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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C assignments and secondary structure identification for full-length ribosomal protein L11 from *Thermus thermophilus*

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Biological context

Ribosomal protein L11 is a strongly conserved component of the large subunit. L11 is hypothesized to be part of the molecular switch regulating translation on the ribosome (Porse et al., 1998). The structure of L11 from *Thermotoga maritima*, bound to its RNA target, has been solved by X-ray crystallography (Wimberly et al., 1999). Strikingly, electron density for this complex is missing in the map for the large subunit at 2.4 Å (Ban et al., 2000). L11 is organized into two domains, one essential for RNA binding and the other implicated in antibiotic binding (Xing and Draper, 1996). We have previously studied the RNA binding domain in solution and discovered that a flexible loop within the protein becomes ordered upon binding to RNA and forms a significant part of the RNA binding surface (Hinck et al., 1997; Markus et al., 1997). Studies of full-length L11 are crucial to understanding in detail the mechanism of antibiotic binding and regulation at the GTPase center. As a first step, we report here nearly complete assignments for the *Thermus thermophilus* L11 and compare its secondary structure in solution with that observed for the *T. maritima* protein bound to RNA and for the free *Bacillus stearothermophilus* C-terminal domain.

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Methods and experiments

T. thermophilus L11 was cloned into the pET11a plasmid and overexpressed in *E. coli* strain BL21(DE3), and the previously reported purification was adapted to our equipment (Triantafillidou et al., 1999). Samples of L11 for NMR contain approximately 1.0 mM protein in 10 mM KH_2PO_4, pH 6.5, 70 mM KCl, 0.1 mM azide, and either 8% or 99% $^{2}H_2O$, as appropriate.

Spectra were acquired at 25 °C on Bruker spectrometers: a DMX500, another DMX500 equipped with a cryoprobe, or a DMX750, all with triple resonance probes and gradients. Spectra were processed with NMRDraw and analyzed with PIPP. Sequential backbone assignments were based on the HNCACB, CBCA(CO)NH, HNCA, HNCO and HCACO experiments. Side chain assignments were determined with the C(CO)NH, HCCH-TOCSY, HBHA(CO)NH and H(CCO)NH experiments. $^{3}J_{HNHA}$ couplings were determined with the HNHA experiment. $^{3}J_{NHB}$ and $^{3}J_{HAHB}$ were determined with the HNHB and HAHB experiments and used to determine stereospecific assignments at the β-methylene position. Methionine methyl groups were assigned using information from the long-range CC experiment. NH_2 groups were correlated with specific side chains using the CBCA(CO)NH. Side chain carbonyl carbons were assigned using information in the HNCO and HCACO. Arginine ε positions were assigned via NOEs to γ and δ positions in the chain. Aromatic rings were identi-

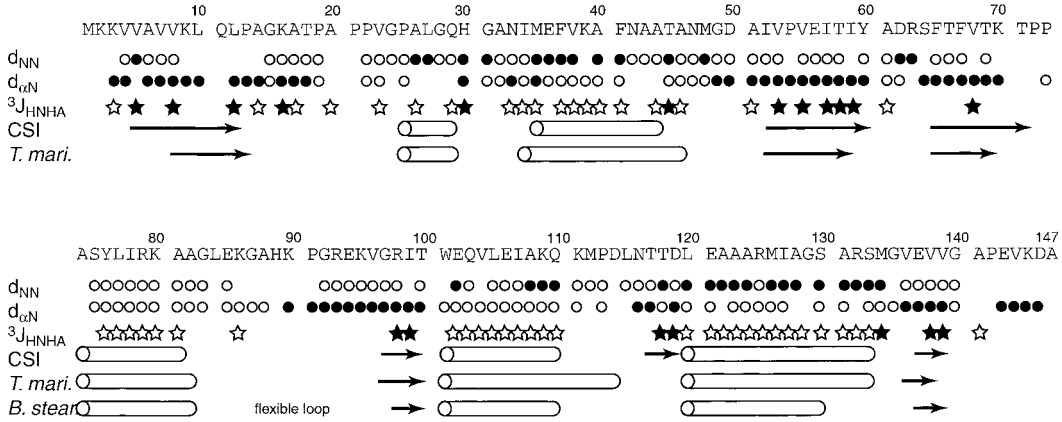

Figure 1. Summary of secondary structure for *T. thermophilus* L11 and a comparison with *T. maritima* L11 and the C-terminal domain of *B. stearothermophilus* L11. The first line gives the amino acid sequence for L11 from *T. thermophilus*. NOEs observed between backbone amide protons at positions i and i+1 are indicated on the next line (d_{NN}), with an empty circle for weaker crosspeaks and a filled circle for stronger cross peaks. Strong HN-HN cross peaks are characteristic of helices. The $d_{\alpha N}$ line contains a circle for all residues where NOEs are observed between Hα of residue i and HN of residue i+1. Strong Hα-HN cross peaks are indicative of extended strands. NOEs were identified in a 3D ^{15}N separated NOESY recorded at 750 MHz. Note that whenever overlap obscures a possible sequential cross peak, no symbol is recorded in the figure. For proline, Hδ replaces HN. The $^3J_{HNHA}$ line records the intraresidue HN to Hα scalar coupling, measured in the HNHA experiment, with an empty star for couplings less than 5.5 Hz (typical for helices) and a filled star for couplings over 8.0 Hz (typical for extended strands). The line labelled CSI shows the secondary structure based on the Hα, Cα, Cβ, and C′ chemical shifts (Wishart and Sykes, 1994), with cylinders indicating α helices and arrows indicating extended strands. The line labelled *T. mari* shows the secondary structure based on the crystal structure of L11 from *Thermotoga maritima* bound to RNA (Wimberly et al., 1999). Note that there are two proteins per asymmetric unit in the crystal and that each protein has different identified regions of secondary structure due to disorder. The maximum extent of secondary structure, determined by comparing the two proteins, is recorded. The line labelled *B. stear*. gives the secondary structure for the C-terminal domain of L11 from *Bacillus stearothermophilus*, based on the previously solved NMR structure (Markus et al., 1997). The top half of the figure corresponds to the N-terminal domain of L11 and the bottom half corresponds to the C-terminal domain of L11.

fied in a 2D ^{1}H-^{1}H TOCSY, the carbon assignments were obtained from a constant time ^{13}C HSQC, and the connections to the backbone spin systems were obtained from a ^{13}C-separated NOESY. Assignments were largely verified with an ^{15}N-separated NOESY. These experiments have been described previously; see Markus et al. (1997).

Extent of assignments and data deposition

For the 147 residues of L11, backbone assignments are essentially complete, except for HN and N of M1 and K2 and N of all 11 prolines. There are no assignments for P21 and P72, which both precede proline residues. Side chain assignments are also mostly complete. Gaps in the side chain assignments include ε for F37 and F67, ε and ζ for F65, Cγ for M48, Hγ, Hδ, and ε for K111, γ for L115, side chain carbonyl for D62 and D119, and ε for R79, R93, and R132. Stereospecific assignments were established for 32 of 79 residues with β-methylene groups. Secondary structure information is summarized in Figure 1.

The ^{1}H, ^{15}N, and ^{13}C chemical shifts at 25 °C, pH 6.5, have been deposited in the BioMagResBank under accession number 4965.

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