Eukaryotic flagellar assembly and the regulation of flagellar motility are complex processes. For example, in the blue-green alga *Chlamydomonas reinhardtii*, more than 25 loci have been characterized that affect flagellar assembly, and over 52 loci have been identified that result in altered motility (1). In contrast to *Chlamydomonas*, only a few of the molecular defects underlying naturally occurring cases of human flagellar immotility have been characterized (2–5). It is likely that the molecular basis of human sperm motility is more complicated than that of *Chlamydomonas* because, in addition to the axoneme, mammalian sperm have several flagellar accessory structures including a fibrous sheath (FS). The FS is a structure found exclusively in the principal piece of the flagellum where it surrounds the outer dense fibers and axoneme. It is believed to play a structural role in sperm motility by restricting the plane of bending of the flagellum (6).

Although many of the target proteins have not been identified, it is well accepted that protein phosphorylation/dephosphorylation events are involved in the initiation and maintenance of mammalian sperm flagellar motility and that phosphorylation occurs via a cAMP-dependent pathway (7–10). In mammalian sperm, the major downstream target of cAMP is protein kinase-A (PK-A), thus making it likely that this enzyme is involved in the regulation of sperm motility (11). Although typically soluble in somatic cells, PK-A also can be found tethered to subcellular organelles via binding of its regulatory (RII) subunit to a-kinase anchor proteins (AKAPs) (12–14). In response to cAMP binding to the RII subunit of PK-A, the catalytic subunit of the kinase is released and becomes free to catalyze phosphorylation of its substrates. By tethering PK-A to close certain substrates, AKAPs may play critical roles in determining the specificity of PK-A action (15–17).

We have cloned and characterized a cDNA encoding mouse AKAP82 (mAKAP82), the major protein of the sperm FS and a member of the AKAP family (11, 18). Mouse AKAP82 is synthesized in the cell body of condensing spermatids as a 97,000 precursor (pro-mAKAP82, GenBank accession number U07423) (18). This precursor polypeptide is transported down the flagellum to the principal piece where it is processed by the proteolytic cleavage of the amino-terminal 179 amino acids to produce pro-mAKAP82 and the free 179 amino acid pro domain (19). Coincident with or following cleavage, mAKAP82 is assembled into the FS. Mouse AKAP82 could tether PK-A close to the axoneme and other components of the flagellum that are involved in sperm motility, thus regulating the action of PK-A.
by directing its activity to specific motility-related targets. With the characterization of mAKAP82 there is now experimental evidence for a functional role of the FS as a mediator of PK-A activity.

Previous work from our laboratories has shown that the human homologue of mAKAP82, hAKAP82, localizes to the FS (20). Additionally, both hAKAP82 and its predicted precursor protein, pro-hAKAP82, bind the RII subunit of PK-A and are the major polypeptides of a limited subset of proteins that become tyrosine-phosphorylated in a time-dependent manner after incubation in a medium supporting capacitation. In human sperm, capacitation is associated with several cellular changes, such as the tyrosine phosphorylation of specific proteins (20–22), that must take place before fertilization can occur. Capacitation also is associated with alterations in sperm motility patterns characteristic of sperm hyperactivation (23).

Because the evidence to date is supportive of both structural and functional roles for pro-mAKAP82 and mAKAP82 in mouse sperm motility, we hypothesize that pro-hAKAP82 and hAKAP82 play central roles in human sperm motility. The hypothesis is based on the observation that these proteins are phosphoproteins that are likely to be involved in the regulation of phosphorylation of other proteins in the sperm flagellum; such phosphorylation events are known to be critical for regulating motility (7–10, 18, 20). Support for this hypothesis requires further characterization of hAKAP82 and its precursor. In this paper, we report that pro-hAKAP82 and hAKAP82 were the human homologues of pro-mAKAP82 and mAKAP82. We provide evidence that two alternative transcripts of the gene were made during both human and mouse spermatogenesis because of the use of different donor/acceptor splice junctions. Additionally, both pro-hAKAP82 and hAKAP82 localized specifically to the entire length of the FS of ejaculated sperm. Finally, we show that the pro-hAKAP82 gene mapped to human Xp11.2, a finding that has significant implications for germ cell development and male infertility.

