DNA-binding ability of RNA polymerase II subunit 5 (RPB5)

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We found that RPB5 retains DNA-binding ability trapped by double-stranded DNA cellulose. The 6 residues critical for binding DNA were identified within the middle part of RPB5, by a three-step alanine scanning with clustered and point substitution libraries. Among them, T111 is solvent-exposed and the nearest neighbor of P112 that is the residue predicted to be closest to DNA. Three residues are important for the structural integrity of the mixed ß-sheet that may indirectly affect DNA-binding ability. We evaluated these residues conserved among human and yeast by introducing a point mutant in yeast in place of its wild-type counterpart. T117A, T117G (hT111) and the glycine substitution of the three residues in the ß-sheet affected cell growth at suboptimal temperatures. Interestingly, most of these residues are also indispensable for RPB5 to bind HBx and/or RAP30. Actually these factors inhibited DNA-binding of RPB5, strongly supporting the notion that these regulators may modulate transcription by inhibiting RPB5 from interacting with DNA.

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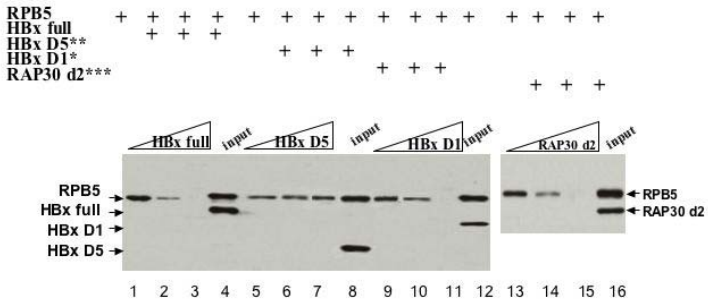
DNA-binding of RPB5 was inhibited by HBx and RAP30



*HBx-D1 harbors aa 51-154 including the coactivation domain and the RPB5-binding region that is responsible to inhibit DNA- binding of RPB5.
**HBx-D5 harbors aa 1-50 including the negative regulatory domain.
***RAP30 d2 harbors aa 1-176 missing the C-terminal region that retains DNA-binding ability.