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Letter to the Editor: Resonance assignments and topology of the ^{15}N, ^{13}C labelled 23 kDa core domain of Xenopus Mob1

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Biological context

At the end of mitosis, a signalling pathway called the Mitotic Exit Network (MEN)/Septation Initiation Network (SIN) respectively in budding and fission yeast regulates the activation of Cdc14, a dual specificity phosphatase. It includes a small GTPase (Tem1) controlling a network of serine-threonine kinase (Cdc15, Dbf2-Mob1) regulating the subcellular location/activation of Cdc14, which controls mitotic exit (for review see Bardin and Amon, 2001). Mutants of the MEN components arrest with segregated chromosomes and cytokinesis defects. In the presence of a misoriented spindle, this pathway prevents mitotic exit. The molecular interactions controlling this pathway are not fully uncovered and it is not established if a similar pathway exists in higher eucaryotes. Nevertheless, database searches identify homologs of MEN/SIN components in higher eucaryotes suggesting that a similar pathway might exist. In the budding yeast, the Mob1 protein is required for Dbf2 protein localisation (Frenz et al., 2000). It binds and activates the Dbf2 kinase in a Cdc15 dependant manner suggesting that it is a kinase regulatory subunit (Lee et al., 2001; Mah et al., 2001). Mob1 belongs to the Mob1/phocein family (Baillat et al., 2001) but its function in controlling mitotic exit is still unresolved. We thought to gain further information about this signal transduction pathway by solving the NMR structure of the *Xenopus laevis* Mob1 protein. Here we report the assignment of the core-conserved domain of Xenopus Mob1.

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Methods and results

Xenopus laevis Mob1 cDNA (Genbank accession AY319763) encoding amino acid 33–215 was PCR amplified then subcloned into pET15b *E. coli* expression vector (Novagen Inc., Madison). This adds a N terminal poly-Histidine sequence as well vector derived sequence (MGSSHHHHHHSSGLVPRGS), which allows a one step purification protocol of the protein. The protein was over-expressed in *E. coli* grown on minimal media containing 3 g/l ^{15}N-ammonium chloride and 10 g/l ^{13}C-glucose and purified on TALON beads (Clontech). A protein ^{15}N-^{13}C labelled on lysine residues was produced using CT8 cells (Waugh, 1996). Upon either Thrombin cleavage, low pH or low ionic strength, the purified protein aggregates as seen by light scattering experiments. For NMR analysis, the tagged Mob1 protein was purified then dialysed and concentrated to 1 mM in a buffer containing 500 mM NaCl, 20 mM Tris-HCl pH 8.0, 5 mM DTT. Approximately 100 mg of uniformly ^{13}C, ^{15}N doubly enriched Mob1 were obtained from 1 l of culture.

NMR spectroscopy

Spectra were recorded on a Brücker Avance 600 MHz and Varian Unity 800 MHz spectrometers. The temperature was maintained at 300 K throughout the NMR experiments. ^{1}H, ^{15}N and ^{13}C chemical shifts were referenced to DSS. The following spectra were recorded for assignment of the backbone and ^{13}Cβ resonances: HNCA and HN(CA)CB at 800 MHz, HN(CO)CA, HNCO, HN(CA)CO and CBCA(CO)NH at 600 MHz. Proton assignments were done using HCCH-TOCSY,^{13}C-^{1}H NOESY-HSQC and ^{15}N-^{1}H NOESY-HSQC at 600 MHz (for review see Sattler et al., 1999). FIDs were processed using the program

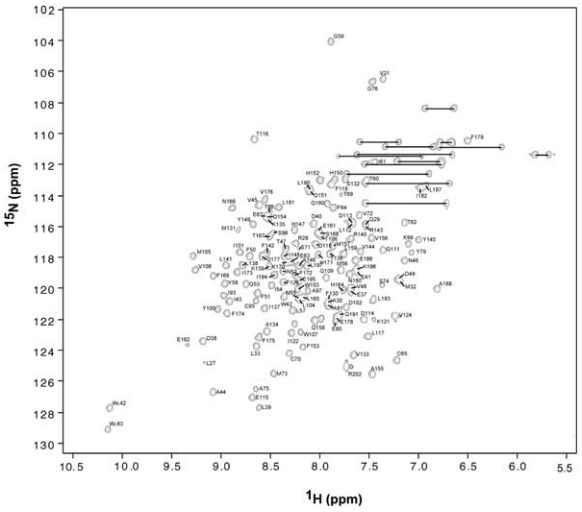

Figure 1. ^{1}H-^{15}N HSQC spectrum of Mob1 from *Xenopus Laevis* at 300 K, pH 8. Assignments of backbone amide peaks are shown by single-letter code and residue number. Gln/Asn sidechain cross peaks are denoted with horizontal lines. The cross peaks originating from the indole NH of tryptophanes is indicated by Wε (signal of Wε107 is outside this region).

Gifa, and data analysis was assisted by the software Garant (Bartels et al., 1997) and the in house software Cindy.

Extent of assignments and topology

Sequence-specific assignments were made primarily via the HNCA/HN(CO)CA pair, using the carbonyl experiments to resolve any ambiguities arising from degeneracy in the ^{13}C frequency. Using this strategy, backbone assignments could be made for the majority of residues from the structured regions of Mob1 and these were confirmed using the HN(CA)CB. Residues had severely attenuated amide signals and as a consequence were barely distinguishable from the noise in the 3-dimensional experiments. The assignment of these residues could only be completed by analysis of the ^{15}N-^{1}H NOESY-HSQC.

Attenuation of these signals could be caused by local conformational exchange processes on an intermediate timescale or by incomplete amide reprotonation due to relatively high pH. The NMR assignment of ^{1}HN, ^{15}N, ^{13}C, ^{13}Cα and ^{13}Cβ of Mob1 is approximately 90% complete over the 183 residues of Mob1 (excluding the tag). The chemical shift data analysis by TALOS (Cornilescu et al., 1999) and ^{1}H-^{1}H NOE were used to identify secondary structure elements. These data clearly show the presence of four long helices from Leu^{39} to Thr^{60}, from Lys^{99} to Asp^{113}, from Phe^{130} to His^{150}, from Ala^{163} to Glu^{178} and two short helices from Leu^{27} to Ala^{30}, from Gln^{191} to Leu^{197}. Two extended streches are found from Met^{73} to Ala^{75}, from Tyr^{79} to Tyr^{81}. Assignments could not be made for the majority of residues within the first extensive interhelices linker sequence (residues 67, 68, 77, 78, 82–90, 92, 94, 98), the second interhelices linker sequence (residues 119, 125, 127–129), at the N-terminus (residues 1–14, 16–26) and also at the C-terminus (residues 198-201) . These regions are likely to be unstructured.

A table of assignment is available as supplementary material and has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5833.

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