

Kinesin-related Proteins in the Mammalian Testes: Candidate Motors for Meiosis and Morphogenesis

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The kinesin superfamily of molecular motors comprises proteins that participate in a wide variety of motile events within the cell. Members of this family share a highly homologous head domain responsible for force generation attached to a divergent tail domain thought to couple the motor domain to its target cargo. Many kinesin-related proteins (KRPs) participate in spindle morphogenesis and chromosome movement in cell division. Genetic analysis of mitotic KRPs in yeast and *Drosophila*, as well as biochemical experiments in other species, have suggested models for the function of KRPs in cell division, including both mitosis and meiosis. Although many mitotic KRPs have been identified, the relationship between mitotic motors and meiotic function is not clearly understood. We have used sequence similarity between mitotic KRPs to identify candidates for meiotic and/or mitotic motors in a vertebrate. We have identified a group of kinesin-related proteins from rat testes (termed here testes KRP1 through KRP6) that includes new members of the bimC and KIF2 subfamilies as well as proteins that may define new kinesin subfamilies. Five of the six testes KRPs identified are expressed primarily in testes. Three of these are expressed in a region of the seminiferous epithelia (SE) rich in meiotically active cells. Further characterization of one of these KRPs, KRP2, showed it to be a promising candidate for a motor in meiosis: it is localized to a meiotically active region of the SE and is homologous to motor proteins associated with the mitotic apparatus. Testes-specific genes provide the necessary probes to investigate whether the motor proteins that function in mammalian meiosis overlap with those of mitosis and whether motor proteins exist with functions unique to meiosis. Our search for meiotic motors in a vertebrate testes has successfully identified proteins with properties consistent with those of meiotic motors in addition to uncovering proteins that may function in other unique motile events of the SE.

INTRODUCTION

The accurate and efficient movement of various macromolecules and organelles within the cell is essential for viability. Motor proteins transport diverse cargoes necessary for the cell's continued existence. Members of the kinesin superfamily of motor molecules produce movement necessary for transport of vesicles, positioning of nuclei, and proper segregation of ge-

netic material to daughter cells (reviewed in Endow, 1991; Goldstein, 1993). KRPs contain a mechanochemical domain that is 30–40% identical to other family members but this sequence conservation does not extend to the remainder of the molecule. These variable regions, often termed tail domains, are thought to specify the target and cellular function of each KRP. The approximately thirty known members of the kinesin superfamily can be subdivided according to functional and structural similarities.

Many KRPs so far identified are involved in some aspect of cell division (reviewed in Gorbsky, 1992;

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Endow, 1993; Sawin and Endow, 1993; Wadsworth, 1993). These movements include spindle morphogenesis, maintenance, and elongation at anaphase b; and chromosome movements at prometaphase, metaphase, and anaphase a. The existence of numerous motor proteins involved in cell division prompts the question of which specific task(s) an individual KRP performs. Although the precise mechanism by which mitotic motors perform their function is not known, enough molecular participants have been identified to propose and test models. Members of one mitotic subfamily, defined by the *bimC* protein from *Aspergillus nidulans*, are required for the separation of centrosomes in the formation of a bipolar spindle. Mitotic KRPs such as *bimC*, *cut7*, *Eg5*, KIP1, and CIN8 are localized to centrosomes and are required early in division to force centrosomes apart to form an intact spindle (Enos and Morris, 1990; Hagan and Yanagida, 1990; Le Guellac *et al.*, 1991; Hoyt *et al.*, 1992; Roof *et al.*, 1992; Saunders and Hoyt, 1992). Members of this subfamily are also found along spindle fibers and are proposed to mediate microtubule sliding during anaphase b.

Although mitotic KRPs display considerable functional diversity, meiotic cell division requires other levels of complexity due to differences in cell type, chromosome behavior, and protein:DNA structures. Meiosis is conducted by a very specific cell population, the germ cells, in a highly regulated way to produce gametes with a haploid DNA complement. The motor functions of an individual testicular cell could be modulated by interaction of constitutive proteins with cell type-specific proteins thus altering the phenotype of the cell. During gametogenesis, germ cells undergo two nuclear divisions including a reductive division at meiosis I. In contrast to the separation of sister chromosomes during mitosis, homologous chromosome pairs (termed a bivalent), exchange genetic material and disjoin during the first meiotic division. Crossing over and the formation of chiasma physically link homologues and result in forces on the bivalent that are markedly different from those experienced by sister chromatids during mitosis. The positioning of chromosomes at the metaphase plate also differs between the two processes with homologues, not sister chromatids, orienting toward the poles at metaphase of meiosis I. These unique chromosome behaviors suggest that specific motor proteins may function in the specialized movements and spindle dynamics of meiosis.

Curiously, although other proteins, such as the tubulins, have isoforms found only in the germ line, no KRP has been detected with exclusive function in meiosis (Fuller *et al.*, 1988). Some motors do appear to have overlapping functions in mitosis and meiosis. Two KRPs from *Drosophila*, *nod* and *ncd*, are required for female meiosis and their molecular characteriza-

tion has provided information about the role of KRPs in meiotic chromosome segregation (Endow *et al.*, 1990; McDonald and Goldstein, 1990; Zhang *et al.*, 1990). *nod* produces the force necessary to maintain nonchiasmatic chromosomes at the midplate and prevent premature disjunction and chromosome loss (Theurkauf and Hawley, 1992). Unlike *nod*, *ncd* acts on all chromosomes and is essential for formation of an intact, bivalent spindle (Endow *et al.*, 1990). Both proteins also function in mitosis as mutations in these genes cause disruption of early embryonic cell division (Komma *et al.*, 1991; Hatsumi and Endow, 1992). Both *Eg5* and *cut7*, members of the *bimC* subfamily, are found in both mitotic and meiotic spindles (Hagan and Yanagida, 1990; Aizawa *et al.*, 1992). Although it is clear that KRPs can have dual functions in both types of cell division, the unique chromosome behavior characteristic of meiosis seems to require the participation of specific motor proteins. In addition, kinesin motors involved in meiosis have only been identified in invertebrates (Endow *et al.*, 1990; McDonald and Goldstein, 1990; Zhang *et al.*, 1990). Therefore, we undertook a systematic analysis of KRPs in a mitotically and meiotically active vertebrate tissue in an effort to identify new meiotic motors and to determine the extent of functional overlap between mitotic and meiotic motor proteins.

We chose testes RNA as starting material for several reasons. In addition to the easy availability of this tissue, the cells of the seminiferous epithelia (SE) of the testes undergo uninterrupted rounds of meiosis throughout the life of the adult animal. This is in contrast to the situation in the ovary where meiosis is initiated prenatally, arrested at prophase of meiosis I for a prolonged period, and resumed only during ovarian maturation just before ovulation. The number of germ cells available for development into oocytes is further reduced by the process of follicular atresia or degeneration, which occurs in most developing follicles. Consequently, the number of meiotically active cells in the ovary, and therefore the amount of meiotic proteins, is much reduced as compared with the testes. We predict that certain meiotic motors identified in testes will also be active in female meiosis and that probes developed in this study may be useful in identifying both universal and gender-specific mitotic motors.

Polymerase chain reaction (PCR) primers specific for mitotic KRPs and for the superfamily in general were used to isolate candidates for meiotic-specific motors. We describe the identification of a family of KRPs in rat testes (KRP1 through KRP6). Two testes KRPs, KRP2 and KRP6, are related to already identified superfamily members, KIF2 and *Eg5*, respectively. Unlike KIF2, however, KRP2 is expressed only in the testes. KRP2, KRP3, and KRP5 appear to be expressed primarily in the meiotically active cells of

Table 1. Degenerate oligonucleotides

1	GCGCTCTAGAAT (C/A)TT(T/C)GC (C/A)TA(T/C)GG (A/T)CA(A/G)AC	5' primer	64-fold degenerate	complementary to the region encoding the ATP-binding site
2	GCGCGAGCTC (A/T)(A/T/G)CC (G/A/C)GCCA (A/G)ATC(A/G/C) AC	3' primer	108-fold degenerate	complementary to the region encoding the conserved VDLAGS sequence
3	GCGCGAGCTC (A/T/G/C)CC (A/T/G/C)(G/C) (A/T)(A/T/G/C) C(G/T)(A/T/G/C) CC(A/C)AT(A/G) TT	3' primer	9216-fold degenerate	complementary to a region encoding the bimC subfamily specific sequence NIGRSG

The sequence of the degenerate oligonucleotides used for RT-PCR are shown. The conserved sequence from the kinesin head domain that each hybridizes to is also indicated.

the seminiferous epithelium, whereas KRP4 and KRP6 show a strikingly different distribution, being expressed at lower levels than KRP2, KRP3, and KRP5, and in a different functional compartment of the epithelium. We have identified at least two KRPs from testes, KRP2 and KRP6, with properties consistent with motor function in meiosis and mitosis, respectively, in addition to new motors with unknown function in spermatogenesis.

MATERIALS AND METHODS

PCR Amplification and DNA Manipulations

First strand cDNA from rat testes poly(A⁺) RNA was template for amplification of rat kinesin-related proteins. cDNA was prepared according to the kit from Stratagene (La Jolla, CA). The reaction contained 2 µg testes poly(A⁺) RNA, 300 ng oligo-dT, 1 mM dNTPs, and murine leukemia virus reverse transcriptase. Amplification of potential kinesin-related proteins was accomplished using two sets of degenerate oligonucleotides. The oligonucleotides used are shown in Table 1.

Recognition sites for the restriction enzymes *Xba*I and *Sac*I were incorporated into the 5' and 3' primers, respectively. Two sets of PCR reactions were conducted: one with primers 1 and 2 and one with primers 1 and 3. Amplification reactions contained testes cDNA, oligonucleotide primers (1 µM each), 50 µM KCl, 100 mM Tris, pH 8.4, 1.5 mM MgCl₂, 0.1% gelatin, and 2.5 U taq polymerase (Perkin-Elmer, Norwalk, CT). PCR was performed according to the following schedule: 94°C for 1.5 min, 56°C (for primers 1 and 2) or 60°C (for primers 1 and 3) for 2.5 min, and 72°C for 3 min for 40 cycles. PCR products were separated by gel electrophoresis and fragments were gel purified, digested with *Xba*I and *Sac*I, and ligated into Bluescript-SK (Stratagene) for sequence analysis. Double-stranded DNA was sequenced by the chain termination method of Sanger using the Sequenase 2.0 kit (United States Biochemical, Cleveland, OH). Alternatively, lambda clones were sequenced using cycle sequencing protocols (Promega, Madison, WI).

