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ARTICLE

Backbone and sidechain ^{1}H, ^{13}C and ^{15}N resonance assignments of the Bright/ARID domain from the human JARID1C (SMCX) protein

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Abstract We have assigned ^{1}H, ^{15}N and ^{13}C resonances of the Bright/ARID DNA-binding domain from the human JARID1C protein, a newly discovered histone demethylase belonging to the JmjC domain-containing protein family.

Keywords Histone demethylation · JARID1C · SMCX · Bright/ARID domain · NMR resonance assignment

Abbreviations

JARID1C	Jumonji/ARID domain-containing protein 1C
ARID	AT-rich interaction domain
SMCX	SMCY homologue X-linked
IPTG	Isopropyl-beta-D-thiogalactopyranoside

Biological context

The JARID1C protein (Jumonji/ARID domain-containing protein 1C), also known as SMCX, is a member of the ARID (AT-rich interaction domain) family of DNA-binding proteins, found in fungi, and invertebrate and vertebrate metazoans. The proteins are involved in a variety of biological processes including embryonic development, cell lineage gene regulation and cell cycle control. Although the specific roles of this domain and of ARID-containing proteins in transcriptional regulation are still unclear, they include both positive and negative transcriptional regulation and modification of chromatin structure. JARID1C acts as a histone H3 lysine 4 demethylase involved in transcriptional repression (Iwase et al. 2007). Mutations in JARID1C are a known cause of X-linked mental retardation (XLMR) and epilepsy, suggesting that JARID1C plays a role in human brain function (Tahiliani et al. 2007; Tzschach et al. 2006, Jensen et al. 2005).

The JARID proteins comprise a JmjN domain, a Bright/ARID domain, a JmjC domain, and two PHD-like zinc finger domains. The approximately 100-residue ARID sequence is present in a series of proteins strongly implicated in the regulation of cell growth, development, and tissue-specific gene expression. Although about a dozen ARID-containing proteins can be identified from database searches, to date, only Bright (a regulator of B-cell-specific gene expression), dead ringer (a Drosophila melanogaster gene product required for normal development), and MRF-2 (which represses expression from the cytomegalovirus enhancer) have been analyzed directly in regard to their DNA binding properties (Herrscher et al. 1995; Iwahara et al. 2002; Yuan et al. 1998). Each binds preferentially to AT-rich sites. In contrast, the human SWI–FSNF complex protein p270 shows no sequence preference in its DNA binding activity, thereby demonstrating that AT-rich binding is not an intrinsic property of ARID domains and that ARID family proteins may be involved in a wider range of DNA interactions.

