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ARTICLE

Backbone ^{1}H, ^{13}C and ^{15}N resonance assignments of the human eukaryotic release factor eRF1

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Abstract Eukaryotic translation termination is mediated by two interacting release factors, eukaryotic class 1 release factor (eRF1) and eukaryotic class 3 release factor (eRF3), which act cooperatively to ensure efficient stop codon recognition and fast polypeptide release. eRF1 consisting of three well-defined functional domains recognizes all three mRNA stop codons located in the A site of the small ribosomal subunit and triggers hydrolysis of the ester bond of peptidyl-tRNA in the peptidyl transfer center of the large ribosomal subunit. Nevertheless, various aspects of molecular mechanism of translation termination in eukaryotes remain unclear. Elucidation of the structure and dynamics of eRF1 in solution is essential for understanding molecular mechanism of its function in translation termination. To approach this problem, here we report NMR backbone signal assignments of the human eRF1 (437 a.a., 50 kDa).

Keywords Termination of protein synthesis · Human polypeptide release factor eRF1 · NMR assignments · Protein domains

Abbreviations

C-domain	eRF1 C-terminal domain
eRF1	Eukaryotic class 1 release factor
eRF3	Eukaryotic class 2 release factor
M-domain	eRF1 middle domain
N-domain	eRF1 N-terminal domain
PTC	Peptidyl transferase center of the ribosome
RF	Prokaryotic release factor
SAXS	Small angle X-ray scattering

Biological context

Translation termination on eukaryotic ribosome occurs in response to a stop codon in the ribosomal A-site and is mediated by the two interacting polypeptide chain release factors eRF1 and eRF3 (Dever and Green 2012; Jackson et al. 2012; Kisselev et al. 2003). Key participant of this process is a class I termination factor, eRF1, which binds to the A site of the ribosome and recognizes all three mRNA stop codons in the decoding site of the small ribosomal subunit. After the transfer of termination signal from the small ribosomal subunit to the peptidyl transferase center (PTC) of the large ribosomal subunit, eRF1 triggers the hydrolysis of pypidyl-tRNA ester bond in PTC which leads to the release of nascent protein. Although analogous in function the molecular mechanism of the translation termination in eukaryotes and prokaryotes are different. Bacteria has two class 1 release factors, RF1 and RF2, and

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each recognizes two out of three stop codons with overlapping specificity. In contrast, eukaryotes employs a single eRF1 to recognize all three stop codons. Translation termination in prokaryotes and eukaryotes also requires class two release factors, RF3 and eRF3, both are ribosome-dependent GTPases but with limited homology and different in fuction (Jackson et al. 2012; Klaholz 2011). The mechanism of stop codon decoding in eukaryotes by eRF1 have been intensively studied by combination of kinetic, biochemical and genetic approaches and several models of eukaryotic stop codon recognition have been proposed (Muramatsu et al. 2001; Kisselev et al. 2003; Kryuchkova et al. 2013). However, there is no established mechanism capable of explaining the potency of eRF1 to decode all three stop codons (UAA, UAG and UGA). Little is known about the molecular mechanism by which the termination signal is transferred from the small to the large ribosomal subunit, to trigger subsequent peptidyl-tRNA ester bond hydrolysis. Principal obstacles to understand details of the molecular mechanism of translation termination in eukaryotes arise from the limited structural data available.

Crystal structure at atomic resolution was recently obtained for ribosomes from low eukaryotes, *S. cerevisiae* (Ben-Shem et al. 2011), however X-ray structure of eukaryotic translation termination complexes has not yet been reported. At present only the crystal structure of the human eRF1 was reported (Song et al. 2000). eRF1 consists of three structurally independent domains, each of which can be assigned with a specific function. The N-terminal domain (residues 1–142) is involved in the stop codon recognition. The middle (M) domain (residues 143–275) contains the strictly conserved GGQ motif that extends into the PTC and is essential in promoting hydrolysis of the peptidyl-tRNA ester bond. The C-domain (residues 277–437) of eRF1 binds to eRF3 and this interaction increases the efficiency of translation termination. However, the crystal structure of eRF1 has relatively low resolution (2.8 Å) and the middle and C-terminal protein domains are poorly defined.

Small angle X-ray scattering studies of human eRF1 (Kononenko et al. 2004) showed that the overall shape of eRF1 in solution is similar to that in crystal, however conformational dynamics arising from the flexible linkers connecting the three domains could not be assessed from the SAXS data. Recently we have determined the high resolution NMR structures of all three domains of human eRF1: N-domain (Polshakov et al. 2012), M-domain (Ivanova et al. 2007) and C-domain (Mantsyzov et al. 2010). Noticeable differences between solution structure and crystal structure were found for all three domains. Among the most important discrepancies are: (1) The orientations of the long helix-loop of the M-domain (residues 176–200), which contains strictly conserved and functionally important GGQ tripeptide, are different in the two structures; and (2) The mini-domain containing residues 329–372 is observable in solution but is missing in the crystal structure, presumably due to mobility or disorder of this protein fragment. In order to elucidate the structure and dynamics of the full length human eRF1, NMR assignments for the protein backbone resonances have been obtained.

