

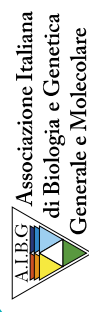


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COMMON AND NOVEL MOLECULAR MECHANISMS OF FRAMESHIFT MUTATIONS CAUSING HSPB8 PATHOLOGY

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The small heat shock protein B8 (HSPB8) is an essential chaperone in maintaining proteostasis in striatal muscle and neuronal cells. By interacting with the co-chaperone BAG3, HSPA family members, and the E3-ubiquitin ligase STUB1, HSPB8 forms the chaperone-assisted selective autophagy (CASA) complex, which promotes the removal of misfolded proteins. Both others and we have reported that frameshift (fs) mutations in the last exon of the HSPB8 gene cause myopathies, with or without neurogenic involvement and cardiomyopathy. These HSPB8 fs mutations can lead to a +1 or +2 nucleotide shift in the open reading frame, resulting in the expression of HSPB8 proteins with approximately 20 or 50 amino acid-long C-terminal extensions. Here, we demonstrate that cell models expressing fs+1 or fs+2 mutants exhibit comparable behavior. Indeed, our analyses reveal that the expression of HSPB8 fs results in aggregation and sequestration of the HSPB8 wildtype along with the CASA members. Consequently, misfolded and ubiquitinated substrates get entrapped in HSPB8 mutant aggregates with autophagy receptors. Thus, through a mechanism based on the gain of toxic function and a dominant negative effect on HSPB8 WT, HSPB8 fs mutants cause a general impairment of proteostasis. To gain insight into the mechanisms regulating HSPB8 fs mutant turnover and degradation, we investigate the crosstalk between HSPB8 and Valosin-containing protein (VCP), a multifunctional member of the proteostasis network. Using an HSPB8 fs+1 as a reference mutant, we observed that the HSPB8 mutant interacts with and sequesters VCP, and that VCP silencing exacerbates HSPB8 aggregation, likely contributing to the pathogenesis of (neuro)myopathies caused by HSPB8. In summary, our results

define pathogenic mechanisms shared among different HSPB8 fs mutations and reveal a novel player in HSPB8 pathology.