Ca²⁺/Calmodulin-dependent Protein Kinase IV Is Expressed in Spermatids and Targeted to Chromatin and the Nuclear Matrix*

Joy Y. Wu and Anthony R. Means‡

From the Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710

Ca²⁺/calmodulin-dependent protein kinase IV and calsperrnin are two proteins encoded by the Camk4 gene. Both are highly expressed in the testis, where in situ hybridization studies in rat testes have demonstrated that CamkIV mRNA is localized to pachytene spermatocytes, while calsperrnin mRNA is restricted to spermatids. We have examined the expression patterns of both CaMKIV and calsperrnin in mouse testis and unexpectedly find that CaMKIV is expressed in spermatogonia and spermatids but excluded from spermatocytes, while calsperrnin is found only in spermatids. CaMKIV and calsperrnin expression in the testes are stage-dependent and appear to be coordinately regulated. In germ cells, we find that CaMKIV is associated with the chromatin. We further demonstrate that a fraction of CaMKIV in spermatids is hyperphosphorylated and specifically localized to the nuclear matrix. These novel findings may implicate CaMKIV in chromatin remodeling during nuclear condensation of spermatids.

Spermatogenesis consists of three phases: mitosis, meiosis, and spermigenesis. Spermatogonia comprise the stem cell population around the periphery of the seminiferous tubules and maintain cell numbers by mitotic divisions. Unknown signals lead to the commitment of some cells to differentiate into spermatocytes, which spend the majority of time in meiotic prophase before undergoing meiosis. Postmeiotic spermatids are closest to the lumen and differentiate into spermatozoa during spermigenesis, a process that includes flagellar formation and nuclear condensation. Within the seminiferous tubule, there are well characterized cell associations that define the histological classification of spermatogenesis in the mouse into 12 distinct stages (1).

Ca²⁺/calmodulin-dependent protein kinase IV (CaMKIV) and calsperrnin (CaS) are two proteins expressed in the testis and encoded by a common gene, Camk4. This gene has three promoters; the first two regulate expression of the COOH-terminal 164 amino acids of CaMKIV, including the calmodulin binding domain (5). CaMKIV is expressed in several tissues in addition to the testis, including brain and thymus (2), whereas calsperrnin is found only in the testis (5). Within the testis, the mRNAs encoding CaMKIV and calsperrnin have distinct expression patterns. In situ hybridization studies in rat testis have demonstrated that Camk4 mRNA is expressed within pachytene spermatocytes (2). On the other hand, calsperrnin mRNA is localized to postmeiotic round spermatids (2, 5). The physiological functions of neither protein are well understood. Several reports have suggested that CaMKIV plays a role in transcriptional regulation in lymphocytes and neurons (6–10). In the testis, CaMKIV has been proposed to regulate calsperrnin expression by phosphorylation and activation of the testis-specific transcription factor CREM (7), which in turn drives transcription of several germ cell genes, including calsperrnin, whose promoters contain cyclic AMP-responsive elements (4, 11, 12). Calsperrnin has no kinase activity and instead may be involved in regulating high levels of calmodulin found in germ cells (13).

Little is currently known about the regulation of CaMKIV in the testis. We have examined the expression and regulation of CaMKIV and calsperrnin in mouse testis. We report that, contrary to predictions based on studies in rat testis, mouse CaMKIV is expressed in spermatogonia and spermatids and excluded from pachytene spermatocytes. In contrast, calsperrnin is restricted to spermatids as expected. The expression of CaMKIV and calsperrnin in seminiferous tubules is stage-dependent and appears to be coordinately regulated. We further demonstrate that CaMKIV is associated with chromatin and the nuclear matrix and may be targeted to the latter structure by phosphorylation. These results may point to a novel function for CaMKIV in posttranscriptional spermatids.

