

Mice Lacking Raf Kinase Inhibitor Protein-1 (RKIP-1) Have Altered Sperm Capacitation and Reduced Reproduction Rates With a Normal Response to Testicular Injury

JEFFREY S. MOFFIT,* KIM BOEKELHEIDE,* JOHN M. SEDIVY,† AND JAN KLYSIK†

From the *Department of Pathology and Laboratory Medicine and †Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI

ABSTRACT: Raf kinase inhibitor protein-1 (RKIP-1) belongs to the phosphatidyl ethanolamine-binding family of proteins (PEBP), which are highly conserved throughout evolution and widely expressed in tissues of mammalian organisms. RKIP-1 is a modulator of extracellular signal-regulated kinase (ERK), nuclear factor-kappa B (NF- κ B), and G protein coupled receptor (GPCR) signaling cascades and is implicated as a factor in numerous physiological processes and disease states including metastasis. Testicular germ cells also express high levels of RKIP mRNA during spermatogenesis, particularly from late pachytene spermatocytes through step 15 elongate spermatids. Therefore, the sensitivity of spermatogenesis to injury was compared in wild-type and RKIP-1^{-/-} mice. Unlike what has been described with tumor suppressors such as p53, RKIP-1^{-/-} and wild-type mice were equally sensitive to germ cell toxicity by x-irradiation as assessed by terminal deoxynucleotidyl transferase biotin-deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL)

positivity 9 hours after a 5 Gy exposure and testicular spermatid head counts 15.5 days after 0.5 Gy exposure. Recent findings also indicate that RKIP is a decapacitation factor receptor on sperm. The present study demonstrates that sperm from RKIP-deficient mice are precociously capacitated compared with their wild-type counterparts. Data from mating experiments indicate decreased reproduction rates between crosses of RKIP-1^{-/-} male mice and either heterozygous or RKIP-1^{-/-} females. Furthermore, RKIP immunolocalization of epididymal sperm supports transfer of the protein from germ cell cytoplasm to the sperm via the cytoplasmic droplet during epididymal transport. Overall, these studies indicate an important role for RKIP in reproduction as a modulator of capacitation but not in the regulation of testicular injury.

Key words: PEBP, testis.

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Raf kinase inhibitor protein-1 (RKIP-1) is an evolutionary conserved member of the phosphatidyl ethanolamine-binding protein (PEBP) family. The expression of RKIP-1 is associated with numerous biological processes, most notably in the regulation of cancer cell metastasis and apoptosis (reviewed by Odabaei et al, 2004; Keller et al, 2005). Decreased expression of RKIP-1 in cancer cells is thought to contribute to chemotherapeutic resistance by modulating survival pathways (Chatterjee et al, 2004), such as signaling cascades (Yeung et al, 2000, 2001; Park et al, 2006). Experiments with prostate and breast cancer cell lines indicate a distinct correlation between RKIP-1 expression and the onset of apoptosis, with down-

regulation of RKIP-1 conferring resistance to chemotherapy-induced apoptosis (Chatterjee et al, 2004).

Spermatogenesis is a complex process in which germ cell proliferation is balanced by apoptosis, optimizing sperm output (reviewed by Holdcraft and Braun, 2004). Furthermore, testicular toxicants such as mono-(2-ethylhexyl) phthalate (MEHP) and x-irradiation are known to activate testicular apoptosis through NF- κ B and other pathways (Rasoulpour and Boekelheide, 2005, 2007) in which RKIP-1 is involved (Yeung et al, 2001). Notably, significant RKIP-1 mRNA expression occurs in the testis during spermatogenesis, particularly from late pachytene spermatocytes through step 15 elongate spermatids (Hickox et al, 2002). Therefore, it is plausible that RKIP-1 may have a role in regulating testicular apoptotic events by modulating ERK and NF- κ B pathways.

In addition to functioning as a metastasis inhibitor gene, RKIP-1 is emerging as an important regulator of male reproduction. Upon release from the male reproductive tract, sperm are incapable of fertilization. Sperm undergo a series of postejaculatory maturational events that culminate in the ability to fertilize an oocyte. This series of biochemical modifications, collectively referred to as capacitation, is a reversible process

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Correspondence to: Jan Klysik, Brown University, Department of Molecular Biology Cell Biology and Biochemistry, Division of Biology and Medicine, 70 Ship St, Providence, RI 02912 (e-mail: Jan_Klysik@Brown.edu).

