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## JOURNAL ARTICLE

# Modification of the rat sperm flagellar plasma membrane during maturation in the epididymis

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Previously, we demonstrated that surface radiolabeling of rat epididymal spermatozoa by lactoperoxidase-catalyzed iodination reveals a major component with an apparent molecular weight of 26,000 to 28,000 daltons (26 kDa) on spermatozoa from the cauda but not the caput epididymidis. To characterize this surface component further, sperm surface constituents radiolabeled by lactoperoxidase-catalyzed iodination were separated by 2-D PAGE. The 26 kDa component was localized by autoradiography and appeared as the major labeled acidic spot on cauda spermatozoa, but neither a radiolabeled spot nor a corresponding stained spot was present on caput spermatozoa. The 26 kDa spot was excised from 2-D gels of plasma membranes from cauda spermatozoa and utilized for immunization. The monospecific antiserum stained a single band of 26 kDa on Western blots of SDS-PAGE-separated plasma membranes from cauda spermatozoa and in a 100,000 X g supernatant fluid of the luminal contents of the cauda epididymidis. Immunohistochemical staining of cauda spermatozoa revealed antigen exclusively on the flagellar domain; the antigen was not seen on caput spermatozoa but first appeared in spermatozoa from the proximal corpus epididymidis. Immunoelectron microscopy confirmed the 26 kDa component was localized to the external face of the flagellar plasma membrane. Immunohistochemical staining of caput spermatozoa incubated in vitro with cauda epididymal luminal fluid revealed the 26 kDa component specifically bound the flagellar domain of immature spermatozoa.

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