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RESEARCH HIGHLIGHT

# RABL-regulated pathways: a new tale in sperm function

**Charles M Allan**

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**Mammalian sperm have a specialized motile flagellum to provide the unidirectional propulsion required to achieve fertilisation of the egg. Defective flagella development and/or function is a significant cause of reduced sperm motility (asthenospermia) and male infertility. Over the past decade, a growing number of distinct proteins with crucial roles in sperm flagella have been identified by genetic loss-of-function approaches in mice, in which the deliberately engineered or spontaneous mutation of individual genes have lead to anomalies in sperm tail assembly, structure and function. In a recent study published in *PLoS Genetics*, Lo *et al.* have utilized random mutagenesis induced by the chemical mutagen *N*-ethyl-nitrosourea to identify a previously uncharacterized protein (RABL2) in murine sperm flagella required for male fertility.**

By combining the *N*-ethyl-nitrosourea-induced random mutagenesis with phenotypic screening of fertility, Lo *et al.*[1] identified a mouse line displaying recessive male infertility associated with a nucleotide mutation localized to the autosomal *Rabl2* gene. The genomic *Rabl2* mutation was an A to G nucleotide substitution predicted to cause a single amino-acid (D73G) substitution in two putative RABL2 protein isoforms derived from alternatively spliced *Rabl2* transcripts.[1] Structural differences between these predicted RABL2 isoforms (designated 1 and 2) were not described, but each would contain distinct amino acid sequences in their C-terminal region. Sterile males homozygous for the *Rabl2* mutation ($Rabl2^{Mot/Mot}$) exhibited mature testes 85% of normal size which produced 50% less sperm. In addition to oligospermia, epididymal sperm length was 17% shorter than normal, and functional analysis revealed an 83% reduction in progressive sperm motility.[1] Female mice carrying the *Rabl2* mutation remained fertile. The oligoasthenospermia observed in infertile $Rabl2^{Mot/Mot}$ males suggested that the *Rabl2* gene encodes a factor essential for the functional development and motility of mouse spermatozoa.

Lo *et al.*[1] found that *Rabl2* isoform 1 and 2 transcripts were enriched in male germ cells, and detected in many other tissues, including the ovary and those containing motile flagella/cilia (interchangeable terms), such as brain, kidney, lung and trachea. Further characterisation of RABL2 was directed to isoform 2, possibly due to its higher mRNA expression levels during testicular development compared to isoform 1. Two human homologues, *RABL2B* and a paralogue *RABL2A* encoding a predicted protein with four amino acid changes, have been previously identified.[2] Human RABL2B shares 89% identity with murine RABL2 isoform 2. While the function of human RABL2 homologues are not known, gene expression databases (EST) suggested *RABL2A/RABL2B* are expressed in a wide range of tissues including testis, brain, kidney and uterus.[1] Inspection of another gene expression database (NCBI GEO Profiles) for human *RABL2A*/*RABL2B* supports a broad tissue expression pattern, including the testis, and further suggests both genes are highly expressed in epididymis and trachea (http://www.ncbi.nlm.nih.gov/geoprofiles: *RABL2A:* GDS596, GDS829, GDS3113; *RABL2B:* GDS832), consistent with roles in spermatozoa and cilia containing tissues.

Mouse *Rabl2* encodes an evolutionarily conserved member of the Rab-like Ras GTPase superfamily.[3] Previous studies have shown that related Rab-like proteins have key roles in flagella development and function in diverse species including *Chlamydomonas reinhardtii* (green algae) and *Trypanosoma brucei* (flagellate protozoan). The murine $Rabl2^{Mot/Mot}$ asthenospermia phenotype suggested that conserved RABL-mediated actions extend to mammalian motile flagella. The assembly and function of motile flagella requires bidirectional intraflagella transport (IFT) of large protein complexes along a central microtubule-based structure known as the axoneme. Delivery of cargo proteins along the flagella axoneme involves components of IFT complex A or complex B machinery. In *Chlamydomonas*, targeted RNA interference showed that the Rab-like protein RABL4 (IFT27) forms part of the IFT complex *B* during flagella tip development.[4] Knock-down studies of RABL5 (IFT22) in *Trypanosoma brucei* produced stunted flagella packed with IFT proteins.[5] Predicting that mammalian RABL2 may exhibit related functions, Lo *et al.*[1] used immunoprecipitation to show that RABL2 interacts with complex B components including RABL4 (IFT27), IFT81 (Cdv1) and IFT172. Immunofluorescent microscopy colocalized RABL2 with these IFT proteins in the mid-piece of elongated spermatids in the testis. It was not reported if the mutated RABL (D73G) had diminished interaction with these IFT proteins,[1] but the wild-type RABL2-IFT protein interactions combined with the $Rabl2^{Mot/Mot}$ phenotype indicated that RABL2 has a specific role in IFT particles.

RABL2 contains the five consensus motifs characteristic of GTP/GDP binding in the Ras superfamily,[3] suggesting that it may cycle between GTP-active and GDP-inactive states. The $Rabl2^{Mot/Mot}$ D73G mutation occurred in a β-sheet domain, which in other superfamily members is required for interactions with other proteins,[3] including factors coordinating GDP–GTP exchange. To study GTP/GDP involvement in RABL2 function, Lo *et al.*[1] used recombinant RABL2 isoform 2 to identify proteins in testis extracts that preferentially bound to RABL2 in a GTP-bound versus GDP-bound state.[1] Mass spectrometry identified 89 proteins that interacted with the putative active state (GTP-bound) RABL2.