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mediated by binding of decapacitation factors to sperm. As decapacitation factors are lost, numerous molecular changes occur, including a rapid influx of Ca^{2+} , hyperpolarizing the sperm membrane. Uncapacitated sperm maintain low intracellular Ca^{2+} levels through a calmodulin-sensitive Ca^{2+} -adenosine triphosphatase (ATPase), primarily located in the postacrosomal region of the sperm head (Adeoya-Osiguwa and Fraser, 1996). The Ca^{2+} -ATPase becomes inactivated in capacitated sperm, resulting in a rapid increase in intracellular Ca^{2+} . Gradients of Ca^{2+} differ between uncapacitated, capacitated, and acrosomal-reacted sperm, resulting in distinct banding detectible by chlortetracycline (CTC) labeling (DasGupta et al, 1994).

The identification of decapacitation factors has greatly contributed to a mechanistic understanding of the capacitation process. Removal of decapacitation factors from uncapacitated sperm results in the rapid acquisition of fertilizing ability, which is reversible upon reincubation with decapacitation factors (Fraser, 1984). Although the exact identity of decapacitation factors has remained elusive, partially purified factors are isolated through gentle centrifugation of uncapacitated sperm (Fraser et al, 1990; Fraser, 1998). Recently, 2 laboratories independently identified RKIP-1 as a sperm surface protein acting as a decapacitating factor (Nixon et al, 2006) or decapacitation factor receptor (Gibbons et al, 2005). Binding experiments performed using recombinant RKIP-1 demonstrated that decapacitation factors can be competed out from the sperm, resulting in capacitated sperm (Gibbons et al, 2005). RKIP-1 immunolocalization studies indicate extensive staining along the sperm, with prominent staining in the postacrosomal region at the junction of the midpiece and head. These studies clearly demonstrate the importance of decapacitation factors that interact with RKIP-1 in the regulation of sperm capacitation and may have significant implications in reproduction.

The present study uses wild-type and RKIP-1^{-/-} mice to examine the importance of RKIP-1 in the testis following x-irradiation, examining effects on germ cell apoptosis and spermatogenesis. Furthermore, the reproductive implications of RKIP-1 deficiency were explored in these knockout mice by examining sperm capacitation states, reproduction rates, and localization of the RKIP-1 protein on sperm.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich Corp (St Louis, Mo) and were of reagent grade or better.

Animals

Adult wild-type and RKIP-1^{-/-} mice were generated, bred, and maintained at Brown University (Providence, RI) as previously described (Theroux et al, 2007). The mice were housed in community cages within a temperature- and humidity-controlled vivarium with a 12-hour alternating light-dark cycle and had free access to water and Purina Rodent Chow 5001 (Farmer's Exchange, Framingham, Mass). The Brown University Institutional Animal Care and Use Committee approved all experimental animal protocols in compliance with National Institutes of Health guidelines. Testes were either preserved in 10% neutral buffered formalin for histopathologic analysis or placed in 154 mM saline supplemented with 247 nM thimerosal and 0.05% Triton X-100 for determining spermatid head counts. For 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) staining, testes were dissected out, briefly prefixed in 5% formaldehyde, and embedded in 3% low melting temperature agarose before making vibratome sections (Theroux et al, 2007).

Staining With X-gal

Whole testes or vibratome sections were stained as described previously (Theroux et al, 2007). Briefly, testes were dissected out from animals killed with CO and were fixed for 1–2 hours in 2% paraformaldehyde. The samples were further incubated with 2 mM MgCl_2 -0.01%, deoxycholate-0.02% Nonidet-P40 (NP-40)-100 mM phosphate buffer (pH.8.0), 5 mM $\text{K}_4\text{Fe}(\text{CN})_6$, 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$, and 1 mg/mL X-gal for 5 hours at 32°C. Vibratome sections of fixed testes were stained with X-gal reagent for 10 hours.

Irradiation Exposure

Unanesthetized male wild-type and RKIP-1^{-/-} mice were exposed to lower-body x-irradiation using a Philips 250-kVp x-ray machine (New Bedford, Mass). Single exposures were administered of 0.5 or 5.0 Gy at a rate of 0.89 Gy/min, as calculated by a Radcal radiation meter (Monrovia, Calif). Animals were restrained in polypropylene chambers, and the upper two thirds of the body were shielded with 3 mm of lead. At designated time points, animals were killed by CO₂ asphyxiation, and testes were immediately excised.

Detection of Apoptosis

Terminal deoxynucleotidyl transferase biotin-deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) staining was conducted using paraffin-embedded testis sections (7 μm) and stained using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Temecula, Calif) as directed by the manufacturer. Sections were counterstained with methyl green. Percent of seminiferous tubules with 0, 1–3, or more than 3 TUNEL-positive nuclei was assessed by blinded counting of all seminiferous tubules with a major-minor axis less than 1.5:1 in 2 cross-sections using a Zeiss Standard microscope (Carl Zeiss, New York, NY). At least 50 tubules were scored per animal for 0, 1–3, or more than 3 TUNEL-positive nuclei (wild-type, n = 6; RKIP-1^{-/-}, n = 4).