EXPERIMENTAL PROCEDURES

Antibody Production—The cDNA for mouse calsperrnin was cloned by RT-PCR from mouse testis RNA with the following primers: sense 5′-TTCCCATGGGACACTGCTCAGAAGA-3′ and antisense 5′-CTGATCGTAGGGAAGGACAGCTTAG-3′. The sequence was then subcloned into the Ncol and XbaI sites of the pET-30c vector (Novagen, Madison, WI) to generate His₅-tagged calsperrnin. The fusion protein was expressed in Escherichia coli and purified over a nickel resin according to the manufacturer’s protocol. Purified protein was mixed with Freund’s adjuvant and injected into rabbits to raise a polyclonal antibody against calsperrnin (anti-CaS).

Western Blotting—Tests were homogenized in a buffer with 25 μM Hepes, 1 mM EGTA, 1 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 20 μg/ml trypsin inhibitor, and 0.1 μg/ml Pefabloc. 75 μg of protein were subjected to electrophoresis on a 12% gel, transferred to a polyvinylidene difluoride membrane, and blocked with 5% milk in Tris-buffered saline with 0.05% Tween 20. Membranes were incubated with anti-CaS (1:5000), anti-CaMKIV (1:1000) (kindly provided by Dr. Hiroaki Sakagami, Tohoku University School of Medicine, Sendai, Japan), anti-CalMK (1:1000) (Transduction Laboratories, San Diego, CA), or anti-lamin B (1:1000) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), detected with horseradish peroxidase-conjugated antibody, and developed with the ECL chemiluminescence system (Amersham Pharmacia Biotech).
Calmodulin Overlay—75 μg of testis protein were subjected to electrophoresis on a 12% gel, transferred to an Immobilon membrane (Millipore Corp., Bedford, MA), and then incubated with 100 mM imidazole (pH 7.0) for 10 min followed by a 40-min incubation in solution G (20 mM imidazole, pH 7.0, 200 mM KCl, 0.1% bovine serum albumin, 0.02% NaN₃, 1 mM CaCl₂, 0.05% Tween 20) (14). The membrane was then incubated in solution G with 125I-calmodulin (specific activity 1 × 10⁶ cpm/ml) for 2 h and washed twice with solution G for 30 min. Calmodulin binding was detected by autoradiography. Iodination of CaM was performed with Bolton-Hunter reagent (Amersham Pharmacia Biotech) as described (15).

Hormonal Treatment—23-Day-old male mice were injected intraperitoneally with 100 i.u. of pregnant mare serum gonadotropin (Sigma) each day for 7 days and then sacrificed by cervical dislocation. Sections were cut and stained with periodic acid Schiff-hematoxylin (Poly Scientific, Bay Shore, NY), detected with diaminobenzidine (Sigma) or NovaRed substrate (Vector Laboratories, Burlingame, CA), and counterstained with hematoxylin.

Nonidet P-40 Extraction of Testis Proteins—Testis proteins were fractionated by Nonidet P-40 solubility as described by Moroi et al. (16). Briefly, testes were homogenized in 1 ml of Nonidet P-40 buffer (25 mM HEPES, pH 7.4, 150 mM NaCl, 4 mM EDTA, 25 mM NaF, 1% Nonidet P-40, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin), rotated at 4 °C for 30 min, and centrifuged at 10,000 × g for 30 min. The supernatant was designated the Nonidet P-40-soluble fraction, and the pellet was resuspended in 100 μl of Nonidet P-40 buffer (25 mM HEPES, pH 7.4, 4 mM EDTA, 25 mM NaF, 1% SDS, 1 mM Na₃VO₄, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Following the addition of 900 μl of Nonidet P-40 buffer, samples were rotated at 4 °C for 30 min and then centrifuged at 10,000 × g for 30 min. The resulting supernatant was designated the Nonidet P-40-insoluble fraction.

Dephosphorylation with λ-Phosphatase—50 μg of testis protein were incubated with 100, 400, or 1000 units of λ phosphatase (New England Biolabs, Beverly, MA) for 1 h at 30 °C. Phosphatase activity was inhibited by the addition of 50 mM EDTA.

