Subcellular localization of RMP, RNA polymerase subunit 5-mediating protein.

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We previously identified a novel protein, RMP, which associates with RNA polymerase II through RPB5 and negatively modulates activated transcription in vivo. We confirmed that endogenous RMP is mostly in cytoplasm although a minor portion was in nucleus. Therefore, a various versions of RMP constructs fused to GFP were transiently expressed in HLE cells and was cytologically examined by a confocal fluorescent microscopy. The N-terminal half of RMP localizes exclusively in cytoplasm, and the C-terminal half is exclusively nucleus. A NLS at aa 339-344 was identified to be functional since a mutated NLS abolished the nuclear localization of RMP deleting the N-terminal portion. A region responsible for the cytoplasmic localization of RMP was mapped within aa 88 - 118 which well coincided with a predicted coiled-coil domain (aa 83-124). The domain by itself changed subcellular localization of TFIIB from nucleus to cytoplasm when it was fused to GFP-TFIIB. The negative regulatory function of RMP was found to be dependent upon nuclear distribution of RMP since the ability of RMP to corepress the activated transcription by GalVP16 in vivo was abolished by the mutation of NLS, and was much augmented by deleting CLS. These results demonstrate that two sequences, the NLS and the coiled-coil domain, are both important for the subcellular localization of RMP. We recently identified a putative interacting partner with the coiled-coil domain by a yeast two-hybird selection.

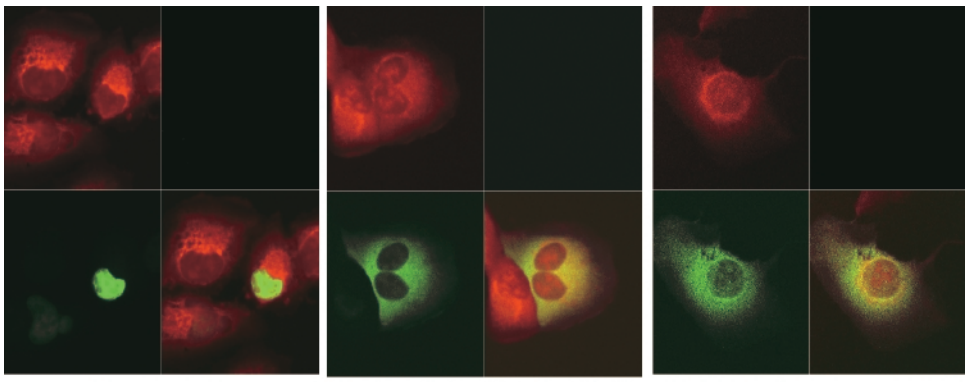

Figure The coiled-coil domain of RMP acts as a cytoplasmic localization sequence.
The N-terminal regions spanning the coiled-coil domain were fused to GFP-TFIIB which was a typical nuclear protein as shown in left panel. Aa 1-118 or the coiled-coil domain alone changed subcellular localization of GFP-TFIIB from nuclear to cytoplasmic as shown in center and right panels. These proteins were transiently expressed in HLE cells and subjected to confocal analyses.