Testicular Spermatid Head Counts

The rate of sperm production (spermatid head counts per gram of testis per day) was assessed as a sensitive measurement of germ cell dysfunction. Both testes from each animal were homogenized individually, and spermatid heads were counted on a hemocytometer as previously described (Blazak et al, 1993). Counts from the 2 testes of each animal were averaged for statistical analysis ($n = 3$ per genotype).

Sperm Capacitation Evaluation

Epididymal sperm were freshly isolated by mincing 2 caudal epididymides in 0.8 mL of 154 mM saline. Following a 5-minute incubation at 37°C, 100- μ L aliquots of sperm were fixed with 8 μ L of 12.5% (wt/vol) paraformaldehyde and treated with 100 μ L of CTC solution as previously described (DasGupta et al, 1993). Briefly, the CTC solution (750 μ M CTC, 130 mM NaCl, 5 mM cysteine, and 20 mM Tris-HCl, pH 7.8) was freshly prepared and stored in the dark at 4°C. Fixed sperm (10 μ L) were imaged on a microscope slide following the addition of an equal volume of 220 mM 1,4-diazobicyclo[2,2,2]-octane diluted in 154 mM saline (9:1) to resist fluorescence quenching. A coverslip was applied and firmly pressed between 2 Kimwipes to remove excess liquid. Sperm were analyzed by fluorescence microscopy using a Carl Zeiss Axiovert 35 microscope with the appropriate fluorescence filters (BP 546, FT 580, LP 590). The CTC banding patterns were compared with previously published images of uncapacitated, capacitated, and acrosomal-reacted sperm (Ward and Storey, 1984; DasGupta et al, 1993). Three hundred sperm were counted per mouse ($n = 3$ mice per genotype).

Sperm Immunolocalization

Sperm were freshly isolated from caput and cauda epididymides as described in "Methods and Materials" and streaked on a Fisher Super+ glass slides (Fisher Scientific, Hampton, NH). After air drying at room temperature, the slides were fixed in a 2% formaldehyde solution, followed by a 0.1% Triton X-100 in phosphate-buffered saline (PBS) wash. The sperm were blocked in 5% normal goat serum (NGS) in PBS supplemented with 0.1% Triton X-100. Antibody raised against RKIP-1 (Upstate Biotechnology, Lake Placid, NY) was diluted in 1% NGS in PBS with 0.1% Triton X-100. Detection of the primary antibody was conducted with a tetramethyl rhodamine isothiocyanate (TRITC) secondary antibody (Sigma, St Louis, MO) incubated in 1% NGS in PBS with 0.1% Triton X-100. Sperm were analyzed by fluorescence microscopy as described above.

Statistical Analysis

Results are expressed as mean \pm standard error of the mean (SEM). Statistical differences for all figures were determined by analysis of variance and Bonferroni post hoc analysis. Statistical differences for breeding studies were assessed using a log-linear model with adjustment for overdispersion of Poisson distribution. Values were considered significantly different with $P < .05$.

Results

Testis RKIP-1 Expression

The mouse strain used in these studies carries a gene-trapped allele expressing β -geo reporter controlled by the native RKIP-1 promoter (Theroux et al, 2007). The β -geo is a fusion protein composed of the neo resistance domain and bacterial β -galactosidase activity, which is detectable upon exposure to X-gal. The tissue expression pattern of β -geo can thus be followed in these animals and is believed to reflect the expression of the native gene into which the gene-trap integration has occurred.

Whole testis isolated from RKIP-1^{-/-} and wild-type males were stained with X-gal. Seminiferous tubules from RKIP-1^{-/-} testis stained more intensely compared with wild-type (Figure 1A). Within the same tubule certain regions stained more strongly than others, suggesting stage-dependent expression of the reporter during spermatogenesis (Figure 1B). In contrast, wild-type testis revealed some degree of nonspecific staining detectable within the interstitium but not in seminiferous tubules (Figure 1C). This staining can be attributed to the weak activity of endogenous β -galactosidase. Higher-power magnification (Figure 1D) revealed that the layer of cells adjacent to the basal lamina did not stain at any stage of spermatogenesis. This layer consists of spermatogonia and early spermatocytes. More advanced spermatocytes and spermatids revealed various levels of reporter expression. The overall stage-dependent expression was very similar to that reported previously (Frayne et al, 1998; Hickox et al, 2002). These staining experiments confirm previously reported high expression levels of RKIP-1 in male testis and suggest its potential role in spermatogenesis (Jones et al, 1983; Araki et al, 1992; Rankin et al, 1992; Frayne et al, 1998; Theroux et al, 2007).