Isolation and Characterization of cDNA Clones

Recombinant phage containing KRP2 sequences were identified by hybridization screening of a lambda-gt10 testes cDNA library (Clon-

tech, Cambridge, UK) using radiolabeled KRP2 cDNA fragment as probe. The probe was labeled using the random-primed method with the Boehringer-Mannheim kit (Indianapolis, IN). C600 cells (Clontech) were infected with phage and plated to achieve about 30,000 plaques per 100-mm plate. Approximately 300,000 plaques were screened by hybridization to the KRP2 fragment. Sixteen positive plaques were identified after autoradiography and comparison of duplicate filters. Positive plaques were purified by reinfection and replating twice. DNA was prepared according to established procedures. Briefly, phage were precipitated from liquid lysates with PEG and NaCl, protein was removed by extraction with phenol/chloroform and chloroform, and phage DNA was precipitated with ethanol. Phage DNA was sequenced using the fmole thermo-cycle sequencing kit from Promega using primers synthesized by the oligonucleotide synthesis services at University of Texas Southwestern Medical Center. Both strands of KRP2 DNA were sequenced using overlapping primers. The head domain within each clone was mapped with PCR using oligonucleotide primers to the lambda gt-10 vector and to the borders of KRP2 head fragment.

Slot and Northern Blot Analysis

Five to ten micrograms of poly(A⁺) from each tissue, isolated using the Ultraspec-II procedure (Biotecx), were denatured and applied to Zetaprobe (Bio-Rad, Richmond, CA) or Nytran (S&S, Keene, NH) membrane using a slot blot apparatus. For Northern analysis, 10 µg total RNA for each tissue was applied to a 1% agarose formaldehyde gel and separated by electrophoresis. Blots were hybridized individually to KRP probes radiolabeled by random priming. After overnight hybridization, the blots were washed with increasing stringency, ending with 0.2× SSC/0.1% SDS at 65°C. Blots were exposed to an activated phosphor screen and the signal was analyzed with a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

Localization of KRP mRNAs by In Situ Hybridization

For hybridization to thick, frozen sections, RNA was transcribed from Bluescript vectors containing ~500 bp of KRP DNA between the conserved FAYGQT and VDLAGS sequences for each of the six KRPs. For thin, paraffin sections, a 1623 nt fragment of the 2015 nt KRP2 open reading frame lacking 392 nt at the 5'-end was used to detect KRP2 message. [³⁵S]UTP was incorporated into the tran-

scripts and labeled probes were hybridized to testes sections obtained from adult Sprague-Dawley rats essentially according to the method of Simmons *et al.* (1989). Tissue was fixed for sectioning by perfusion with 4% paraformaldehyde and 0.05% glutaraldehyde. Sections were prepared for hybridization by paraformaldehyde fixation followed by protease digestion, acetylation, and dehydration. Paraffin-embedded tissues were processed in essentially the same manner following deparaffinization with xylene. After incubation with 0.5×10^6 cpm of labeled KRP probe, the sections were washed with increasing stringency with a final wash in $0.1 \times$ SSC at 55°C , treated with RNase, dehydrated, and exposed to film for estimation of hybridization. Slides were then defatted, dehydrated, dipped in NTB-2 liquid emulsion (Kodak, Rochester, NY), and silver grains were developed after 3–21 days.

RESULTS

Identification of a Group of Kinesin-related Proteins in Rat Testes

A slight variation of the typical reverse transcriptase (RT)-PCR approach was used to identify KRPs potentially involved in chromosome segregation. All members of the kinesin superfamily contain stretches of conserved amino acids in their motor domains. Degenerate oligonucleotide primers corresponding to these sequences were used to amplify all superfamily

members. Members of kinesin subfamilies also contain characteristic, conserved sequences that can be used to selectively amplify these messages as well.

Rat testes poly(A+) RNA was used as template for cDNA synthesis because this tissue undergoes rapid cell division and is a rich and easily available source of both meiotic and mitotic messages. Two sets of degenerate oligonucleotide primers were used to amplify KRPs from testes cDNA: one pair, complementary to sequences that encode the conserved *FAYGQT* and *VDLAG* motifs, was designed to amplify all members of the kinesin superfamily whereas the other set selects for members of the mitotic *bimC* subfamily. PCR products of about 500 bp, a size consistent with KRPs, were gel purified and subcloned into a Bluescript vector for sequence analysis. Forty-four of the 53 subclones sequenced contained conserved sequences characteristic of the kinesin superfamily. The translated sequence of each clone was distinct from the rat kinesin heavy chain; therefore, they were classified as new rat KRPs.

The clones fell into six different sequence classes (see Figure 1). Two PCR fragments, KRP2 and KRP6, were

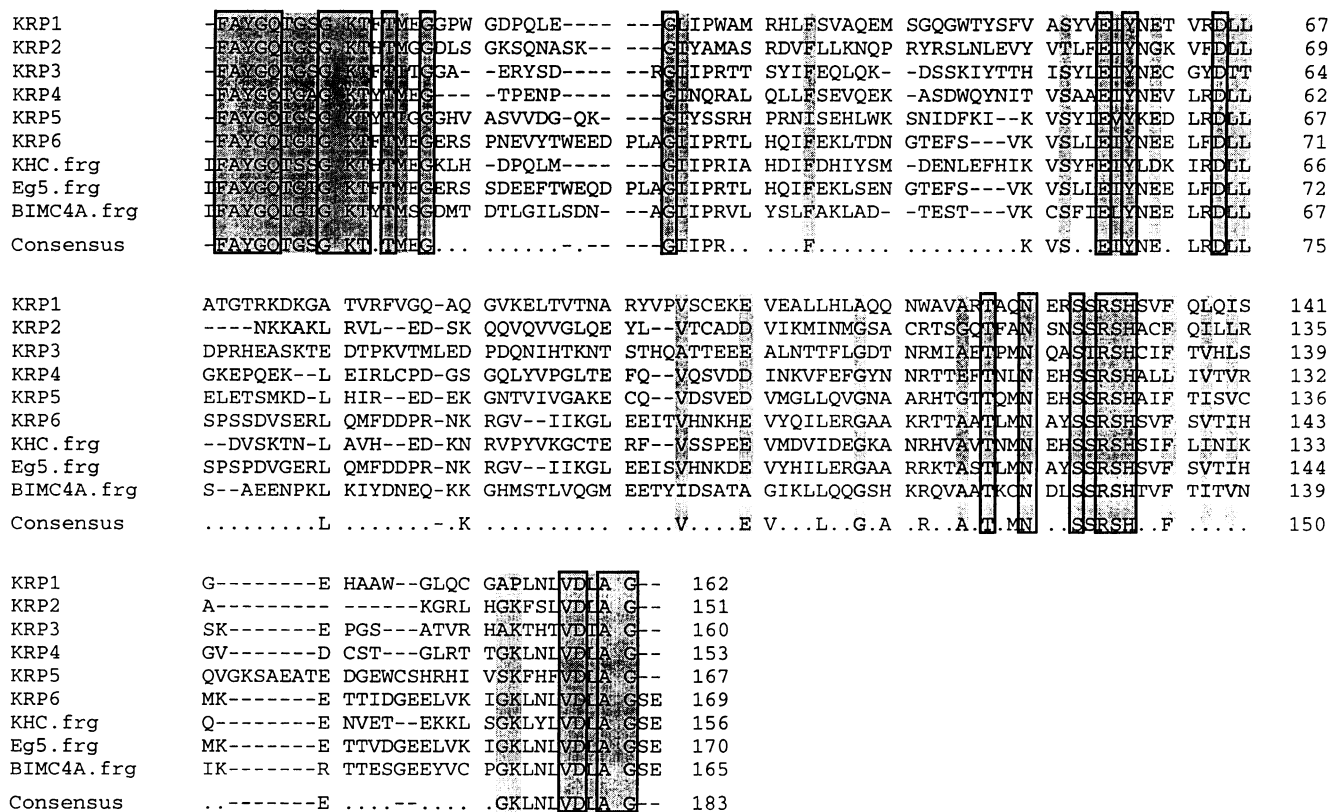


Figure 1. Sequences of the six KRP PCR fragments. PCR was performed using testes cDNA (as described in the text) and the resultant fragments were purified, subcloned, and sequenced. The sequences of the isolated fragments are shown. Sequences were aligned using the Geneworks program from Intelligenetics (Mountain View, CA). All contain sequence motifs characteristic of the kinesin superfamily.

amplified with the bimC specific primer; KRP2 was also isolated with the kinesin universal primers. KRP2 and KRP6 show significant homology to previously identified KRPs involved in mitosis. The KRP6 fragment is 90% identical to a corresponding region from the head domain of *Xenopus* Eg5 (Le Guellac *et al.*, 1991). The KRP2 head fragment is 74% identical to mouse KIF2 and 88% identical to hamster MCAK (Aizawa *et al.*, 1992; Wordeman and Mitchison, 1995).

Four of the KRPs isolated from testes, KRP1, KRP3, KRP4, and KRP5, did not show high levels of homology to known motor proteins in database searches. These proteins may represent new motors with potential roles in motile events in the testes.

Isolation and Sequencing of the Complete cDNA for KRP2

KRP2 and KRP6 were isolated with the bimC specific primers and therefore were obvious candidates for mitotic/meiotic KRPs. The high sequence identity of the motor domains of KRP6 and Eg5 (90%) confirmed our strategy of using subfamily specific primers and suggests that KRP6 is a new member of the bimC subfamily. The nonmotor domains of KRP6 are currently being cloned and sequenced to determine whether these regions are also highly conserved. High conservation of these domains would strongly support a function for KRP6 in spindle morphogenesis and maintenance similar to that of Eg5 (Sawin *et al.*, 1992).

Unlike KRP6, the KRP2 head domain is more related to that of mouse KIF2 than to members of the bimC subfamily. We focused our attention on KRP2 because numerous members of the bimC subfamily are currently under investigation. KIF2 was the first central motor KRP identified and belongs to a growing kinesin subfamily whose members are implicated in chromosome and spindle dynamics (Aizawa *et al.*, 1992).

