The Marsupial Sperm Tail Cytoskeleton: A Morphological and Biochemical Study

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CHAPTER 1 LITERATURE REVIEW AND RESEARCH PROPOSAL

ABSTRACT

The motile apparatus of the sperm tail contains, like that of flagella and cilia, an axoneme composed of microtubules. However, unlike cilia and flagella, it also contains additional, unique, cytoskeletal structures which are thought to play important roles in sperm motility and stability. In eutherian mammals, these cytoskeletal structures, the outer dense fibres and fibrous sheath, are composed of multiple, highly insoluble, proteins. Marsupials diverged from eutherians over 100 million years ago and their sperm tails appear to have morphologically similar cytoskeletal structures, however almost nothing is known of their chemical composition or morphogenesis. For my PhD, I have investigated the formation, and protein composition, of the outer dense fibres and fibrous sheath of a model marsupial species, the brush-tail possum (*Trichosurus vulpecula*). Twelve spermatid steps of spermiogenesis were identified in the possum by transmission electron microscopy. Outer dense fibre and fibrous sheath morphogenesis were found to be lengthy, multi-step, processes extending over a large part of spermiogenesis. The major proteins in the outer dense fibres and fibrous sheath were determined by first developing procedures for isolating and solubilizing the proteins, whose molecular weights were then determined by SDS-PAGE. The outer dense fibres were found to have seven major proteins of kDa 73, 58, 55, 54, 52, 41 and 16, whereas the fibrous sheath had twelve major proteins of kDa 106, 76, 66, 62, 55, 53, 52, 46, 40, 30, 28 and 16. A polyclonal antibody was prepared against a major protein fraction of the fibrous sheath and, with this antibody, the morphogenesis of the fibrous sheath was found to be restricted to steps 7-12 of spermiogenesis. This antiserum did not react with the proteins extracted from the outer dense fibres thus indicating little homology between proteins of these two structures.

Nevertheless, this antibody showed strong cross-reactivity with the 76 and 62 kDa proteins of the fibrous sheath of several other species of marsupials from three other families, as well as with those from the laboratory rat, therefore indicating conservation of at least these two proteins across marsupial and eutherian subclasses. Furthermore, antisera obtained from an overseas laboratory prepared against the eutherian (laboratory mouse) GAPDS (47.5 kDa) fibrous sheath protein was found to label the possum fibrous sheath by immunofluorescence and immunogold electron microscopy, further indicating conservation of fibrous sheath proteins across both infraclasses of mammals. In addition, an antibody to a laboratory rat outer dense fibre protein, ODF2 (84 kDa), was found to cross-react with the possum outer dense fibre proteins of molecular weights 55 and 28 kDa, suggesting that proteins of this cytoskeletal structure may be similarly conserved, albeit of different molecular weights. The results of this study, thus indicate that, despite over 100 million years of divergence between marsupials and eutherians, there is conservation of several major proteins across these two extant infraclassess of mammals even though differences in their molecular weights occur. These findings indicate that, prior to divergence into the two major lineages of extant mammals, an increased complexity of the cytoskeleton of the sperm tail evolved that included incorporation of several additional proteins over and above those that make up the axoneme.