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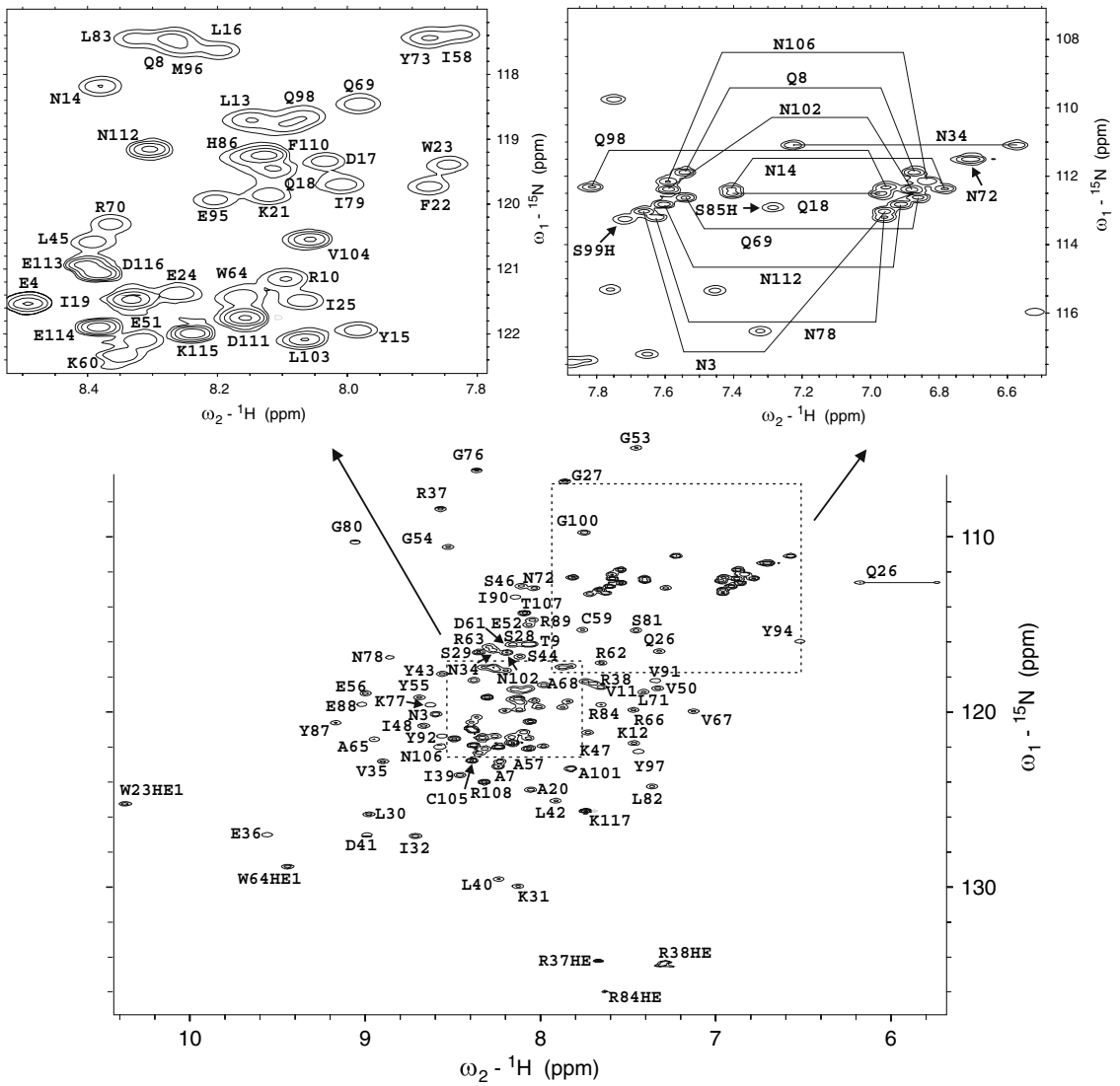

Fig. 1 Assigned ^{15}N–^{1}H HSQC spectrum of the Bright/ARID domain of the human JARID1C protein acquired at 750 MHz and 297 K

Methods and experiments

Cloning, expression and purification of human JARID1C Bright/ARID domain

The Bright/ARID domain comprising residues 73–188 of the human JARID1C protein (NCBI GenBank GI:11321605, Swissprot, P41229) was cloned into the homemade pNIC28-Bsa4 vector, which adds a TEV-cleavable hexahistidine tag to the N-terminus of the protein, cleavage of which leaves two additional residues, SM on the construct N-terminus. Uniformly ^{15}N- and ^{15}N, ^{13}C-labelled samples of the His-TEV-SM-Bright/ARID domain were grown in *E. coli* BL21(DE3)-Rosetta cells (Novagen) containing the above plasmid, in M9 minimal medium supplemented with 25 μg/ml kanamycin, 34 μg/ml chloramphenicol and containing 0.5 g/l $^{15}NH_4Cl$ and either 2 g/l (w/v) $^{12}C_6$-glucose or $^{13}C_6$-glucose, respectively, as the sole nitrogen and carbon sources. Cultures were grown at 37°C and transferred to 18°C when the OD_{600} reached 0.6. Expression was induced with 1 mM IPTG and cells were left to grow at 18°C overnight. Cells were harvested by centrifugation, washed with ice-cold 150 mM NaCl and frozen at −80°C. Frozen cell pellets from 2 l culture (~6.3 g wet mass) were resuspended in a 25 ml lysis buffer (20 mM Tris–HCl pH 8.0, 500 mM NaCl, 5 mM imidazole, 1 mM β-mercaptoethanol, Complete® protease inhibitors EDTA-free (1 tablet/50 ml), 2 mM $MgCl_2$, 10 units Benzonase/ml (>90% purity Novagen), 5 mM arginine and glutamate). The cells were broken by four passes at 16,000 p.s.i. through a high pressure homogeniser (EmulsiFlex-C3/Avestin) followed by centrifugation for 60 min at 60,000*g*. The supernatant was further clarified by filtration (0.45 μm). Nickel affinity purification was carried out on a workstation

