000) in this junctional complex become disorganized following hypophysectomy (Muffly et al, 1993, 1994). In this study, we have found that espin mRNA expression decreases significantly after hypophysectomy. Therefore, it is possible that the Sertoli cell becomes unable to maintain the actin bundles distributed at the ectoplasmic specialization after hypophysectomy because of a concomitant decrease in the cross-linking activity of espin as a result of lowered gene expression. However, more research will be needed to determine whether this decrease in espin mRNA expression is a cause or a result of ectoplasmic specialization disorganization following withdrawal of gonadotrophic hormones.

This study has demonstrated that withdrawal of gonadotrophic hormones by means of hypophysectomy results in a decrease in the amount of F-actin in the rat Sertoli cell. This change is not brought about by the loss of expression of the β-actin gene or protein, but potentially by the elevation of the cellular critical concentration of G-

actin required to maintain steady-state actin dynamics. Decreases in the expression of the Sertoli cell–specific profilin II transcript and the actin bundling protein espin support this model. Disruptions in Sertoli cell actin dynamics and in the integrity of actin-based junctions found at points of Sertoli–germ cell adhesion could alter the ability of Sertoli cells to successfully support spermatogenesis in the absence of gonadotrophic hormones.

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