Title:	The use of the Yeast Two Hybrid system to detect direct binding partners of Hs-SDE2
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Abstract:	SDE2 [silencing defective 2] protein in the fission yeast Schizosaccharomyces pombe emerges to be conserved among various eukaryotic organisms, and is weakly homologous to ubiquitin family proteins (see below). The absence of SDE2 in S. pombe causes defective silencing at the telomeres. SDE2 mutant phenotypes also indicate that the protein could also contribute to the preservation of genome integrity by participating to respond and repair DNA double-strand breaks. Biochemically the protein also co-purifies with a wide range of splicing factors, suggesting that it is associated with RNA splicing. Ongoing studies in SK Mishra's lab suggest that SDE2 is a Ubl and seems to be associated with RNA splicing. The covalent attachment of ubiquitin-like proteins (Ubls) confers diverse functions on their target proteins. The modified substrates might be involved in various biological processes, which include DNA replication, signal transduction, cell cycle control, embryogenesis, cytoskeletal regulation, metabolism, stress response, homeostasis and RNA processing. The splicing reaction that assembles exonic (protein-coding) regions in eukaryotic mRNAs from their much longer precursors provides a uniquely flexible means of genetic regulation. Thus, resolving mechanism of RNA splicing at the molecular level is not only important for understanding gene expression, but it is also of medical importance, as aberrant pre-mRNA splicing is the basis of many human diseases or contributes to their severity. The identification of protein-protein interactions (PPIs) is essential for a better understanding of biological processes, pathways and functions. The aim of my thesis was to detect interactions between Homo sapiens (Hs) SDE2 and other unknown proteins using yeast two hybrid system using HIS3 reporter gene so that function of Hs-SDE2 in eukaryotes can be deciphered. Yeast two hybrid assay was performed using the cDNA library screen approach. Two separate screens were performed for Hs-SDE2 full length protein and the ubiquitin fold region containing N-terminus of Hs-SDE2 protein. Each part of SDE2 sequence was fused into the pGBKT7 vector, generating bait clones. The expression of vector constructs were verified through sequencing, and the constructs were transformed into Y2HGold yeast strain. The high-complexity cDNA libraries made from 3 different tissues or cells, expressed in yeast strain Y187 were used for the screening. The binding partners of SDE2 reported in this study belong to various pathways like signaling and growth regulation. Thus results shown in this thesis indicate that SDE2 might have additional functions other than RNA splicing.
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