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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of Mlc1p from *Saccharomices cerevisiae*

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Biological context

The budding yeast *Saccharomices cerevisiae* provides a good model for the study of the molecular machinery involved in vesicle trafficking and cytokinesis. In this organism, the unconventional class V myosin Myo2p is implicated in vesicle transport and polarized growth. All myosins have at least one type of light chain bound to the myosin heavy chain via a light chain binding motif called an IQ site (May et al., 1998). Binding of light chains can affect both myosin head and light chains orientation. Calmodulin (CaM) was the first myosin light chain discovered in *S. cerevisiae*, and subsequently, a previously uncharacterized protein, Mlc1p, was shown to be a second myosin light chain of Myo2p (Stevens and Davis, 1998).

Myo2p is not the only target of Mlc1p. It was found that Mlc1p also binds to a class II myosin (Myo1p) in late mitosis and to Iqg1p, an IQGAP-like protein, during cytokinesis (Boyne et al., 2000; Shannon and Li, 2000). Localization of Mlc1p occurs before and independently of Iqg1p, Myo2p, actin and Myo1p, suggesting that there is yet another target of Mlc1p, possibly septin dependent. More recently, it was shown that Mlc1p is involved in two pathways during cytokinesis: one that is essential and requires Myo2p and a second that is non-essential and involves Myo1p and Iqg1/Cyk1p (Wagner et al., 2002). Moreover, Myo2p associates with vesicles via the formation of a complex with the Rab/Ypt Sec4p protein, that by cycling between a GTP- and GDP-bound state and between cytosol and membranes, acts as a molecular switch regulating the timing and specificity of vesicle tethering and docking. In this context, it cannot be excluded that Mlc1p can form a complex either with Myo2p and/or Sec4p during its trip on secretory vesicle (Wagner et al., 2002).

Systematic protein–protein interaction studies, including mass spectrometry, two-hybrid analysis and tandem-affinity purification combined with mass spectrometry (Ito et al., 2001; Ho et al., 2002; Gavin et al., 2002), found Mlc1p in complexes containing the protein Ded81, a cytosolic asparaginyl-tRNA synthetase required for protein synthesis; Cmd1, a master regulator of calcium mediated signalling; and She3 and Myo4, required for mother-specific HO expression. Although the role of Mlc1p in these complexes is still not known, they already anticipate a pivotal role for Mlc1p in a significant number of cellular events.

Mlc1p is a small protein (149 aa) that belongs to the EF-hand protein family (May et al., 1998). No structure is available for the isolated Mlc1p protein, neither in the crystalline state nor in solution. Very recently, the structure of complexes of Mlc1p with two of the six Myo2p IQ motifs were solved by X-ray crystallography (Terrak et al., 2003). Analysis of the bound form of Mlc1p showed the presence of two homologous domains, the N- and C-lobes, connected by a linker loop. Each lobe contains two helix-loop-helix (EF-hand) motifs. In contrast to CaM, the EF-hand motifs of Mlc1p do not bind Ca^{2+},

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due to substitutions of several amino acids involved in the coordination of Ca^{2+} in CaM.

In the present work, we report the Mlc1p backbone and side-chain assignments. They can be of great utility to tackle interaction studies using NMR with different IQ motifs present in the molecular partners of Mlc1p, like Myo2p, Myo1p, Iqg1p and yet undiscovered proteins involved in cytokinesis.

Methods and experiments

The Mlc1p gene was cloned into the pET-15b expression vector. The recombinant protein was overexpressed as a N-terminal His6-tagged fusion protein in *Escherichia coli* strain, BL21(DE3). Uniformly labelled $^{13}C/^{15}N$- and ^{15}N-isotopically enriched protein samples were prepared by growing the cells in M9 minimal media containing $^{15}NH_4Cl$ and $^{13}C_6$-D-glucose. The protein was purified by affinity chromatography on a Ni-NTA column (Qiagen). Further purification of the protein was achieved by gel filtration chromatography (prepSuperdex 75, Amersham). The final NMR sample contained the His6-tag as well as the thrombin cleavage site.

NMR samples contained 0.8 mM of Mlc1p in 60 mM phosphate buffer, pH 6.9, 0.33 M NaCl and 0.01% NaN3. All NMR spectra were recorded at 25°C on a Bruker Avance 700 MHz spectrometer equipped with pulsed field gradient triple-resonance probes (Figure 1). Assignments of the ^{1}H, ^{15}N, ^{13}CO and ^{13}C resonances were based on the following experiments: CBCA(CO)NH, HBHA(CO)NH, CBCANH, HNCO, HNCA, HN(CO)CA, (HACA)CONH, 3D ^{15}N-edited TOCSY-HSQC, HCCH-TOCSY, HCCH-COSY and HACACO. Chemical shifts were confirmed by inspection of intraresidue and sequential NOEs in the ^{1}H, ^{15}N-NOESY-HSQC and ^{1}H, ^{13}C-NOESY-HMQC experiments.

Extent of assignments and data deposition

^{1}H, ^{13}C and ^{15}N backbone resonances of 144 residues out of the 147 non-proline residues of Mlc1p were assigned. Three HN groups could not be detected in the $^{1}H-^{15}N$ HSQC spectra, belonging to K8, Q41 and S57. These residues appear at the surface of the available structures of Mlc1p complexes, suggesting that a relatively high exchange with solvent is the main reason of signal

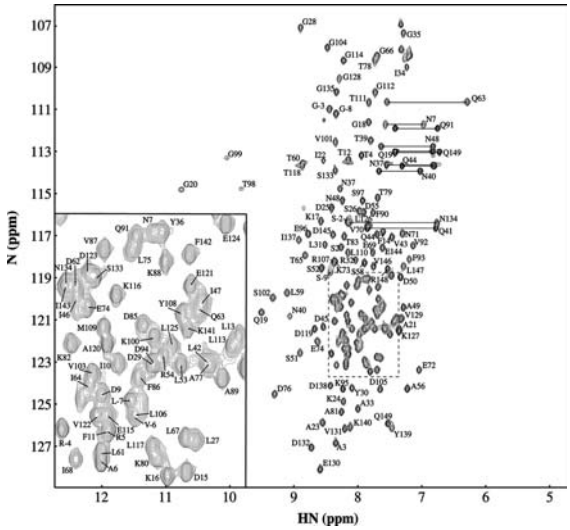

Figure 1. $^{1}H-^{15}N$ HSQC spectrum of 0.8 mM Mlc1p in phosphate buffer pH 6.9, collected at 700 MHz. Residues belonging to the His6-tag and the thrombin cleavage site are numbered with negative values.

disappearance. About 96% of Mlc1p side chains resonances were assigned. Data are still missing for the aromatic ring of F93; H^{ζ} protons of all phenylalanines, except for F11; the N71 $N^{\delta}H_2$ group of, which could not be detected in the $^{1}H-^{15}N$ HSQC spectrum, and Hγ protons of L27, L31, L59, L126 and L147. The forthcoming NOE analysis is expected to help in the determination of the yet unassigned chemical shifts. The ^{1}H, ^{13}C and ^{15}N chemical shifts of Mlclp have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6332.

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