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# Letter to the Editor: Assignment of $^{1}H$, $^{13}C$ and $^{15}N$ resonances of the ARID domain of P270

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## Biological context

ARID is a family of homologous DNA-binding domains. Because the initial ARID proteins to be characterized interact with specific AT-rich sequences, the motif was named the *A*T-*r*ich *i*nteraction *d*omain or ARID (Herrscher et al., 1995). ARID domains occur in a wide variety of species ranging from yeast to nematodes, insects, mammals and at least one species of plant (for a review, see Kortschak et al., 2000). Using solution NMR methods, the structures of human Mrf-2 (Yuan et al., 1998) and the *Drosophila Dri* protein (Iwahara et al., 1999) have been solved. These two domains share similar structures in the central region, but *Dri* forms two additional helices in the C- and N-terminal flanking sequences. The structural diversity between the Mrf-2 and Dri domains indicates that other structural subfamilies that are distinct from those represented by Mrf-2 and Dri ARID are likely to exist.

SWI1 is a member of the SWI/SNF complex found in yeast and human. This complex appears to alter chromatin structure and facilitates the binding of chromosomal DNA to transcription factors and RNA polymerases (Quinn et al., 1996). SWI1 is clearly involved in DNA-binding, because it can cross-link to DNA by UV-activated cross-linkers in the SWI/SNF complex with DNA. Although some ARID domains, such as Mrf-2, *Dri* and Bright are sequence-specific double-stranded DNA-binding proteins (Kortschak et al., 2000), the ARID domain of human SWI1 (also known as p270) interacts with double-stranded DNA with no sequence specificity (Dallas et al., 2000). Because the DNA-binding activity of the p270 ARID is different from that of the other well-characterized ARID domains, we are using NMR spectroscopy to investigate the structural mechanism of DNA-binding by the p270 ARID. Here we report the assignment of $^{1}H$, $^{13}C$ and $^{15}N$ resonances of the p270 ARID. The assignments obtained from the present study will be used for the determination of the three-dimensional structure and further understanding of the DNA binding mechanism of p270.

## Methods and experiments

The p270 ARID was expressed in *E. coli* for structural studies by NMR. The c-DNA encoding residues 1003 to 1123 of p270 followed by a His-tag was subcloned into PET28a (Novagen, Inc). The expression plasmid was transformed to *E. coli* strain BL21DE3. Uniformly $^{15}N$-labeled protein or $^{15}N/^{13}C$ doubly labeled protein was produced by growing the cells in M9 media using $^{15}NH_4Cl$ and $^{13}C$-glucose as the sole nitrogen and carbon source. The protein was purified using a $Ni^{2+}$-NTA column.

NMR samples contained approximately 0.5–1 mM of protein in 100 mM sodium phosphate buffer (pH 6.0) containing 95%$H_2O$/5%$^{2}H_2O$ or

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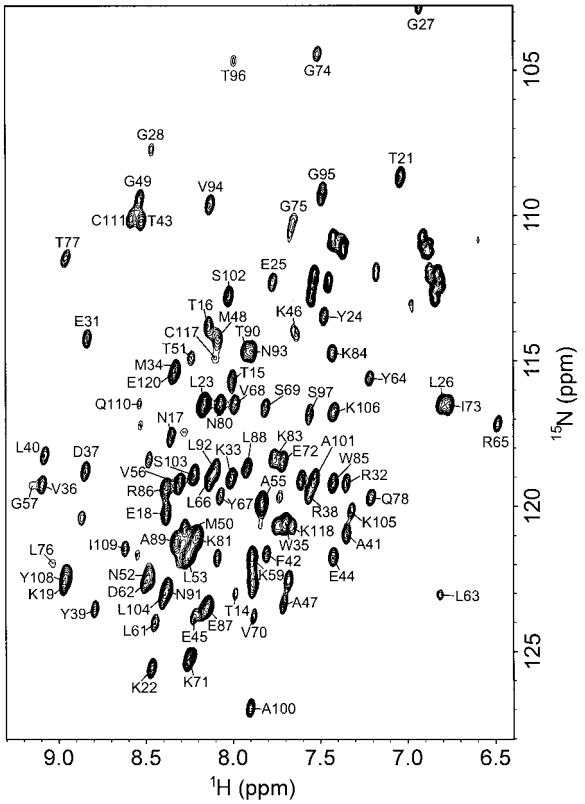


*Figure 1.* $^{1}$H-$^{15}$N HSQC spectrum of the p270 ARID domain. The spectrum was recorded in the absence of $^{13}$C decoupling. Sequential assignments are indicated in the figure.

100%$^{2}H_2O$. All NMR experiments were performed at 20 °C on a Varian Unity-plus 500 NMR spectrometer equipped with pulse-shaping and pulsed-field gradient capabilities. Felix98 (Molecular Simulations Inc., San Diego, CA) was used for NMR data processing and analysis. $^{1}$H, $^{15}$N and $^{13}$C resonance assignments were obtained using the following experiments: HNCACB, CC-TOCSY-(CO)NH, CBCA(CO)NH, HCCH-TOCSY, $^{15}$N-edited TOCSY-HSQC, H(CC-TOCSY-CO)NH, HBHA(CO)NH (for reviews on NMR methodology, see Bax et al., 1994; Clore and Gronenborn, 1994).

## Extent of assignments and data deposition

Nearly all $^{1}$H, $^{13}$C and $^{15}$N resonances have been assigned. The$^{1}H_N$-$^{15}$N HSQC spectrum of the p270 ARID is shown in Figure 1. Assignments of backbone $^{1}H_N$ and $^{15}$N resonances are indicated in the spectrum. Except for residues in the histidine-tag at the C-terminus, the resonances of amide N, $H_N$, $C_\alpha$, and $H_\alpha$ of all residues have been obtained. Almost all side-chain resonances have been assigned. Overall, 98% of backbone resonances and greater than 96% of the side chain resonances have been assigned. A list of the $^{1}$H, $^{13}$C and $^{15}$N chemical shifts has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-7349. The secondary structure of the P270 ARID contains seven helices and no β-sheets. In addition to the six helices commonly observed in the Mrf-2 and Dri ARID domains (Yuan et al., 1998; Iwahara et al., 1999), an additional short helix is identified at the N-terminus.

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