Dokumenttyp: journal article
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Abstract: We have explored a modified cytosolic yeast-two-hybrid Sos-recruitment system (SRS) in order to test for membrane localization of a protein. In this system, membrane localization is assessed by rescue of a yeast strain carrying a temperature-sensitive mutation in the CDC25 gene (cdc25-2) at restrictive temperature. The homologous human Sos (hSos) is capable to replace cdc25-2 provided that it is attached to the membrane because only then hSos is functional. This can be achieved when hSos is artificially fused to a protein containing trans-membrane domains (Tms). GFP/YFP fusion construct analyses of the Arabidopsis thaliana PEPINO/PASTICCINO2 (PEP/PAS2) protein have previously shown disparate cellular localizations although this protein possesses clear Tms. Analysis of N-terminal and C-terminal hSos-PEP/PAS2 fusions respectively suggests, that PEP/PAS2 is an integral membrane protein with cytosolic N- and C-termini. This implies that the protein has an even number of Tms and that the first Tm, a signal peptide, is not cleaved off. Our study shows that SRS is suitable to test for protein membrane localization and possibly for more detailed topological analysis of membrane proteins.
Zeitschriftentitel / Abkürzung: Mol Genet Genomics
Jahr: 2010