To determine whether KRP2 shares the central motor domain arrangement of KIF2 and sequence homology throughout the polypeptide, we determined the complete cDNA sequence and translated sequence for rat testes KRP2. A rat testes cDNA library in lambda-gt10 was screened using the KRP2 PCR fragment as probe. Sequence analysis of the isolated clones identified a 2015 nt open reading frame encoding a protein of 75.9 kDa calculated MW (Figure 2A). Sequence comparison of KRP2 with rat kinesin heavy chain shows 36% sequence identity to the motor domain (our unpublished observations). In contrast to the kinesin heavy chain, the motor domain of KRP2 is centrally located from amino acid number 193 to 542 and flanked by between 100 and 200 aa on either side. This location is consistent with the high (74%) sequence identity of the KRP2 motor domain with that of KIF2, a central motor domain KRP.

Secondary structure analysis programs confirm that KRP2 has a different domain structure than classical kinesin but shares some structural features with KIF2. The amino terminal and central motor domains contain alternating structural motifs suggestive of a globular structure whereas the carboxyl terminal 100 amino acids are predicted to be largely α -helical (Figure 2B). KRP2 lacks the extended α -helical stalk characteristic of many KRPs. The KRP2 molecule was analyzed with the Coils 2 program to identify regions of KRP2 that may form α helical coiled-coil (Figure 2C; Lupas *et al.*, 1991). In contrast to the kinesin heavy chain where coiled-coil is located in the central domain of the molecule, KRP2 contains stretches of about 36 amino acids flanking the head domain that are predicted to assume a coiled-coil conformation.

KRP2 Is a Member of an Emerging Subclass of Kinesin-related Proteins

Sequence analysis of KRP2 showed high sequence identity of its head domain to that of mouse KIF2 (Aizawa *et al.*, 1992). KRP2 is homologous to another protein with high sequence identity to the motor domain of KIF2: hamster MCAK (mitosis centromere associated kinesin), which is localized to centromeres during mitosis (Wordeman and Mitchison, 1995). This homology and structural similarity suggest that this group, like the bimC subfamily, are closely related proteins with similar, but not identical, functions. Members of the kinesin superfamily can be grouped into subfamilies of structurally and functionally related proteins. Members of these groups have a higher degree of sequence homology with each other than with members of other subfamilies, share sequence homology in their nonmotor domains, and possess a similar domain arrangement. Subfamily members can have related cellular function but, unlike true orthologs, these proteins can have different cellular localizations and cargo specificities.

To investigate the relationship between KRP2 and members of the KIF2 subfamily, we compared the entire coding sequence of KRP2 with that of other KIF2 family members and examined the tissue distribution of KRP2 message. The motor domain of KRP2 is 74% identical to that of mouse KIF2 but this homology drops to only 36–38% identity in the nonmotor domains (Figure 3A). This relationship is typical of homologous subfamily members and suggests that KRP2 and KIF2 may have different cellular functions.

While completing the analysis of KRP2, the complete sequence of hamster MCAK became available (Wordeman and Mitchison, 1995). The primary sequence of KRP2 is 83% identical to MCAK and this high level of homology is not restricted to the motor domain. The nonmotor domains of KRP2 and MCAK are between 72–77% identical (Figure 3B). This is in

A

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901 gagagccaactggcgtaggtctgctgtggacttctgtgtgctgctgtctgcttctc 960
961 tccacctcagtcctcactcagtcctcccttggtctggaactggaaccattccatct 1020
1021 agATGATTGACATTGACGATGTGGCTGCCATAAACCCAGAGCTGTACAGCTTCTTCCCT 1080
1 M I D I D D V A A I N P E L V Q L L P L 20
1081 TACGACCGAAGGACGACTACTGCCACTGCAGGAGAACGTAACCATCCCAAAGCAAAAACGC 1140
21 R P K D S T A T A G E R N H P K A K T Q 40
1141 AAGTCCGTCAACTCCAAAATTCAGGTCCAAAAGAAGGCCTTCGAAGCGTTCCACTCGCA 1200
41 V R Q L Q N S R S K R R P S K R S T R I 60
1201 TATCCACTGTCTCCGAAGTTCGAATCCCTGCTCAGGAGAATGAAATGGAGTGGAGCTGC 1260
61 S T V S E V R I P A Q E N E M E V E L P 80
1261 CTGTGTCCACAACTCTCGCAAGCCATTTCCCATTCACACAGGCCACCCTAGGCCCTCT 1320
81 V S T N S R K P F P I H T G H P R P S C 100
1321 GCTCCACAGTGACAGAATTACCGTTGTGTGATGATCAGCGAGGAGGCAGAANGCAAGCCC 1380
101 S T V T E L P L L M I S E E A E X Q A H 120
1381 ACTCCACCCGAAGCACCTCTCTGCAAAACCCTGGGAATTCAGTTCGGAGGAAATCATGTA 1440
121 S T R S T S S A N P G N S V R R K S C I 140
1441 TTGTGAAGGAGATGGAGAAAATGAAGAACAAGCGAGAAGAGAAGAGGGCCAGAACTCGG 1500
141 V K E M E K M K N K R E E K R A Q N S E 160
1501 AGATAAGAATAAAGCGAGCTCAGGAATATGACAACAGCTTTCCAAACTGGGAATTTGCC 1560
161 I R I K R A Q E Y D N S F P N W E F A R 180
1561 GGATGATTAAGGAGTTTCGGGTTACTATGGACTGTAATCCTCTCACTGTGACAGACCCTA 1620
181 M I K E F R V T M D C N [P L T V T D P I 200
1621 TTGAAGAACACAGGATCTGTGTCTGTGTGTCAGGAAACGCCCACTGAATAAACAAAGACTGG 1680
201 E E H R I C V C V R K R P L N K Q E L A 220
1681 CCAAGAAAAGAAATCGATGTGATTTCTGTTCCAGCAAGTGTCTCTCTCTGGTGCATGAAC 1740
221 K K E I D V I S V P S K C L L L V H E P 240
1741 CCAAGCTGAAAGTGGACTTAAACGAAGTACCTGGAGAACCAGGCTTTTGTCTCGACTTTG 1800
241 K L K V D L T K Y L E N Q A F C F D F A 260
1801 CCTTTGATGAAACTGCTTCCAATGAGGTCGTCTACAGGTTACAGCAAGGCCACTGGTAC 1860
261 F D E T A S N E V V Y R F T A R P L V Q 280
1861 AGACGATTTTGAAGGGGGAAAGCAACGTGCTTTGCCATGGGCAGACAGGCAGTGGAA 1920
281 T I F E G G K A T C F A Y G O T G S G K 300
1921 AGACACACAAATGGGTGGAGACCTGTCTGGTAAAAGTCAGAACGCATCTAAAGGATCT 1980
301 T H T M G G D L S G K S Q N A S K G I Y 320
1981 ACGCAATGGCTTCCCGGATGTCTTCTTGAAGAATCAGCCTCGCTACCGGAGCTTAA 2040
321 A M A S R D V F L L K N Q P R Y R S L N 340
2041 ACCTGGAAGTTTATGTGACGTTCTTCGAGATCTACAATGGGAAGTATTTGAAC TGCTCA 2100
341 L E V Y V T F F E I Y N G K V F E L L N 360
2101 ACAAGAAGGCAAGTTACGTGTGCTGGAGGACAGCAAGCAGCAGGTGAGGTTGTGGGGC 2160
361 K K A K L R V L E D S K Q Q V Q V V G L 380
2161 TGCAGGAGTACCTGGTTACCTGTGCCGATGAATGTCATCAAGATGATCAACATGGGCAGC 2220
381 Q E Y L V T C A D E C H Q D D Q H G Q R 400
2221 GCCTGCCAGGAACTTCGGGACAAACATTTGCCAACTCCAATTCCTCCCGCTCCCAGCCT 2280
401 L P G T S G Q T F A N S N S S R S H A C 420
2281 GCTTCCAGATTCCTCTCGAGCTAAAGGGAGATTACACGGCAAATTCCTTTGGTGGATC 2340
421 F Q I L L R A K G R L H G K F S L V D L 440
2341 TGGCAGGGAATGAACGAGGAGCCGACGCTAGTGCTGACCGACAGACTCGCATGGAGG 2400
441 A G N E R G A D T S S A D R Q T R M E G 460
2401 GTGCAGAAATTAACAAGTCTCTCTGGCTCTGAAGGAATCGATCCGGGGCGCTGGGACAGA 2460
461 A E I N K S L L A L K E S I R A L G Q N 480
2461 ACAAGGCTCATACCCATTTCCGTGAGAGCAAGCTGACTCAGGTGCTGAGGGACTCCTTCA 2520
481 K A H T P F R E S K L T Q V L R D S F I 500
2521 TTGGGAGAACTCGAGGACATGCATGATCGCCATGATCTCACCGGGGATAAGCTCCTGTG 2580
501 G E N S R T C M I A M I S P G I S S C E 520
2581 AATACACTTTAAACACTGAGATACGACAGAGTCAAGGAGCTGAGCCCCACAGCG 2640
521 Y T L N T L R Y A D R V K E L S P H S G 540
2641 GGGCCAGTGGGAGGAACAGGGCAGTCCAGATGGAGACAGAGGAGATGGACGCATCTCAT 2700
541 A Q] W E E Q G S P D G D R G D G R I S W 560
2701 GGGCCCTCTCTACAGGCAATGAAGAGGAGGAGCTTCTCCAGATGTCCAGCTTTAATG 2760
561 G L S T G N E E E E L S S Q M S S F N E 580
2761 AAGCCATGACTCAGATCAGGGAGCTGGAAGAGAGGGCCATGGAAGAGCTCAGAGAGATCA 2820
581 A M T Q I R E L E E R A M E E L R E I I 600
2821 TACAGCAAGGACCAAGCTGGCTTGAGCTCTCTGAGATGACGGATCAGCCAGACTATGACT 2880
601 Q Q G P S W L E L S E M T D Q P D Y D L 620
2881 TGGAGACCTTCGTGAACAAGGAGAAATCTGCCCTGACACAGCAGGCTAAGCAGGCTAAGC 2940
621 E T F V N K A E S A L T Q Q A K Q A K H 640
2941 ACTTCTCAGCCCTTCAAGAAGTCATCAAGGCCCTACGCCCTGCCATGCAACTGGAAGAAC 3000
641 F S A L Q E V I K A L R L A M Q L E E Q 660
3001 AGGCTAGCAAACAAATAAACAGCAAGAACGGCACCAATgatgacttcaataaagatct 3060
661 A S K Q I N S K K R H Q * 680
3061 gaaaggtatcac 3072

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Figure 2.