Germ Cell Susceptibility to Ionizing Radiation-Induced Apoptosis

Wild-type and RKIP-1^{-/-} mice were exposed to 5 Gy lower-body x-irradiation and killed 9 hours after exposure (Figure 2). This dosing regimen is associated with significant increases in germ cell apoptosis (Hasegawa et al, 1997). Based on the quantification of seminiferous tubules with 0, 1–3, or more than 3 TUNEL-positive cells, no differences in susceptibility to apoptosis were detected between wild-type and RKIP-1^{-/-} mice. These results suggest that RKIP-1 does not regulate apoptotic changes in murine germ cells following x-irradiation.

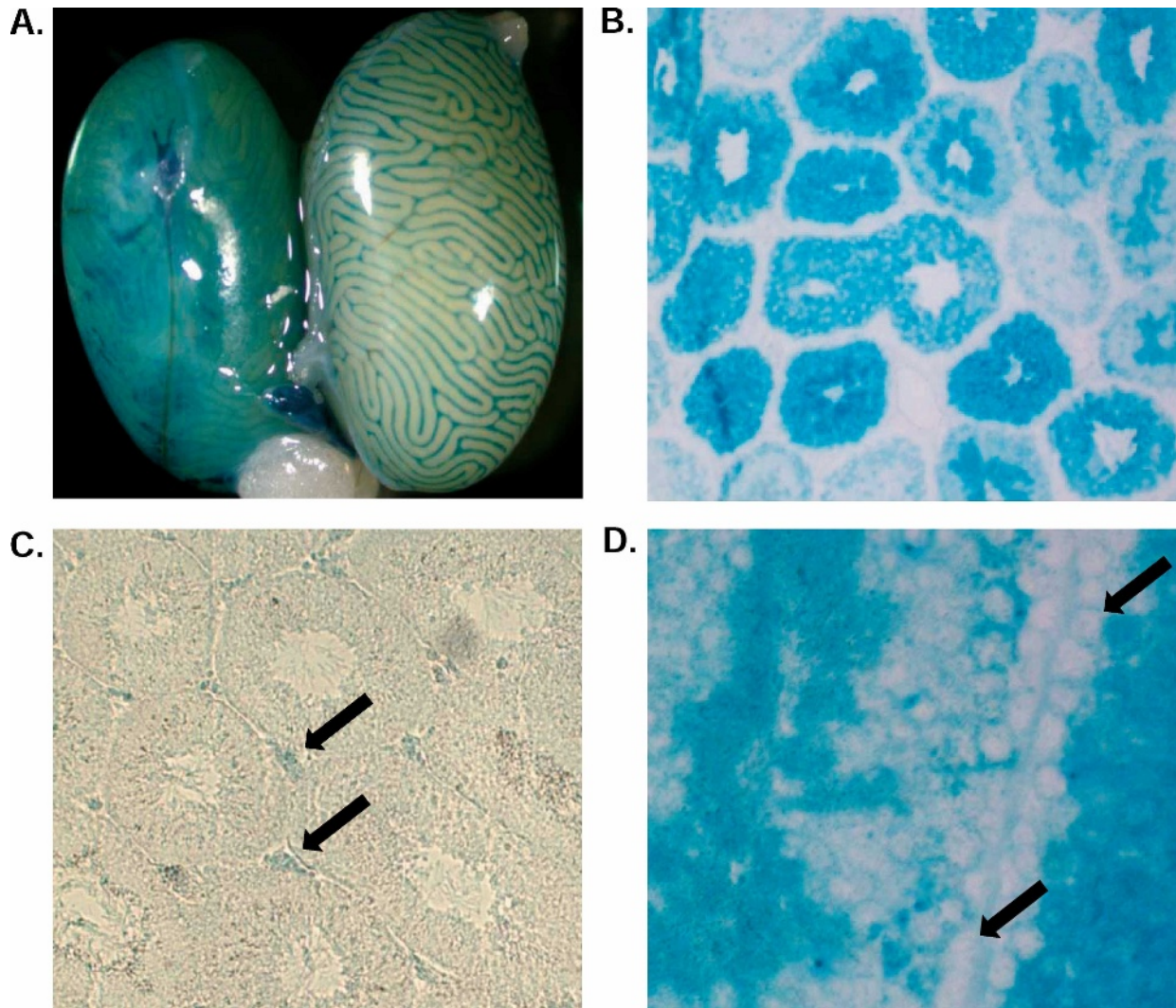


Figure 1. X-gal staining. **(A)** Whole testes from 10-week-old RKIP-1^{-/-} (left) and wild-type (right) males. The seminiferous tubules of the RKIP-1^{-/-} testicle stain to differing degrees, suggesting stage-dependent expression of the reporter. **(B)** Low-magnification cross-section of 10-week-old heterozygous testis stained for LacZ. **(C)** Low-magnification cross-section of 10-week-old wild-type testis stained for LacZ. Arrows indicate minor staining within the interstitial space. **(D)** Higher-power magnification of a cross-section through the reporter-positive testis. Arrows indicate cells adjacent to basal lamina that do not stain with X-gal.

Meiotic Germ Cell Sensitivity to Ionizing Radiation Exposure

Wild-type and RKIP-1^{-/-} mice were exposed to 0.5 Gy lower-body irradiation and killed 15.5 days after exposure (Figure 3). The x-irradiated meiotic germ cells were allowed to develop over 15.5 days into elongate spermatids, and the apoptotic effect of the exposure was quantified by spermatid head counts as a sensitive measure of spermatogenesis. The similarity in testicular spermatid head counts between wild-type and RKIP-1^{-/-} mice indicated that the meiotic spermatocytes in these 2 genotypes were equally susceptible to ionizing radiation.

RKIP-1^{-/-} Mice Undergo Premature Capacitation

