10. Abstract

Huntington`s Disease (HD) is an inherited, progressive neurodegenerative disorder caused by an elongated polyglutamine repeat located at the N-terminus of huntingtin. The pathomechanism of HD as well as the normal function of huntingtin are unclear. HD exon 1 proteins with a polyglutamine tract in the pathological range (> 37 glutamines) form protein aggregates *in vitro*. In addition, the accumulation of insoluble polyglutamine-containing protein aggregates in intranuclear and perinuclear inclusions has been detected in brains of HD transgenic mice and HD patients, leading to the hypothesis that HD is caused by the accumulation of protein aggregates in neuronal inclusions. To study the aggregation of huntingtin in more detail, stable, inducible 293 Tet-Off cell lines producing HD exon 1 proteins with 20, 51 and 83 glutamines were established in this work. In this cell model, the huntingtin exon 1 proteins with a polyglutamine tract in the pathological range (51 and 83 glutamines), but not in the normal range (20 glutamines), form perinuclear inclusions. These structures contain aggregated, ubiquitinated huntingtin exon 1 protein with a fibrillar morphology. The inclusion bodies are formed at centrosomes and are surrounded by vimentin filaments. Immunofluorescence and electron microscopy revealed that the 20S, 19S and 11S subunits of the 26S proteasome, the molecular chaperones BiP, Hsp70, and Hsp40 as well as the RNA-binding protein TIA-1, the potential chaperone 14-3-3, and α-synuclein colocalize with the perinuclear inclusions. Inhibition of the proteasome activity resulted in a 2-fold increase in the amount of aggregates, indicating that the inclusion bodies accumulate when the capacity of the ubiquitin-proteasome system is exhausted. In 293 Tet-off cells, inclusion body formation also resulted in cell toxicity and ultrastructural changes such as indentations and disruption of the nuclear envelope. Together these findings suggest, that under normal conditions mutant HD exon 1 fragments are transported to the proteasome for proteolytic digestion. HD exon 1 fragments with a long polyQ repeat polymerize and resist degradation, instead recruiting additional proteins to the inclusion bodies and forming the „degrasome“.

To gain insight into the normal function of huntingtin we have screened for huntingtin interacting proteins using the yeast two-hybrid system and discovered the huntingtin interacting protein 1 (HIP1). It is shown that HIP1 is a multidomain protein containing an N-terminal ENTH domain, a central coiled-coil forming region and a C-terminal actin-binding domain. By affinity chromatography three HIP1 associated proteins were identified, clathrin heavy chain, α-adaptin A and C. *In vitro* binding studies revealed that the central coiled-coil domain is required for the interaction of HIP1 with clathrin, whereas DxF-motifs located

upstream to this domain are important for the binding of HIP1 to the C-terminal „appendage“ domain of α-Adaptin A and C. Expression of full-length HIP1 in mammalian cells resulted in a punctate cytoplasmic immunostaining characteristic of clathrin-coated vesicles. In contrast, when a truncated HIP1 protein containing both the DxF-motifs and the coiled-coil domain was overexpressed, large, perinuclear vesicle-like structures containing HIP1, huntingtin, clathrin and internalized transferrin were observed, indicating that HIP1 is an endocytic protein. The structural integrity of HIP1 seems to be crucial for the maintenance of a normal vesicle size *in vivo*.