



King's Research Portal

DOI:

[10.1007/s12104-015-9605-3](https://doi.org/10.1007/s12104-015-9605-3)

Document Version

Publisher's PDF, also known as Version of record

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Martino, L., Salisbury, N. J. H., Brown, P., Kelly, G., Atkinson, R. A., & Conte, M. R. (2015). (1)H, (15)N and (13)C chemical shift assignments of the La motif and RRM1 from human LARP6. *Biomolecular NMR Assignments*, 9(2), 337-340. <https://doi.org/10.1007/s12104-015-9605-3>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

^1H , ^{15}N and ^{13}C chemical shift assignments of the La motif and RRM1 from human LARP6

Luigi Martino^{1,3} · Nicholas J. H. Salisbury^{1,4} · Paul Brown¹ · Geoff Kelly² · R. Andrew Atkinson¹ · Maria R. Conte¹

Received: 11 December 2014 / Accepted: 11 April 2015 / Published online: 22 April 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract We report here the nearly complete ^1H , ^{15}N and ^{13}C resonance assignment of the La motif and RNA recognition motif 1 of human LARP6, an RNA binding protein involved in regulating collagen synthesis.

Keywords LARP6 · La motif · RRM1 · La related protein · RNA binding protein

Biological context

LARP6 is a member of the La related proteins (LARP) superfamily and it has been implicated in several developmental events including myogenesis, neurogenesis and possibly metastasis (Bayfield et al. 2010; Bousquet-Antonelli and Deragon 2009). In vertebrates, LARP6 regulates collagen synthesis by binding to a conserved stem-loop in the 5' untranslated region (UTR) of the mRNAs encoding the collagen $\alpha 1(\text{I})$ and $\alpha 2(\text{I})$ subunits, thereby coordinating their translation into the heterotrimeric collagen type I (Blackstock et al. 2014; Cai et al. 2010a, b). This

interaction is mediated by a conserved RNA binding unit present in LARP6, named the La module, which comprises two domains, a La motif (LaM) and an RNA recognition motif (RRM1). The La module was first discovered in the founding member of theLARPs, the La protein, where the LaM and RRM1 were shown to work in synergy to recognise 3' UUU_{OH} RNA targets (Alfano et al. 2004; Bayfield et al. 2010; Kotik-Kogan et al. 2008). Although the La module is conserved across the LARP superfamily, the recognised RNA targets are not, and this RNA binding versatility is thought, at least in part, to account for the different cellular processes in whichLARPs are involved (Bayfield et al. 2010). Contrary to the archetype La protein, for which high resolution structures of several domains in the apo and bound form, as well as biophysical insights into its RNA binding properties, have been reported (Jacks et al. 2003; Alfano et al. 2004; Teplova et al. 2006; Kotik-Kogan et al. 2008; Martino et al. 2012), theLARPs are much less well understood and the mechanism by which La modules ofLARPs can recognise a great variety of RNA molecules, with different shapes and sequences, is still elusive.

To understand in detail the RNA recognition mechanism ofLARPs we embarked on a structural and biophysical analysis of the LaM and RRM1 from human LARP6 (HsLARP6). Interestingly, this study revealed that the relative orientation of the LaM and RRM1, mainly dictated by the sequence and structure of the interdomain linker, could play a key role in RNA target discrimination by the La module (Martino et al. 2015). These investigations illustrate the complexity of protein-RNA recognition by underscoring the importance of modular types of interaction in achieving binding specificity and affinity.

Since malfunction of collagen production is connected to a number of fibroproliferative disorders the investigation of the RNA binding mechanism of human LARP6 may be

✉ Maria R. Conte
sasi.conte@kcl.ac.uk

¹ Randall Division of Cell and Molecular Biophysics, King's College London, New Hunt's House, Guy's Campus, London SE1 1UL, UK
² MRC Biomedical NMR Centre, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
³ Present Address: Division of Molecular Structure, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
⁴ Present Address: Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK

exploited in the future for rational drug design. Here we report the backbone and the sidechain NMR assignment of the LaM and RRM1 of human LARP6.

