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Inteactions between *Drosophila* USP and ECR-A in Yeast Using the Two-hybrid system

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ultraspiracle gene product(USP) is one of several orphan receptors in *Drosophila*, sharing significant homology with the mammalian retinoid X receptor. In *Drosophila* the response to the hormone is mediate in part by USP and ecdysone receptors(ECR), which are members of the nuclear receptor superfamily. Heterodimers of these proteins bind to ecdysone response elements(EcRE) and ecdysone to modulate transcription. We used the yeast two-hybrid assay for detection of protein-protein interactions *in vivo* to screen for novel partners of USP. The GAL4 DNA-binding domain fused to USP was used as bait to screen a *Drosophila* embryonic cDNA library in which the cDNA was fused to the GAL4 activation domain. Several cDNA clones encoding proteins that interact with USP were isolated, one of which corresponded to the ecdysone receptor A isoform(ECR-A). Domain analysis on USP revealed that the ligand binding domain is required for heterodimerization with ECR-A. Given the ability of USP to dimerize preferentially with ECR-A, this strategy should be useful for cloning novel partners for USP from a variety of cell types.

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The phenotype of a temperature-sensitive mutant of *Saccharomyces cerevisiae* ARS-Binding Factor 1 gene is suppressed by TRP1 gene at high-copy number

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ABFI is a DNA binding protein that recognizes the motif, RTCRYN$_5$ACG, at many sites in the yeast genome including autonomously replicating sequences (ARSs), mating type silencers and promoter elements. YEP24-SAB1 plasmid containing a suppressor gene for temperatute-sensitive lethal mutant in the ABFI gene was isolated by their ability to permit growth of *abf1-5 ts*-mutant at non-permissive temperature. Hybridization to Chromoblot and to prime clone grid filters revealed that SAB1 gene was located on chromosome IV. To localized the suppressor activities on the cloned plasmids, some deletions were made and analyzed. The suppressor activity was narrowed down to 1 kbp yeast genomic DNA. The nucleotide sequence of the 1 kbp DNA was determined and turned out to contain TRP1 gene encoding phosphoribosylantranilate isomerase.