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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignment of the amino-terminal domain of the Tfb1 subunit of yeast TFIIH

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Biological context

TFIIH is a multi-subunit complex involved in both transcription of mRNA-coding genes and DNA repair (for a review see Zurita and Merino, 2003). TFIIH is recruited to the transcription pre-initiation complex (PIC) through interactions with TFIIE where it plays a key role in the transition from the initiation phase to the elongation phase of transcription by virtue of its ability to phosphorylate the carboxyl-terminal domain (CTD) of the largest subunit of RNAP polymerase II (RNAP II) (Ohkuma et al., 1995). In addition, both human and yeast TFIIH has been shown *in vitro* and *in vivo* to directly interact with a number of activator proteins (Zurita and Merino, 2003). This conservation of TFIIH binding across species emphasizes the potential biological importance of these interactions, although the precise functional roles of these interactions are not completely understood.

Currently, there are only a limited number of high-resolution structures available for the individual nine subunits of TFIIH, and there are no high-resolution structures available for Tfb1. There are low-resolution structures based on the electron crystal structure of the five-subunit core yeast TFIIH complex (Chang and Kornberg, 2000) and electron microscopy studies of the complete nine-subunit human TFIIH complex (Schultz et al., 2000), both of which contained Tfb1 and p62, respectively. However in both of these studies, the resolution of the structures was insufficient to accurately position either the Tfb1 subunit or the p62 subunit relative to the other subunits in the complex.

Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignment of the amino-terminal 115 amino acids of the yeast Tfb1 protein ($Tfb_{1–115}$). This domain corresponds to a similar domain from p62 for which NMR assignments and structure have been previously reported (Gervais et al., 2001, 2004). Despite the homologous functions of the amino-terminal domain of Tfb1 and p62, the two domains share a relatively low sequence identity (17.4%) and similarity (34.0%) within their first 120 amino acids (Henikoff and Henikoff, 1992).

Methods and experiments

The Tfb1 amino-terminal domain ($Tfb1_{1–115}$) was expressed from a GST-2T vector (Amersham Bioscience) as a GST-fusion protein in an *E. coli* host strain TOPP2. The purified protein includes two additional amino acids (G, S) at the amino-terminal from the thrombin cut site, and four additional amino acids (G, N, T, S) at the carboxyl-terminal from the cloning procedure. Uniform (>98%) ^{15}N- and $^{15}N/^{13}C$-labeling was obtained by growing the cells in a modified minimal medium containing $^{15}NH_4Cl$ and $^{12}C_6$-glucose or $^{13}C_6$-glucose as the sole nitrogen and carbon sources, respectively. The protein was cleaved from the GST-tag

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with thrombin and the purification procedure was essentially identical to the procedure used for purifying the carboxyl-terminal domain of RAP74 (Nguyen et al., 2003). Samples for NMR studies contained 1.2 mM of $Tfb1_{1–115}$ (^{15}N- or $^{15}N/^{13}C$-labeled), in NMR buffer (20 mM sodium phosphate buffer pH = 6.5 and 1 mM EDTA) in either 90% H_2O/10% D_2O or 99.9% D_2O. All the NMR spectra were collected at 300 K on Varian ^{Unity}Inova 500 MHz, 600 MHz and 800 MHz spectrometers equipped with z pulsed-field gradient units and HCN triple resonance probes. The temperature of all probes was calibrated to 300 K using a methanol standard. The backbone and aliphatic side chains resonances of $Tfb1_{1–115}$ (^1H, ^{15}N and ^{13}C) were assigned using a combination of experiments, including 2D $^1H–^{15}N$ HSQC, 2D $^1H–^{13}C$ CT–HSQC, 3D HNCO, 3D HNCACB, 3D (HB)CBCA(CO)NNH, 3D C(CO)NNH, 3D H(CCO)NNH, 3D HCCH–COSY, 3D ^{15}N-edited NOESY–HSQC and 3D ^{13}C-edited HMQC–NOESY. The aromatic side chains ^1H, ^{13}C and ^{15}N resonances were assigned using a combination of 2D (Hβ)Cβ (CγCδ)Hδ, 2D (Hβ)Cβ(CγCδCε)Hε, 2D $^1H–^1H$ DQF–COSY, 2D $^1H–^1H$ NOESY and 3D ^{13}C-edited HMQC–NOESY experiments (For details of original references for experiments see Nguyen et al., 2003). Chemical shifts of all proton, carbon and nitrogen nuclei were referenced externally to that of DSS at 0 ppm. NMR data were processed using the NMRPipe/NMRDraw package (Delaglio et al., 1995) and analyzed with NMRView (Johnson and Blevins, 1994).

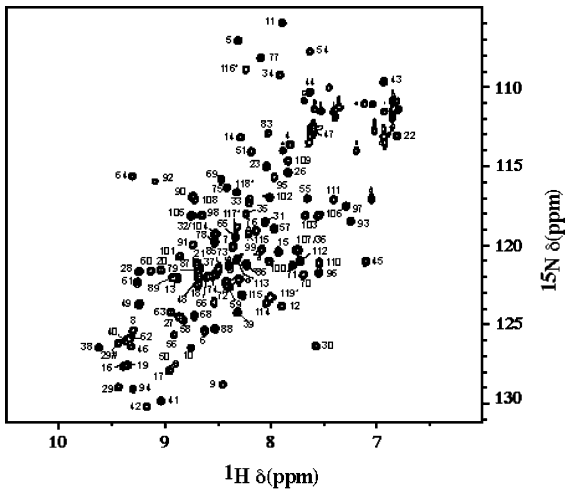

Figure 1. Two-dimensional $^1H–^{15}N$ HSQC spectrum of uniformly ^{15}N-labeled $Tfb1_{1–115}$ at 300 K and pH = 6.5 (see Methods and experiments). The numbers correspond to sequence residue numbers and numbers with asterisks (116–119) correspond to additional residues introduced during the cloning procedure.

Extent of assignments and data deposition

The 2D $^1H–^{15}N$ HSQC spectrum of $Tfb1_{1–115}$ is characterized by very good dispersion both in the ^1H and ^{15}N chemical shift dimensions (Figure 1), this domain adopts a stable conformation in solution, and we are currently determining its three-dimensional structure. For the ^1H, ^{15}N and ^{13}C backbone nuclei of residues 2–115 of $Tfb1_{1–115}$, nearly complete assignment was achieved: 98% of the $^{13}C^{\alpha}$, 97% of the $^1H^{\alpha}$, 95% of the $^1H^N$, 95% of the ^{15}N, and 92% of the $^{13}C'$ resonances were assigned. The extent of the assignment for the aliphatic and aromatic side chains (^1H-bound ^{13}C, ^1H-bound ^{15}N and ^1H nuclei, except for $^1H–^{15}N$ and ^{15}N of Lys and Arg) was 93%: 90% of the side chains were completely assigned; 7% were partially assigned, and 3% were unassigned. Three amino acids (Met1, Val80, and Val81) remained completely unassigned. The ^1H, ^{13}C and ^{15}N chemical shift assignment for $Tfb1_{1–115}$ has been deposited in the BioMagnResBank (www.bmrb.wisc.edu) under the Accession Number # 6225.

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