Vision (Applied Biosystems). The soluble fraction was loaded on an 8 ml Ni-affinity MC-Poros column with a starting buffer composition of 20 mM Tris–HCl buffer pH 8.0, 500 mM NaCl and 5 mM imidazole at a flow rate of 1 ml/min. Bound protein was eluted with 20 mM Tris–HCl buffer pH 8.0, 500 mM NaCl, 5–500 mM imidazole gradient at 5 ml/min. The extinction at 280 nm was monitored and fractions were collected and analyzed by SDS-PAGE.

The His-tag was cleaved with 1 mg TEV per 40 mg target protein in a dialysis bag (3.5 kDa MW cutoff) and dialysed against 5 l of: 20 mM Tris–HCl 8.0, 500 mM NaCl, 1 mM β-mercaptoethanol, at 15°C overnight. Once cleavage was complete, the sample was supplemented with 5 mM arginine and glutamate and concentrated to 3 ml on a 5 kDa Amicon Ultra-15 centrifuge concentrator. The desired JARID1C Bright/ARID domain was separated from other cleavage products on a 320 ml Superdex 75 column with PBS, pH 7.4, and concentrated using Amicon Ultra-15 concentrators with 5 kDa cutoff. The protein was then exchanged into NMR buffer and concentrated to 1 mM in NMR buffer: 50 mM phosphate, pH 6.0, 50 mM NaCl, 5 mM d-DTT, 0.02% azide, with 10% (v/v) D_2O. Protein molecular weights were confirmed by mass spectrometry.

NMR spectroscopy

NMR spectra were acquired at 297 K, using Bruker DRX 600 and DMX 750 spectrometers in standard configuration with triple resonance probes equipped with self-shielded triple axis gradient coils. Spectra for the resonance and NOE assignment were recorded essentially as described in the original references. A 1 mM ^{13}C, ^{15}N-labelled sample of JARID1C in 90% H_2O/10% D_2O (NMR buffer; pH 6.0) was used for all H_N-detected triple resonance experiments; 3D CBCA(CO)NNH, 3D CBCANNH, 3D CC(CO)NNH, 3D H(CCCO)NNH, 3D HBHA(CBCACO)NNH, 3D HNCO, 3D HN(CA)CO, 3D ^{15}N-separated NOESY-HSQC, for ^{15}N T1 and ^{15}N T2 relaxation measurements, for heteronuclear ^{15}N–^{1}H NOE experiments and for a 3D ^{13}C-separated, aliphatic-centred NOESY-HSQC spectrum. The sample was then freeze-dried and redissolved in 100% D_2O for acquisition of 3D ^{13}C-separated HMQC-NOESY, 3D HCCH-COSY, and 3D HCCH-TOCSY spectra. Data were processed using the program XWIN-NMR (version 2.6) of Bruker BioSpin GmbH (Rheinstetten, Germany). Assignment of ^{13}C, ^{15}N and ^{1}H resonances was carried out using standard assignment procedures on an Intel Dual Xeon 3GHz PC with the program SPARKY v. 3.1 (Goddard and Kneller).

Extent of assignment and data deposition

Figure 1 shows the assigned ^{15}N HSQC spectrum of the Bright/ARID domain from the human JARID1C protein. Ninety-four percent of all backbone and sidechain ^{1}H, ^{15}N and ^{13}C resonances were assigned. One hundred percent of Trp Nε1/Hε1, Asn/Gln NH2 and His sidechains were assigned, as well as 75% Phe, 87% Tyr and 30% Arg Nε, Hε sidechains. The assignments were deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) with accession code BMRB-15348.

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