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Letter to the Editor: Resonance assignment and secondary structure of the La motif

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Biological context

Processing of nascent RNA polymerase III (pol III) transcripts is a complex mechanism that involves the La protein, an abundant and evolutionarily conserved RNA-binding phosphoprotein first identified as an autoantigen in systemic lupus erythematosus and Sjogren's syndrome patients (Maraia and Intine, 2001; Wolin and Cedervall, 2002). La acts by binding specifically to the 3′ poly (U) ends of pol III precursors in an interaction mediated by the highly-conserved N-terminal domain (NTD) of the protein. La proteins of higher eukaryotes also possess a C-terminal domain responsible for RNA nuclear retention and recognition of the 5′ terminus of nascent RNA transcripts (Jacks et al., 2003; Maraia and Intine, 2001).

The NTDs of La proteins contain two RNA binding domains, both crucial for poly (U) RNA binding. These domains are predicted to be RNA recognition motifs (RRM) that bind with RNA via a four-strand β-sheet (Hall, 2002); however, whereas the domain spanning residues 105–194 has been shown to adopt an RRM-like structure ((Alfano et al., 2003) and unpublished data), some authors contest that the first domain (residues 1–103) folds into a predominantly helical structure that has been termed a 'La-motif' (Wolin and Cedervall, 2002). Interestingly, the La motif is also conserved in other proteins otherwise unrelated to bona fide La proteins, but its role remains uncertain (Wolin and Cedervall, 2002). In this note we present the essentially complete assignment for the La motif of human La (hLa) and the delineation of its secondary structure.

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Methods and experiments

An N-terminal fragment of human La encompassing the residues 1–103 was subcloned by PCR into pET-30 expression vector using the LIC methodology (Novagen). The hexahistidine-tagged recombinant protein was overexpressed in BL21(DE3) pLysS *E. coli* cells grown minimal media containing 0.8 g·L^{-1} ^{15}N-ammonium chloride and 2g·L^{-1} ^{13}C glucose. Cell pellets were lysed in 20 mM Tris-HCl, 300 mM NaCl, 10 mM Imidazole, pH 8 and purified by affinity chromatography on a Ni-NTA resin (Qiagen), following the manifacturer's protocol. The protein eluted was dialysed in 50 mM Tris-HCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 10% (v/v) glycerol pH 7.25 (buffer A), loaded on a 5 ml Hi-Trap Heparin column (Amersham-Pharmacia Biotech) and eluted with a linear 0–2.0 M KCl gradient in buffer A. After dialysis in 50 mM Tris-HCl, 100 mM KCl, 5 mM Ca_2Cl, pH 8 and cleavage with factor Xa (Novagen) to remove the N-terminal hexahistidine tag, the samples were re-loaded onto the Ni-NTA column and the tag-free protein was collected in the flow-through. NMR samples were prepared dialysing the purified protein against a buffer containing 20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, pH 7 which was then concentrated to 0.9 mM in 700 μl. NMR spectra were recorded at 293 K on Varian Inova spectrometers operating at 14.1 and 18.8 T. Sequence specific assignments for the backbone were accomplished using HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNCO experiments (Bax and Grzesiek, 1993). Side chain

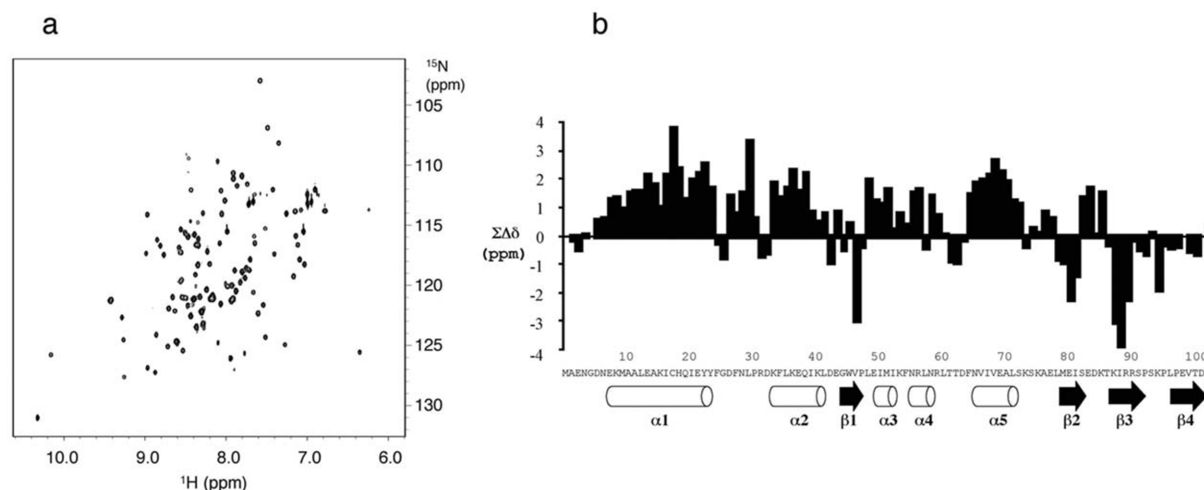

Figure 1. (a) ^{15}N-^{1}H HSQC spectrum of recombinant La1-103 recorded at 14.1 T and 293 K. (b) Plot of the chemical shift deviation from random coil for the La motif. The chemical shift deviation from random coil was obtained by subtracting the experimental values from the random coil chemical shifts and then deriving a weighted consensus value ($\Sigma\Delta\delta$) using $(4\Delta\delta_{H\alpha} + \Delta\delta_{C\beta} - \Delta\delta_{C\alpha} - \Delta\delta_{C'})$/number of assignments. The secondary structure features are also indicated.

resonances were assigned by combining data from the following experiments: ^{15}N-edited TOCSY-HSQC and NOESY-HSQC (Fesik and Zuiderweg, 1988), HCCH-TOCSY and ^{13}C-edited NOESY-HSQC.

Backbone dihedral angles were predicted using TALOS software (Cornilescu et al., 1999). NMRPipe/NMRDraw (Delaglio et al., 1995) was employed to process all spectra which were then analysed using XEASY (Bartels et al., 1995).

Extent of assignment, secondary structure and data deposition

Near complete assignment for 101 out of the 103 backbone resonances of the La motif was achieved (i.e., not including the 2 N-terminal amino acids): 99% of ^{1}H$_{N}$, 99% of ^{15}N (excluding five Proline residues), 100% of ^{13}C$_{\alpha}$, 99% of ^{13}C$_{\beta}$, 92% of ^{13}C$'$ and 99% of ^{1}H$_{\alpha}$have been unambiguously assigned. HNCO experiments were used to obtain ^{13}C$'$ resonances and therefore all ^{13}C' N-terminal to proline residues remain unassigned. Side chain assignments are also almost complete. Missing aliphatic side-chain assignments arise from the N-terminal stretch encompassing amino acids 1–4, N7, E9, N56 and E70 whereas of the 9 aromatic residues 90% of the ^{1}H and ^{13}C resonances have been unambiguously assigned.

Secondary structure elements were determined by the analysis of the chemical shifts of backbone atoms and ^{13}C$_{\beta}$, by characteristic NOE patterns from the ^{15}N-edited NOESY-HSQC and by the results from TALOS software (Figure 1). By revealing that the La motif possesses a ααβαααββ fold, quite different from the archetypal βαββαβ fold found in RRM modules, these data resolve a long-standing debate and unveil the novel nature of this RNA binding domain.

The chemical shifts for backbone and side chain assignments have been deposited in the BioMagResBank (accession number 6044).

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