Methods and experiments

The full-length cDNA encoding human eRF1 with the C-terminal His_6-tag fusion was cloned into pET23b(+) vector (Novagen) under the control of phage T7 RNA polymerase promoter. The full size eRF1 (residues 1–437) was overproduced in *E. coli*, strain BL21(DE3)/pUBS, in M9 medium. For uniform ^{2}D, ^{13}C and ^{15}N isotope labeling, [$^{13}C_6$,$^{2}D_7$]-D-glucose (Isotec Stable Isotopes) and $^{15}NH_4Cl$ (Cambridge Isotope Laboratories Inc.) were used as the isotope sources in M9 minimal medium in 99.9 % D_2O solvent. Protocol for bacterial growth in D_2O was similar to that described earlier (Gardner and Kay 1998) with minor modifications. These include two-step increase of D_2O concentration (50 and 75 %) during the initial stage of the growth of bacterial colony in small (50 ml) volume, before replanting of the cells in larger volume of M9 media in 100 % D_2O. Such gradual increase allows easier adaptation of the *E. coli* cells to deuterium oxide. Typically, level of expression of the ^{2}D,^{13}C,^{15}N-labelled human eRF1 was ~40 mg/L. The protein was isolated using Ni–NTA resin (Qiagen) and additionally purified by cation exchange chromatography using HiTrap SP columns (Pharmacia). The protein sample was concentrated to 0.2 mM and dialyzed against 25 mM sodium phosphate buffer (pH 6.8) and 100 mM NaCl. The protein was found to be unstable and precipitates at concentration above 0.3 mM or ionic strength below 100 mM NaCl. Even at 0.2 mM concentration and high ionic strength condition human eRF1 still precipitated with a half time of ~24 h at 305 K. Noticeable enhancement in stability was found when L-Arg and L-Glu was added to the NMR sample solution to a final concentration of 50 mM each that allowed us to record several heteronuclear multidimensional NMR spectra for backbone resonance assignments (Golovanov et al. 2004).

The NMR samples were prepared in 95 % H_2O/5 % D_2O, 100 mM NaCl and 25 mM sodium phosphate buffer (pH 6.8), with or without 50 mM Arg + Glu mixure. Dithiothreitol in concentration of 2 mM was added to the final solution in order to prevent oxidation of the four free cysteine residues (C97, C127, C302 and C335). Spectra were acquired at 305 K on a Bruker AVANCE 850 MHz spectrometer equipped with a triple resonance z-gradient

