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JOURNAL ARTICLE

Molecular cloning and developmental expression of the major fibrous sheath protein (FS 75) of rat sperm

M. El-Alfy, D. Moshonas, C. R. Morales and R. Oko
Department of Anatomy and Cell Biology, McGill University.

The fibrous sheath (FS) is a cytoskeletal structure that encases the axoneme in the principal piece of the spermatozoon tail. In the rat, it is composed of several proteins, of which a 75-kDa polypeptide (FS 75), as estimated by PAGE, is the most prominent. The objectives of this study were to clone and sequence this protein and to characterize its transcriptional and translational origins during spermatogenesis. Initially, we isolated two overlapping cDNA segments that encoded a large part of the FS 75 protein but lacked the initiation codon for translation. Both clones were obtained by screening a rat testicular phagemid cDNA library with an anti-FS 75 polyclonal antibody. An upstream portion of the FS 75 mRNA containing the initiation codon was obtained by polymerase chain reaction with a pair of specific primers. Accounting for the overlap in all segments, 2786 nt of an approximately 3-kb FS 75 mRNA was obtained. The amino acid sequence of the longest possible open reading frame of the rat FS 75 was found to be similar to two previously cloned variants of the major FS polypeptide of mouse spermatozoa. Sequence analysis of the rat FS cDNA revealed anchoring kinase A protein domains and several kinase phosphorylation sites, supporting the idea that this protein plays a crucial role in the motility of spermatozoa. The presence of a potential N-myristoylation site suggests that this protein may covalently bind to the inner leaflet of the plasma membrane (PM), which in turn may explain the close association of the FS and PM from early development. Developmental northern blot analysis and in situ hybridization revealed that the FS 75 mRNA is haploid expressed, with an abundant level of mRNA in late round spermatids. Maximum levels of the FS 75 polypeptide, as determined by immunocytochemistry, correlated with a rapid decline in corresponding mRNA levels in step 14-16 spermatids. Since transcription termination occurs several steps earlier, the bulk of FS 75 mRNA appears to be translationally regulated.

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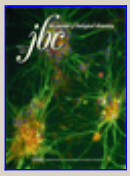

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