

61. PRESENCE OF ACTIN AND GTPASE RHO A IN EPIDIDYMAL SPERM FROM THE FAT-TAILED DUNNART (*SMINTHOPSIS CRASSICAUDATA*)

E.K. Richards, M. Lin and R.J. Aitken

School of Biological Sciences, University of Newcastle, NSW 2287, Australia.

Marsupial sperm are released from the testis with an immature morphology. Whilst in transit through the epididymis the sperm cell undergoes radical structural changes (1). In somatic cells morphological change occurs through reorganisation of the actin cytoskeleton, a process regulated by RhoA protein. It is known that actin is found in the sperm head of a range of mammalian species, and there is evidence that actin is involved in sperm maturation in the tammar wallaby (2). To localise F-actin and RhoA, the epididymis from freshly killed fat-tailed dunnarts was dissected into caput, corpus and caudal regions. Sperm were diffused into PBS and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, and membranes were permeabilised in -20°C acetone. Presence and distribution of F-actin on sperm was tested by incubation with 50 µg/mL Phalloidin-FITC conjugate in PBS with 1% DMSO for 60 min at RT. Presence of RhoA was tested by immunofluorescence: Binding sites were blocked with 10% BSA and 5% NGS in PBS, before exposure to the primary antibody – 1:50 mouse monoclonal anti-human RhoA. Secondary antibody was 1:100 FITC-conjugated anti-mouse IgG. F-actin was found in epididymal sperm of the fat-tailed dunnart. Sperm from the caput and corpus epididymis displayed bright fluorescence on the head and midpiece of the cell whereas fluorescence subsided from all regions but the midpiece of cauda sperm. RhoA distribution coincided with F-actin, and strong fluorescence was seen in the head and midpiece of caput and cauda sperm. Low levels of RhoA were also detected along the flagellum of mature sperm. Control treatments showed that the fluorescence was not due to autofluorescence or non-specific binding of the FITC conjugate. F-actin and RhoA were spatially and temporally localised to those regions of the sperm that are undergoing morphological change. It appears that the proteins may be involved with the processes of sperm epididymal maturation. RhoA found on the flagellum is thought to act through RhoGTPase, Ropporin and ACAP3 on the microtubules to regulate motility (3).

(1) Harding, H.R. *et al.* (1979) 'The Spermatozoan.' (Urban & Schwarzenberg: Baltimore.) (2) Lin, M. *et al.* (2002) *Reproduction* **124**: 107–117. (3) Eddy, E.M *et al.* (2003) *Micros. Res. Tech.* **61**: 103–115.