

Functional Genetics of Spastin

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Mutations in the human spastin gene (SPG4) cause the most prevalent form of autosomal dominant hereditary spastic paraplegia (HSP), a neurodegenerative disorder characterized by progressive weakness and spasticity of the lower limbs. Human spastin is ubiquitously expressed and encodes a member of the AAA (ATPases associated with various cellular activities) protein family which is characterized by a conserved domain with ATPase activity. In a previous study, using polyclonal antibodies against the N-terminal spastin sequence, we have shown that the native protein is localized in both the perinuclear cytoplasm and the nucleus. Furthermore, using a reporter system based on four in-frame fused copies of green fluorescent protein we demonstrated that spastin carries two nuclear localization sequences both independently functional in mediating nuclear entry. We suggest a dual function for the spastin protein: one involving it in cytoplasmic trafficking and another, still unknown function in the nucleus. The precise localization of spastin may be a regulated process involving import into the nucleus as well as export back into the cytoplasm. Supporting this notion, we have identified two potential nuclear export sequences (NES) within the spastin amino acid sequence. Our present study was aimed at characterizing domains in the N-terminal part of spastin that impede nuclear entry of transiently expressed spastin in cultured cells. Using appropriate deletion constructs with a GFP reporter, we were able to identify a short sequence motif of approximately 25 amino acids in size to be responsible to retard spastin in the cytoplasm of the cell. Ongoing experiments are aimed at investigating whether functionality of NES or rather abolition of tubulin binding and sequestration of spastin in the cytosol is the mechanism underlying this observation. Spastin is widespread among different organisms and highly conserved between species. Thus, animal models provide an excellent tool for functional and localization studies. Studies focus on the distribution of spastin in different stages of mouse brain development as well as the identification and knock-down of zebrafish spastin and its consequences for early development.