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Erlin-2 is a human endoplasmic reticulum (ER) lipid raft protein that belongs to the SPFH (Stomatin, Prohibitin, Flotilin and HflC/K) family of mammalian proteins. Erlin-2 is a type-II ER-membrane protein anchored to the ER membrane by its N-terminus. Erlin-2 and its homolog Erlin-1 associate to each other forming a heteromeric complex that binds to inositol 1,4,5-trisphosphate receptors (IP₃Rs) immediately after their activation, thus being essential to the ER associated degradation (ERAD) pathway of proteins. When Erlin-2 expression is down-regulated, the polyubiquitination and subsequent degradation of IP₃Rs is inhibited. This leads to pathological changes in Ca²⁺-signaling, and is related to abnormal growth and cancer development. In this work, we created two functionally different constructs of Erlin-2 based on their sequence homology with other members of the SPFH family: construct 22-172 that contains an SPFH domain, and construct 22-339 that comprises both the SPFH and the coiled coil domains. Both constructs were amplified by PCR reactions from the plasmid pET17b containing the Erlin-2 gene. Both constructs are currently being cloned into the plasmid RP1B, which codes for a 6xHis tag followed by a TEV protease cleavage site. The recombinant proteins will be expressed in E. coli BL21DE3 cells and purified by niquel affinity followed by size exclusion chromatography. The purified recombinant proteins will have their three-dimensional structures determined by NMR and/or X-ray crystallography. The atomic resolution structure of Erlin-2 will bring new insights into the mechanism by which proteins are degraded in the ER as well as the molecular basis of cancer pathogenesis.


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