Chromatin and Nuclear Matrix Preparation—Isolation of chromatin and nuclear matrix was performed as described (17) with modifications. Testes were homogenized in cytoskeletal buffer (CSK; 10 mM Pipes, pH 6.8, 100 mM NaCl, 500 mM sucrose, 3 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 0.5% Triton X-100, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄, and 25 mM NaF). Following incubation at 4 °C for 30 min, samples were centrifuged at 5000 × g for 3 min. Chromatin was released by digestion with 1000 units/ml RNase-free Dnase 1 (Roche Molecular Biochemicals) in CSK buffer for 15 min at 37 °C. Ammonium sulfate in CSK buffer was added to a final concentration of 0.25 M. Samples were incubated at 4 °C for 5 min and centrifuged. The pellet was extracted with 2 M NaCl in CSK buffer for 5 min at 4 °C and then centrifuged. The resulting nuclear matrix fraction was solubilized in urea buffer (8 M urea, 0.1 M NaH₂PO₄, 0.1 M Tris, pH 8).

RESULTS

Although the rat and mouse forms of CaMKIV are highly conserved in their amino termini, they diverge significantly at the carboxyl terminus (18). To characterize the patterns of CaMKIV and calspermin expression in the mouse, we cloned and expressed mouse calspermin as a His-tagged fusion protein (his-CaS; Fig. 1A, lanes 1 and 2). Purified His-tagged calspermin is recognized at high concentrations by an antibody raised against rat calspermin (lane 3) and binds 125I-CaM on overlay (lane 4), confirming the presence of a functional calmodulin binding domain in the recombinant protein. This protein was used to raise a polyclonal rabbit antibody that detects both CaMKIV and calspermin (Fig. 1B).

We used the polyclonal rabbit antibody to examine the age-dependent expression patterns of CaMKIV and calspermin in the mouse testis during postnatal development. The first wave of spermatogenesis after birth proceeds synchronously. For the first two postnatal weeks, seminiferous tubules are populated only by Sertoli cells and spermatogonia, with pachytene spermatocytes appearing on day 14 and round spermatids on day 21 (19). We unexpectedly found that CaMKIV protein is already expressed at day 9, although at low levels (Fig. 1C, upper panel). The appearance of CaMKIV in the testis occurs earlier than would be expected for localization in pachytene spermatocytes and suggests that CaMKIV may in fact be expressed instead in spermatogenesis. CaMKIV protein levels first peak at day 15, with a second, more pronounced increase after day 25. In contrast, calsermin protein does not appear until day 25, as predicted if expressed in postmeiotic spermatids (Fig. 1C, lower panel). Western blotting of testis extracts from germ cell-deficient W/Wo mice, which contain only Sertoli cells, confirmed that CaMKIV is restricted to germ cells (Fig. 1D).

To examine the cell types expressing CaMKIV and calspermin, we performed immunohistochemical analysis of testes from mice of varying ages. Positive staining can be seen in numerous cells at the periphery of the seminiferous tubule by day 8, when Western blot results indicate that only CaMKIV is expressed (Fig. 2A). Since CaMKIV is not expressed in Sertoli cells, these cells must be spermatogenesis. Sections incubated with preimmune serum or with antibody preabsorbed with antigen show no positive staining of spermatogonia (Fig. 2B and data not shown). By day 15, CaMKIV-positive cells are clearly restricted to peripheral spermatogonia, with no staining visible in newly emerging pachytene spermatocytes (Fig. 2C). Immunostaining of testes at day 25, when calsermin is first expressed as determined by Western blotting, revealed the appearance of positive staining in round spermatids as they

![Fig. 1. Expression of mouse calsermin and production of anti-calspermin antibody.](http://www.jbc.org/)
reached stages IV and V, confirming that calspermin is produced in haploid postmeiotic germ cells (Fig. 2D). The adult pattern of immunohistochemical staining was established by day 30 (Fig. 2E). Interestingly, there is a clear stage dependence in staining of both spermatogonia and spermatids. Both of these cell types first stain positive at stages IV and V and maintain expression through stages VIII and IX (Fig. 2F). These results establish a novel cellular localization for CaMKIV as well as an apparently coordinated stage-dependent pattern of expression for both CaMKIV and calspermin.