Given the recent findings that RKIP-1 may act as a decapacitation factor (Nixon et al, 2006) or as a receptor for decapacitation factors (Gibbons et al, 2005), epididymal sperm from wild-type and RKIP-1^{-/-} mice were evaluated for capacitation status using CTC imaging (Ward and Storey, 1984; Lee et al, 1987; DasGupta et al, 1994; Fuller and Whittingham, 1997). Wild-type sperm were evenly distributed between uncapacitated (44.9%) and capacitated (46.8%) states after a 5-minute incubation in saline (Figure 4). However, RKIP-1^{-/-} mice had a significantly higher percentage of capacitated (80.3%) sperm and fewer

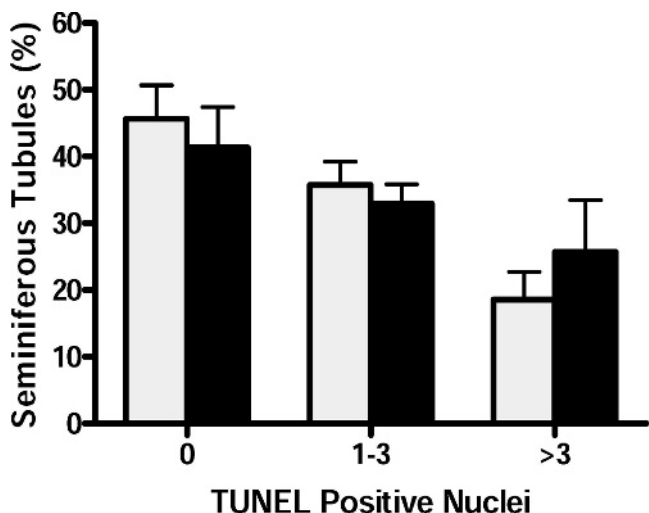


Figure 2. Quantification of seminiferous tubules with 0, 1–3, or more than 3 TUNEL-positive cells following exposure to 5 Gy ionizing radiation. Wild-type (gray bar, $n = 6$) and $RKIP-1^{-/-}$ (black bar, $n = 4$) mice were killed 9 hours after lower-body x-irradiation to assess susceptibility to germ cell apoptosis. No statistical differences were detected between the genotypes ($P > .05$).

uncapacitated (10.3%) sperm. There were no appreciable differences in acrosome-reacted sperm measured between genotypes (8.3% wild-type; 9.4% $RKIP-1^{-/-}$). These results indicate that sperm from $RKIP-1^{-/-}$ mice undergo precocious capacitation.

RKIP-1^{-/-} Mice Exhibit Reduced Reproduction Rates

Because premature capacitation would be expected to lead to reduced reproduction efficiency, the breeding rate was evaluated within the $RKIP-1$ colony. The lack of embryonic lethality from $RKIP-1$ deficiency was previously demonstrated by the normal mendelian distribution of genotypes (Theroux et al, 2007). Comprehensive analysis of the progeny genotypes of 10 breeding pairs per possible genotype pairing revealed that $RKIP-1^{-/-}$ male mice exhibited a reduction in reproduction rates when mated with females lacking either 1 ($P = .02$) or both $RKIP-1$ wild-type alleles ($P = .0002$) (Table). Interestingly, heterozygous male mice mated to $RKIP-1^{-/-}$ females demonstrated normal reproduction rates compared with wild-type.