ANZAC Research Institute, Concord Hospital, University of Sydney, Sydney, NSW 2139, Australia
Correspondence: Dr CM Allan (charles@anzac.edu.au)

Five proteins (ATP6VE1, EB21, HK1, HSP4AL and LDHC) known to have roles in ciliated tissue, microtubule function, or localized to the fibrous sheath surrounding the axoneme, were verified to interact with RABL2 by co-immunoprecipitation analysis. Western blotting and immunolocalisation studies indicated that these five IFT effector proteins were less abundant in defective *Rabl2*$^{Mot/Mot}$ compared to normal *Rabl2* sperm tails, despite the presence of comparable levels of mutated RABL2 (D73G) versus normal RABL2 protein. In normal sperm, these effector proteins were present in the principal piece of sperm tails (as well as the peri-acrosome), beyond RABL2 localized to the mid-piece of the sperm tail. In *Rabl2*$^{Mot/Mot}$ sperm, the residual immunolocalisation of HK1 to the mid- and not principal piece of the tail further suggested that the D73G mutation reduced RABL2-mediated delivery of effector proteins in the growing sperm tail. Collectively, these findings provide evidence that RABL2 plays an important role in sperm tail assembly and intraflagellar movement of cargo protein complexes at least up to the mid-piece region.

Many mouse models with reported anomalies in sperm flagella have been established using strategies to target genes with known or predicted functions, several to study genetic causes of human ciliopathy.[6–8] Ciliopathies are heterogeneous systemic disorders that may involve male infertility, such as primary ciliary dyskinesia and Bardet–Biedl syndrome, caused by a range of unrelated genes that affect ciliary/flagella function. For instance, primary ciliary dyskinesia presents a wide spectrum of sperm anomalies, most arising from loss of dynein motors that drive flagella/cilia beating.[9] Mouse models with abnormal sperm flagella may also exhibit defects in sperm head structure, as well as the systemic phenotypes of ciliopathies.[6,10] The oligoasthenospermia observed in the *Rabl2*$^{Mot/Mot}$ mice[1] may represent a motile flagella-specific defect with few or mild systemic characteristics of ciliopathy, although the overall health of *Rabl2*$^{Mot/Mot}$ mice has yet to be reported.[1]

Disruption of factors involved in the regulation of IFT can lead to asthenospermia and male infertility. The identification of mouse RABL5 protein interactions in sperm flagella presents a new molecular target in a crucial IFT pathway for future investigations of sperm development and function. At present, the clinical significance of RABL2-specific dysfunction in humans is unknown. While strategies such as ICSI can overcome many pathologies in the mid-piece of spermatozoa,[11] the identification of RABL2 and factors involved in oligo/asthenospermia may provide new clinical markers for screening to reduce potential transmission of mutant (e.g., ciliopathic) genes to the offspring. Further characterisation of such factors and associated pathways may also provide new opportunity for the development of contraceptive strategies which target spermatozoa.

1 Lo JC, Jamsai D, O'Connor AE, Borg C, Clark BJ *et al*. RAB-like 2 has an essential role in male fertility, sperm intra-flagellar transport, and tail assembly. *PLoS Genet* 2012; **8**: e1002969.
2 Wong AC, Shkolny D, Dorman A, Willingham D, Roe BA *et al*. Two novel human RAB genes with near identical sequence each map to a telomere-associated region: the subtelomeric region of 22q13.3 and the ancestral telomere band 2q13. *Genomics* 1999; **59**: 326–34.
3 Rojas AM, Fuentes G, Rausell A, Valencia A. The Ras protein superfamily: evolutionary tree and role of conserved amino acids. *J Cell Biol* 2012; **196**: 189–201.
4 Qin H, Wang Z, Diener D, Rosenbaum J. Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. *Curr Biol* 2007; **17**: 193–202.
5 Adhiambo C, Blisnick T, Toutirais G, Delannoy E, Bastin P. A novel function for the atypical small G protein Rab-like 5 in the assembly of the trypanosome flagellum. *J Cell Sci* 2009; **122**: 834–41.
6 Escalier D. Knockout mouse models of sperm flagellum anomalies. *Hum Reprod Update* 2006; **12**: 449–61.
7 Inaba K. Sperm flagella: comparative and phylogenetic perspectives of protein components. *Mol Hum Reprod* 2011; **17**: 524–38.
8 Zhang Q, Seo S, Bugge K, Stone EM, Sheffield VC. BBS proteins interact genetically with the IFT pathway to influence SHH-related phenotypes. *Hum Mol Genet* 2012; **21**: 1945–53.
9 Mitchison HM, Schmidts M, Loges NT, Freshour J, Dritsoula A *et al*. Mutations in axonemal dynein assembly factor DNAAF3 cause primary ciliary dyskinesia. *Nat Genet* 2012; **44**: 381–9, S1–2.
10 Yan W. Male infertility caused by spermiogenic defects: lessons from gene knockouts. *Mol Cell Endocrinol* 2009; **306**: 24–32.
11 Chemes HE, Alvarez Sedo C. Tales of the tail and sperm head aches: changing concepts on the prognostic significance of sperm pathologies affecting the head, neck and tail. *Asian J Androl* 2012; **14**: 14–23.