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Proteomic Characterization of the 3Physiological Role of CPR1 in *Saccharomyces cerevisiae* KNU5377 Against the Menadione Stress

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In order to understand the functional role of CPR1 in *Saccharomyces cerevisiae* KNU5377 showing multi-tolerance against high temperature, inorganic acid, and oxidative stress, whole cellular proteins were analyzed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). This was followed by two-dimensional (2D) gel electrophoresis. Under the menadione stress, the 23 up-regulated proteins could be clearly identified only in the wild-type KNU5377 strain. Among the proteins, Sod1p, Tsa1p, Ahp1, Cpr1p, Cpr3, Ssb2p and Hsp12p were classified as being a part of antioxidant systems or protein-folding related systems. The protein of CPR1 could not be completely detected in the *cpr1△* mutant of KNU5377 and the other up-regulated proteins in the wild-type strain showed a clear correlation with the results of an immunoblot analysis. Moreover, a reduction in growth patterns (about 50%) could be seen in the *cpr1△* mutant, as compared with that of the wild-type strain under the mild MD stress. These results suggest that the up-regulation of CPR1 might contribute to tolerance against MD as an oxidative stress.

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SF3a3, a Splicing Factor, is a Repressor of Constitutive Androstane Receptor (CAR)

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Constitutive Androstane Receptor (CAR) is a member of a nuclear receptor superfamily andplays an important role in degradation of xenobiotics in the liver. Using yeast two-hybrid screening, we have identified SF3a3, a 60KD subunit of splicing factor 3a, as a CAR-interacting protein. Recently, it has been shown that transcription factors such as steroid hormone receptors and transcriptional coactivators interact with splicing factors and function as regulators of the partners' functions. In the present work, we investigated the physiological implications of the interaction of CAR with SF3a3 with regard to splicing and transcriptional activation activities, the two main functions of these proteins. First, we confirmed this interaction by both co-immunoprecipitation and a GST-pull down assay. Functional studies showed that overexpression of SF3a3 specifically inhibited the reporter activity driven by a promoter containing CAR binding sequences more than 2-fold whereas reduced expression of SF3a3 activated the same reporter activity up to 4-fold. In addition, SF3a3shifted alternative splicing of the CD44 reporter gene regulated by CAR to the production of the inclusion form. These data suggest that SF3a3 functions as a regulator of genes under the control of CAR at least at transcription level.

Key words: SF3a3, CAR, splicing factor