Sperm Acquire RKIP-1 From the Cytoplasmic Droplet

Immunostaining of caput epididymal sperm showed that $RKIP-1$ was exclusively localized within the cytoplasmic droplet (Figure 5A). The cytoplasmic droplet is residual germ cell cytoplasm, which is reabsorbed during maturation of sperm within the epididymis. Analysis of caudal epididymal sperm (Figure 5B) showed that nearly all sperm had complete resorption of the cytoplasmic droplet with extensive $RKIP-1$ staining in the postacrosomal

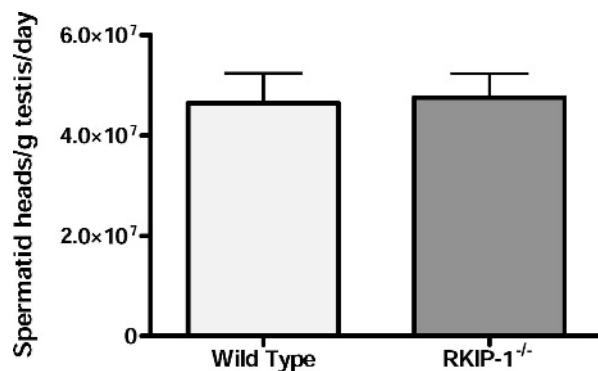


Figure 3. Analysis of wild-type and $RKIP-1^{-/-}$ meiotic germ cell sensitivity to ionizing radiation exposure as measured by sperm production. Wild-type (gray bar, $n = 3$) and $RKIP-1^{-/-}$ mice (black bar, $n = 3$) were exposed to 0.5 Gy lower-body ionizing radiation, and spermatocytes were allowed to age 15.5 days after exposure, developing into elongate spermatids. No statistical differences were detected between the genotypes ($P > .05$).

region and along the tail. Thus, these results suggest that $RKIP-1$ may be transferred from germ cell cytoplasm to mature sperm via the cytoplasmic droplet.

Discussion

Given the abundant presence in epididymal fluids (Jones et al, 1983) and high expression during spermatogenesis (Frayne et al, 1998; Hickox et al, 2002), $RKIP-1$ is suspected to play an important role in the male reproductive tract. Spermatogenic germ cell proliferation is associated with apoptotic changes that regulate sperm output (reviewed by Holdcraft and Braun, 2004). Upon exposure to testicular toxicants, apoptosis occurs through induction of $NF-\kappa B$ (Rasoulpour, 2005, 2007), which also interacts with $RKIP-1$ (Yeung et al, 2001), and through activation of other cell signaling systems (Embree-Ku et al, 2002). In tumor cell lines, $RKIP-1$ sensitizes cells to apoptotic death upon exposure to chemotherapeutic drugs (Chatterjee et al, 2004). Therefore, we investigated the involvement of $RKIP-1$ in germ cell apoptosis following x-irradiation in wild-type and $RKIP-1^{-/-}$ mice. Lower-body exposure of 5 Gy irradiation produced similar levels of germ cell apoptosis in both mouse genotypes 9 hours after exposure. Similarly, wild-type and $RKIP-1^{-/-}$ mice exposed to 0.5 Gy irradiation showed no differences in testicular spermatid head counts 15.5 days after exposure, a sensitive measure of germ cell loss. Taken together, these results indicate a similar susceptibility of wild-type and $RKIP-1^{-/-}$ germ cells to acute or delayed-onset irradiation-induced apoptosis. These findings were somewhat surprising, given the known importance of $RKIP-1$ in sensitizing cancer cells to drug-induced apoptosis