There is a significant increase in CaMKIV protein levels after day 25 (Fig. 1C). This is coincident with the time when mice begin to attain sexual maturity and may simply reflect the increase in germ cell numbers at puberty. However, there is a surge in gonadotropin levels at this time as well, raising the possibility that CaMKIV might be hormonally regulated. To determine whether repeated administrations of FSH could increase CaMKIV levels in the testis, 23-day-old mice were injected with 5 IU of FSH every day for a week. Testis lysates from hormone-treated and control mice were then immunoblotted for CaMKIV, with no detectable change in CaMKIV levels (data not shown). Hypophysectomy has also been used to study hormonal effects on gene regulation in rat testis (21, 22). Postpubertal mice were hypophysectomized and sacrificed 1 week later. Again, no change in CaMKIV levels was evident (data not shown). These results indicate that CaMKIV is not acutely regulated by hormonal stimuli.

Another explanation for the observed increase in CaMKIV levels in postpubertal mice would be the expression of CaMKIV in a new population of cells. Although immunohistochemistry results confirm that CaMKIV is expressed in spermatogonia while calspermin is found in spermatids, they do not rule out the possibility that CaMKIV is also expressed in spermatids, since the antibody cannot distinguish between the two proteins. To address this question, we turned to germ cell fractionation, in which relatively pure populations of germ cells can be isolated by passing them through a density gradient (23). Western blotting of isolated pachytene spermatocyte, round spermatid, and condensing spermatid fractions demonstrated that CaMKIV is indeed expressed at high levels in round and condensing spermatids but is completely absent in pachytene spermatocytes (Fig. 3A). Therefore, contrary to in situ hybridization studies to detect Camk4 mRNA in rat testis, our results establish that mouse CaMKIV protein is excluded from pachytene spermatocytes but expressed in both spermatogonia and elongating spermatids.

Proteins expressed in elongating spermatids are frequently subjected to translational regulation. For instance, the protamine genes are transcribed in round spermatids and then stored for 1 week as cytoplasmic ribonucleoprotein particles before being translated in late elongating spermatids (24). More extensive analysis has demonstrated that the increase in CaMKIV protein levels occurs at approximately day 27 (Fig. 4B and data not shown). If CaMKIV were translationally regulated, one might predict an up-regulation of mRNA levels before the increase in protein levels at day 27. Northern blot analysis of testis RNA from mice of increasing ages revealed that there is a significant increase in mRNA levels at day 27, along with an apparent decrease in the length of the CaMKIV message (Fig. 3B). The increase in mRNA levels occurs concomitantly with the increase in CaMKIV protein, indicating
CaMKIV localization to nuclear matrix in spermatids

**FIG. 3. CaMKIV is expressed in spermatids.** A, purified pachytene spermatocytes (PS), round spermatids (RS), and condensing spermatids (CS), were isolated by passing through a density gradient, lysed in sample buffer, and immunoblotted for CaMKIV along with whole testis lysate (TS). B, Camk4 mRNA levels increase, and calserpin mRNA appears at day 27. Testes from mice of varying ages were homogenized, and total RNA was extracted. Testis RNA was probed with an antibody specific for CaMKIV and calserpin.

**FIG. 4. Phosphorylated CaMKIV is present in the detergent-insoluble fraction of spermatids.** A, CaMKIV is found in both Nonidet P-40-soluble and -insoluble fractions. Testis proteins were separated by solubility in Nonidet P-40 as described under “Experimental Procedures.” 100 μg of Nonidet P-40-soluble (NP) and 20 μg of Nonidet P-40-insoluble (SDS) proteins were resolved by SDS-PAGE and probed for CaMKIV expression. B, a CaMKIV band of higher electrophoretic mobility appears after day 25. Testes were isolated from mice of varying ages, and one testis from each was separated into Nonidet P-40-soluble (NP) and -insoluble (NS) fractions. CaMKIV levels were detected by Western blot. C, ND40-insoluble (N) and -soluble (S) proteins from adult testes were immunoblotted with an antibody specific for CaMKIVb. D, the electrophoretic mobility shift is due to phosphorylation of CaMKIVα. Testis lysate was incubated with 0 units (lane 1), 100 units (lane 2), 400 units (lane 3), or 1000 units (lane 4) of λ-phosphatase for 1 h at 30°C. Phosphatase activity (400 units) was inhibited by the addition of 50 mM EDTA (lane 5).

that CaMKIV expression in spermatids is not translationally regulated. Likewise, calserpin mRNA appears on day 27, the same day at which calserpin protein is first expressed.

