Direct interaction between the subunit RAP30 of TFIIF and RNA polymerase subunit 5 (RPB5) which contributes to the association between TFIIF and RNA polymerase II

Wenxiang Wei, Dorjbal Dorjsuren, Yong Lin, Weiping Qin, Takahiro Nomura, Naoyuki Hayashi and Seishi Murakami

General transcription factor TFIIF, heteromeric tetramer of RAP30 and RAP74, assembles in initiation complex and associates with Pol II but Pol II subunit responsible for the interaction remains unclear. We examined whether TFIIF interacts with RPB5, the exposed domain of which binds HBx and a novel regulatory protein, RPB5-mediating protein (RMP).

The results demonstrate that RPB5 directly binds RAP30 in vitro using the purified recombinant proteins, and in vivo in COS1 cells transiently expressing recombinant RAP30 and RPB5. The RAP30-binding region was mapped within the middle part (aa 47-120) of RPB5, which partly overlaps the HBx-binding region. Although the middle parts (aa 101-170) and the N-terminal part of RAP30 independently bind RPB5, the latter was not involved in the RPB5-binding when RAP30 was present in TFIIF complex. By scanning the middle part of RAP30 by clustered alanine-substitution mutants, then point alanine-substitutions pinpointed two residues critical for the RPB5-binding in in vitro and in vivo assays. Wild, but not Y124A and Q131A, in GST-RAP30 forms coexpressed with FLAG-RAP74 efficiently recovered endogenous RPB5 to the FLAG-RAP74-bound anti-FLAG M2 resin. The recovered endogenous RPB5 is assembled in pol II as demonstrated immunologically. Interestingly, coexpression of the middle part of RPB5 and wild RAP30 in GST-forms inhibited recovery of endogenous pol II to the FLAG-RAP74-bound M2 resin, strongly suggesting that the RAP30-binding region of RPB5 inhibited the association of TFIIF and pol II. Taken together, the exposed domain of RPB5 interacts with RAP30 of TFIIF and is important for the association between pol II and TFIIF.

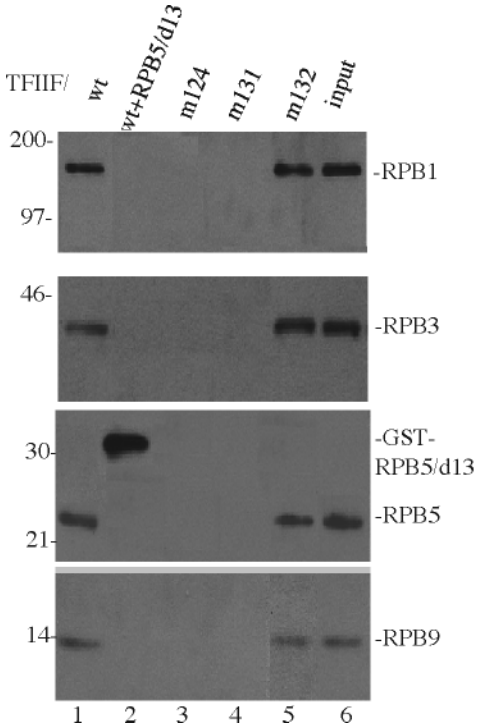

Figure RAP30 is involved in the association of Pol II and TFIIF *in vivo*.

TFIIF mutants defect in binding with endogenous Pol II. COS1 cells were transfected with mammalian expression plasmids of pNKFLAG-RAP74 and pNKGST-RAP30 or its mutants as indicated on the top. In lane 2 additional expression vector of pNKGSTRPB5/d13 was co-transfected. About 2.5mg of total lysates were immunoprecipitated with 20 μl of anti-FLAG M2 antibody-bound resin. After being washed, the recovered proteins were eluted, fractionated by 12.5% SDS-PAGE and detected by western blot analysis with antibodies against RPB1, RPB3, RPB5 and RPB 9 as indicated to the right Lane 6 shows 3% of input of lysate used for lane 1.