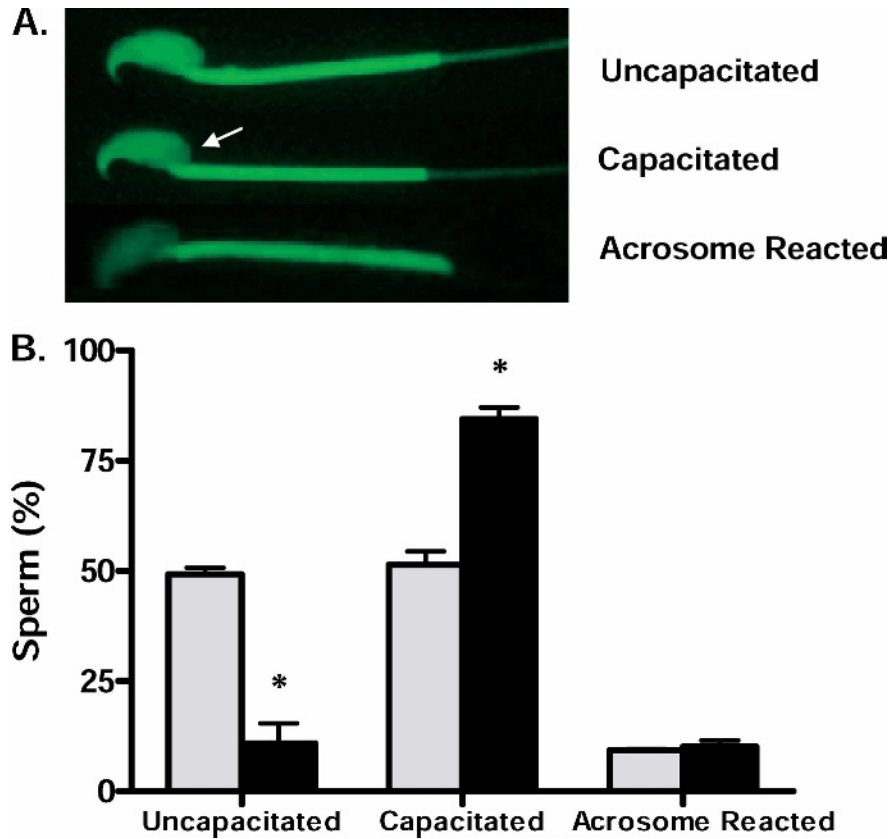


Figure 4. Sperm capacitation states differ in wild-type and RKIP-1^{-/-} mice. (A) Epididymal sperm were imaged with chlortetracycline to identify sperm banding patterns consistent with uncapacitated, capacitated, and acrosome-reacted states. Arrow indicates darker banding pattern associated with capacitated sperm. (B) The percent of capacitated sperm was significantly increased in RKIP-1^{-/-} mice (black bar, n = 3) compared with wild-type mice (gray bar, n = 3). Asterisks indicate significant differences from wild-type ($P < .05$).

(Chatterjee et al, 2004), and indicate that RKIP-1 does not participate in the complex regulation of germ cell apoptosis following ionizing radiation in the testis (Embree-Ku et al, 2002; Rasoulpour and Boekelheide, 2007). Another explanation is that RKIP-2, an RKIP-1 homolog that is prominently expressed within the testis

(Hickox et al, 2002), may provide some compensatory sensitization to apoptosis in the RKIP-1^{-/-} mice.

Capacitation is a highly regulated process in sperm maturation requiring removal of surface decapacitation factors within the female reproductive tract. The loss of decapacitation factors influences changes in sperm motility and enables the acrosomal reaction required for fertilization. Recent reports indicate that RKIP-1 is either a decapacitation factor or a surface receptor for sperm decapacitation factors (Gibbons et al, 2005; Nixon et al, 2006), suggesting that sperm from RKIP-1^{-/-} mice may undergo premature capacitation. Indeed, using the CTC staining technique, significantly higher levels of capacitated sperm were seen in cauda epididymides from RKIP-1^{-/-} mice compared with wild-type. Although many functional aspects of the capacitation process remain controversial, a significant body of evidence suggests that capacitation influences sperm motility, chemotactic responsiveness, and fertilization (Cohen-Dayag et al, 1995; Jaiswal et al, 1998; Zhu et al, 2006). Therefore, we examined the effects of RKIP-1 deficiency on mating success. Conceivably, if capacitation is a critical regulator of sperm maturation,

The number of pups obtained from 10 mating pairs with defined genotypes

Male	Female	# of Pups in 16 Weeks	# of Litters	Average # of Pups per Litter
+/+	+/+	130*	17	7.64
+/+	+/-	139*	17	8.17
+/+	-/-	152*	21	7.23
+/-	+/+	131†	17	7.70
+/-	+/-	130†	21	6.19
+/-	-/-	121†	17	7.11
-/-	+/+	141‡	18	7.83
-/-	+/-	63‡§	10	6.30
-/-	-/-	11‡	3	3.66

Totals are *421, †382, and ‡215.

Statistical differences compared with the number of pups born in 16 weeks from wild-type x wild-type crosses (+/+ x +/+): § $P = .02$; || $P = .0002$.

