Mutational analysis of the central part of human RNA polymerase II subunit 5 (RPB5) by two-step alanine scanning: the residues critical for interactions with TFIIF subunit RAP30 and Hepatitis B Virus X protein and those for DNA-binding ability.

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RPB5 is close to DNA downstream of initiation site and interacts with several regulators. HBx binds the central part of RPB5 to modulate activated transcription, and TFIIF subunit RAP30 interacts with the same part of RPB5 that is critical for the association between TFIIF and RNAPII. By introducing systematic mutagenesis of the central part of RPB5 using two-step alanine scanning libraries to pinpoint critical residues for its binding to RAP30 in the TFIIF complex and/or to HBx, and identified these residues in both mammalian cells and in an *in vitro* binding assay. Four residues, F76, I104, T111 and S113, are critical for both TFIIF- and HBx-binding, indicating the overlapping nature of the sites of interaction. In addition, V74 and N98 are required for HBx-binding, and T56 and L58 are needed for RAP30-binding. Interestingly the residues exposed to solvent, T111 and S113, are very close to the DNA, implying that two factors may modulate the interaction between DNA and RPB5[1].

Reference 1: Le TTT, Zhang S, Hayashi N, Yasukawa M, Delgermaa L, Murakami S. (2005) J. Biochem (Tokyo), 138(3): 215-224.

Summary of RPB5-binding abilities

Mutated humanRPB5 residue	HBx	RAP30 (TFIIF)	Mutated humanRPB5 residue	HBx	RAP30 (TFIIF)
T56A	++ [a]	-	I99A	++	++
D57A	++	++	T100A	++	++
L58A	++	-	R101A	++	++
D70A	++	++	L103A	++	++
Q71A	++	++	I104A	-	-
M72A	++	++	V105A	++	++
F73A	++	++	M110A	++	++
V74A	-	++	T111A	-	-
F75A	++	++	P112A	++	++
F76A	-	-	S113A	-	-
N98A	-	++			

[a] (++): binding positive; (-): binding negative