6. Abstract

Huntington´s Disease (HD) is an inherited, progressive neurodegenerative disorder caused by an elongated polyglutamine stretch in the protein huntingtin (htt). The prolonged polyglutamine sequence triggers aggregation of mutant htt and causes the formation of neuronal inclusions in HD patient brains. Neither the cellular function of htt nor the pathomechanism of HD are currently known. Because of its interactions with a variety of characterized proteins, htt is believed to be involved in different cellular processes including transcription, endocytosis and cell signaling. To further investigate the function of htt, this study placed htt in a comprehensive network of protein interactions. The network was generated from data obtained by yeast two-hybrid (Y2H) cDNA-library and array screens. The htt network includes 186 physical interactions among 64 proteins. 165 of these interactions have not been described before. To evaluate the quality of the Y2H data, 54 interactions were tested by *in vitro* binding experiments and 35 interactions (65 %) were confirmed. In total 19 direct interaction partners of htt were identified by Y2H screens, most of which were associated with transcriptional regulation. The HD network provides additional clues to htt function and moreover is a valuable starting point to identify proteins involved in HD pathogenesis.

An important novel interaction partner of htt identified in this study is GIT1, a G protein-coupled receptor kinase-interacting protein. *In vitro* aggregation experiments have shown that GIT1 is crucial for htt aggregation. Moreover, C-terminal GIT1 fragments recruit mutant htt into vesicle-like perinuclear structures which probably is the mechanism underlying GIT1-mediated enhancement of htt aggregation. Immunohistochemical studies revealed that GIT1 is present in neuronal inclusions that typically appear in the brain of HD patients. Furthermore, a reduced amount of full-length GIT1 was observed in the brains of diseased patients while significant amounts of C-terminal GIT1 fragments were found to be accumulated. These findings suggest that during progression of HD GIT1 is processed. An abnormal cellular distribution and function of GIT1 may contribute to HD pathogenesis.

Like HD, also the prion diseases belong to the family of neurodegenerative disorders. Prion diseases result from the misfolding of a single protein, the prion protein PrP. The mechanisms leading to misfolding of PrP and to toxicity are unclear. In this study

the spontaneous appearance of prions in the yeast *S. cerevisiae* was investigated. Yeast is as a simple and well-established model system for studying the properties of prions. The yeast prion [*PSI* $^{+}$] results from misfolding and aggregation of the translation termination factor Sup35. The spontaneous appearance of [*PSI* $^{+}$] requires the presence of other prions such as [*PIN* $^{+}$]. This study shows that [*PSI* $^{+}$] can form spontaneously in the absence of pre-existing prions, when the intrinsic propensity of Sup35 to form aggregates is enhanced by the addition of aggregation-prone htt exon1 fragments containing 54 or 92 glutamines. Expression of the Sup-htt fusion proteins led to the accumulation of aggregates and stimulated the conversion of endogenous Sup35 from the soluble into the insoluble state. Likewise expression of PrD-Htt fusion proteins consisting of N-terminal Sup35 and htt exon1 fragments formed aggregates and induced [*PSI* $^{+}$]. The efficiency of the *de novo* appearance of [*PSI* $^{+}$] was dependent on the length of the polyglutamine expansion and the aggregation rate of the fusion proteins. Once acquired, the [*PSI* $^{+}$] state was stably transmitted from mother to daughter cells even upon loss of the Sup-htt fusion proteins, indicating that Sup-htt aggregates are required for induction but not for propagation of [*PSI* $^{+}$]. In line with the *in vivo* data, *in vitro* studies revealed that purified PrD-htt fusion proteins spontaneously assemble into amyloid-like fibers which in turn initiate the polymerisation of purified soluble Sup35. The polyglutamine-dependent induction of the prion [*PIN* $^{+}$] in the absence of pre-existing prions supports the hypothesis that also other prions can be induced by the addition of polyglutamine tracts. Thus, the presented data suggest that spontaneous aggregation of the nonprion protein is the molecular basis for the *de novo* appearance of yeast prions.