Diverse functions of SHIPPO-domain proteins in flagellar assembly

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Abstract:

The flagellum of the African trypanosome is essential for their ability for infect new hosts and their transmission via tsetse. The flagellum has 3 major structural domains – the basal body (BB) that templates new flagellar growth, the axoneme that "beats" for cellular propulsion, and the transition zone (TZ) that separates the two. The TZ is the structural intermediate between the BB and the axoneme and is fundamental for flagellar assembly and function. For example, the TZ harbours the "basal plate", an electron dense structure at the distal TZ-axoneme boundary that nucleates axonemal microtubules essential for flagellar beating. Despite its importance, assembly of the TZ is not well understood.

Our previous work revealed the TZ to be extraordinarily complex, comprised of >70 proteins. We showed that one of these, TZP250 (transition Zone Protein 250kD) has an RNAi phenotype that includes severe axoneme defects, suggesting an important role in flagellar assembly. Here, we show that the primary defect in TZP250 mutants is TZ length dysregulation and basal plate mispositioning, revealing a key role for TZP250 in TZ morphogenesis. To investigate TZP250 function further, we used ultra-expansion microscopy (UExM) on bi-terminally tagged TZP250 to show that while the N terminus of TZP250 encircles the TZ at the (proximal) BB-TZ boundary, the C terminus lies at the (distal) TZ-axoneme boundary. Hence, TZP250 molecules lie along the entire ~350nm length of the TZ. Combining structural predictions, sophisticated phenotypic assays and super-resolved domain localisation, we propose that TZP250 acts as a molecular ruler that defines the length of the TZ.

Protein domain analysis shows that the central part of TZP250 is dominated by repeated 'SHIPPO' domains, a Pro-Gly-Pro-Gly-X-Tyr motif that was recently shown to mediate binding to the outer wedge of the axonemal doublet microtubules and are likely to represent key biochemistry for TZP250 function. Interestingly, we reveal that, despite representing a complex, multi-copy gene family, SHIPPO proteins are found only in ciliated organisms, and diversified in metazoa, such as humans and zebrafish. Using only the presence of this domain, we identify a second SHIPPO-containing protein in trypanosomes, which localises to the axoneme and is essential for its complete assembly, suggesting a role in maintaining axoneme stability.

In summary, we show the SHIPPO domain to represent a highly conserved but diverse protein family with different functions within a ciliary context.