Protein expression and purification

For our NMR studies, two human LARP6 domains, the LaM, encompassing residues 70–183, and RRM1, residues 180–295, were prepared as follows. Both domains were cloned into pET-Duet1 vector (Novagen) with an N-terminal histidine tag followed by a TEV-cleavage site. ^{15}N and $^{15}\text{N}/^{13}\text{C}$ labelled recombinant proteins were produced in *Escherichia coli* Rosetta II, growing transformed bacteria in minimal media enriched with 0.8 g L^{-1} ^{15}N -ammonium chloride and 2 g L^{-1} ^{13}C glucose, and induced at $18\text{ }^\circ\text{C}$ for 14 h. Cell pellets were resuspended in 50 mM Tris, pH 8.0, 300 mM NaCl, 10 mM imidazole, 5 % glycerol, 2 mM PMSF (phenylmethanesulfonyl fluoride) and lysozyme, and lysed by sonication. Following centrifugation, the proteins were purified by nickel affinity chromatography on a 5 mL HisTrap column (GE Healthcare) following the manufacturer's protocol. The N-terminal histidine tag was removed by overnight incubation with TEV protease (TEV^{pro}) (at TEV^{pro}:HsLARP6 molar ratio of 1:50) at $4\text{ }^\circ\text{C}$ in 50 mM Tris, pH 8.0, 100 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol (DTT). The reaction mixture was subsequently applied to a Ni-NTA column (Qiagen) to remove the cleaved tags, the His₆-tagged TEV^{pro} and any undigested product, and the cleaved LARP6 proteins were dialysed overnight in 50 mM Tris pH 7.25, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT. The proteins were finally purified on a 5 mL Hi-Trap heparin column (GE Healthcare) and eluted with a linear 0–2 M KCl gradient in 25 mM Tris pH 7.25, 10 % glycerol, 1 mM DTT. The LaM and RRM1 were dialysed in 20 mM Tris pH 7.25, 100 mM KCl, 50 mM arginine glutamate salt (Golovanov et al. 2004), 1 mM DTT and 20 mM Tris pH 7.25, 100 mM KCl, 1 mM DTT respectively.

NMR spectroscopy

NMR samples contained $\sim 0.5\text{ mM}$ protein in 95 % $\text{H}_2\text{O}/5\text{ } \%$ D_2O or 99 % D_2O at pH 7.25 in 100 mM KCl, 50 mM arginine glutamate salt, 1 mM DTT for the LaM and in 20 mM Tris pH 7.25, 100 mM KCl, 1 mM DTT for RRM1. All NMR spectra for backbone and sidechain resonance assignment were collected at 298 K on a Varian Inova spectrometer operating 18.8 T and on Bruker Avance spectrometers at 14.1 and 16.4 T equipped with triple resonance cryoprobes. Backbone resonances were

assigned in a sequential manner using $[\text{}^1\text{H}, \text{}^{15}\text{N}]$ -HSQC, HNCA, HN(CO)CA, HNCACB, HN(CO)CACB and HNCO experiments (Grzesiek and Bax 1993). Sidechain resonances were obtained using $[\text{}^1\text{H}, \text{}^{15}\text{N}]$ -HSQC, $[\text{}^1\text{H}, \text{}^{13}\text{C}]$ -HSQC HCCH-TOCSY ^{15}N -edited NOESY-HSQC and ^{13}C -edited NOESY-HSQC spectra (Fesik et al. 1988). NMR data were processed using NMRPipe/NMRDraw (Delaglio et al. 1995) and visualised/assigned using CcpNMR Analysis 2.2 (Vranken et al. 2005) software and/or CARA/Neasy software (Bartels et al. 1995). Chemical shifts were referenced to internal 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS).

Extent of the assignment and data deposition

Figure 1 shows the assigned $[\text{}^1\text{H}, \text{}^{15}\text{N}]$ -HSQC spectra for the LaM (a) and RRM1 (b) acquired at 298 K. The dispersion of the resonances in both cases suggests that both domains are well folded. The LaM is made of 114 residues of which 6 are prolines and 3 are glycines. For the backbone, 97/107 NH, 102/114 H α , 84/114 CO, 106/114 C α and 100/111 C β resonances were successfully assigned. This corresponds to 90, 89, 74, 92 and 90 % of the NH, H α , CO, C α and C β resonances respectively and implies an 87 % complete backbone assignment. Around 78 % of aliphatic sidechain (position γ onwards) and 65 % of the aromatic side chains ^1H and ^{13}C assignments have also been made. The RRM1 has 116 residues with 7 prolines and 7 glycines. Here the backbone assignment was 88 % complete: assignments have been obtained for 104/108 NH (95 %), 97/116 H α (84 %), 93/116 CO (80 %), 111/116 C α (96 %) and 93/109 C β (85 %) resonances. In addition, around 82 % and 72 % of aliphatic and aromatic ^{13}C and ^1H sidechain resonances have been assigned respectively.

