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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the AT-rich interaction domain from the Dead Ringer protein

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Biological context

The AT-rich interaction domain (ARID) is a sequence-specific DNA-binding module found in a variety of proteins involved in eukaryotic gene regulation (Herrscher et al., 1995; Gregory et al., 1996). The domain consists of a highly conserved ∼130 amino acid segment that has been shown to interact with the minor groove of DNA (Herrscher et al., 1995; Valentine et al., 1998). The ARID containing protein Dead Ringer participates in the establishment of the dorsoventral axis in *Drosophila melanogaster*, by converting the maternal morphogen Dorsal from a transcriptional activator to a repressor. The Dorsal and Dead Ringer proteins bind to neighboring sites upstream of genes containing a conserved promoter element called the ventral repression region (VRR). These genes are repressed when both Dorsal and Dead Ringer bind to the VRR and cooperatively recruit the transcriptional co-repressor protein Groucho (Valentine et al., 1998). The Dead Ringer protein binds to AT-rich sites within the ventral repression region and in vitro selection studies suggest that it preferentially binds to sites containing the nucleotide sequence PuATTAA (Gregory et al., 1996). Surprisingly, this specificity is similar to that of the unrelated homeodomain family, suggesting that two structurally distinct DNA-binding domains have evolved to interact with the same nucleotide sequence. We have initiated NMR studies of the Dead Ringer protein in order to gain insights into the molecular basis of DNA recognition and its function in embryogenesis.

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Methods and results

The AT-rich interaction domain from the Dead Ringer protein (residues 262–398 + two amino-terminal residues from the cleavage site; 139 residues) was overexpressed as a GST-fusion in *E. coli* strain BL21 and purified by glutathione-affinity chromatography, thrombin proteolytic cleavage, gel-filtration and cation-exchange chromatography. NMR spectra were acquired at 303 K on Bruker DRX-500 and -600 spectrometers. All measurements were performed in $^{1}H_2O$ on either ^{15}N or ^{15}N and ^{13}C labeled Dead Ringer protein (1.5 mM Dead Ringer protein, 20 mM Tris-HCl (pH 6.7), 100 mM NaCl, 0.01% NaN_3, 1.5 mM $ZnCl_2$, 2 mM DTT and 5% $^{2}H_2O$). Spectra used for resonance assignments were as follows: 2D homonuclear NOESY, 2D homonuclear TOCSY, 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{15}N HSQC, 3D HCACO, 3D HNCO, 3D HNCA, 3D HN(CO)CA, 3D CBCA(CO)NH, 3D double ^{15}N-edited HMQC-NOESY-HSQC, 3D ^{15}N-edited NOESY-HSQC, 3D HNHA, 3D HNHB, 3D ^{13}C-edited NOESY-HSQC, 3D HCCH-COSY, and 3D HCCH-TOCSY. In addition, 3D ^{15}N-edited TOCSY-HSQC, 3D ^{15}N-edited ROESY-HSQC, ^{15}N- and ^{13}CO- ^{13}C spin-echo difference 2D CT ^{1}H-^{13}C HSQC spectra and a long-range ^{13}C-^{13}C correlation spectrum were used for stereospecific assignments. Detailed descriptions of these experiments, along with their original references, have been reviewed elsewhere (Bax et al., 1994; Cavanagh et al., 1996). In the 2D ^{1}H-^{13}C HSQC and 3D ^{1}H-^{13}C NOESY-HSQC experiments, ^{13}C-WURST inversion pulses were incorporated to achieve simultaneous detection of both aromatic and aliphatic resonances with good sensitivity (Ogura et al., 1996). Pulse field gradients and ^{1}H purge pulses were utilized for water

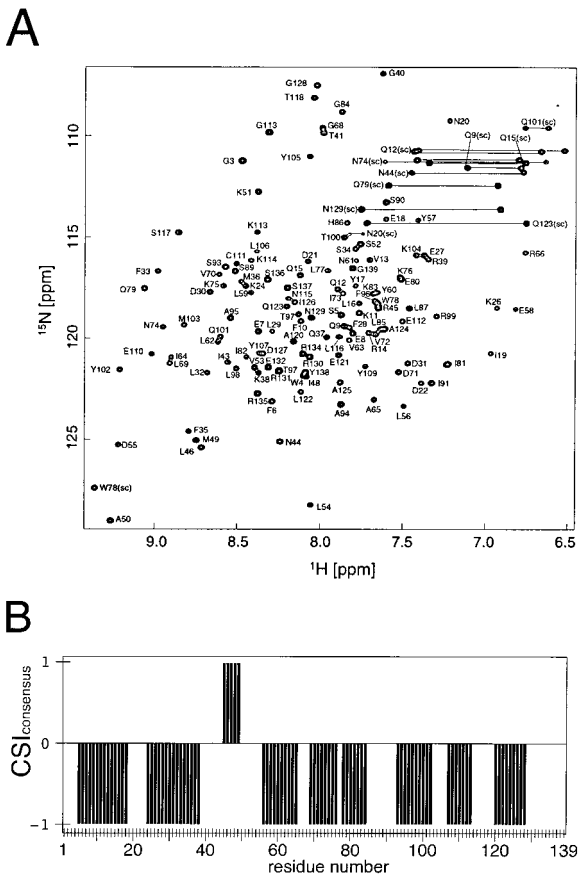

Figure 1. (A) 2D ^{1}H-^{15}N HSQC spectrum of the Dead Ringer protein with assigned cross peaks labeled (sc denotes side-chain resonances). Signals due to G67 ^{15}N-^{1}H and W4 ^{15}Nϵ1-^{1}Hϵ1 nuclei are located outside the shown region. (B) Plot of the composite Chemical Shift Index (CSI, $^{13}C_{\alpha}$, $^{13}C_{\beta}$, $^{13}C_{CO}$, $^{1}H_{\alpha}$) (Wishart and Sykes, 1994) as a function of residue number for the Dead Ringer DNA-binding domain. An index of -1 indicates helical structure, 0 indicates coil, and 1 indicates β-sheet/strand structure.

suppression. ^{1}H chemical shifts were referenced to external DSS in $^{2}H_2O$ (0.00 ppm), while ^{15}N and ^{13}C chemical shifts were referenced indirectly to the absolute frequency ratios $^{15}N/^{1}H = 0.101329118$ and $^{13}C/^{1}H = 0.251449530$ (Live et al., 1984). NMR data were processed with NMRPipe software (Delaglio et al., 1995) and spectra were analyzed with the program NMRView (Johnson and Blevins, 1994).

Extent of assignments and deposition

Figure 1A shows that the NMR spectra of this fragment are well dispersed, enabling nearly complete assignment of the ^{1}H, ^{13}C and ^{15}N resonances. Assignments were obtained for ~88% and ~94% of the ^{13}C and ^{15}N resonances, respectively. Of the hydrogen atoms, 96% were assigned and are complete enough for a structure determination of this domain (manuscript in preparation). Analysis of the secondary chemical shifts indicates that nearly all of the polypeptide is structured and primarily helical (Figure 1B) . The secondary structure consists of as many as 8 helices, with a small portion of the protein adopting a β-sheet or extended strand structure. The ^{1}H, ^{13}C and ^{15}N chemical shifts and the $^{3}J_{HN-H\alpha}$ coupling constants have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4334.

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