TMC5-CIB1 Complex at the Spermatid Plasma Membrane is essential for Spermiogenesis

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Spermiogenesis is the developmental process during which haploid round spermatids transform into sperm by undergoing a series of unique morphogenetic events including nuclear condensation, flagellum formation, and excess cytoplasmic elimination. The molecular mechanisms driving these processes are poorly understood, representing a significant knowledge gap. To address this gap, we examined an understudied member of the 'transmembrane channel-like' (TMC) protein family with potential roles in mechanotransduction, TMC5. We found TMC5 resides in a complex with the spermiogenesis-essential protein 'calcium and integrin binding protein 1' (CIB1). Indeed, both TMC5 and CIB1 co-localized at the plasma membrane in round, elongating, and condensing spermatids, in TMC5-mCherry knockin mice and by immunostaining, respectively. A direct interaction between TMC5 and CIB1 vas validated by co-immunoprecipitation. To define its role in spermiogenesis, we generated *Tmc5* knockout (KO) mice; these mice exhibited disrupted spermatid elongation resulting in azoospermia and male infertility. Strikingly, CIB1 was also mislocalized from the spermatid plasma membrane. Although the precise role of TMC5 is unknown, based on known roles for founding members of the TMC family (TMC1-2) in cytoskeletal regulation and plasma membrane homeostasis in other systems, we hypothesized a similar role for TMC5 in spermiogenesis. In support of this, comparative super-resolution microscopy of *Tmc5* WT and KO spermatids revealed: 1) extensive F-actin network dysregulation, including loss of F-actin at spermatid plasma membrane; 2) disassembly of the spermatid-containing acrosome-acroplaxome-manchette complex that modulates spermatid nuclear re-shaping; and 3) aberrant phosphatidyl serine externalization. In summary, we identified a novel membrane protein that is essential for spermiogenesis. Current efforts are focused on defining the molecular function of the TMC5-CIB1 complex, and results will provide new insight into the complex molecular mechanisms that regulate spermatid morphogenesis