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

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RPB5 is a RNA pol II subunit, which is located at the tip of lower jaws of pol II. Recent studies show that RPB5 is in close contact to promoter DNA when Pol II is recruited into the preinitiation complex. It interacts with the basal transcription factors IIB and IIF, and transcriptional regulatory factors such as Hepatitis B Virus X protein (HBx) and a novel regulatory protein, RMP (RPB5-mediating protein), through the middle part. We examined whether RPB5 bind with non-specific DNA.

The results of DNA binding assay using non-specific DNA cellulose demonstrated that RPB5 bound double stranded DNA in vitro, but not single stranded one. By analyzing DNA-binding of truncated forms of RPB5, the DNA-binding required the middle part (aa 47-120) of RPB5. Scanning of the middle part of RPB5 by clustered alanine-substitutions and further point alanine-substitutions pinpointed three sequences and finally 4 residues, V74, V82, T111 and S113, are critical for the DNA-binding. The former two residues may not contribute directly to DNA-binding since the residues seem to be not exposed according to the crystal model. Interestingly, two proline residues, P80 and P112, predicted to be closer to DNA, have no contribution to the DNA-binding. The binding ability is not stronger compared with RAP30's DNA binding in the same experiment condition. DNA-binding of RPB5 can be drastically inhibited in the presence of RAP30 missing the C-terminal DNA-binding domain, RMP and HBx. Interestingly, all of them can interact with RPB5 through the same domain for the DNA-binding, suggesting a possibility that these proteins may regulate DNA-binding ability of RPR5 in transcription

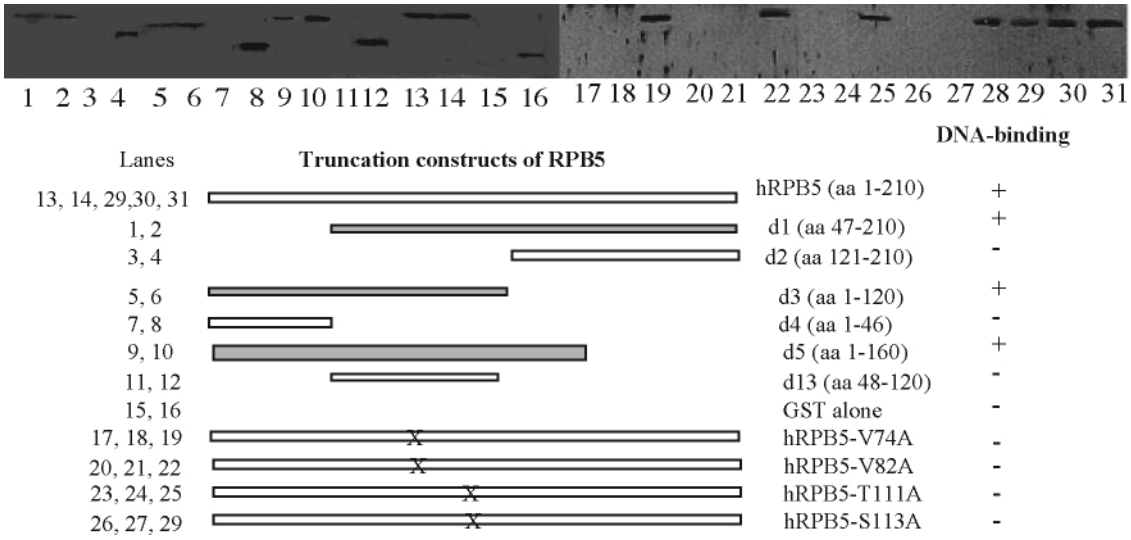

Figure DNA-binding ability of RPB5

Binding ability to double stranded DNA-cellulose was examined. Lanes 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 25, 28, 31, are 5% of input proteins, respectively. For the experiments with the point mutant proteins were independently repeated twice. Bound proteins were applied 12.5% SDS-PAGE and subjected to Western blotting with anti-RPB5 antibody.