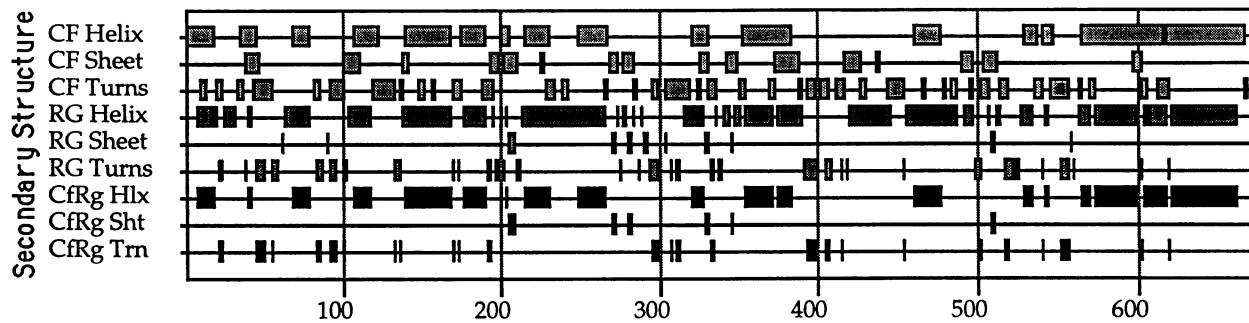
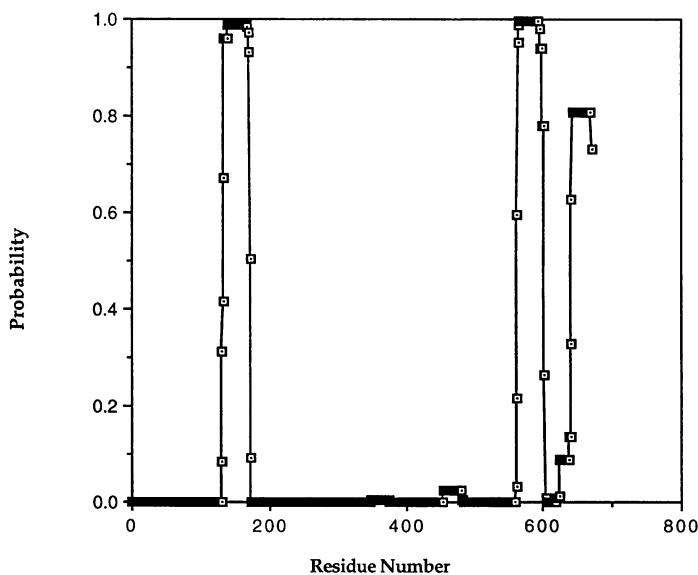
B**C**

Figure 2 (cont). Sequence analysis of KRP2 (GenBank accession number U44979). The nucleotide and predicted amino acid sequence of KRP2 is shown (A). The sequence encoding the head domain is bracketed and the locations of the degenerate oligonucleotides used for RT-PCR are underlined. The predicted secondary structure for KRP2 is shown in panel B. Panel C shows the position of putative coiled-coil regions in the KRP2 polypeptide as predicted by the Coils 2 program (Lupas *et al.*, 1991).

contrast to the relationship between the KRP2 and KIF2 polypeptides, which are less homologous outside their motor domains. Sequence alignment of KRP2, MCAK, and KIF2 demonstrates the strong sequence identity between KRP2 and MCAK that is present throughout these polypeptides (Figure 3C). These observations support the view that KRP2 is related but not identical to KIF2. The strong sequence identity between KRP2 and MCAK, a motor associated with the mammalian centromere, suggests that KRP2 may function in chromosome segregation.

Our comparisons of KRP2 with members of the KIF2 subfamily included recently described proteins. These include two proteins from *Xenopus*: XKCM1 and XKIF2 (xenopus central motor kinesin and xenopus kinesin family 2; Walczak *et al.*, 1996). Like MCAK, XKCM1 has been immunolocalized to the centromere region of mitotic chromosomes. The head domain of KRP2 is 81% and 73% identical to XKCM1 and XKIF2, respectively. In the case of XKCM1 the homology

extends to the nonmotor domains, indicating a possible functional similarity. Another central motor kinesin has been recently identified in the diatom *Cylindrotheca fusiformis*. DSK1 (diatom spindle kinesin 1) is located along spindle microtubules and is less homologous to the other members of this family (Wein and Cande, personal communication).

The tissue distribution of the KRP2 message was examined to determine whether its pattern of expression is consistent with that of a meiotic motor. KRP2 mRNA is found only in the testes, being undetectable in other tissues, including ovaries (Figure 4, A and B). The expression pattern of KRP2 is quite different from that reported for KIF2; KRP2 expression is restricted to testes whereas KIF2 was detected in all adult tissues examined (Aizawa *et al.*, 1992). The exclusive expression of KRP2 in testes is consistent with a role for this protein in meiosis. Comparison of primary sequence and expression patterns strengthens the argument that KRP2 and KIF2 are not orthologs but are related pro-

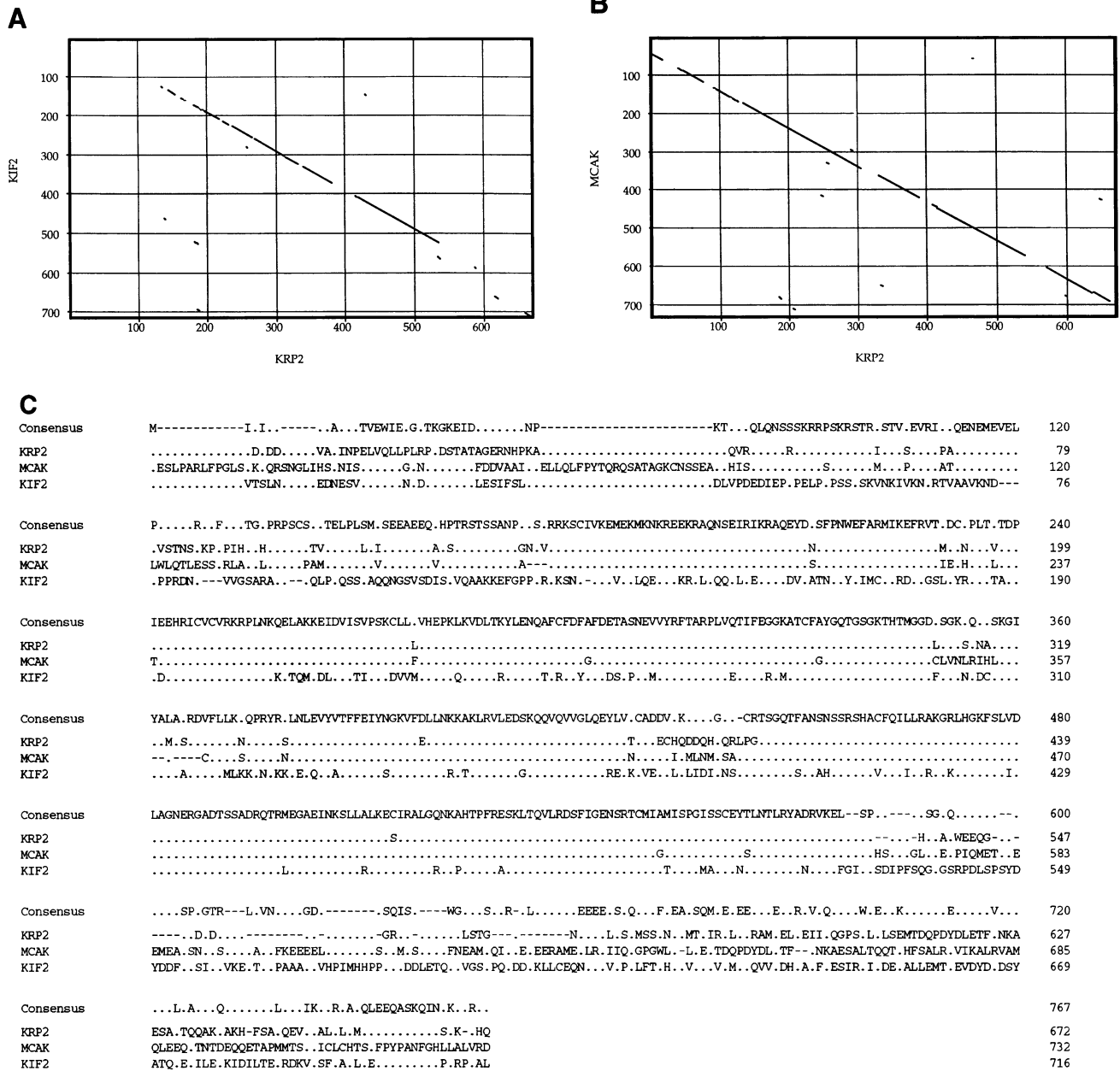


Figure 3. Comparison of KRP2 with KIF2 and MCAK. The sequence KRP2 was compared with two members of the KIF2 subfamily using dot-matrix: KIF2 (A) and MCAK (B). The relationship between KRP2, MCAK, and KIF2 is also displayed by sequence alignment (C). Identical sequence is indicated with dots, only variant residues are shown in the sequence of each of the three polypeptides. The consensus is shown above the aligned sequences.

teins with different, perhaps tissue-specific, functions. It would be helpful to compare the tissue distribution of KRP2 with that of MCAK, given their high level of sequence identity; however, this information was not available for analysis.

The other testes KRPs isolated in our screen are expressed primarily but not exclusively in the testes.

KRPs 3, 4, 5, and 6 are also expressed in the ovary, to varying degrees (Figure 4A). Additionally, KRP4 is expressed at a low level in the brain. This expression pattern is confirmed by Northern analysis, which shows a smaller KRP4 mRNA species specifically found in testes that is not observed in the ovary and brain (Figure 4B). This indicates possible

alternative splicing of the KRP4 message specific to the testes. The other KRP messages found in the ovary, KRPs 3, 5, and 6, are not visible on a Northern blot of 10 μg total RNA. This could be due to low abundance of the messages or the species heterogeneity.

Testes KRPs Are Localized to the Seminiferous Epithelium

Specific cell types are stratified within the SE (as shown in Figure 5). This ordered arrangement allows us to identify cell types that may be enriched in individual KRP gene products. Figure 5 shows a schematic of a representative tubule in cross-section. Mitotically active spermatogonia are located at the periphery of the epithelia (Figure 5, region a) while cells undergoing meiosis are located in a more interior area of the tubule. Spermatids are located even closer to the apical surface of the epithelia (Figure 5, region c) and are released into the lumen (Figure 5, region d) when their maturation is complete.