By immunohistochemical staining at higher magnifications, CaMKIV appears to be present in the nucleus of both spermatogonia and spermatids, where CaMKIV might be expected to localize if it is involved in transcriptional regulation. The acidic COOH terminus of CaMKIV may be characteristic of chromatin-associated proteins (25). To determine whether CaMKIV might be bound to chromatin, we first examined whether CaMKIV in a testis lysate is resistant to detergent extraction (16). CaMKIV is present in both Nonidet P-40-soluble and -insoluble fractions (Fig. 4A). Furthermore, it exists as a doublet in both, although the upper band runs with slower electrophoretic mobility in the Nonidet P-40-insoluble fraction. Western blotting of testis lysates from mice of varying ages revealed that the higher band in the insoluble fraction does not appear until day 27 (Fig. 4B), suggesting that this might represent a spermatid-specific pool of CaMKIV. In support of this possibility, the upper band comigrates with a band of higher electrophoretic mobility seen in purified elongating spermatids (Fig. 3A and data not shown). The appearance of the higher band accounts for the increased protein levels after day 25 seen in Fig. 1C.

The doublet bands seen in both Nonidet P-40-soluble and -insoluble fractions might represent different CaMKIV isoforms or posttranslational modification. Two isoforms of CaMKIV have been identified in the cerebellum, with the larger β-isofrom distinguished by a 28-amino acid NH2-terminal extension (26, 27). A Western blot was performed with an antibody specific for the CaMKIVβ isoform to determine whether the two bands of CaMKIV seen in the testis correspond to the two different isoforms. Although CaMKIVβ is clearly expressed in the cerebellum, there is no discernible expression in the testis, suggesting that the upper band is not the CaMKIVβ isoform (Fig. 4C). Activation of CaMKIV requires phosphorylation by an upstream Ca2+/calmodulin-dependent protein kinase kinase (28, 29). To establish whether the upper band represents a posttranslational modification of CaMKIVα, testis lysates were treated with increasing concentrations of λ-phosphatase (Fig. 4D). This resulted in the collapse of the doublet into a single band of faster electrophoretic mobility, indicating that the electrophoretic mobility shift is due to phosphorylation. This shift could be prevented by the addition of 50 mM EDTA to inhibit the phosphatase (Fig. 4D, lane 5).

To determine whether CaMKIV is indeed associated with chromatin, the detergent-insoluble pellet was treated with DNase I followed by 0.25 M ammonium sulfate to release chromatin-bound proteins (17). The remaining pellet was then washed with 2 M NaCl and resuspended in urea buffer to obtain the nuclear matrix. Western blotting of these fractions demonstrated that CaMKIV is associated with both chromatin and the nuclear matrix (Fig. 5A, upper panel). The presence of nuclear matrix proteins was confirmed by blotting for lamin B, a matrix-specific marker (Fig. 5A, lower panel) (27). Interestingly, the CaMKIV present in each fraction appears to be differentially phosphorylated, with the higher band present only in the nuclear matrix. To examine whether this might be spermatid-specific hyperphosphorylated CaMKIV, testes from mice at different ages were fractionated and blotted for CaMKIV. CaMKIV is not present in the nuclear matrix until day 27 (Fig. 5B, lane 12), although it can be found in the chromatin at any age (Fig. 5B, lanes 2, 6, and 10). These results suggest that at least a portion of CaMKIV expressed in elongating spermatids is hyperphosphorylated and targeted to the nuclear matrix.