**EXPERIMENTAL PROCEDURES**

**Isolation of Human and Mouse cDNA and Genomic Clones Encoding Pro-hAKAP82**—To isolate a cDNA clone corresponding to pro-hAKAP82, a random-primed human testis cDNA library in a λgt11 vector (CLONTECH, Palo Alto, CA) was screened by filter hybridization with a radiolabeled 1.9-kilobase pair cDNA fragment representing the 5′-end of the pro-mAKAP82 cDNA (24). After multiple rounds of screening, a 1.4-kilobase pair partial pro-hAKAP82 cDNA clone homologous to the 5′-UTR and the first 1222 bp of coding sequence of pro-mAKAP82 was isolated and subcloned into the EcoRI site of pGEM-3™ (Promega, Madison, WI).

A human testis Expressed Sequence Tag (EST) clone containing an approximately 750-bp insert, which was over 90% homologous to bases 1948–2520 of the pro-mAKAP82 cDNA coding region and the pro-mAKAP82 3′-UTR, was identified by performing a BLAST (basic local alignment search tool (25)) search on the GenBank EST data base with the pro-mAKAP82 cDNA sequence. The human EST clone was obtained from the I.M.A.G.E. consortium (LNLN) (I.M.A.G.E. consortium clone 728916, Research Genetics, Inc. Huntsville, AL) (26, 27).

To map the chromosomal location of the pro-hAKAP82 gene by fluorescence in situ hybridization (FISH), a genomic clone containing pro-hAKAP82 was isolated from a genomic human bacterial artificial chromosome (BAC) library (in vector pBeloBAC 11) by Research Genetics using a PCR-based technique. PCR primers were designed based on the cDNA sequence for pro-hAKAP82. Sequence analysis of the BAC clone showed that it contained pro-hAKAP82.

A genomic clone containing the 5′-flanking region of pro-mAKAP82 was isolated from a mouse genomic library in λgt11 (Promega) using a hybridization with a radiolabeled PCR fragment from the mouse cDNA as a probe (24). A second genomic clone containing the full-length genomic sequence of pro-mAKAP82 together with its 5′- and 3′-flanking regions was isolated from a mouse P1 embryonic stem cell library using a PCR-based technique and primers derived from the pro-mAKAP82 cDNA sequence (Genome Systems Inc., St. Louis, MO).

**Generation and Analysis of PCR Products**—A 1.6-kilobase pair PCR product corresponding to the region of pro-hAKAP82 that was not included in the human cDNA or EST clones (homologous to bases 437 to 2092 of the pro-mAKAP82 cDNA) was amplified by PCR from a human testis cDNA library using primers based on the pro-hAKAP82 cDNA sequence (corresponding to bases 437 to 457 of the pro-hAKAP82 cDNA coding region) and the pro-hAKAP82 EST sequence (corresponding to bases 2907 to 2077 of the pro-hAKAP82 cDNA coding region).

To assay for the presence of an alternative splice variant of pro-hAKAP82, a DNA fragment was amplified by PCR from a human testis cDNA library using primers corresponding to a region in the 5′-UTR of mouse Fas (GenBank accession number U10341 (28)) and to a region in the 5′-end of the coding region of the pro-hAKAP82 gene. PCR products were purified with the Wizard™ PCR Prep kit (Promega) before sequencing.

**DNA Sequencing and Computer Analysis**—All sequencing was done with the AmpliTaq@, F5 dye terminator cycle sequencing kit chemistry or the BigDye™ terminator cycle sequencing kit chemistry and the appropriate primers using a 373A DNA sequencer (PE Applied Biosystems, Foster City, CA). Ambiguities were resolved by sequencing the opposite strand. DNA and protein sequence analyses were performed using the MacVector™ (Kodak Scientific Imaging Systems, New Haven, CT) and Sequencer™ (Gene Codes Corp., Ann Arbor, MI) software programs.

**Preparation of Sperm and Sperm Proteins**—Samples of human semen were obtained from normal, healthy donors with good sperm motility (total sperm motility greater than 75%, progressive motility greater than 60%). Ejaculate volume, percentage of total and progressively motile sperm, and sperm concentration were determined for each ejaculate. Sperm were washed 3× in PBS. Before final centrifugation, the volume, sperm concentration, and total sperm numbers again were determined for each sample. After the final wash, sperm pellets were dissolved in SDS sample buffer containing 40 mM dithiothreitol and boiled for 5 min. The amount of protein in each sample was determined by the Amido Black procedure (29).