We conducted in situ hybridization experiments to determine whether individual KRPs are expressed in specific cell types of the testes (Figure 6). Anti-sense RNA probes specific for the head domain of each KRP were hybridized to 25- to 30- μm frozen testes sections. KRPs 2, 3, and 5 were expressed in the same region of the SE whereas KRP3 was expressed to a lower degree than KRPs 2 and 5 (Figure 6, A–D, G, and H). Expression of these three genes is localized to a region in the interior of the epithelia containing meiotic cells. KRPs 4 and 6 are also expressed in the SE but in a pattern slightly different from that of KRPs 2, 3, and 5 (Figure 6, E, F, I, and J). KRPs 4 and 6 appear to be expressed primarily in the basal area of the epithelium rather than closer to the lumen as seen for KRPs 2, 3, and 5. In addition, the reduced intensity of labeling indicates that KRPs 4 and 6 may be less abundant messages. The signal associated with the KRP4 message is less localized than that of KRP6 but appears enriched in the basal region of the SE. KRP1 does not appear to localize specifically to the tubules and is found uniformly throughout the testes (our unpublished observations).

The low level of resolution provided by the thick frozen sections used in this study does not make it possible to assign each KRP to a specific cell type. Such determinations require using thin paraffin sections. However, it is clear from these studies that the pattern of expression of KRPs 2, 3, and 5 is different from that of KRPs 4 and 6. KRP2 mRNA, and possibly 3 and 5, is localized to a meiotically active region of the SE whereas KRPs 4 and 6 appear localized to mitotically active regions of the epithelium.

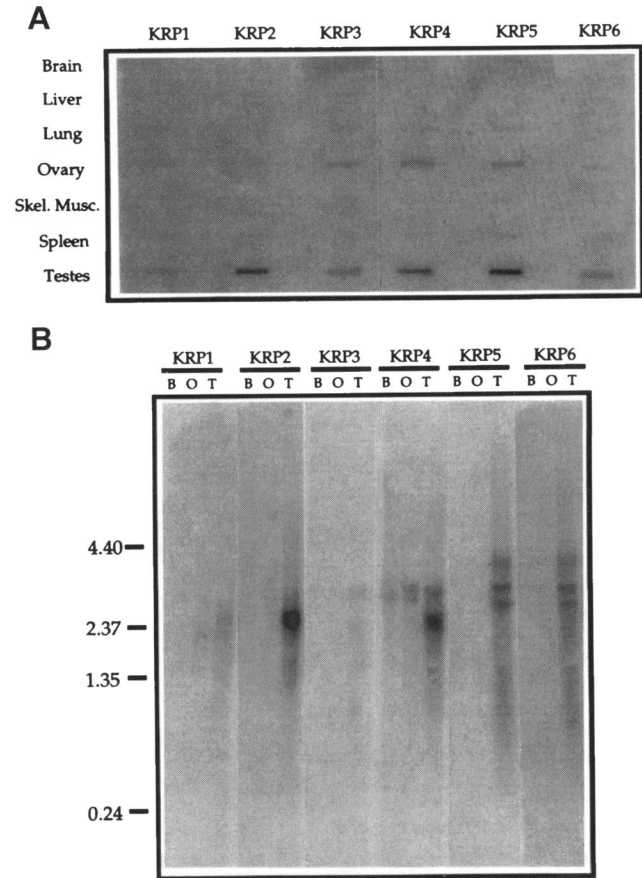


Figure 4. Tissue distribution of KRP gene expression. (A) Slot blot analysis of KRP gene expression. Ten micrograms of total RNA from each of the tissues indicated was applied to membrane using a slot blot apparatus. Each strip was then hybridized individually with labeled probe specific for each KRP. KRPs 2, 3, and 5 are testes specific whereas KRPs 4 and 6 are enriched in the testes but also found in other tissues. (B) Northern analysis of KRP gene expression. Ten micrograms of total RNA isolated from brain, ovary, and testes was separated by electrophoresis through a formaldehyde gel, transferred to nylon membrane, and hybridized to labeled DNA probes specific for each KRP.

KRP2 Is Expressed in a Subset of the Stages of the Seminiferous Epithelium

The cyclical process of spermatogenesis takes 48–58 days in the rat for stem cells to differentiate into spermatozoa. A new cycle is initiated every 12–13 days resulting in the overlapping of lineages and the association of cells from different developmental stages with one another within the epithelium. The rat seminiferous epithelium consists of 14 histologically distinct stages or cell combinations (Leblond and Clermont, 1952). Each stage contains a different collection of developing cell types and is classified primarily by the morphology of the developing spermatid (Leblond and Clermont, 1952). Due to the convoluted nature of the tubules within the testes, a cross-section reveals

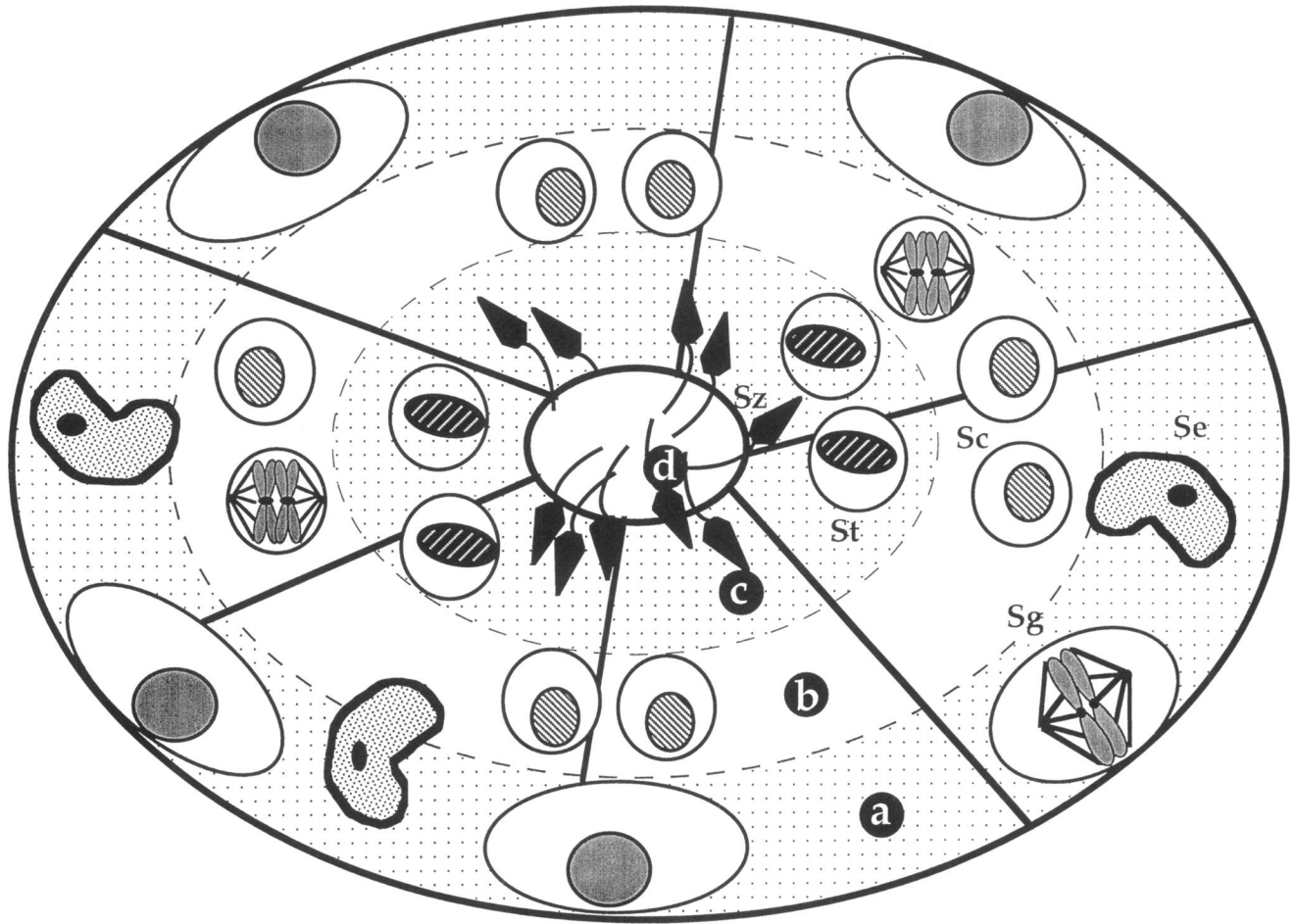


Figure 5. Schematic of the seminiferous epithelium. The SE of the testes contains several different cell types: rapidly dividing cells of the spermatogenic lineage, post meiotic cells, and supporting cells. Spermatogenesis involves a well-defined developmental sequence whereby germ cells undergo reduction in chromosome number and dramatic morphological changes to form active spermatozoa. During their maturation, developing spermatocytes move toward the lumen of the tubule where they are eventually released. The almost stratified organization of cell types within the SE permits determination of whether KRPs are expressed in actively dividing cells. The different cell types of the SE and their relative positions within the epithelium are shown. The epithelium is arbitrarily divided into four zones to indicate regions of enrichment of certain cell types: (a) basal region where spermatogonia (Sg) reside, (b) internal zone rich in spermatocytes (Sc), (c) region of maturing spermatids (St), and (d) the lumen with released spermatozoa (Sz). The mitotic and meiotic activity of cells in each zone is indicated by the presence of mitotic/meiotic figures. Cell division occurs in zones a and b containing mitotically active spermatogonia and meiotically active spermatocytes, respectively. Sertoli cells, the somatic cells of the tubule, are shown as pyramidal cells with oddly shaped nuclei (Se).

tubules in each of the 14 stages. Cross-sections containing meiotically active cells have a characteristic histology with views of secondary spermatocytes, occasional meiotic figures, and spermatids with a bent head morphology residing deep in the epithelium. If KRP2 is indeed a meiotic motor, we predict its presence in tubes with this appearance and not in tubes with cells from other developmental stages such as later stage spermatids arranged at the apical edge of the epithelium.

To examine whether KRP2 is expressed in all tubule cross-sections or in only a subset, we used thin ($3\ \mu\text{m}$) paraffin sections of rat testes for in situ hybridization.

