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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignment of the N-terminal domain of human eRF1

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Biological context

In translation termination processes, an in-frame stop codon (either UAG, UGA or UAA) is recognized directly by a class-I release factor (RF1/RF2 in prokaryotes and eRF1 in eukaryotes). The eukaryotic release factor eRF1 recognizes all three of those stop codons, but not the Trp codon UGG. This discrimination must require some conformational changes in the recognition domain of eRF1, because the UGG codon cannot be eliminated if eRF1 recognizes the stop codons by simply binding to A/G as the second letter and A/G as the third. Indeed, although human eRF1's crystal structure was reported (Song et al., 2000), the recognition mechanism has not been clearly illustrated. Muramatsu et al. published the hypothesis that the stop codon triplet is bound in the region containing the C-terminus of α2-helix of eRF1 (Glu55-Asn61) and that the discrimination of the three stop codons from the Trp codon requires a structure consisting of two α-helices (α2 and α3) and two β-strands (β1 and β4), which regulate the conformational flexibility of the tip region (Muramatsu et al., 2001). Recently, cross-linking experiments between eRF1 and mRNA in ribosome suggested that the entire domain is involved in the recognition (Chavatte et al., 2003). Thus, to address the mechanism underlying stop codon recognition, it is important to investigate the dynamic structure of this domain in solution. Here we report ^{1}H, ^{15}N and ^{13}C resonance assignments for the N-terminal domain [Met_1-Asp_{142}] of human eRF1.

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Methods and experiments

The cDNA region coding for the N-terminal domain [Met_1-Asp_{142}] of human eRF1 was amplified by RT-PCR using human stomach total RNA (BD Biosciences Clontech, Palo Alto, CA) as a template and was then subcloned into pTWIN1 (New England Biolabs, Beverly, MA). This domain, accompanied by chitin-binding domains at both the N- and C-termini, was overproduced in *E. coli* Origami(DE3)pLacI (Novagen, Madison, WI). Then both of the chitin binding domains were removed by the affinity purification procedure described by the supplier (New England Biolabs), and the N-terminal domain of human eRF1 was further purified by cation exchange chromatography. The recombinant protein obtained by this protocol has additional N-terminal residues (Gly-Arg) and an additional C-terminal residue (Met). ^{15}N-labeled and $^{13}C/^{15}N$-labeled proteins were obtained by the same expression system described above in C.H.L. media (^{15}N: 95% and ^{15}N, ^{13}C: 95%, respectively) (Shoko, Tokyo). These purified proteins were dialyzed against NMR buffer (20 mM Na-phosphate (pH 6.0), 100 mM NaCl, 2 mM DTT and 0.02% NaN_3 in 90% H_2O/10% D_2O) and concentrated to approximately 0.5 mM with size-exclusion filters (Orbital Biosciences, Topsfield, MA).

All NMR data were recorded at 298K on Unity Inova 600 spectrometers (Varian Inc., Palo Alto, CA). HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH spectra were used to attain assignments of backbone resonances. All data were processed using NMRPipe (Delaglio et al., 1995) and analyzed using Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco). ^{1}H chemical shifts

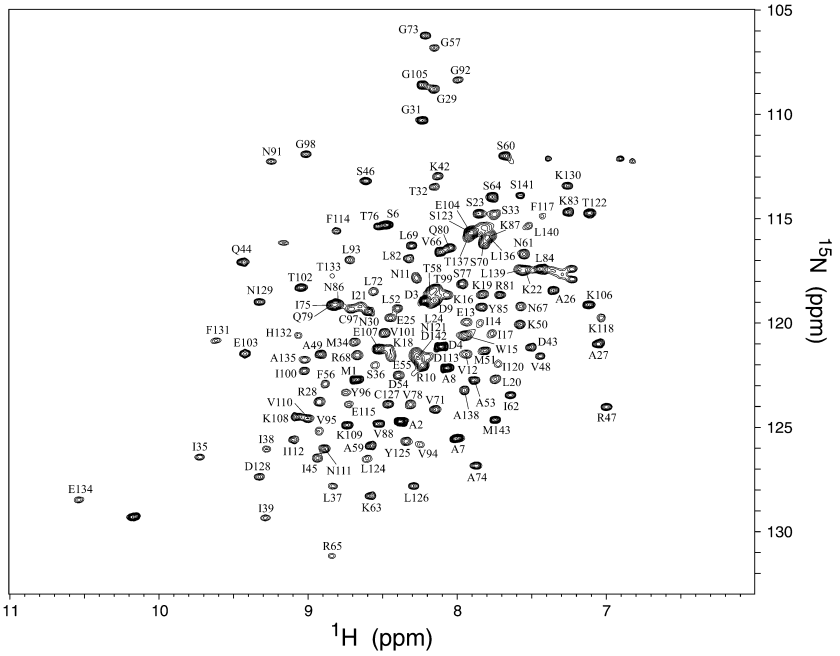

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum with assignments of N-terminal domain of human eRF1 in 20 mM Na-phosphate (pH 6.0), 100 mM NaCl, 2 mM DTT and 0.02% NaN_3 in 90% H_2O/10% D_2O at 298K. The numbering of the residues follows the residue numbers of native eRF1 except for the non-native additional residue M143. Side chain peaks are not labeled.

were referenced to the ^{1}H resonance frequency of DSS (Wishart et al., 1995).

Extent of assignment and data deposition

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum of the N-terminal domain [Met_1-Asp_{142}] of human eRF1. All $^{1}H^{N}$ and ^{15}N resonances of native non-proline backbone residues have been assigned. Definitive assignments for the two N-terminal non-native residues have not been obtained due to the rapid amide proton exchange with water. The assigned ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 6116.

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