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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N assignments of the ribosome recycling factor from *Thermus thermophillus*

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Biological context

Termination of protein synthesis and the release of polypeptide products are signaled by the presence of a stop-codon in the ribosomal A-site. In Eubacteria, release factors (RF1, RF2 and RF3) recognize the stop-codon, trigger peptidyl-tRNA hydrolysis and peptide release, and dissociate from the ribosome (Nakamura et al., 2000). Following termination the ribosome is left in a 'post-termination' state composed of the 70S ribosome, the RNA message, the final P-site deacylated tRNA and an empty A-site. Ribosomal release factor (RRF) orchestrates the disassembly of the post-termination complex (Hirokawa et al., 2002; Ito et al., 2002; Janosi et al., 1996). 70S or 50S dissociation promotes a 'recycling' of the 30S and 50S subunits allowing translation to initiate on other RNA-messages; requirements for cell growth and efficient protein synthesis. Structures of ribosome recycling factors (Kim et al., 2000; Selmer et al., 1999; Toyoda et al., 2000) indicate RRF proteins adopt an L-shape consisting of a three-helix coil and a $\beta/\alpha/\beta$ sandwich separated by two loops. A similar shape to tRNA suggests RRF functions through tRNA mimicry. Ribosome recycling proteins are found only in prokaryotes and their vestiges (chloroplasts and mitochondria), making ribosome recycling an ideal anti-microbial target.

Although structures of RRF are known, little is known about structural features responsible for the recycling action. Genetic studies suggest that ribosome recycling is dependent upon the structural or dynamic properties of the linker (or 'hinge' regions) between the two domains (Toyoda et al., 2000). Here we report backbone ^{1}H, ^{15}N and ^{13}C resonance assignments for the RRF from *Thermus thermophillus* (ttRRF) which are paramount for structural and dynamic comparisons of genetic variants that modulate RRF function.

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Methods and experiments

Expression of ttRRF has been previously described (Toyoda et al., 2000). Proteins were expressed in minimal media containing either ^{15}N-NH_4Cl (2 g/l) or ^{15}N-NH_4Cl and ^{13}C-D-glucose (2 g/l). Purification of ttRRF followed previously described methods (Toyoda et al., 2000). NMR samples were in 50 mM potassium phosphate (pH 6.40), 50 mM NH_4Cl and 10% $^{2}H_2O$, with protein concentrations of 1.05 mM.

NMR experiments were performed at 37 °C on Varian INOVA 750, 600 and UNITY 500 MHz spectrometers. For backbone sequential assignments, the following experiments were used: 2D ^{1}H,^{15}N-HSQC, CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, (H)C(CO)-C-TOCSY-NH, ^{13}C-edited NOESY-HSQC, and ^{15}N-edited NOESY-HSQC. Assignments were confirmed using U-^{2}H/^{13}C/^{15}N labeled ttRRF and the following experiments: HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, (H)C(CO)-C-TOCSY-NH and a ^{15}N-edited 3D NOESY-HSQC (for details see Gardner and Kay, 1998). NMR spectra were processed in FELIX 97 (MSI) and analyzed for resonance assignment in Sparky (T.D. Goddard and D.G. Kneller, UCSF). Spectra were referenced directly (^{1}H) and indirectly (^{13}C, ^{15}N and ^{2}H) to DSS (Wishart et al., 1995).

Extent of assignments and data deposition

For ttRRF, backbone amides were unambiguously assigned for 177 residues (Figure 1; 185 amino acids

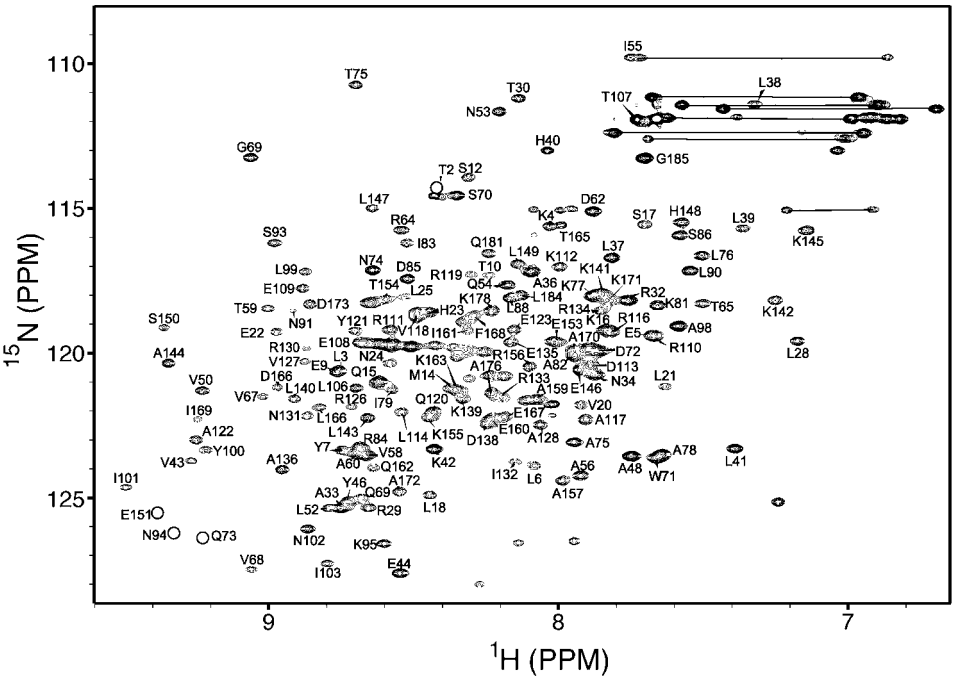

Figure 1. The assigned ^{1}H-^{15}N HSQC of the ribosome recycling factor from *Thermus thermophillus* at 37 °C, pH 6.4. For clarity several assignments are omitted or lie outside the spectral view. Gln/Asn sidechain cross peaks are denoted with horizontal lines. The cross peaks for 2, 151, 94 and 73 are visible at lower contours at positions indicated by circles.

minus seven prolines and the N-terminal methionine). Transverse relaxation rates differ remarkably within ttRRF, ranging from 19 S^{-1} in the three-helix coil domain to 10 S^{-1} in the $\beta/\alpha/\beta$ sandwich. Complete assignments for the protonated protein were not possible without reference to assignments in the perdeuterated ttRRF. Amide signals for several amino acid residues are weak in the triple-resonance spectra (Figure 1), presumably due to conformational or rapid solvent exchange at these amide protons. Since D62 lies between two prolines, backbone shifts could not be assigned uniquely from triple-resonance data, but inter-residue NOEs support these assignments. Missing Cβ resonances (10, 17 and 154) were confirmed in triple resonance experiments of the perdeuterated ttRRF. All carbonyl and Cα assignments have been made with the exceptions of M1 and P104. Secondary structural elements were identified using chemical shift indices (Wishart and Sykes, 1994) and inter-residue NOEs and were similar to other RRF structures. The backbone resonance assignments (Cα, Cβ, C′, N and H_N) for ttRRF have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5458. The assignments provide a platform to study structural and dynamic changes associated with functional alterations about the hinge regions in the ribosome recycling factor.

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