**Immunological Reagents**—An antiserum against the predicted pro-domain of pro-hAKAP82 (anti-hpro) was prepared as follows. A peptide corresponding to residues 131–145 (NH2-VGDTGEDYHRSSENCOOH, the hpro peptide) of the pro-hAKAP82 protein was synthesized with a cysteine added to the amino terminus (Quality Control Biologicals, Hopkinton, MA). The peptide was conjugated to keyhole limpet hemocyanin via the cysteine and then used for immunization. The antiserum was characterized by immunoblotting using protein extracts from ejaculated human sperm. A portion of the antiserum was affinity-purified, eluted, neutralized, and dialyzed in PBS.

**Immunoblot Analysis of Sperm Proteins**—Proteins from ejaculated human sperm were separated under reducing conditions by SDS-polyacrylamide gel electrophoresis on a 10% gel and electrophoretically transferred to nitrocellulose membranes. Equal amounts of protein were analyzed in each lane. The blots were blocked, probed with anti-hpro (1:2000 (v/v)), processed, and developed using an ECL kit (Amersham Pharmacia Biotech) as described previously (19) before being exposed to X-ray film. In the preabsorption experiments, the antiserum was incubated with the hpro peptide (1 mg/ml) in PBS containing 0.1% (v/v) Tween 20 and 3% (w/v) bovine serum albumin for 1 h at room temperature and then used to probe the immunoblots as described above.

**Indirect Immunofluorescence of Sperm**—One-ml aliquots of sperm diluted in PBS to 3 × 106 cells/ml were permeabilized in 0.1% (v/v) Triton X-100 for 15 min and then washed once in PBS. Pellets were resuspended in 1 ml of PBS, and the cells were transferred onto coverslips. After settling, cells were fixed in 4% (w/v) paraformaldehyde, incubated in –20 °C methanol, washed, and blocked in normal goat serum as described previously (19). Sperm were then incubated in anti-hpro diluted 1:10 (v/v) in 10% goat serum overnight at 4 °C and washed in PBS. Sperm were incubated for 1 h at 37 °C in the secondary antibody (fluorescein isothiocyanate-conjugated goat anti-rabbit IgG, Jackson Immunoresearch Laboratories, Inc., West Grove, PA), diluted 1:50 (v/v) in 10% goat serum, and again washed in PBS before being mounted on slides with mounting media (Fluoromount-G, Southern Biotechnology Associates Inc., Birmingham, AL). Slides were viewed with a Zeiss Photomicroscope III equipped with epifluorescence. Photomicrographs were taken with a Zeiss Photomicroscope III equipped with epifluorescence. Photomicrographs were taken with a Zeiss Photomicroscope III equipped with epifluorescence. Photomicrographs were taken with a Zeiss Photomicroscope III equipped with epifluorescence. Photomicrographs were taken with a Zeiss Photomicroscope III equipped with epifluorescence.
translation and hybridized to metaphase chromosome spreads prepared from peripheral blood lymphocytes from a normal man. Labeled probe (300 ng) was incubated overnight at 37 °C with 3 µg of human cot-1 DNA (Amersham Pharmacia Biotech) in Hybrisol VII (Oncor Inc., Gaithersburg, MD). The probe then was denatured at 72 °C for 5 min and pre-annealed for 30 min at 37 °C. Slides were dehydrated and denatured before hybridizing overnight at 37 °C with the probe in a humidified chamber. Slides were washed in 50% formamide,1× SSC (0.15 M NaCl and 0.015 M sodium citrate) for 10 min and then twice in 2× SSC for 4 min each. All washes were done at 40 °C with gentle shaking. Slides were transferred to a solution of 1× phosphate-buffered detergent (Oncor). Detection was performed with rhodamine-labeled anti-digoxigenin antibody, and chromosomes were counterstained with diamidino-phenylindole. Metaphase chromosome spreads were visualized using a Zeiss universal microscope with a Photometrics™ cooled-CCD camera and Quips™ Imaging Software (Vysis™ Inc., Downers Grove, IL). Twenty metaphase spreads with signals on both chromatids at the same band position were used to determine chromosomal location.