**DISCUSSION**

These studies have focused on the characterization of CaMKIV regulation and expression patterns in mouse testis. They have led to the unexpected finding that CaMKIV is not
expressed in pachytene spermatocytes as had been predicted based on previous studies in rat testis (2). CaMKIV is instead expressed initially in spermatogonia, and then again in elongating spermatids. There are several potential explanations for the observed differences. One reason is that conclusions about CaMKIV localization in rat testis were based only on in situ hybridization to detect mRNA, whereas our experiments have examined the localization of the protein in mouse. It is possible that the mRNA is synthesized early in spermatocytes, then stored until the spermatid stage for translation, as occurs with the protamines (24). However, although Camk4 mRNA is already present at day 9 in mouse testis, there is a significant increase in mRNA level at day 27 coincident with the appearance of CaMKIV in spermatids, suggesting that CaMKIV expression in spermatids is not translationally regulated. This increase is accompanied by a reduction in the length of the Camk4 mRNA. Human testis has been reported to have CaMKIV mRNAs of several different lengths (30). The decrease in length may be due to use of an alternate polyadenylation site, shortening of the poly(A) tail, or perhaps even alternative splicing. In the rat brain, use of alternate polyadenylation sites results in CaMKIV mRNAs of differing lengths (31). Another explanation for the discrepancy in expression patterns is that there may be some species differences in CaMKIV expression patterns. Further studies on the localization of CaMKIV protein in rat testis will be required to clarify this issue.

We have also found that CaMKIV expression is stage-dependent in the adult mouse testis and appears to be coordinated with that of calserpin. To our knowledge, this is the first demonstration of coordinated stage-dependent expression for two proteins encoded by the same genetic locus. In the testis, germ cell-specific isoforms of proteins are often produced by the use of alternative promoters and/or splicing (32, 33). However, CaMKIV and calserpin are unusual in that the proteins are functionally distinct and both are expressed in the testis. Intriguingly, several examples of functionally unrelated gene products involve other calmodulin-dependent protein kinases. These include three proteins lacking kinase activity, telokin/KRP (34), øKAP (35), and CARP (36), derived from the loci encoding myosin light chain kinase, CaM-dependent protein kinase II, and CaM-dependent protein kinase VI, respectively. The coordinated expression of CaMKIV and calserpin in stages IV through IX suggests a common regulation, although the mechanism is not clear. However, the expression of calserpin is not dependent on CaMKIV, since calserpin production is unaffected in mice lacking CaMKIV.

The appearance of CaMKIV in elongating spermatids is temporally coincident with the increase in CaMKIV protein and mRNA levels at day 27 as well as the appearance of a slower migrating band of CaMKIV resistant to detergent extraction. This shift in electrophoretic mobility is due to phosphorylation, since the testis is devoid of the CaMKIVβ isoform and phosphatase treatment of testis lysates results in the presence of only a single band of faster electrophoretic migration. Taken together, these findings suggest the presence of a previously unidentified fraction of hyperphosphorylated CaMKIV in elongating spermatids. This hyperphosphorylated CaMKIV is exclusively targeted to the nuclear matrix.

The nature of the phosphorylation events targeting CaMKIV to the nuclear matrix has not been elucidated. Activation of CaMKIV is thought to require a unique three-step mechanism: 1) binding of Ca2+/CaM, 2) phosphorylation of Thr196 by Ca2+/calmodulin-dependent protein kinase kinase, and 3) autophosphorylation of Ser12 and Ser13 in the amino terminus (8). Expression of CaMKIV in BJAB cells (18) or rabbit reticulocyte lysates (8) results in the appearance of two closely migrating bands. Mutations that abrogate kinase activity abolish the upper band (8), suggesting that phosphorylation of one or more of these sites may be responsible for a shift in electrophoretic mobility. There is also a phosphorylated band in the soluble CaMKIV fraction that migrates somewhat faster than phosphorylated detergent-insoluble CaMKIV and may reflect phosphorylation on fewer or perhaps alternate sites. Identification of the target residues in each case may offer further insights into the regulatory mechanisms governing CaMKIV expression and localization in germ cells.