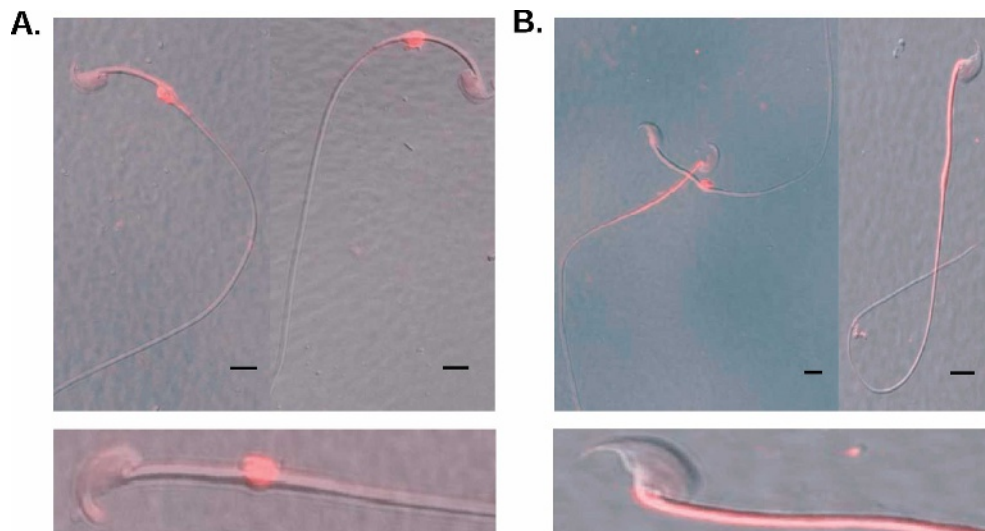


Figure 5. RKIP-1–immunostained caput and cauda epididymal sperm from wild-type mice. Merged fluorescent and differential interference contrast digital images of sperm are shown. **(A)** Caput epididymal sperm with RKIP-1 staining localized to the cytoplasmic droplet. Magnified representative image of a caput epididymal sperm is shown in lower panel. **(B)** Cauda epididymal sperm with RKIP-1 staining localized to the cytoplasmic droplet or diffuse staining along the sperm flagellum. Immunostaining of sperm from *RKIP-1^{-/-}* mice produced no detectable fluorescence (data not shown). Bar = 20 μ m. Magnified representative image of a cauda epididymal sperm is shown in lower panel.

functional differences between genotypes may result in different mating efficiencies. Interestingly, reproduction rates for all mating combinations were unremarkable except for *RKIP-1^{-/-}* males mated to either heterozygous or *RKIP-1^{-/-}* females.

An unanswered question has been the source of RKIP-1 protein expression on sperm. Mature sperm lack the cellular machinery to produce proteins; therefore, RKIP-1 must be acquired either from an earlier germ cell developmental stage or from another source within the male reproductive tract. Previous reports surmised that the “phosphatidyl binding protein” may be transferred from the residual germ cell cytoplasm (Vierula et al, 1992; Saunders et al, 1995). The present study confirms the localization of RKIP-1, demonstrating probable transfer of RKIP-1 from the cytoplasmic droplet to the postacrosomal region of the sperm head and midpiece during maturation in the epididymis. This staining differs slightly from a previously published report (Gibbons et al, 2005), which showed more extensive RKIP staining on the sperm head. The present RKIP staining differences are likely explained by the use of different RKIP antibodies or perhaps the method of epididymal sperm isolation.

The apparent capacity of sperm to accept and integrate RKIP-1 from the cytoplasmic droplet is consistent with our observations of reproductive efficiency. Because the reproduction rates are only decreased in crosses between *RKIP-1^{-/-}* males and either heterozygous or *RKIP-1^{-/-}* females, the RKIP-1–deficient sperm could be acquiring RKIP-1 protein within the female reproductive tract when

insufficient RKIP-1 protein is available to the male sperm during spermatogenesis. A biological role for RKIP-1 secretion in the female could be to provide a dynamic equilibrium for the removal of decapacitation factors from the sperm. As sperm travel within the female reproductive tract, RKIP-1 generated by the female (Frayne et al, 1999) could facilitate the disassociation of decapacitation factors away from the sperm, thus initiating capacitation. Conversely, the equilibrium could also favor sperm reacquiring decapacitation factors in the case of *RKIP-1^{-/-}* sperm in a wild-type or heterozygous female, facilitating a dynamic process of cycling between capacitated and uncapacitated sperm as previously demonstrated in vitro (Gibbons et al, 2005). Although this unique form of protein transfer fits with the current data, further research is necessary to confirm this potential mechanism.

The present study demonstrates similar susceptibility between wild-type and *RKIP-1*–deficient mice to ionizing radiation in the testis. However, important differences exist between these genotypes with regard to sperm capacitation status. These findings suggest that RKIP-1 is an important component in male reproduction and may be an important therapeutic target for improving success rates of male infertility or providing male contraception.

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