RESULTS

Pro-hAKAP82 Is the Human Homologue of pro-mAKAP82—We determined the full-length sequence of the pro-hAKAP82 cDNA by aligning and sequencing the pro-hAKAP82 cDNA clone, PCR product, and EST clone (see “Experimental Procedures”; GenBank accession number AF072756). The composite cDNA sequence contained an initiator methionine with an in-frame, upstream stop codon. The coding region of pro-hAKAP82 was 2535 bases long and contained one open reading frame, which predicted a protein of 845 amino acids and concluded with an in-frame stop codon. Although no consensus polyadenylation signals (AATAAA) were present upstream of the putative poly(A) tract, two less well conserved potential polyadenylation signals (AATAAC) were present at bases 2807 and 2827. The predicted molecular weights for the various forms of the human protein were 93,500 for pro-hAKAP82 and, assuming that the cleavage site in pro-hAKAP82 is the same as in pro-mAKAP82, 73,272 for hAKAP82 and 20,244 for the pro domain (hpro). Two cDNA sequences, pro-mAKAP82 and Fsc1, have been reported for the major mouse FS protein (18, 28). The predicted amino acid sequence of pro-hAKAP82 was highly homologous to both of these proteins. Specifically, at least 79% of the amino acids were identical, and 91% were conserved between the mouse (both pro-mAKAP82 and Fsc1) and human sequences. Critical functional domains that have been defined previously in pro-mAKAP82, 73,272 for hAKAP82 and 20,244 for the pro domain (hpro).

Both the Mouse and Human Genes for pro-AKAP82 Are Alternatively Spliced—The protein coding regions and 3′-UTRs of the pro-mAKAP82 and Fsc1 cDNAs are essentially identical; however, the 5′-UTRs of the two sequences share no homology. Furthermore, the Fsc1 5′-UTR contains an alternative in-frame start codon (27 bases upstream of the start codon for pro-mAKAP82) that could result in a protein containing an additional 9 amino acids at its amino terminus. These observations suggested that the two transcripts arise from alternative splicing of the same gene. To determine whether this was the case, genomic clones of pro-mAKAP82 and its 5′- and 3′-flanking regions were isolated and sequenced. The 5′-UTRs of both pro-mAKAP82 and Fsc1 were present in a single genomic clone, and were separated from each other by 585 bp (Fig. 1A and B), indicating that the two cDNA clones (pro-mAKAP82 and Fsc1) resulted from alternative splicing of the pro-mAKAP82 gene. The coding region of pro-mAKAP82 contained 5 exons and 4 introns with consensus splice donor/acceptor sites present at most exon/intron boundaries (Table 1; GenBank accession numbers AF087516 and AF087517). An overview of the structure of pro-mAKAP82 is shown in Fig. 1A.

The 5′-UTR of the pro-hAKAP82 cDNA was highly homologous to the 5′-UTR of pro-mAKAP82 but shared no homology with the 5′-UTR of Fsc1. To determine whether the pro-hAKAP82 gene, like the mouse homologue, was alternatively spliced, we used a PCR-based approach to search for a human cDNA sequence homologous to the Fsc1 5′-UTR. Using primers corresponding to regions in the 5′-UTR of Fsc1 and the 5′ end of the coding region of the pro-hAKAP82 cDNA, an approximately 400-bp product (5′-UTR) was amplified from a human testis cDNA library. The sequence of 5′-UTR, which was highly homologous to the 5′-UTR5′ end of the coding region of Fsc1 (67% identical bases, Fig. 1B). The 5′-UTR sequence, like the sequence of Fsc1, contained an alternative in-frame start codon 27 bp upstream of the start codon for pro-hAKAP82, which could result in a protein containing an additional 9 amino acids at its amino terminus compared with pro-hAKAP82. Five of these 9 deduced amino acids were identical to the 9 predicted additional amino acids of Fsc1. This finding is strong support for the concept that, like the mouse, there are at least two alternative splice variants of the pro-hAKAP82 gene, one with a 5′-UTR homologous to pro-mAKAP82 and one with a 5′-UTR homologous to Fsc1.

