RNA polymerase II initiation factor α from rat liver is almost identical to human TFIIB

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Transcription initiation by RNA polymerase II (Pol II) from the core region of TATA box-containing promoters is a multi-stage process requiring the action of at least five initiation factors (α, βγ, δ, ε, τ) in the rat liver system (1). Rat transcription factor α (TFIIB) consists of a 35-kDa polypeptide essential for accurate initiation by Pol II at the core regions of many promoters (2). Studies of promoter selection indicate that rat α functions in concert with other transcription factors, βγ (TFIIF or RAP30/74), to promote selective binding of Pol II to an initial complex (3). The precise role of α in this process, however, has not yet been established. To expedite mechanistic studies of rat α's function in transcription initiation, we have isolated cDNA clones encoding this factor.

PCR amplification with two degenerate primers for LDALPR (position 7–12) and ETSVDLI (position 203–209) in human TFIIB (4) gave rise to a product of 550 base pairs from rat liver mRNA. A rat liver cDNA library in λZAP II was screened with the fragment derived from the PCR product. The complete nucleotide sequence was determined with the longest cDNA clone isolated. The DNA sequence predicts a long open-reading frame encoding a polypeptide of 316 amino-acid residues with a molecular weight of 34.8 kDa. We concluded that the cDNA clone encodes rat α protein on the basis of a partial amino-acid sequence (LDALPR, position 7–12) as well as the reactivity of the recombinant product to an anti-α antibody (data not shown).

Comparison of the rat α cDNA sequence with the sequence for human TFIIB (4) revealed striking similarity (90%) in the coding region. Among 948 nucleotides corresponding to 316 amino-acid residues, only 94 nucleotides in the rat α sequence are different from the human sequence (4). Surprisingly, almost all the base substitutions in the rat α sequence are silent at the amino-acid level; only one amino-acid residue (Ser, position 302) in the rat α sequence is different from the human TFIIB sequence (Thr, ref. 4). This indicates that TFIIB is strongly conserved in rat and human.

The rat α cDNA was expressed in *E.coli* using a T7-expression vector, pET-11a (Novagen). *E.coli* BL21(DE3), transformed with pRTα containing the rat α cDNA, was broken by sonication after induction with IPTG. The supernatant by centrifugation was loaded onto a DEAE Sepharose column and the flow-through fraction was applied onto a phosphocellulose column. The recombinant α protein, eluted with 0.6 M KCl from the column, was able to direct basal levels of transcription from the adenovirus-2 major late promoter (AD-MLP) in a dose-dependent manner, as in the case of the natural protein purified from rat liver (Figure 1). This indicates that the recombinant protein produced in *E.coli* can replace the native rat α to reconstitute Pol II-dependent transcription initiation together with the other factors.

Rat α does not bind stably to the initial complex containing the native TATA factor, but instead may actively promote entry of Pol II into the preinitiation complex (3). Mutational analysis of the rat α cDNA will help us to elucidate its interactions with other initiation factors including Pol II and its function in transcription initiation.

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REFERENCES

1. Conaway,J.W. and Conaway,R.C. (1991) *J. Biol. Chem.* **266**, 17721–17724.
2. Conaway,J.W., Bond,M.W. and Conaway,R.C. (1987) *J. Biol. Chem.*, **262**, 8293–8297.
3. Conaway,R.C., Garrett,K.P., Hanley,J.P. and Conaway,J.W. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 6205–6209.
4. Ha,I., Lane,W.S. and Reinberg,D. (1991) *Nature*, **352**, 689–695.

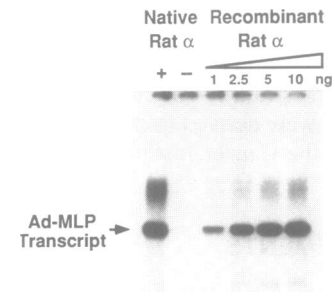

Figure 1. Recombinant rat α (TFIIB) replaces native rat α in reconstituted run-off transcription reactions. Transcription reactions were carried out as described previously (3). Reaction mixtures were reconstituted with α purified from rat liver or with recombinant rat α. Ad-MLP indicates the position of the 254-nucleotide run-off transcript from the promoter on pDN-ADML (2).