The J domain of Tpr2 regulates its interaction with the proapoptotic and cell-cycle checkpoint protein, Rad9

Shuang-Lin Xiang, Tomoyasu Kumano, Shu-ichi Iwasaki, Xiangao Sun, Kastuji Yoshioka, and Ken-chi Yamamoto

Human Rad9 is a key cell-cycle checkpoint protein that is postulated to function in the early phase of cell-cycle checkpoint control through complex formation with Rad1 and Hus1. Rad9 is also thought to be involved in controlling apoptosis through its interaction with Bcl-2. To study the biochemical functions of mammalian Rad9 as both a checkpoint and a possible apoptotic molecule, we used a yeast two-hybrid method with full-length human Rad9 as bait, and identified Tpr2 (Tetratricopeptide repeat protein 2) as a novel Rad9-binding factor. Tpr2 was first identified as a factor interacting with the GAP-related domain of neurofibromin in two-hybrid screening, and is a member of the growing Tpr family. All members of this family contain various numbers of a degenerate 34-amino-acid repeated motif called a tetratricopeptide repeat (tpr). The tpr's are protein-protein interacting motifs, and function in diverse cellular processes such as cell-cycle control, transcription repression, stress response, protein kinase inhibition, mitochondrial and peroxisomal protein transport, and neurogenesis. Tpr2 contains seven tpr motifs in its N-terminus and has been reported to bind Hsc70 through these tpr motifs. In addition, in its C-terminus, Tpr2 has a typical J domain found among members of the Hsp40/DnaJ co-chaperone family. The Hsp40/DnaJ family members regulate the chaperone activity of Hsp70/Hsc70 by stimulating their intrinsic ATPase activity; therefore, it is plausible that Tpr2 also functions as a co-chaperone in various cellular processes. However, the precise cellular functions of Tpr2 remain to be clarified. In the present study, we show that Tpr2 binds not only to Rad9, but also to Rad1 and Hus1, in vivo. However, unlike the interactions of Tpr2 with Rad1 or Hus1, both the in vivo Tpr2/Rad9 interaction and the cellular localization of Rad9 are influenced by the J domain of Tpr2. These results indicate a critical role for the J-domain of Tpr2 in functional interactions between Tpr2 and Rad9.