Most of the missing assignments in the two domains correspond to residues T70, A71, S72, Q83, R120, R121, N122, K123, Y126, K130, K136 for the LaM and Q204, K205, N206, G207, S251 for the RRM1, for which peaks in the $[\text{}^1\text{H}, \text{}^{15}\text{N}]$ -HSQC spectra could not be observed. None of the exchangeable sidechain protons of Arg and Lys residues was identified, nor the sidechain amide groups of most Asn and Gln.

Secondary structures were derived from backbone chemical shifts and estimates for ψ/ϕ dihedral angles were obtained using TALOS+ (Shen et al. 2009). As expected, the secondary structure elements predicted for HsLARP6 LaM closely resemble what was previously found for human La LaM (Alfano et al. 2003). Interestingly, the topology for the HsLARP6 RRM1 was found to be $\beta 1\alpha 0'\alpha 1\beta 2\alpha 1'\beta 3\alpha 2\beta 4$; this domain therefore contains two non-canonical secondary structure elements located in the loop between $\beta 1$ and $\alpha 1$ ($\alpha 0'$) and the loop between $\beta 2$ and

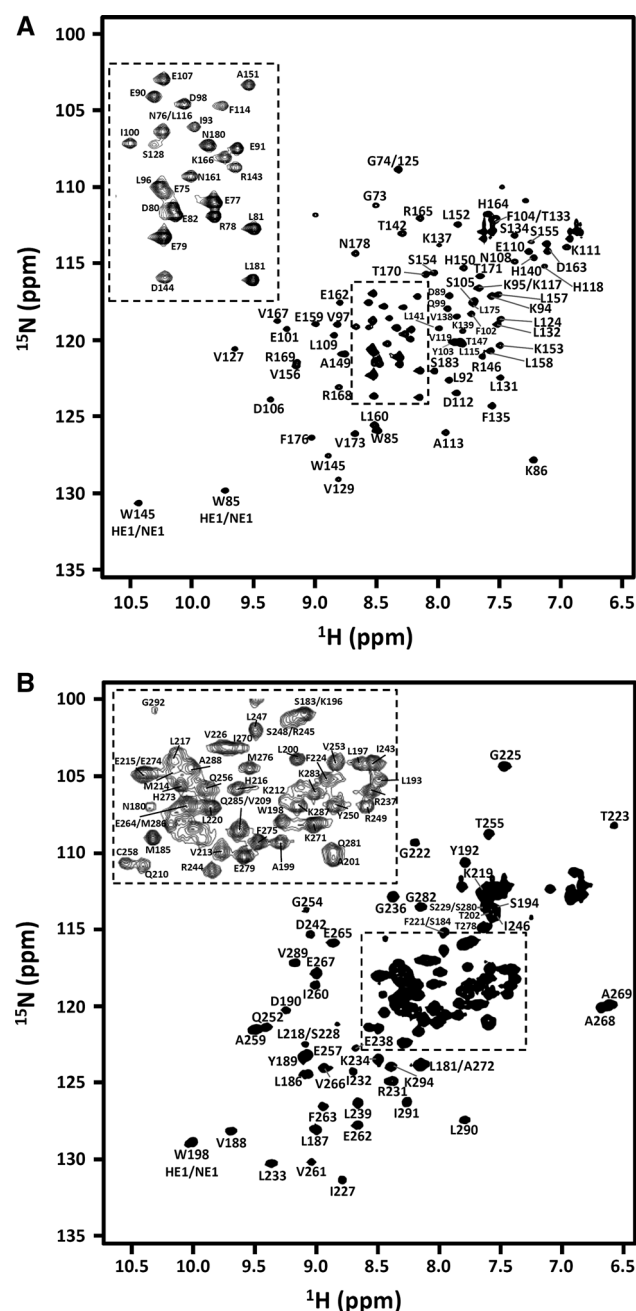


Fig. 1 [^1H , ^{15}N]-HSQC spectra for human LARP6 LaM (a) and RRM1 (b) recorded at 298 K on a Bruker Avance spectrometer working at 16.4 T. Backbone chemical shifts assignments are labelled for both domains. Residue types and numbers are indicated. Residue numbering corresponds to native sequence. For both spectra, top left panels in dotted squares show an expansion of the central crowded [^1H , ^{15}N] HSQC region for clarity. The difference in linewidths between the two domains reflects a different sample behavior in the experimental conditions used, confirmed by relaxation analysis (Martino et al. 2015)

$\beta 3$ ($\alpha 1'$) in addition to the expected four β -strands ($\beta 1$ – $\beta 4$) and two α -helices ($\alpha 1$ and $\alpha 2$) typical of the RRM fold. These findings were confirmed in the high resolution structure recently obtained (Martino et al. 2015).

