A tale of two actins: significant functional overlap of divergent actin isoforms in the unicellular green alga *Chlamydomonas reinhardtii*

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A variety of organisms express multiple actin isoforms encoded by different genes which are sometimes tissue specific or developmentally regulated. However, the selective localization and segregation of cytoskeletal functions of these genes are understudied. Here we consider two actins found in the genome of the green alga *Chlamydomonas reinhardtii* which share ~63% sequence identity: the conventional actin IDA5 and the divergent actin NAP1. The behavior and functions of these actins have been elusive due to the difficulty in visualizing filaments with traditional methods and mild phenotypes seen in null mutants of these genes.

Through adjusted fluorescence protocols and *in situ* cryo electron tomography, we are now able to visualize filaments of these actin polymers near the nuclear envelope, ER, and Golgi apparatus. When subsets of the actin network are perturbed (in mutants of a formin or ARP2) or in gametes, we see a strong increase in signal of the remaining filamentous actins and reorganization of perinuclear filaments. IDA5 disruption with the actin-depolymerizing drug latrunculin B (Lat B), known to increase in Lat B-insensitive NAP1 expression, results in formation of discrete NAP1 rings surrounding the nucleus.

We also find that mild phenotypes in individual genetic mutants are due to a significant amount of functional redundancy between IDA5 and NAP1. Actin functions were uncovered using individual mutants as well as latB to selectively target IDA5 on the *nap1* mutant background (simultaneous acute perturbation of both actins). We find that actins play multiple roles in the growth of motile flagella in these cells. Either IDA5 or NAP1 expression is an absolute requirement for flagellar assembly from newly synthesized proteins. Flagellar protein synthesis at normal levels also requires one form of actin during induced flagellar assembly. Finally, these actins are required for protein organization of the region essential for gating at the base of flagella called the transition zone. Ultimately, IDA5 appears to be able to perform all of the identified functions of NAP1 with equal efficiency, but not vice versa. These experiments provide broader insight into the behaviors of multiple actin isoforms within a single cell.