KRP2 is clearly not expressed to the same degree in all stages (Figure 7). Expression varies from almost entirely absent in some tube cross-sections to quite intense in others (Figure 7, A and C). We observed that the tubules with the most intense signal had few spermatozoa at the apical aspect of the epithelium whereas those tubes lacking signal contained abundant sperm ready for release. The morphology of these tubes is suggestive of a stage containing meiotically active spermatocytes. This is consistent with our model that KRP2 functions in meiotic chromosome segregation. In addition, no radioactive signal was detected in the nongerminal pseustratified epithelia of the epididy-

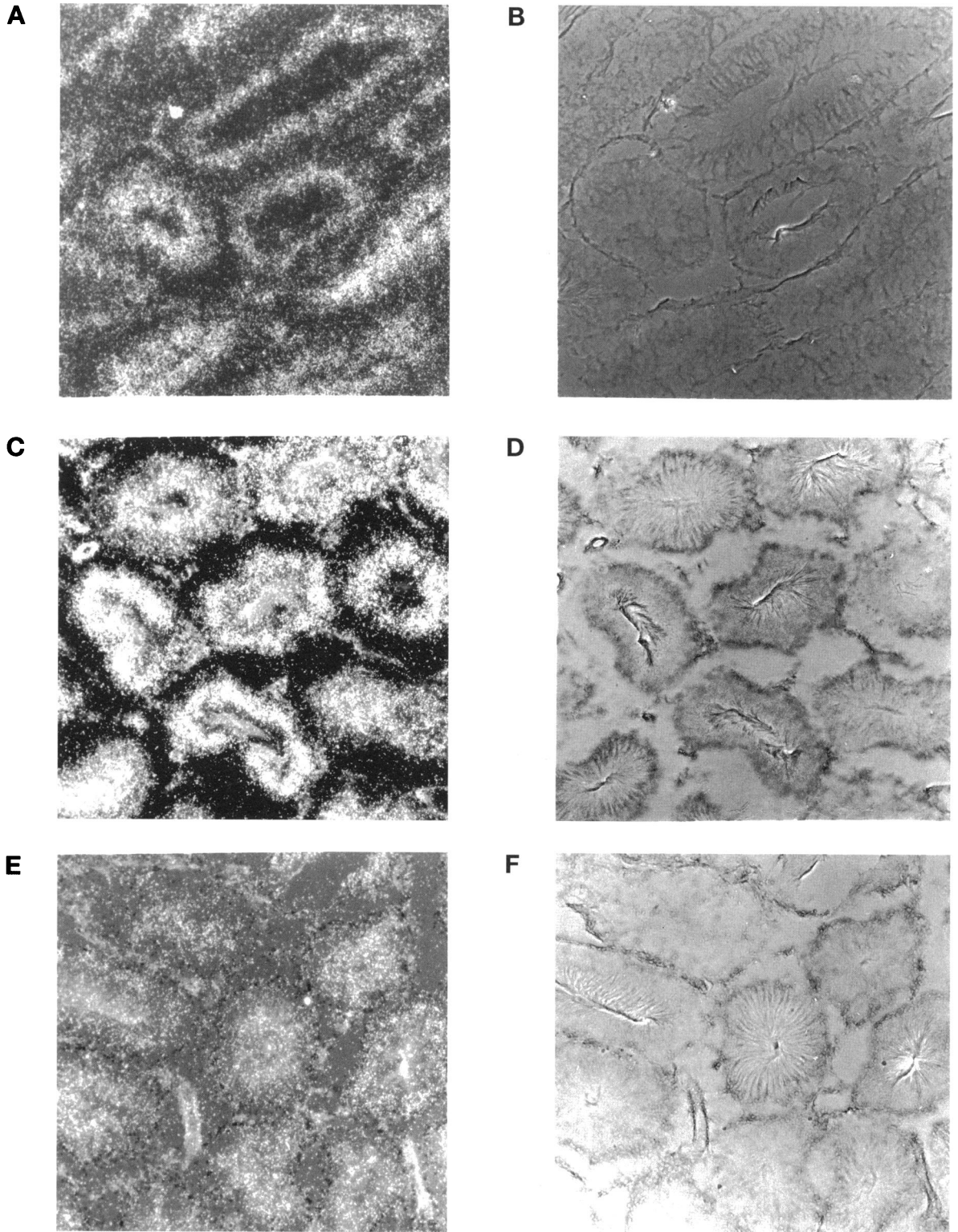
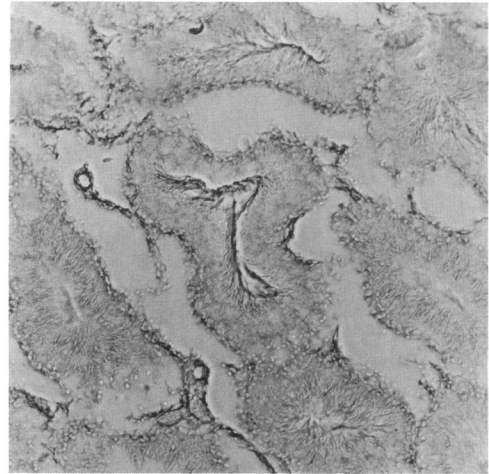


Figure 6.

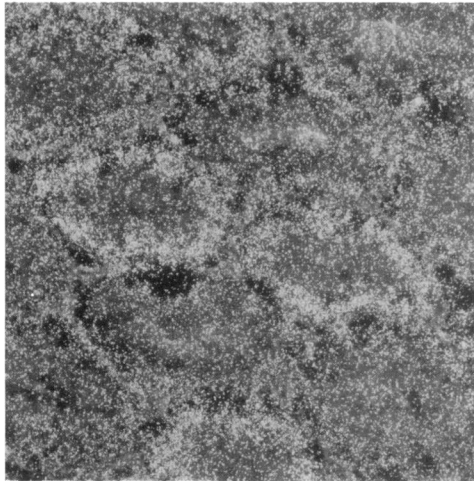
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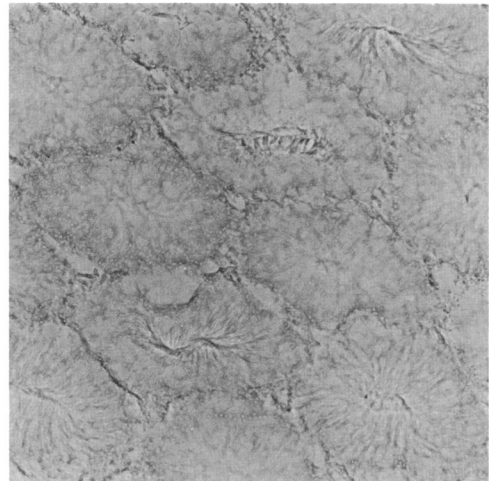
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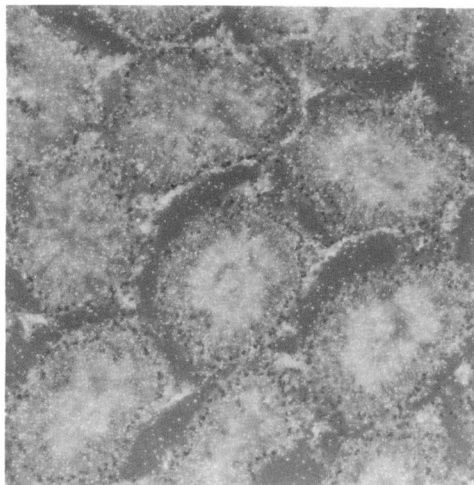
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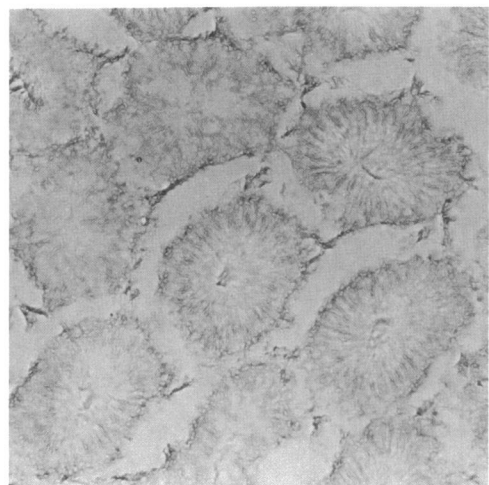


Figure 6 (continued).

mis, the convoluted duct that connects the testes to the ductus deferens, which is mitotically active.

DISCUSSION

An individual eukaryotic cell contains numerous motor proteins to conduct the movements necessary for cell viability. Kinesin motors participate in quite diverse cellular functions including cell division, organelle transport, and nuclear movement. Although many KRPs have roles in mitotic cell division, no KRP has been identified with exclusive function in meiosis. One might expect to find specialized motors designed for the unique chromosome dynamics of meiotic cell division; however, a systematic search for such proteins has not yet been reported.

Meiotic tissues exhibit unique subcellular movements that may require specialized motors. Chromosome behavior during meiosis, particularly during the first division, differs markedly from that seen during mitosis. Alignment and disjunction of chromosomes during meiosis I occurs by orientation of bivalents to the pole, not sister chromatids as in mitosis. Somatic cells of the testes may also contain specialized motor proteins. Sertoli cells contain an atypical microtubule array that may involve unique microtubule motors and may mediate the movement and positioning of spermatids within the SE (Redenbach and Vogl, 1991).

We conducted a PCR screen in an effort to identify new KRPs involved in meiosis and other motile events unique to germinal tissue. Rat testes contains at least six kinesin-related proteins, three of which are expressed exclusively in the SE. Two of these, KRP2 and KRP6, are homologous to mitotic motors. The remaining KRPs do not match any known KRP and may represent motors with previously unknown functions. These new KRPs are candidates for testes-specific motors with roles in movements unique to spermatogenesis and spermiogenesis including chromosome segregation, nuclear shaping of the sperm head, flagellar movements, and transport of spermatozoa within Sertoli cell crypts.

KRP2 is a new member of an emerging kinesin subfamily of central domain KRPs (Aizawa *et al.*,

1992). This group contains proteins shown primarily to be associated with the mitotic spindle, centrosome, and the centromere. The central domain subfamily appears analogous to the bimC subfamily; both groups are comprised of proteins with related functions and divergent tail domains.

KRP2 and MCAK are closely related throughout their sequence and may represent functional homologues. The close relationship between KRP2 and a mitotic KRP coupled with its localization to the SE make KRP2 a good candidate for a motor active in meiosis. This idea is further strengthened by the finding that KRP2 expression is restricted to a subset of spermatogenic stages including those containing meiotic cells. MCAK has been shown to localize to the mammalian centromere (Wordeman and Mitchison, 1995). Although assignment of KRP2 as a centromere-associated kinesin awaits development of specific antibodies, the extensive homology of KRP2 with MCAK suggests a possible association with the centromere in dividing spermatocytes.

