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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the conserved core of hAsf1 A

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Biological context

The Anti Silencing function 1 protein (Asf1) is a highly conserved protein involved in chromatin assembly processes (Tyler et al., 1999) which plays important roles in DNA replication, recombination, and repair. A model for Asf1 function in the cellular response to genotoxic stress was proposed from studies in *S. cerevisiae* (Emili et al., 2001). In the absence of DNA damage, Asf1 interacts with Rad53, a checkpoint kinase involved in DNA repair. The complex Rad53-Asf1 is recruited to sites of DNA damage. Upon Rad53 phosphorylation, the complex is dissociated. Asf1 binds histones H3 and H4, and assists in nucleosome assembly.

Other proteins have been identified as Asf1 partners in different organisms, such as the human protein Tlk implicated with Asf1 in a DNA damage response (Groth et al., 2003), Hir1p involved in chromatin remodelling (Sharp et al., 2001), and the transcription factor CCG1 (Munakata et al., 2000). In the context evoked above, the involvement of Asf1 in various identified multiprotein assemblies constitutes an advantage for dissecting the related binding motifs and analysing their possible concerted combinations. Furthermore, investigating the conserved interactions between organisms should provide useful additional information. The *Saccharomyces cerevisiae ASF1* encodes a protein of 279 amino acids (Le et al., 1997). In humans, two ASF1 isoforms have been found, hASF1A and hASF1B, which encode proteins of 204 and 202 amino acids respectively (Munakata et al., 2000; Sillje and Nigg, 2001). Remarkably, 59% of the first 150 residues are conserved between yeast and humans whereas hAsf1 lacks the C-terminal acidic stretch present in scAsf1. In order to obtain further insights into structure – function relationships of Asf1, we carried out NMR studies of the entire hAsf1A protein (1-204) and the conserved core (1–156). Here we report the complete ^{1}H, ^{15}N and ^{13}C resonance assignments of hAsf1A 1–156.

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Methods and experiments

Recombinant $(His)_6$ – GST – Tev site – $_h$Asf1 A 1–156 and 1–204 were produced from the pETM30 – $_h$Asf1A 1-156 and 1-204 constructs. *Escherichia coli* strain Bl21 gold (DE3) was grown in M9 minimal media supplemented with $(^{15}NH4)_2SO_4$ (Eurisotop, 0.5 g l^{-1}) as the sole nitrogen source and/or ^{13}C-glucose (Eurisotop) as the sole carbon source. The soluble $(His)_6$-tagged GST fusion proteins were immobilized on GSH agarose (Sigma) and then eluted with an excess of glutathione (Sigma). They were cleaved using a $(His)_6$-tagged TEV protease (1% w/w of protease / fusion protein). A Ni-NTA agarose column (Qiagen) was used to trap the $(His)_6$-tagged TEV protease and the $(His)_6$-tagged GST. A last step of anion exchange chromatography was done (source Q Applied Biosystems, binding conditions: Tris 50 mM pH 8.0, elution with 250 mM NaCl). Purified proteins were concentrated to $\sim$1 mM in a 10 kDa limit concentrator (Millipore) and exchanged into NMR buffer, Tris-d_{11} 20 mM pH 7.4 (Eurisotop),

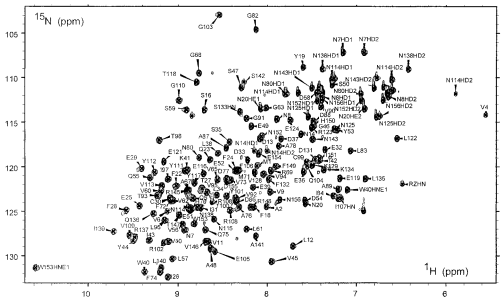

Figure 1. $^{1}H^{N}-^{15}N$ HSQC spectrum of $_{h}$Asf1A 1–156 at 303 K pH 7.4. Assignments of resonances are indicated using one-letter amino acid code. The highly shifted resonance corresponding to residue K3 (N: 114.65 ppm, HN: 3.608 ppm) is not shown.

in either H_2O/D_2O 90%/10% or in pure D_2O, containing 0.1 mM EDTA, 0.1 mM DSS and 0.1 mM NaN_3.

NMR experiments were carried out on a Bruker DRX-600 spectrometer equipped with a triple-resonance probe at 303 K. The sequential backbone resonance assignments were achieved using standard ^{15}N-^{1}H – HSQC, ^{15}N-edited NOESY-HSQC, HNCA, HN(CO)CA, CBCA(CO)NH, HNCO, HBHA(CO)NH, CBCACOHA and HNHA experiments. Side chain assignments were achieved using HCCH-TOCSY (mixing time of 14 and 28 ms), HCCH-COSY, and ^{13}C edited NOESY-HSQC experiments. Proton chemical shifts (in ppm) were referenced relative to internal DSS and ^{15}N and ^{13}C references were set indirectly relative to DSS using frequency ratios (Wishart et al., 1995). Aromatic protons were assigned using a HCCH-TOCSY experiment centered on aromatic protons (mixing time of 6 ms) and two ^{13}C edited NOESY-HSQC experiments: one centered on aromatic protons (mixing time of 150 ms) and the other centered on aliphatic protons (mixing time of 120 ms). All NMR data were processed using Xwinnmr (Bruker) and analyzed using Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco).

Extent of assignment and data deposition

In a first step, $^{1}H^{N}-^{15}N$-HSQC spectra of the two Asf1 constructs 1–156 and 1–204 were recorded and superimposed. This comparison readily revealed that the C-terminal tail 157–204 is not structured and does not influence the NH chemical shifts of the conserved core 1–156. Further heteronuclear NOE experiments confirmed that the C-terminal segment 157-204 is highly flexible. Therefore, only the hAsf1A 1–156 region has been considered for the structural study.

Figure 1 shows the $^{1}H^{N}-^{15}N$-HSQC spectrum of the conserved core of $_{h}$Asf1 A (residues 1–156) that exhibits a nice dispersion (168 isolated peaks instead of 180 expected). All $^{1}H^{N}$, ^{15}N $^{13}C^{\alpha}$ and $^{1}H^{\alpha}$ backbone resonances have been assigned except for the T120 amide nitrogen and $^{1}H^{N}$. Most ^{13}CO assignments were determined (95%), apart from those of residues G63, V65, P66, E119, N125, P126, K129, A141, N143 and N156.

All non-aromatic side chain protons and carbons have been assigned except Nε/Hε of R69, R108, R123, R137, R145 and R148, Cγ/Qγ of R137, Cδ/Qδ, Cγ of P17 and Cδ/Qδ of P85. 85% of the aromatic side chain atoms have been assigned. The chemical shift assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the temporary accession number (132.166.55.169.27469).

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