Subcellular Localization of RPB5-Mediating Protein, RMP, and its putative functional partner[1].

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We previously identified a novel cellular protein RMP that retains corepressor activity and functionally antagonizes transcriptional modulation by Hepatitis B virus X protein (Dorjsuren D. et al., Mol. Cell. Biol., 18: 7546-7555, 1998). Here, subcellular localization of RMP was examined with GFP-fused forms. We found that a nuclear localization signal (NLS) and a coiled-coil (CC) domain functioning as a cytoplasmic localization signal (CLS), are both important for the subcellular localization of RMP. The CLS apparently acts dominantly since RMP is mostly localized in cytoplasm with weak and diffuse signals in nuclei, and the NLS is indispensable for the nuclear localization of RMP only in the absence of the CLS. Using a yeast two-hybrid method, we isolated a putative corepressor, DNA methyltransferase 1 (DNMT1) associating protein, DMAP1, which was demonstrated to bind the CC domain of RMP. DMAP1 facilitates nuclear localization of RMP and the corepressor activity of RMP in a dose-dependent manner through the interacting with the CC domain of RMP. The results were discussed in light of the recent paper showing a novel evolutionary conserved role of RMP/URI in TOR signaling (Gstaiger, M. et al., Science, 302: 1208-1212, 2003).

Reference 1: Delgermaa L, Hayashi N, Dorjsuren D, Nomura T, Thuy LT, Murakami S. (2004) Mol. Cell. Biol., 24(19):8556-8566.

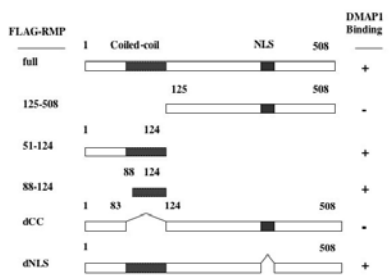

Figure illustrates the summary of the DMAP1-binding region of RMP that is within the coiled-coil domain by GST pull-down *in vitro* using the partially purified proteins. The result is consistent with that using extracts of cells transiently expressing two differentially tagged proteins[1].