The novel finding of CaMKIV expression in elongating spermatids raises the question of the function of CaMKIV in these cells. In lymphocytes and hippocampal neurons, there is evidence to suggest that CaMKIV plays a role in cyclic AMP-responsive element-binding protein-mediated transcription (6–10). This led to the proposal that in the testis CaMKIV has a similar function in regulating CREM, another member of the B-ZIP family of transcription factors (11). However, the results we report here cast doubt on the role of CaMKIV as a physiologically relevant activator of CREM. CaMKIV is not expressed in pachytene spermatocytes or early round spermatids, where it might colocalize with CREM. In addition, CaMKIV is not produced until spermatids begin to elongate, after transcriptional activity has ceased (37). Indeed, we have recently demonstrated that cyclic AMP-responsive element-dependent gene transcription is intact in mice with a targeted deletion of CaMKIV.

Some clues to the function of CaMKIV in spermatids might be found in its localization to the chromatin (an observation previously noted in neurons by Jensen et al. (42)) and nuclear matrix. One of the hallmarks of spermiogenesis is nuclear condensation, during which DNA is compacted by the sequential replacement of histones by transition proteins and protamines (38). DNA packaging in sperm differs from that of other cell types in that sperm DNA is organized into linear, side-by-side arrays rather than into nucleosomes as found in somatic cells (39). These linear arrays allow for greater packing of DNA, with the result that sperm DNA is 6-fold more condensed than DNA in mitotic chromosomes (39, 40). Of particular note is that CaMKIV appears to be targeted to the nuclear matrix only in elongating spermatids. In mammalian sperm nuclei, the nuclear matrix is believed to organize DNA further into loop

\[2 \text{ J. Y. Wu and A. R. Means, submitted for publication.}\]
CaMKIV Localization to Nuclear Matrix in Spermatids