Pro-hAKAP82 and the Free pro Domain Localize to the Entire Length of the Principal Piece of Human Sperm—A polyclonal antibody generated against the mature mAKAP2 protein recognizes two bands in ejaculated human sperm; one at 82,000 (hAKAP82) and another at 97,000 (20). These findings suggest that, like the mouse, hAKAP82 is formed by proteolytic cleavage of a higher Mr precursor, pro-hAKAP82. An antibody (anti-hpro) raised against a peptide sequence in the predicted processed (hpro) region of pro-hAKAP82 (a region presumed to be absent from mature hAKAP82) was used to probe immunoblots of ejaculated human sperm protein. Anti-hpro identified a polypeptide at 97,000, which is pro-hAKAP82 (Fig. 2A). In addition, a 18,000 protein was detected. This protein approximates the size predicted for the hpro domain of pro-hAKAP82 (Mr 20,244) and indicated that some of this fragment persisted in mature sperm. As expected, because the hpro peptide has been removed from the mature protein, no band was recognized at Mr 82,000 (hAKAP82). Preabsorption of anti-hpro with the hpro peptide abolished the immunoreactivity, demonstrating that the antisera reacted specifically with the hpro sequence. The sizes of hAKAP82 (20), pro-hAKAP82, and hpro are consistent with the hypothesis that the pro-hAKAP82/hAKAP82 cleavage site is similar, if not identical, to that of the mouse.

Immunoreactivity was seen along the entire length of the principal piece when human sperm were probed with anti-hpro (Fig. 2, B and C). All sperm labeled in a similar fashion. This result is in contrast to the findings in mature cauda epididymal mouse sperm in which pro-mAKAP82 and mouse pro are found only in the proximal portion of the principal piece (19). Anti-hpro was specific for the FS as it reacted exclusively with the immunoactive, demonstrating that the antiserum reacted specifically with the hpro sequence. The sizes of hAKAP82 (20), pro-hAKAP82, and hpro are consistent with the hypothesis that the pro-hAKAP82/hAKAP82 cleavage site is similar, if not identical, to that of the mouse.

The pro-hAKAP82 Gene Maps to Xp11.2—To map the chromosomal location of the pro-hAKAP82 gene and to determine whether the gene is a candidate for any previously mapped human genetic diseases involving male infertility, we analyzed chromosomes from a normal man by FISH using a digoxigenin-11-dUTP-labeled BAC genomic clone of pro-hAKAP82. Results showed that pro-hAKAP82 mapped to Xp11.2, adjacent to the
TABLE I

Nucleotide sequences of splice junctions in the pro-mAKAP82 gene

The sequences of exons and introns are indicated by capital and small letters, respectively.

| Number | Size (bp) | Sequence |
|--------|-----------|----------|
| 1'     | 142       | ACAAAG   |
| 1*     | 115       | ATCAAG   |
| 2      | 96        | AAAGTG   |
| 3      | 48        | GAAGAT   |
| 4      | 102       | TCTAAG   |
| 5      | 2115      | GAGAAG   |

| Number | Size (bp) | Sequence |
|--------|-----------|----------|
| 1'     | 585       | gttagag  |
| 1*     | >1 kbp    | -        |
| 2      | 1308      | gttaga   |
| 3      | 360       | tcaaca   |
| 4      | 1985      | ttcgac   |
| 5      | 354       | tcaag    |

| Sequence |
|----------|
| ATGTCT   |
| ATGTCT   |
| ATATGC   |
| AAAGAT   |
| ACGGAG   |

| Number | Sequence |
|--------|----------|
| mAKAP82| 5' UTR   |
| hAKAP82| 5' UTR   |

This intron contains a highly repetitive region that has been intractable to sequencing. kbp, kilobase pairs.
centromere (Fig. 3). This region of the human X chromosome is syntenic to the most proximal end of the mouse X chromosome, the location of the pro-mAKAP82 gene (30).