The kinetochore and its associated chromatin display strikingly different properties during meiosis I than do their counterparts in mitosis. The centromeres of sister chromatids must remain fused or very closely associated during meiosis I to prevent premature separation and nondisjunction. This behavior is likely to require meiosis-specific centromere components. KRP2 could represent a tissue-specific variant of MCAK designed for the unique chromosomal behavior during meiosis. KRP2 located at the centromere could provide the force necessary to assure the continued adherence of sister chromatids during anaphase of meiosis I. Other possible roles for the KRP2 motor consistent with its sequence homology and tissue localization include movement of bivalents during meiosis I and sister chromatids during meiosis II. Future experiments will determine whether KRP2 is found only in meiotic cells. Such a localization would implicate KRP2 in meiosis-specific events such as orientation and poleward movements of bivalents and/or bivalent stabilization/adhesion during meiosis.

One interesting question raised by our studies is whether gender-specific motors exist in vertebrates. Several of the motors isolated from testes are also expressed in ovaries although one motor, KRP2, seems testes specific. It is clear from studies in *Drosophila* that meiosis in female and male flies progresses by quite different mechanisms (Hawley *et al.*, 1994). There are hints that such differences may exist in higher organisms as well. For example, the rate of nondisjunction during female meiosis in mammals is significantly higher than that observed in males (Nijhoff and De Boer, 1981). Certainly, the two sexes have quite different problems to contend with during gametogenesis including the partitioning of different chromosome

Figure 6 (cont). In situ localization of KRP gene expression. Perfusion fixed testes were frozen, sliced, and hybridized to ³⁵S-labeled RNA probes complementary to each KRP mRNA. Testes sections were viewed by dark-field (A, C, E, G, I, and K) and bright field (B, D, F, H, J, and L) microscopy. KRP genes show varying patterns of expression. KRPs 2 (A and B), 3 (C and D), and 5 (G and H) are expressed primarily in an internal zone of the SE. KRP6 message (I and J) is found more basally, at the outer edge of the SE while KRP4 gene expression (E and F) is found in the SE but is not well localized, being slightly enriched in the basal region. Panels K and L show dark field and phase images of negative controls: tissue hybridized to labeled probe corresponding to the sense strand of KRP2. In situ hybridization with the KRP1 probe gave a very low signal that was not discernible above background and therefore is not shown.

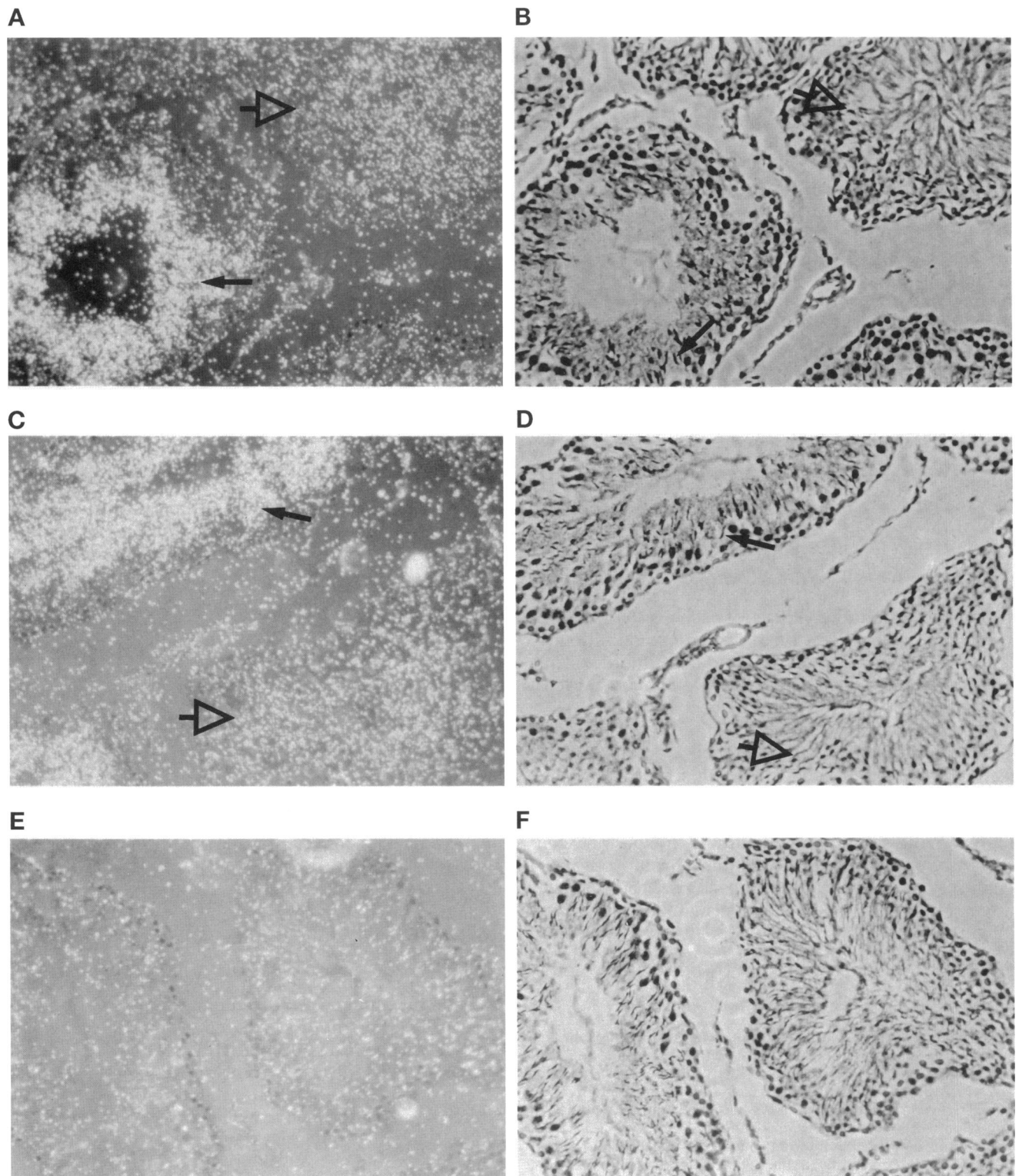


Figure 7. Localization of KRP2 message in thin sections of rat testes. Testes fixed by perfusion were embedded in paraffin, cut into 3 μm thick sections and hybridized to ^{35}S -labeled, anti-sense RNA probes complementary to KRP2. Testes sections were viewed by both dark field (A, C, and E) and bright field (B, D, and F) microscopy. These panels show views representative of data from several experiments. KRP2 message appears specific to one or a small number of spermatogenic stages as it is not uniformly localized to all tubule cross-sections.

complements and different modes of meiotic regulation. The specific mechanisms underlying many of these differences are poorly understood. We believe it is quite likely, as is the case in *Drosophila*, that vertebrates will employ gender-specific motor proteins during meiosis. Further exploration of the motors involved should greatly facilitate our understanding of these processes.

The mitotic members of the KIF2 subfamily continues to expand with the recent report of two central motor KRPs in *Xenopus* egg extracts and DSK1, a spindle-associated kinesin from diatom. The two *Xenopus* proteins have motor domains with high (70%) sequence identity to KIF2 (Wolczack and Mitchison, personal communication). One of these, XKCM1, localizes to the centromere early in mitosis then shifts to a polar location at later times. Diatom DSK1 has been localized to the spindle overlap zone (Wein and Cande, personal communication). DSK1 is the most divergent member of this group with 45% sequence identity to the KRP2 head domain.

Most of the central motor KRPs appear to form a subfamily analogous to the bimC family. Both groups contain structurally related proteins with similar cellular functions. The exception is mouse KIF2, which has been reported to function in the movement of small vesicles in fast axonal transport (Noda *et al.*, 1994). The existence of a kinesin subfamily member that is structurally homologous but functionally divergent from other members of its class is unusual. KIF2 shares high sequence identity with KRP2 and MCAK but that homology declines outside the motor domain. The expression pattern of KIF2 is also quite different from KRP2 with KRP2 expression restricted to testes whereas KIF2 is found in all adult tissues examined. Characterization of additional members of the central motor subfamily and determination of the cellular function of already identified members should clarify the relationships between these proteins. It is possible that during the process of evolution KIF2 retained motor functions similar to those of other subfamily members while gaining different functionalities by divergence of its nonmotor domains.

Like KRP2, KRP6 is homologous to a previously characterized mitotic KRP; however, KRP6 has a dif-

ferent pattern of expression from KRP2 indicative of a disparate function. The KRP6 PCR fragment is 90% identical to the analogous region from *Xenopus* *Eg5*. Such high sequence conservation suggests that KRP6 functions in mitosis in a manner similar to *Eg5*. *Eg5* is located along spindle pole fibers and appears more concentrated at the centrosome. It has been proposed that *Eg5* facilitates depolymerization at microtubule minus ends, thus producing the poleward tubulin flux seen in the mitotic spindle (Sawin *et al.*, 1992). The distribution of KRP6 message within the testes is consistent with a role in mitosis: KRP6 mRNA is located in a region of the SE where mitotically active spermatogonia reside.

The observation that KRP6 message is found in other tissues, although enriched in testes, suggests that KRP6 may be a more general mitotic protein. The in situ experiments described here are not of high enough resolution to determine whether KRP6 is found in spermatocytes as well as spermatogonia. It is clear, however, that KRP6 is expressed at a much lower level in the meiotically active area of the tubule than is KRP2. Although KRP2 and KRP6 are homologous to mitotic motors, their distinct expression patterns in the SE indicate functions in different cell types and at separate stages in the development of haploid sperm.

The remaining KRPs isolated in our screen, KRP1, KRP3, KRP4, and KRP5, display interesting localizations and may represent motors designed for the unique motile properties of the testes, both in chromosome segregation and other intracellular movements. This is particularly true for the testes-specific KRP messages. The PCR fragment from the head domain of the testes KRP3 gene is 56% identical to a similar region from a recently discovered kinesin-related gene from *Chlamydomonas reinhardtii* (Bernstein and Rosenbaum, personal communication). This new KRP was identified by its homology to the kinesin superfamily and its up-regulation after deflagellation. Another KRP identified in the same screen, Klp1, has been characterized further and is located on one of the central pair microtubules of the *Chlamydomonas* flagella (Bernstein *et al.*, 1994). Klp1 is proposed to exert force against the central microtubule, thus producing a twisting movement. The presence of a homologous gene in a vertebrate is tantalizing and suggests that KRP3 may be important for the structure and function of the flagellar axoneme in spermatozoa. It is certainly possible, given the high level of expression of spermatozoa proteins in testes, that some of the other testes KRPs identified in our screen may also participate in flagellar movement.