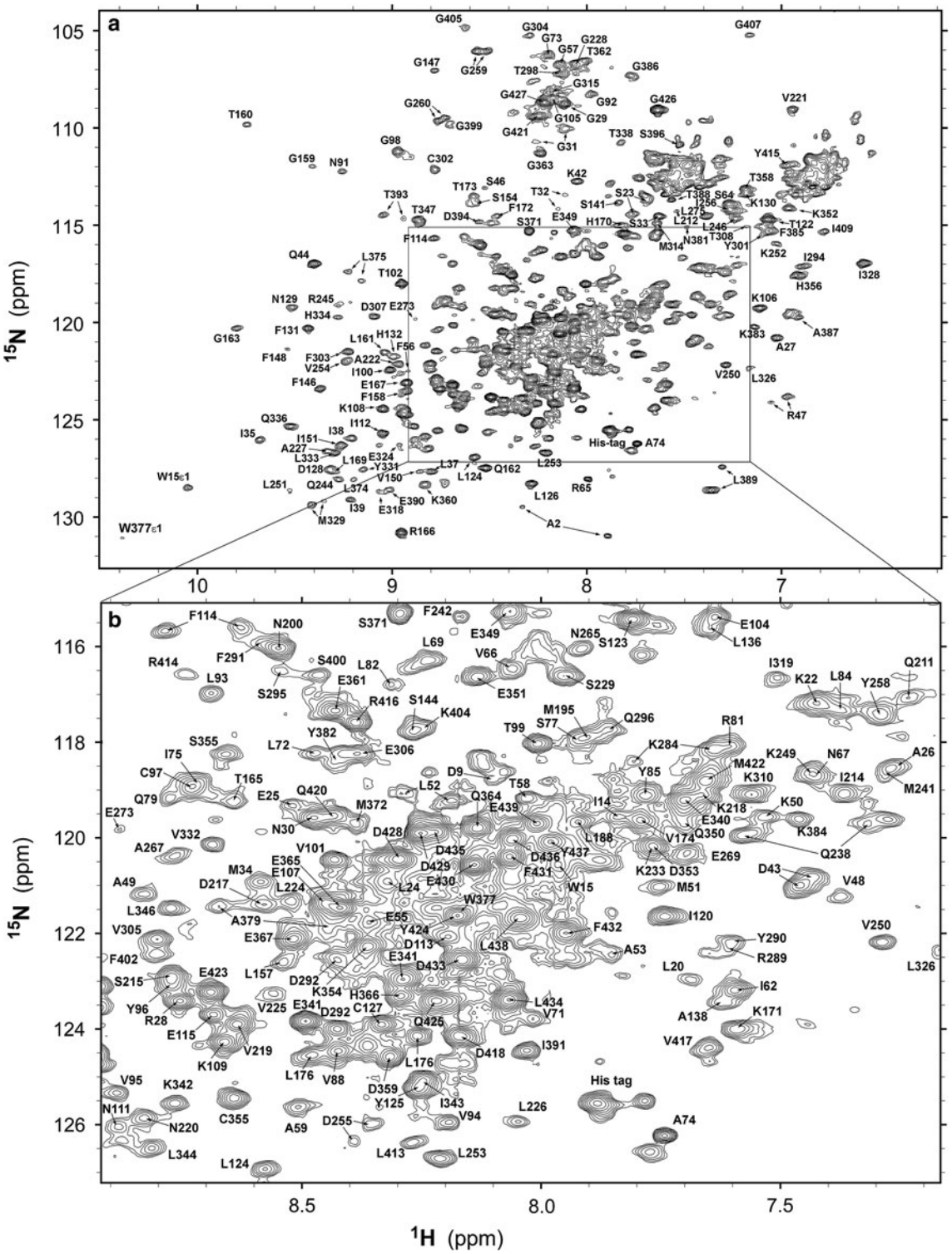

Fig. 1 The ^{1}H,^{15}N-TROSY spectrum of the human eRF1 recorded at 850 MHz (**a**) and expanded view of the most crowded *central region* (**b**). Drawn are representative resonance assignments including residues existing in two conformational states

cryoprobe. Acquiring spectra at lower temperatures with an aim of increasing protein stability resulted in substantial broadening of the resonances and deteriorated the spectral quality. ^{1}H, ^{13}C and ^{15}N resonance assignments were determined using the following set of triple-resonance experiments: 2D ^{15}N–^{1}H TROSY (Pervushin et al. 1998)

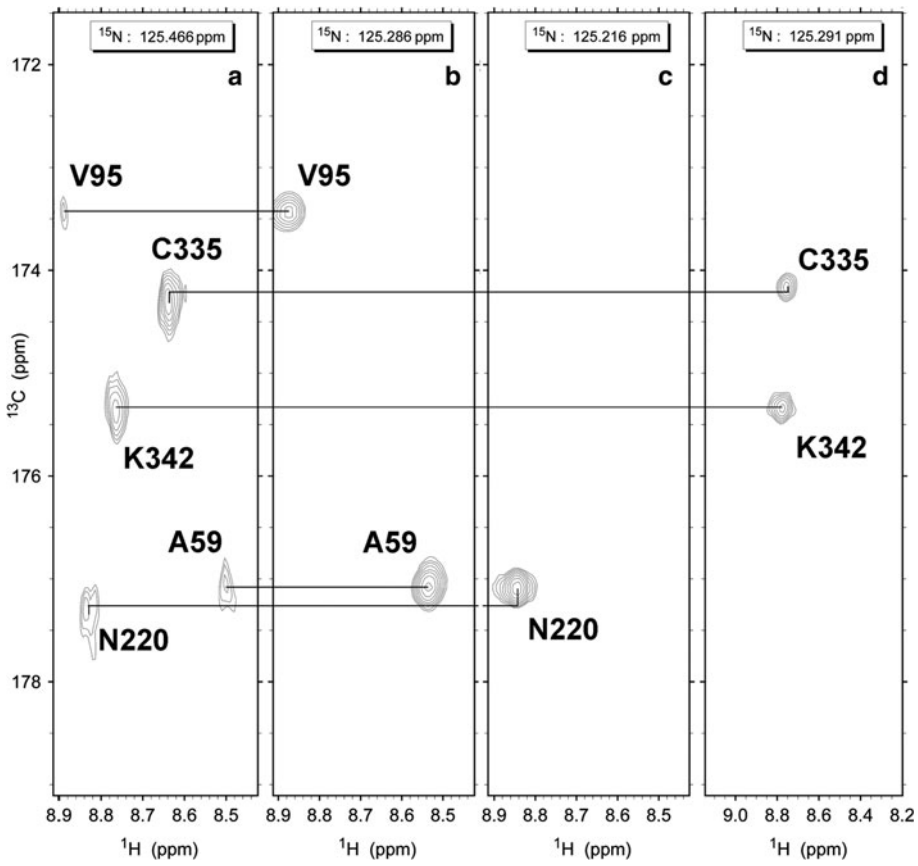

Fig. 2 Comparison of strips from the 3D TROSY-HNCO spectrum of the full length human eRF1 (**a**) and corresponding strips from 3D HNCO spectra of individual eRF1 domains: N-domain (**b**), middle domain (**c**) and C-terminal domain (**d**)