DISCUSSION

The high homology of the amino acid sequences of pro-hAKAP82, pro-mAKAP82/Fsc1, and a 75-kDa rat FS protein (78% identical to and 89% conserved with pro-hAKAP82 at the amino acid level) indicates that the structure and function of AKAP82 is highly conserved in sperm of a number of mammalian species. Of particular interest is the finding that the amino acid sequence of the RII binding site of pro-mAKAP82 (11) is identical in pro-hAKAP82 (14 of 14 identical amino acids) and is highly conserved in the 75-kDa rat FS protein (13 of 14 identical amino acids). Furthermore, when human sperm proteins were probed with radiolabeled RII, prominent bands were identified at $M_r$ 97,000 and $M_r$ 82,000 that corresponded to pro-hAKAP82 and hAKAP82, respectively (20). The binding was eliminated by preincubation of the RII subunit with a synthetic RII-binding peptide (11) corresponding to the sequence of the pro-mAKAP82 RII binding site but not by preincubation of the RII subunit with a peptide containing a scrambled version of the sequence of the RII-binding peptide (data not shown). These results confirmed that pro-hAKAP82 and hAKAP82 are AKAPs and that the RII binding site was conserved between the mouse and human proteins. The ability of pro-hAKAP82 and hAKAP82 to bind the RII subunit of PK-A together with the importance of protein phosphorylation events for sperm motility makes it likely that hAKAP82 anchors PK-A to the sperm FS and directs the signal transduction pathways that control human sperm motility.

Although the ability to bind the RII subunit of PK-A has historically been used to identify and define AKAPs, recent evidence indicates that AKAPs function as scaffolding proteins for a variety of signal transducing molecules (31–35). Thus, multifunctional kinases and phosphatases can be targeted to...
subcellular locations via anchoring to a common AKAP. The highly polarized structure of the flagellum together with a paucity of cytoplasm in sperm lends itself to the idea of a scaffolding protein serving to sequester typically soluble proteins. In the future, it will be important to determine whether AKAP82 functions as a scaffold for other proteins involved in sperm motility.

In addition to the ability of pro-hAKAP82 and hAKAP82 to bind RII, the processing of pro-hAKAP82 into hAKAP82 represents another possible mechanism through which these proteins may be involved in the regulation of sperm motility. Compared with mouse sperm (18), human sperm contained a relatively large amount of pro-hAKAP82 in addition to hAKAP82 (20). Also, although pro-mAKAP82 is located only in the proximal principal piece of mouse cauda epididymal sperm, reactivity to the anti-hpro antibody persisted throughout the entire length of the principal piece of mature human sperm. These data indicated that the processing of pro-hAKAP82 to hAKAP82 is different from, and possibly less efficient than, the processing of pro-mAKAP82 to mAKAP82 (19). One hypothesis is that a decrease in processing of pro-hAKAP82 to hAKAP82 might be associated with sperm with poor motility. However, anti-hpro appeared to immunoreact similarly against pro-hAKAP82 in all sperm, suggesting that pro-hAKAP82 is not associated preferentially with subpopulations of immotile or poorly motile sperm in normal human ejaculates. Immunofluorescence experiments in this report were performed on ejaculates obtained from normal, fertile donors, and all samples had very high percentages of motile sperm. Thus, differences in the efficiency of processing may be found in patients with more dramatic reductions in sperm motility.

Taken together, the data on mAKAP82 and hAKAP82 suggest several potential ways in which pro-hAKAP82 and hAKAP82 could be associated with sperm motility. First, these proteins, by their ability to bind the RII subunit of PK-A, may function to direct signal transduction pathways by channeling the activity of the kinase. Other studies have suggested that the interaction of RII with sperm AKAPs may itself be important for the regulation of sperm motility independent of the activity of the catalytic subunit of PK-A (36). Second, the processing of pro-hAKAP82 into hAKAP82 may be associated with alterations in flagellar motility. Third, the capitation-dependent tyrosine phosphorylation of pro-hAKAP82 and hAKAP82 (20) may regulate an undefined function important to sperm motility. Fourth, pro-hAKAP82/hAKAP82 may also be important to the cytoskeletal integrity of the FS. And finally, the persistence of the free pro domain in both mature mouse and human sperm suggests that the pro domain itself may have some as yet unknown function in the FS. Future studies will focus on the potential association of alterations in processing or phosphorylation of pro-hAKAP82 and hAKAP82 with changes in human sperm motility.

Alternative splicing of the pro-AKAP82 gene resulted in the generation of two transcripts in both the mouse (pro-mAKAP82 and Fsc1) and the human (pro-hAKAP82 and the transcript represented by 5’-UTRγ). The Fsc1 and 5’-UTRγ sequences predict proteins that contain 9 more amino acids at their amino termini than do the pro-mAKAP82 and pro-hAKAP82 proteins. Recently, a human testis-specific cDNA (termed hi) encoding a predicted protein sequence that is 92% identical to and 95% conserved with that of pro-hAKAP82 was reported (37). The 5’-UTR of hi is 97% identical to the sequence of 5’-UTRγ, indicating that the hi sequence encodes the alternative splice variant of pro-hAKAP82. Surprisingly, and with no direct experimental evidence, the predicted hi protein is described as a sperm surface glycoprotein.