KRP4 and KRP5 show no significant homology to protein sequences entered into available databases and therefore speculation on possible functions must rely upon cellular localization. KRP5 is expressed in

(Figure 7 cont.) Instead, some tubules (A and C, filled arrows) are much more intensely stained than others (A and C, empty arrowheads). Tubule cross-sections with maximum signal share several histological characteristics including sperm heads embedded deep within the epithelia with few visible at the apical surface (B and D, filled arrows). In contrast, less well stained tubules were observed to have a lumen filled with sperm tails and sperm heads lining the apical side of the epithelia (B and D, empty arrowheads). The similar morphology of KRP2 positive tubules further confirms the idea that KRP2 is a stage-specific message. Panels E and F are sections hybridized to the sense strand of KRP2 as a negative control.

the same region of the SE at about the same level as KRP2 but unlike KRP2 is expressed in the ovaries as well. Such a restriction to germinal tissue could indicate specific motor function in gametogenesis. It is unlikely that KRP5 is involved in mitosis in that its pattern of expression within the testes is quite dissimilar from that of KRP6.

Unlike KRP2, expression of KRP4 is not restricted to testes, being found in the ovary and brain. Interestingly, the testes appear to express two forms of the KRP4 message: a constitutive species found in brain, ovary, and testes and a smaller testes-specific species. KRP4 message is enriched in a region of the SE more basally located than where KRPs 2, 5, and 3, are found and its pattern of expression is far less localized.

Three KRP messages are enriched in the seminiferous epithelium of the testes, suggesting a role for these proteins in intracellular motility unique to this tissue. Motors participating in chromosome segregation during meiosis would be predicted to localize to the meiotically active interior of the epithelium. This appears to be the case for the KRP2 gene, and possibly for KRP3 and KRP5 as well. Motor proteins involved in spermiogenesis would also be localized to the interior of the tubule and closer examination at higher resolution is required to define the cell types expressing these KRPs. KRP3, due to its identity to a flagellar KRP, is a good candidate for a motor involved in the formation and functioning of motile sperm.

Mitotic motor proteins should be expressed in the mitotically active spermatogonia and in Sertoli cells. Spermatogonia divide only during a small portion of the developmental cycle of the tubule and are not subject to the clonal expansion of spermatocytes. Therefore, mitotic motors should be found in a smaller percentage of tubules than meiotic motors or motors involved in latter stages of spermiogenesis. KRP6 fits this expectation with its localization to the basal area of the tubule in a fraction of the tubules examined.

We have identified new proteins, some with potential roles in mitosis and meiosis and others implicated in the unique motile events of the testes. KRP2, a testes-specific motor identified in our screen, has properties consistent with its function as a motor in chromosome segregation during meiosis. KRP2 is homologous to a group of proteins located in the mitotic apparatus and is expressed in a region of the SE containing meiotically active cells. KRP6 is also related to a mitotic motor but has a pattern of expression distinct from that of KRP2 and consistent with expression in mitotically active spermatogonia. The other KRPs identified in our screen may have roles in chromosome segregation or in the later maturation of spermatids into spermatozoa.

The description of a new class of testes-specific motors is important in understanding the complex development of mammalian sperm. The proper regulation

and activity of meiotic motors is essential for formation of viable gametes. Misregulation of motors necessary for chromosome segregation and for successful completion of spermiogenesis could result in abnormal gametes, abnormal progeny, and infertility. Identification of motors specific for gametogenesis represents a first step in understanding the role of motor proteins in the differentiation of germ cells to functional gametes.

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Note added in proof. Further comparison of the KRP1 sequence with those in available databases revealed high sequence identity to HSET, a kinesin-related protein from human (Ando *et al.*, 1994) and CHO2 from hamster (Kuriyama *et al.*, 1995). Although the function of HSET has not yet been demonstrated, CHO2 is a minus-end directed motor associated with the interphase centrosome and mitotic spindle.

REFERENCES

- Aizawa, H., Sekine, Y., Takemura, R., Zhang, Z., Nangaku, M., and Hirokawa, N. (1992). Kinesin superfamily in murine central nervous system. *J. Cell Biol.* 119, 1287-1296.
- Ando, A., Yara-Kikuti, Y., Kawata, H., Okamoto, N., Imai, T., Eki, T., Yokoyama, K., Soeda, E., Ikemura, T., Abe, K., and Inoko, H. (1994). Cloning of a new kinesin-related gene located at the centromeric end of the human MHC region. *Immunogen.* 39, 194-200.
- Bernstein, M., Beech, P.L., Katz, S.G., and Rosenbaum, J.L. (1994). A new kinesin-like protein, Klp1, localized to a single microtubule in the *Chlamydomonas* flagellum. *J. Cell Biol.* 125, 1313-1326.
- Endow, S.A. (1991). The emerging kinesin family of microtubule motor proteins. *Trends Biochem. Sci.* 16, 221-225.
- Endow, S.A. (1993). Chromosome distribution, molecular motors and the claret protein. *Trends Genet.* 9, 52-55.
- Endow, S.A., Henikoff, S., and Soler-Niedziela, L. (1990). Mediation of meiotic and early mitotic chromosome segregation in *Drosophila* by a protein related to kinesin. *Nature* 345, 81-83.
- Enos, A.P., and Morris, N.R. (1990). Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. *Cell* 60, 1019-1027.
- Fuller, M.T., Caulton, J.H., Hutchens, J.A., Kaufman, T.C., and Raff, E.C. (1988). Mutations that encode partially functional $\beta 2$ tubulin subunits have different effects on structurally different microtubule arrays. *J. Cell Biol.* 107, 141-152.

- Goldstein, L.S.B. (1993). With apologies to Scheherazade: tails of 1001 kinesin motors. *Ann. Rev. Genet.* 27, 319–351.
- Gorbisky, G.J. (1992). Chromosome motion in mitosis. *BioEssays* 14, 73–80.
- Hagan, I., and Yanagida, M. (1990). Novel potential mitotic motor protein encoded by the fission yeast *cut7⁺* gene. *Nature* 347, 563–566.
- Hatsumi, M., and Endow, S.A. (1992). Mutants of the microtubule motor protein, nonclaret disjunctional, affect spindle structure and chromosome movement in meiosis and mitosis. *J. Cell Sci.* 101, 547–559.
- Hawley, R.S., Frazier, J.A., and Rasooly, R. (1994). Separation anxiety: the etiology of nondisjunction in flies and people. *Human Mol. Genet.* 3, 1521–1528.
- Hoyt, M.A., He, L., Loo, K.K., and Saunders, W.S. (1992). Two *Saccharomyces cerevisiae* kinesin-related gene products required for mitotic spindle assembly. *J. Cell Biol.* 118, 109–120.
- Komma, D.J., Horne, A.S., and Endow, S.A. (1991). Separation of meiotic and mitotic effects of *claret* non-disjunctional on chromosome segregation in *Drosophila*. *EMBO J.* 10, 419–424.
- Kuriyama, A.T., Kofron, M., Essner, R., Kato, T., Dragas-Granoic, S., Omoto, C.K., and Khodjakov, A. (1995). Characterization of a minus end-directed kinesin-like motor protein from cultured mammalian cells. *J. Cell Biol.* 129, 1049–1059.
- Leblond, C.P., and Clermont, Y. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. NY Acad. Sci.* 55, 548–573.
- Le Guellac, R., Paris, J., Couturier, A., Roghi, C., and Philippe, M. (1991). Cloning by differential screening of a *Xenopus* cDNA that encodes for a kinesin-related protein. *Mol. Cell. Biol.* 11, 3395–3398.
- Lupas, A., Dyke, M.V., and Stock, J. (1991). Predicting coiled coils from protein sequences. *Science* 252, 1162–1164.
- McDonald, H.B., and Goldstein, L.S.B. (1990). Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila*. *Cell* 61, 991–1000.
- Nijhoff, J.H., and De Boer, P. (1981). Spontaneous nondisjunction in mammals: a study evaluating the various experimental approaches. *Genetica* 56, 99–121.
- Noda, Y., Soto-Yoshitake, R., Kondo, S., Nangaku, M., and Hirokawa, N. (1994). KIF2 is a new anterograde microtubule-based motor which transports membranous organelles distinct from those carried by KHC and KIF3A/B. *Mol. Biol. Cell* 5, 32a.
- Redenbach, D.M., and Vogl, A.W. (1991). Microtubule polarity in Sertoli cells: a model for microtubule-based spermatid transport. *Eur. J. Cell Biol.* 54, 277–290.
- Roof, D.M., Meluh, P.B., and Rose, M.D. (1992). Kinesin-related proteins required for assembly of the mitotic spindle. *J. Cell Biol.* 118, 95–108.
- Saunders, W.S., and Hoyt, M.A. (1992). Kinesin-related proteins required for structural integrity of the mitotic spindle. *Cell* 70, 451–458.
- Sawin, K.E., and Endow, S.A. (1993). Meiosis, mitosis and microtubule motors. *BioEssays* 15, 399–407.
- Sawin, K.E., LeGuellec, K., Philippe, M., and Mitchison, T.J. (1992). Mitotic spindle organization by a plus-end-directed microtubule motor. *Nature* 359, 540–543.
- Simmons, D.M., Arriza, J.L., and Swanson, L.W. (1989). A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radiolabeled single-stranded RNA probes. *J. Histochem. J.* 12, 169–181.
- Theurkauf, W.E., and Hawley, R.S. (1992). Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* 116, 1167–1180.
- Wadsworth, P. (1993). Mitosis: spindle assembly and chromosome motion. *Curr. Opin. Cell Biol.* 5, 123–128.
- Walczak, C.E., Mitchison, T.J., and Desai, A. (1996). XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell (in press)*.
- Wordeman, L., and Mitchison, T.J. (1995). Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* 128, 95–105.
- Zhang, P., Knowles, B.A., Goldstein, L.S.B., and Hawley, R.S. (1990). A kinesin-like protein required for distributive chromosome segregation in *Drosophila*. *Cell* 62, 1053–1062.