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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the DNA binding domain of transcription factor Mbp1 from *S. cerevisiae* in both its free and the DNA bound forms, and ^{1}H assignments of the free DNA

Pauline B. McIntosh[a], Ian A. Taylor[b], Steven J. Smerdon[b], Thomas A. Frenkiel[c] & Andrew N. Lane[a,*]
[a]*Division of Molecular Structure,* [b]*Division of Protein Structure and* [c]*Biomedical NMR Centre, National Institute of Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.*

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Biological context

In the budding yeast *Saccharomyces cerevisiae* the commitment to DNA synthesis in preparation for mitosis occurs late in the G1 phase of the cell cycle, at the point called Start (Murray and Hunt, 1993). The regulation of genes for DNA synthesis requires the action of a multimer of the transcription factors Swi6 and Mbp1 (Mlu 1-box binding protein) which binds to a DNA sequence known as the Mlu I cell cycle box (MCB) element which is found in the promoters of numerous genes involved in DNA synthesis (Price et al., 1991). Mbp1 is a large (833 residues) protein comprising three domains: an N-terminal DNA binding domain, a series of ankyrin repeats, and a C-terminal heterodimerisation domain.

The X-ray structure of the DNA binding domain of Mbp1 (residues 1–124) revealed a globular molecule consisting of a twisted, six-stranded β-barrel packed against two pairs of α-helices (Taylor et al., 1997), similar to the winged helix-loop-helix family of proteins. The C-terminal residues (102–124) showed no electron density, indicating disorder in this region in the crystal state. However, biochemical studies show that these residues confer substantial DNA binding interactions (I.A.T. and S.J.S., unpublished results).

Here we report the NMR resonance assignments for the DNA binding domain of Mbp1 (residues 2–124) in both the free state and complexed with a DNA duplex containing the DNA target (MCB) site, and the ^{1}H NMR assignments of the DNA duplex.

*To whom correspondence should be addressed. E-mail: alane@nimr.mrc.ac.uk

Methods and results

$Mbp1_{2-124}$ was expressed as a C-terminal hexaHis fusion protein containing a seven-residue linker, and purified from *E. coli* as previously described (Taylor and Smerdon, 1997). For ^{15}N-labelled and ^{15}N/^{13}C-labelled protein, the growth medium was replaced by M9 minimal medium supplemented with $(^{15}NH_4)_2SO_4$ (1 g L^{-1}) and ^{12}C or $^{13}C_6$ D-glucose (2 g L^{-1}) as the sole nitrogen and carbon sources.

All samples were prepared in 40 mm Na-phosphate buffer, 100 mM NaCl, 10% D_2O/90% H_2O at pH 7.6. The DNA duplex d(TTTG**ACGCGTCA**A)$_2$ (MCB12T, recognition sequence in bold) was prepared by annealing from 95 °C. The solution was cooled to 4 °C and dialysed extensively against the same buffer.

All NMR spectra were recorded at 15 °C at 11.75 or 14.1 T. For unlabeled protein, spectra were recorded at 1.3 mM (free protein) or 0.5 mM (complex). For the ^{15}N/^{12}C protein the concentration was 0.9 mM (free) or 0.8 mM (complex), and for the ^{15}N/^{13}C protein, the concentration was 1.0 mM (free) or 0.8 mM (complex).

All spectra were acquired with solvent suppression by WATERGATE (Piotto et al., 1992). NMR spectra were processed using the program NMRpipe (Delaglio et al., 1995) and analysed on screen using the XEASY software (Bartels et al., 1995).

The heteronuclear spectra recorded for Mbp1 were: ^{15}N/^{1}H 2D HSQC, 3D TOCSY-HSQC with an isotropic mixing time of 40 ms, NOESY-HSQC, using NOE mixing times of 100 and 200 ms, HNHB

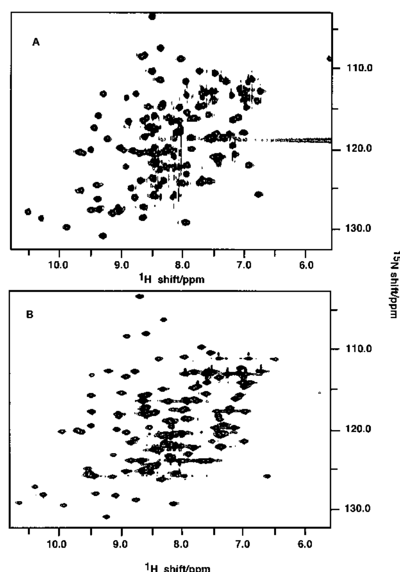

Figure 1. 2D ^{15}N/^{1}H HSQC spectra of free Mbp1(2–124) (A) and Mbp1(2–124) complexed with the MCB12T DNA duplex (B). Both spectra were recorded at 15 °C and 11.75 T and illustrate the changes in amide proton and nitrogen shift induced in Mbp1 on binding to DNA.

(Archer et al. 1991), HNHA, ^{13}C/^{1}H 2D HSQC, 3D HNCA, CBCANH and CBCA(CO)NH (Cavanagh et al. (1996) and references therein).

The heteronuclear spectra recorded for the Mbp1/DNA complex were: ^{15}N/^{1}H 2D HSQC, 3D TOCSY-HSQC, with isotropic mixing times of 40 and 80 ms; NOESY-HSQC using a mixing time of 100 ms; ^{13}C/^{1}H 2D HSQC, HSQC-NOESY using a mixing time of 100 ms, 3D HNCA, HN(CO)CA and C(CO)NH (Cavanagh et al. (1996) and references therein). 2D ^{15}N/^{1}H HSQC spectra of the free and complexed Mbp1 are shown in Figure 1.

Extent of assignments and data deposition

For the purposes of the assignment statistics the six His residues of the histidine tag are not included. In addition the N-terminus (-NH_3^+) of Ser 2 is unassigned.

Free protein. The ^{15}N and $^{1}H_N$ resonances for 114 of 123 possible (non-Pro residues) backbone amides were assigned. In addition all of the Asn and Gln NH_2 side-chain signals were assigned. 93.8% of the Cα and 92.7% of the Cβ signals were assigned from the triple resonance experiments together with all of the Gln Cγ signals. 83% of the Hα and 67.5% of the Hβ resonances in the protein were unambiguously assigned.

Mbp1–DNA complex. The assignment of ^{15}N and $^{1}H_N$ resonances for 115 of 123 possible (non-Pro residues) backbone amides was performed. In addition all of the Asn and Gln NH_2 sidechain signals were assigned. 93% of the Cα signals were assigned from the triple resonance experiments together with the Cγ signals for 4 of the 6 Gln residues. 73% of the Hα and 33.3% of the Hβ resonances in the bound protein were unambiguously assigned.

Free DNA. Of the non-exchangeable protons, only the H4′ and H5′/H5″ were not assigned. All of the imino protons, and the amino protons of the three cytosines were assigned.

All available ^{15}N, ^{13}C and ^{1}H chemical shifts for Mbp1 in its free and complexed form and the ^{1}H assignments for the free DNA have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession numbers 4254, 4256 and 4258, respectively.

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