Alternative splicing has been reported in other AKAP genes including the Drosophila AKAP, DAKAP50, and another human/mouse male germ cell AKAP, S-AKAP84 (38, 39). In mouse AKAP-KL, alternative splicing together with the use of alternative translation initiation codons, results in six different protein isoforms (40). The expression of these isoforms is differentially regulated and results in different isoforms predominating in different tissue types. These data suggest that the various isoforms of AKAP-KL may play different physiologic roles. At this time, we do not know the significance of the two alternative splice products of pro-hAKAP82 or if both of the two alternative forms of the protein even exist. However, conservation of the alternative splice event between mouse and human lends support to the hypothesis that each of the two pro-AKAP82 alternative splice products are functionally significant.

We previously showed that the gene for mouse pro-AKAP82 maps to the proximal end of the X chromosome (30). In the current study, the human gene for pro-AKAP82 mapped to the region of the human X chromosome (Xp11.2) that is syntenic to the proximal X chromosome of the mouse. In the mouse, pro-mAKAP82 is expressed post-meiotically in late spermiogenesis (18, 28). During this haploid phase, the X and Y chromosomes are generally considered inactive. This means that, in the mouse, at least some regions of the X chromosome are actively transcribed even in late spermiogenesis. In addition, because the cells are haploid at the time that the transcript first appears, yet the protein is present in all cells, mRNA and/or protein for mAKAP82 must be shared between conjoined spermids via their intercellular bridges. Our data suggest that a similar event may occur in humans.

Although several genes and genetic diseases have been mapped to Xp11.2, the reported phenotypes are not suggestive of a defect in a sperm FS protein. Thus, clinically significant mutations occurring in the hAKAP82 gene have not yet been mapped or genetically characterized. Because pro-hAKAP82 is on the X chromosome, defects in the gene in males would necessarily be maternally inherited. If men with mutations in pro-hAKAP82 were sterile, then the defect would be self-limiting and thus would be rare. Candidates for a defect in this gene are men with dysplasia of the fibrous sheath. Affected individuals have more than 95% immotile sperm. The sperm tails are short and thick, and the FS is disorganized (41). It will be important to determine whether or not hAKAP82 is involved in this or any other form of male factor infertility associated with reduced sperm motility.

Acknowledgments—We gratefully acknowledge the assistance of Dr. Deborah Driscoll, Ms. Holly Mensch, and Ms. Bea Sellinger with the FISH techniques and FISH data interpretation. We thank Drs. Gregory S. Kopf, Bayard T. Storey, and Alexander Travis for critical reading of this manuscript.

REFERENCES

1. Bell, C. (1994) Trends Biochem. Sci. 19, 427–429
2. Afzelius, B. A., and Eliasson, R. (1979) J. Ultrastruct. Res. 69, 43-52
3. Eliasson, R., Massberg, B., Canner, P., and Afzelius, B. (1977) N. Engl. J. Med. 297, 1–6
4. Narayan, D., Krishnan, S. N., Upender, M., Ravikumar, T. S., Mahoney, M. J., Dolan, T. F., Teebi, A. S., and Haddad, G. G. (1994) J. Med. Genet. 31, 493–496
5. Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Bhuta, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagner, M., Levi-Acobas, F., Largent-Piet, D., Munuch, A., Steel, K. P., Brown, S. M., and Petit, C. (1995) Nature 374, 60–61
6. Lindemann, C. B., Orlando, A., and Kanous, K. S. (1992) J. Cell Sci. 102, 249–260
7. Tash, J. S., and Means, A. R. (1982) Biol. Reprod. 26, 745–763
8. Brokaw, C. J. (1987) J. Cell. Biochem. 35, 175–184
9. Tash, J. S., Kribes, M., Patel, J., Means, R. L., Klee, C. B., and Means, A. R. (1988) J. Cell Biol. 106, 1626–1633
10. Tash, J. S., and Bracho, G. E. (1994) J. Androl. 15, 505–509
11. Visconti, P., Johnson, L., Oyaski, M., Fornes, M., Moss, S., Gerton, G., and Kopf, G. (1997) Dev. Biol. 192, 351–363
hAKAP82 in Human Sperm