The chemical shift data were deposited in the BioMagResBank (<http://www.bmrb.wisc.edu/>) under the accession numbers 25159 and 25160 for the LaM and RRM1 respectively.

Acknowledgments This work was supported by the Wellcome Trust through a Capital Award for the Centre for Biomolecular Spectroscopy to MRC (085944/Z/08/Z). LM was recipient of a long-term EMBO Fellowship and NJHS of a MRC studentship funding his MRes in Molecular Biophysics.

Conflict of interest None declared.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Alfano C, Babon J, Kelly G, Curry S, Conte MR (2003) Resonance assignment and secondary structure of an N-terminal fragment of the human La protein. *J Biomol NMR* 27:93–94
- Alfano C, Sanfelice D, Babon J, Kelly G, Jacks A, Curry S, Conte MR (2004) Structural analysis of cooperative RNA binding by the La motif and central RRM domain of human La protein. *Nat Struct Mol Biol* 11:323–329
- Bartels C, Xia TH, Billeter M, Guntert P, Wuthrich K (1995) The program XEASY for computer-supported NMR spectral analysis of biological macromolecules. *J Biomol NMR* 6:1–10
- Bayfield MA, Yang R, Marais RJ (2010) Conserved and divergent features of the structure and function of La and La-related proteins (LARPs). *Biochim Biophys Acta* 1799:365–378
- Blackstock CD, Higashi Y, Sukhanov S, Shai SY, Stefanivic B, Tabony AM, Yoshida T, Delafontaine P (2014) Insulin-like growth factor-1 increases synthesis of collagen type I via induction of the mRNA-binding protein LARP6 expression and binding to the 5' stem-loop of COL1a1 and COL1a2 mRNA. *J Biol Chem* 289:7264–7274
- Bousquet-Antonelli C, Deragon JM (2009) A comprehensive analysis of the La-motif protein superfamily. *RNA* 15:750–764
- Cai L, Fritz D, Stefanovic L, Stefanovic B (2010a) Binding of LARP6 to the conserved 5' stem-loop regulates translation of mRNAs encoding type I collagen. *J Mol Biol* 395:309–326
- Cai L, Fritz D, Stefanovic L, Stefanovic B (2010b) Nonmuscle myosin-dependent synthesis of type I collagen. *J Mol Biol* 401:564–578
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6:277–293
- Fesik SW, Luly JR, Erickson JW, Abad-Zapatero C (1988) Isotope-edited proton NMR study on the structure of a pepsin/inhibitor complex. *Biochemistry* 27:8297–8301
- Golovanov AP, Hautbergue GM, Wilson SA, Lian LY (2004) A simple method for improving protein solubility and long-term stability. *J Am Chem Soc* 126:8933–8939
- Grzesiek S, Bax A (1993) Measurement of amide proton exchange rates and NOEs with water in $^{13}\text{C}/^{15}\text{N}$ -enriched calcineurin B. *J Biomol NMR* 3:627–638

- Jacks A, Babon J, Kelly G, Manolaridis I, Cary PD, Curry S, Conte MR (2003) Structure of the C-terminal domain of human La protein reveals a novel RNA recognition motif coupled to a helical nuclear retention element. *Structure* 11:833–843
- Kotik-Kogan O, Valentine ER, Sanfelice D, Conte MR, Curry S (2008) Structural analysis reveals conformational plasticity in the recognition of RNA 3' ends by the human La protein. *Structure* 16:852–862
- Martino L, Pennell S, Kelly G, Bui TT, Kotik-Kogan O, Smerdon SJ, Drake AF, Curry S, Conte MR (2012) Analysis of the interaction with the hepatitis C virus mRNA reveals an alternative mode of RNA recognition by the human La protein. *Nucleic Acids Res* 40:1381–1394
- Martino L, Pennell S, Kelly G, Busi B, Brown P, Atkinson RA, Salisbury NJH, Ooi ZH, See KW, Smerdon SJ, Alfano C, Bui TT, Conte MR (2015) Synergic interplay of the La motif, RRM1 and the interdomain linker of LARP6 in the recognition of collagen mRNA expands the RNA binding repertoire of the La module. *Nucleic Acids Res* 43:645–660
- Shen Y, Delaglio F, Cornilescu G, Bax A (2009) TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. *J Biomol NMR* 44:213–223
- Teplova M, Yuan YR, Phan AT, Malinina L, Ilin S, Teplov A, Patel DJ (2006) Structural basis for recognition and sequestration of UUU(OH) 3' termini of nascent RNA polymerase III transcripts by La, a rheumatic disease autoantigen. *Mol Cell* 21:75–85
- Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, Laue ED (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins* 59:687–696