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REFERENCES

1. Russell, L. D., Ettlin, R. A., Sinha Hikim, A. P., and Clegg, E. D. (1990) Histological and Histopathological Evaluation of the Testis, 1st Ed., pp. 119–161, Cache River Press, Clearwater, FL.
2. Means, A. R., Cruzalegui, F., LeMagueresse, B., Needleman, D. S., Slaughter, G. R., and Ono, T. (1991) Mol. Cell. Biol. 11, 3960–3971.
3. Means, A. R., Ribar, T. J., Kane, C. D., Hook, S. S., and Anderson, K. A. (1997) Recent Prog. Horm. Res. 52, 389–406.
4. Sun, Z., Sassone-Corsi, P., and Means, A. R. (1995) Mol. Cell. Biol. 15, 561–571.
5. Ono, T., Slaughter, G. R., Cook, R. G., and Means, A. R. (1989) J. Biol. Chem. 264, 2081–2087.
6. Ensen, H., Sun, P., Brickey, D., Soderling, S. E., Klamo, E., and Soderling, T. R. (1994) J. Biol. Chem. 269, 15520–15527.
7. Sun, P., Ensen, H., Myung, P. S., and Maurer, R. A. (1994) Genes Dev. 8, 2527–2539.
8. Chatila, T., Anderson, K. A., Ho, N., and Means, A. R. (1996) J. Biol. Chem. 271, 21542–21548.
9. Anderson, K. A., Ribar, T. J., Illario, M., and Means, A. R. (1997) Mol. Endocrinol. 11, 725–737.
10. Bito, H., Deisseroth, K., and Tsien, R. Y. (1996) Cell 87, 1203–1214.
11. Sun, Z., and Means, A. R. (1996) in Proceedings of the 9th European Testis Workshop (Hansson, V., ed) pp. 29–32, Springer-Verlag, Berlin.
12. Foulkes, N. S., Schletter, F., Pevet, P., and Sassone-Corsi, P. (1993) Nature 362, 264–266.
13. Means, A. R., and Cruzalegui, F. (1993) Recent Prog. Horm. Res. 48, 79–97.
14. Glennay, J. R., Jr., and Weber, K. (1986) J. Biol. Chem. 255, 10551–10554.
15. Chafoulias, J. G., Dedman, J. R., Munjai, R., and Means, A. R. (1979) J. Biol. Chem. 254, 10262–10267.
16. Morii, S., Saitou, M., Fujimoto, K., Sakakibara, A., Furuse, M., Yoshida, O., and Tsukita, S. (1998) Am. J. Physiol. 274, C1708–C1717.
17. Reyes, J. C., Muchardt, C., and Yaniv, M. (1997) J. Cell Biol. 137, 263–274.
18. Mosialos, G., Haninian, S. H., Jewahar, S., Vara, L., Kieff, E., and Chatila, T. A. (1994) J. Virol. 68, 1697–1705.
19. Don, J., Winer, M. A., and Wolgemuth, D. J. (1994) Mol. Reprod. Dev. 38, 16–23.
20. Grasso, P., Ronhavskaya, M., and Reichert, L. E., Jr. (1997) Endocrinology 138, 4215–4219.
21. Nazian, S. J., Brewer, L. D., and Ncss, G. C. (1991) J. Androl. 12, 264–272.
22. Krummen, L. A., Toppari, J., Kim, W. H., Morelos, B. S., Ahmad, N., Swardloff, R. S., Ling, N., Shimasaki, S., Esch, F., and Bhasin, S. (1989) Endocrinology 125, 1630–1637.
23. Belloe, A. R., Cavicchia, J. C., Millette, C. F., O’Brien, D. A., Bhatnagar, Y. M., and Dym, M. (1977) J. Cell Biol. 74, 68–85.
24. Kibbe, K. C., Dietel, R. J., and Hecht, N. B. (1984) Dev. Biol. 105, 71–79.
25. Earnshaw, W. C. (1987) J. Cell Biol. 105, 1479–1482.
26. Ohmstede, C. A., Jensen, K. F., and Sahyoun, N. E. (1989) J. Biol. Chem. 264, 5866–5875.
27. Sakagami, H., and Kondo, H. (1993) Brain Res. Mol. Brain Res. 19, 215–218.
28. Tokumitsu, H., Ensen, H., and Soderling, T. R. (1995) J. Biol. Chem. 270, 19230–19234.
29. Edelman, A. M., Mitchellhill, K. I., Selbert, M. A., Anderson, K. A., Hook, S. S., Stapleton, D., Goldstein, E. G., Means, A. R., and Kemp, B. E. (1996) J. Biol. Chem. 271, 10806–10810.
30. Bland, M. M., Monroe, R. S., and Ohmstede, C. A. (1994) Gene (Amst.) 142, 191–197.
31. Ohmstede, C. A., Bland, M. M., Merrill, B. M., and Sahyoun, N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5784–5788.
32. Erickson, R. P. (1999) Trends Genet. 6, 204–209.
33. Eddy, E. M. (1998) Semin. Cell Dev. Biol. 9, 451–457.
34. Collinge, M., Matrisian, P. E., Zimmer, W. E., Shattuck, R. L., Lukas, T. J., Van Eldik, L. J., and Wattersson, D. M. (1992) Mol. Cell. Biol. 12, 2359–2371.
35. Bayer, K. U., Lohler, J., and Harbers, K. (1996) Mol. Cell. Biol. 16, 29–36.
36. Vreugdenhil, E., Datson, N., Engels, B., de Jong, J., van Koningsbruggen, S., Schaaf, M., and de Kloet, E. R. (1999) J. Neurobiol. 39, 41–50.
37. Soderstrom, K. O., and Parvinen, M. (1976) Mol. Cell. Endocrinol. 5, 181–199.
38. Meistrich, M. L. (1989) in Histones and Other Basic Nuclear Proteins (Hnilica, L. S., Stein, G. S., and Stein, J. L., eds) pp. 165–182, CRC Press, Inc., Boca Raton, FL.
39. Ward, W. S., and Coffey, D. S. (1991) Biol. Reprod. 44, 569–574.
40. Balhorn, R. (1982) J. Cell Biol. 93, 298–305.
41. Ward, W. S., Partin, A. W., and Coffey, D. S. (1989) Chromosoma 98, 153–159.
42. Jensen, K. F., Ohmstede, C. A., Fisher, R. S., and Sahyoun, N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2650–2653.
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Joy Y. Wu and Anthony R. Means

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