12. Pawson, T., and Scott, J. D. (1997) Science 278, 2075–2080
13. Rubin, C. S. (1994) Biochim. Biophys. Acta 1224, 407–479
14. Scott, J. D., and McCartney, S. (1994) Mol. Endocrinol. 8, 5–11
15. Coghlan, V. M., Bergeson, S. E., Langeberg, L., Nilaver, G., and Scott, J. D. (1993) Mol. Cell. Biochem. 127/128, 309–319
16. Rubin, C. S. (1994) Biochim. Biophys. Acta 1224, 467–479
17. Mechaly-Rosen, D. (1995) Science 268, 247–251
18. Carrera, A., Gerton, G. L., and Moss, S. B. (1994) Dev. Biol. 165, 272–284
19. Johnson, L., Foster, J., Haig-Ladewig, L., VanScoy, H., Rubin, C., Moss, S., and Gerton, G. (1997) Dev. Biol. 192, 340–350
20. Carrera, A., Moos, J., Ning, X., Gerton, G., Tesarik, J., Kopf, G., and Moss, S. (1996) Dev. Biol. 180, 284–296
21. Visconti, P. E., Bailey, J. L., Moore, G. D., Pan, D., Olds-Clarke, P., and Kopf, G. S. (1995) Development 121, 1129–1137
22. Galantino-Homer, H. L., Visconti, P. E., and Kopf, G. S. (1997) Biol. Reprod. 56, 707–719
23. Morales, P., Overstreet, J. W., and Katz, D. F. (1988) J. Reprod. Fertil. 83, 119–128
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
26. Lenson, G., Auffray, C., Polymeropoulos, M., and Soares, M. B. (1996) Genomics 33, 151–152
27. Adams, M. D., Kelley, J. M., Gecayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merril, C. E., Wu, A., Old, B., Moreto, R. F., Kerlavage, A. R., McCombie, W. R., and Venter, J. C. (1991) Science 252, 1651–1656
28. Fulcher, K. D., Mori, C., Welch, J. E., O’Brien, D. A., Klappper, D. G., and Eddy, E. M. (1995) Biol. Reprod. 52, 41–49
29. Schaffner, W., and Weissman, C. (1973) Anal. Biochem. 56, 502–504
30. Moss, S., VanScoy, H., and Gerton, G. (1997) Mamm. Genome 8, 37–38
31. Carr, D. W., Stofko-Hahn, R. E., Fraser, I. D. C., Cone, R. D., and Scott, J. D. (1992) J. Biol. Chem. 267, 16816–16823
32. Coghlan, V. M., Perrino, B. A., Howard, M., Langeberg, L. K., Hicks, J. B., Gallatin, W. M., and Scott, J. D. (1995) Science 267, 108–111
33. Klauke, T. M., Faux, M. C., Labudda, K., Langeberg, L. K., Jaken, S., and Scott, J. D. (1996) Science 271, 1589–1592
34. Huang, L. J., Durick, K., Weiner, J. A., Chun, J., and Taylor, S. S. (1997) J. Biol. Chem. 272, 8057–8064
35. Burton, K. A., Johnson, B. D., Hausken, Z. E., Westenbroek, R. E., Idzerda, R. L., Scheuer, T., Scott, J. D., Catterall, W. A., and McKnight, G. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11067–11072
36. Vijayaraghavan, S., Goueli, S. A., Davey, M. P., and Carr, D. W. (1997) J. Biol. Chem. 272, 4747–4752
37. Mohapatra, B., Verma, S., Shanker, S., and Suri, A. (1998) Biochem. Biophys. Res. Commun. 244, 540–545
38. Han, J.-D., Baker, N. E., and Rubin, C. S. (1997) J. Biol. Chem. 272, 26611–26619
39. Lin, R.-Y., Moss, S. B., and Rubin, C. S. (1995) J. Biol. Chem. 270, 27894–27810
40. Dong, F., Feldmesser, M., Casadevall, A., and Rubin, C. (1998) J. Biol. Chem. 273, 6533–6541
41. Chemes, H. E., Brugo, S., Zanchetti, F., Carrere, C., and Lavieri, J. C. (1987) Fertil. Steril. 48, 664–669