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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the region 1463-1617 of the mouse p53 Binding Protein 1 (53BP1)

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Biological context

Human 53BP1 (p53 Binding Protein 1) was initially identified in a yeast two-hybrid screen as a protein that binds to the central DNA binding domain of the tumor suppressor p53 and that enhances p53-mediated transcriptional activation (Iwabuchi et al., 1998). This 1972 amino acids protein encompasses a BRCT tandem in the C-terminus region. BRCT domains are found in numerous proteins involved in DNA damage responses (cell cycle control, recombination and DNA repair) (Callebaut et al., 1997). 53BP1 binds p53 through its first BRCT domain and through the linker between the two BRCT domains, as shown recently by X-ray cristallography (Derbyshire et al., 2002).

Several recent reports showed that 53BP1 is involved in DNA damage responses. In the absence of genotoxic stress, 53BP1 nuclear localization is diffuse. After exposure to ionizing radiations, 53BP1 is hyper-phosphorylated in an ATM-dependant pathway and is rapidly relocalized to distinct nuclear foci corresponding to DNA double strand break sites (Rappold et al., 2001). Other proteins known to be involved in the DNA damage signalling pathway were found to colocalize with 53BP1 after irradiation. In particular phosphorylated H2AX (γ–H2AX) seems to be essential for 53BP1 recruitment (Fernandez-Capetillo et al., 2002) and these two proteins were shown to interact physically (Ward et al., 2003). Furthermore it seems that the recruitment of 53BP1 to γ–H2AX nuclear foci is crucial for the phosphorylation of numerous ATM substrates, including SMC1, p53 and BRCA1 (Di Tullio et al., 2002, Wang et al., 2002). The region 1052–1639 of human 53BP1 was shown to be necessary and sufficient for the nuclear foci formation after IR and for the interaction with γ–H2AX (Ward et al., 2003). This region is essentially constituted of low complexity sequences. The fragment 1487–1532 is however predicted by PSI-BLAST to adopt a Tudor fold, a fold found in several RNA binding proteins (Ponting, 1997). We applied NMR techniques in order to determine the solution structure of a 155 residues domain encompassing this potential Tudor domain. Here we report the ^{1}H, ^{15}N and ^{13}C assignment of the 1463–1617 region of mouse 53BP1 which is 99% identical to the 1478–1632 region of human 53BP1.

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Methods and experiments

The region 1463–1617 of mouse 53BP1 was expressed in *E. coli* strain BL21-Gold(DE3) transformed with plasmid pDEST15 (Invitrogen) encoding the 53BP1 fragment in fusion with gluthatione-S-transferase (GST) and a TEV protease (Tobacco Etch Virus protease) cleavage site between 53BP1 and GST. The protein was purified using glutathione-agarose beads (SIGMA), once to separate the fusion protein from the other bacterial proteins, and another time after cleavage with the TEV protease to separate the 53BP1 fragment from GST. Uniformly labeled ^{15}N protein was produced in minimal medium M9 containing 1 g l^{-1} of $(^{15}NH_4)_2SO_4$ (Boehringer) as the nitrogen source. Uniformly labelled ^{13}C/^{15}N protein was produced in a rich medium prepared

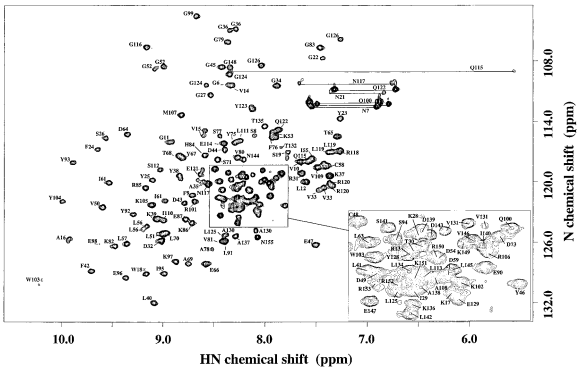

Figure 1. $^{1}H^{N}$-^{15}N HSQC spectrum of the region 1463–1617 of mouse 53BP1.

from uniformly labelled $^{13}C/^{15}N$ *Spirulina maxima* cyanobacteria. NMR samples (about 0.8 mM) were prepared in Tris-HCl 50 mM buffer pH 7.0 containing 150 mM NaCl in either 90% H_2O/10% D_2O or in 100% D_2O. 1mM EDTA, a protease inhibitor cocktail (SIGMA), 1mM NaN_3 and 3-(trimethylsilyl)[2,2,3,3-$^{2}H_4$] propionate (TSP) were added as a chemical shift reference. ^{13}C and ^{15}N chemical shifts were referenced indirectly to TSP, using the absolute frequency ratios. All experiments were performed at 27 °C on Bruker DRX-500 or DRX-600 spectrometers equipped with triple-resonance probes. Sequential backbone resonance assignments were achieved using data from the following experiments: ^{15}N-^{1}H HSQC, HNCO, HNCA, HN(CO)CA, CBCA(CO)NH, CBCANH, CBCACOHA, HNHA, (HCA)CO(CA)NH. Side chain assignments were obtained using HBHA(CO)NH, HCCH-TOCSY, HCCH-COSY and ^{13}C-HSQC NOESY experiments. All spectra were processed with the programs Xwinnmr (Bruker) or NMRPipe (Delaglio et al., 1995) and analysed using Felix (Molecular Simulations).

Extent of assignments and data deposition

Figure 1 shows the ^{15}N-^{1}H HSQC spectrum of the domain 1463–1617 of mouse 53BP1. The residues are numbered from 1 to 155. 154 backbone crosspeaks are observed while only 151 (155 minus 4 prolines) are expected from the amino acid sequence. Backbone and side chain assignments, and 3D ^{15}N HSQC-NOESY patterns revealed that 15 spin systems split in two (V15, T30, V33, G36, G52, K53, L56, L119, R120, E121, G124, L125, G126, A130, V131). For each couple of peaks we were able to identify a major form (called chain A in the list of chemical shifts). In their major form, residues G124, L125 and G126 are located before trans P127. We suppose that the minor form observed for these residues (Chain B) correspond to the *cis* proline P127. Thus one hypothesis to explain the splitting of the other spin systems is that these residues are close in space to P127.

Thirteen backbone ^{15}N-^{1}Hn crosspeaks are not observed. The topology of the protein confirmed that half of these residues are localised in loops. The others are in the N-terminus part of the protein. Chemical or conformational exchange processes presumably explain this absence. Finally 94% of $^{1}H_N$ and ^{15}N, 99% of $^{13}C_\alpha$, 98% of $^{1}H_\alpha$ and 96% of ^{13}CO backbone chemical shifts were assigned.

Side-chain assignments are also almost complete (92%). The non assigned side-chain nuclei correspond to the whole side chains (except for $^{13}C\beta$ and $^{1}H\beta$) of 8 residues and a few atoms of 11 other residues.

The ^{1}H, ^{13}C and ^{15}N chemical shifts of the region 1463–1617 of mouse 53BP1 have been deposited in the BioMagResBank (http://bmrb.wisc.edu) under accession number 5878.

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