

C-Terminal 6-His Tag on LC8 Impairs Generation and Motility of *Chlamydomonas* Flagella

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LC8 is a small, approximately ninety amino acid protein ubiquitously present in eukaryotes. The amino acid sequence is more than 90% identical across species, indicating evolutionary pressure to maintain the entire molecule. It is found in many vital complexes, binding such diverse proteins as dynein motor subunits, nitric oxide synthase, transcription factors, and viral proteins, and sequestering apoptotic factors to microtubules. Mutants of several species defective in the LC8 gene display serious pleiotropic phenotypes.

Structural studies have shown that LC8 forms homodimers, with the two interfacial grooves binding sites. Bivalent binding with independent proteins in complexes is crucial for complex stability and proper assembly. However, recent structural analyses and our in-vivo evidence from *Chlamydomonas* demonstrates that current binding models are insufficient to explain LC8's sequestering functions. In-situ, LC8 appears to form a disulfide bond with a 50 kDa, tubulin-like molecule. Also, our studies have revealed that C-terminal extensions cause severe phenotypic defects. We postulate that LC8 is a multi-facet adaptor, capable of interacting with various proteins at the C-terminus or peripheral cysteine in addition to the interfacial grooves. This would allow formation of potentially large molecular complexes and tethering of targets to the cytoskeleton.

To test this and identify disulfide-bound proteins, we generated *Chlamydomonas* strains expressing LC8 with a 6-His tag at the C-terminus (LC8-6His) and strains with wild-type and his-tagged LC8. The LC8-6His strains are barely distinguishable from null-LC8 strains, displaying short and paralyzed flagella. The hybrid strains can grow full-length flagella and swim, albeit in an erratic and uncoordinated manner. These results reveal the detrimental effects of a positively charged tag that are only partially alleviated by wild-type LC8. The hybrid LC8 strain will be used to isolate the disulfide-linked complex of LC8-6His and the unknown 50 kDa protein.