Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network

Craig Blackstone, MD, PhD
Cellular Neurology Unit, Neurogenetics Branch, NINDS, NIH, Bethesda, MD, USA

Hereditary spastic paraplegias (HSPs; SPG1–45) are inherited neurological disorders characterized by lower extremity spastic weakness. Over half of all HSP cases result from autosomal dominant mutations in atlastin-1 (SPG3A), REEP1 (SPG31), or spastin (*SPG4*). The atlastin-1 dynamin-related GTPase interacts with spastin, a microtubule-severing ATPase, as well as with the DP1/Yop1p and reticulon families of ER shaping proteins, and SPG3A caused by atlastin-1 mutations has been linked pathogenically to abnormal ER morphology. We investigated SPG31 by analyzing the distribution, interactions, and functions of REEP1. We found that REEP1 is structurally related to the DP1/Yop1p family of ER-shaping proteins and distributes to the ER in cultured rat cerebral cortical neurons, where it colocalizes with spastin and atlastin-1. When overexpressed in COS7 cells, REEP1 forms protein complexes with atlastin-1 and spastin within the tubular ER, and these interactions required the hydrophobic hairpin domains in each of these proteins. REEP proteins were required for ER network formation in vitro, and REEP1 also bound microtubules and promoted ER alignment along the microtubule cytoskeleton in COS7 cells. A SPG31 truncation mutant REEP1 lacking the C-terminal cytoplasmic region did not interact with microtubules and disrupted the ER network. These data indicate that HSP proteins atlastin-1, spastin, and REEP1 interact within the tubular ER membrane in corticospinal neurons to coordinate ER shaping and microtubule dynamics. Thus, defects in tubular ER shaping and network interactions with the microtubule cytoskeleton seem to be the predominant pathogenic mechanism underlying HSP.