Structural determinants of preinitiation complex assembly on human Pol II promoters

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Introduction

Transcription initiation of all protein encoding genes in Eukarya requires the formation of a preinitiation complex (PIC) that consists of RNA polymerase II and the basal transcription initiation factors (TF) IIA, IIB, IID/TATA-box binding protein (TBP), IIE, IIF, and IIH. It is generally accepted that binding of TBP to the TATA-box nucleates the formation of the PIC either through a step-wise assembly of other basal factors or through recruitment of a pre-assembled holoenzyme. It remains unclear, however, what determines the orientation of PIC assembly, and, thus, the direction of transcription initiation [1]. To understand what determines the orientation of PIC assembly on human Pol II promoters, we have determined the 2.65 Å resolution crystal structure of a human TBPc-human TFIIBc complex bound to an idealized and extended adenovirus major late promoter [2].

Methods and Materials

The crystal structure of the human TBPc-TFIIBc-DNA complex was determined by molecular replacement using the previously determined structure of the ternary *A. thaliana* TBP2-human TFIIBc-DNA complex [3] as a search model. The molecular replacement solution was confirmed using data collected on similar crystals of the ternary complex that contained a iodinated oligonucleotide. The large unit cell and the weak diffraction of these crystals required the use of the high-brilliance synchrotron radiation source provided by the Structural Biology Center's (SBC) beamline 19-ID.

Results

The crystal structure reveals five ternary complexes in the asymmetric unit that are linked through a single base-pair overhang at the 5'- end of the DNA used for crystallization. This packing arrangement gives rise to a continuous DNA helix that extends throughout the crystal (Figure 1), giving rise to a total molecular mass of more than 275 kDa for the entire complex. The overall structure of the human ternary complex is very similar to that of the previous determined crystal structure [3]. However, our structure reveals a minor mismatch in the human TBPc-TFIIBc interface that may explain why TBP is interchangeable from yeast to man in *in vitro* transcription assays.

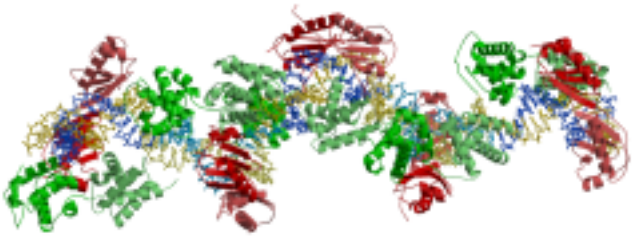

Figure 1: Ribbons diagram representing the content of one asymmetric unit. Human TBPc is shown in pink (N-terminal stirrup) and red (C-terminal stirrup), and human TFIIBc in light green (N-terminal repeat) and dark green (C-terminal repeat). The five complexes are distinguished by alternating dark and light yellow (coding strand), and dark and light blue (noncoding strand) of the DNA.

More importantly, however, our structure shows that human TFIIBc binds to the promoter asymmetrically by recognizing the flanking regions of the TATA-box. Binding of TFIIB is cooperative with TBP; requiring a distorted TATA-box induced by TBP, which, in turn, is stabilized by Lys189 and possibly Arg193 of human TFIIB [2].

Discussion

The crystal structure of the human TFIIBc-TBPc-DNA complex describes the stereochemistry of the interface formed by human TFIIBc and both the major groove upstream and the minor groove downstream of the TATA-box. The interactions are consistent with a unique polarity of the ternary complex, and, therefore, of the assembly of the PIC, which would define the direction of transcription in the absence of other basal factors. The simultaneous binding of the helix-turn-helix in the C-terminal cyclin-like repeat of TFIIBc to the major groove upstream, and the binding of the "recognition-loop" in the N-terminal repeat to the minor groove downstream of the TATA-box, is compatible only with the TBP-induced deformation of the DNA, suggesting that binding of TFIIB and TBP to the promoter is synergistic [2]. Thus, our crystal structure reveals that human TFIIB, aided by the deformation of the TATA-box by TBP, "differentiates" between the major groove upstream and the minor groove immediately downstream of the TATA-box through base-specific contacts and, therefore, provides polarity for the nucleating events in the assembly of a competent PIC on TATA-box-containing promoters.

Acknowledgments

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