and 3D ^{15}N–^{1}H TROSY-HNCO, TROSY-HN(CA)CO, TROSY-HNCA, TROSY-HNCACB and TROSY-HN(CO)CACB (Salzmann et al. 1999). Spectra were processed by NMR Pipe (Delaglio et al. 1995), and analyzed using SPARKY (from Goddard and Kneller).

Assignments and data deposition

Backbone resonance assignments were based on 3D heteronuclear NMR experiments carried out on ^{2}D,^{13}C,^{15}N-labelled human eRF1. Figure 1 presents a TROSY spectrum of the human eRF1 recorded at 305 K and illustrates state of the protein. There are many signals with high intensity due to increased mobility of corresponding residues. These are primarily from unstructured C-terminal tail (residues 414–437) and minidomain 329–372. In contrast, many backbone amide signals have reduced intensity and increased line width, corresponding to residues involved in conformational exchange at ms time scale. There are also signals (mainly from the M-domain of eRF1) which could not be found in 2D and 3D NMR spectra due to unfavorable exchange (either conformational or amide proton exchange with water). Attempts to decrease temperature result in substantial line broadening for many signals. This confirms an assumption about complex conformational behavior of the protein. Unfortunately, human eRF1 appeared to be rather unstable at higher temperature; therefore 305 K was chosen as a compromised temperature. At this temperature it was possible to carry out NMR experiments during ~24 h time span using a single sample. Stability of the protein is enhanced in Arg+Glu media (Golovanov et al. 2004), however this doesn't have impact on the signal line widths.

Previously, nearly complete NMR assignments have been obtained for the N-terminal domain [BMRB-18092 (Polshakov et al. 2012)], the middle (M) domain [BMRB-6763 (Ivanova et al. 2006)] and the C-terminal domain [BMRB-15366 (Mantsyzov et al. 2007)]. For many residues of the full length human eRF1 signal assignments were obtained by comparing the chemical shifts for ^{15}N, $^{1}H_N$, ^{13}C', $^{13}C\alpha$ and $^{13}C\beta$ nuclei with corresponding values for the individual domains. Figure 2 shows an example of such correlations observed in 3D TROSY-HNCO spectrum. HNCO spectrum appeared to be the most informative due to its highest signal-to noise ratio. However, in many cases it was necessary to use complementary information from TROSY-HN(CA)CO, TROSY-HNCA, TROSY-HNCACB and TROSY-HN(CO)CACB experiments to obtain correct signal assignments.

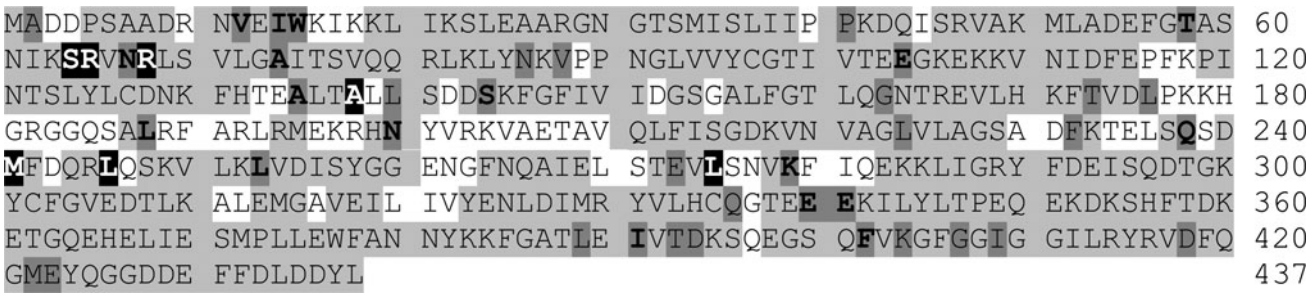

Fig. 3 Primary sequence of the human eRF1 marked according to the completeness of NMR signal assignments and RMSD for chemical shifts between full length protein and individual domains. Assigned residues are *color-coded*. Residues drawn on *white* background are unassigned. *Dark grey* and *black* backgrounds correspond to the residues which have RMSD values greater than the mean RMSD value for the whole protein. Residues drawn by *bold white* on *black* background have the most significant differences (fivefold mean RMSD or greater) in chemical shifts between the full size eRF1 and individual domains

Chemical shift assignments were made for resonances from 370 of 437 residues (85 %) of the human eRF1. 89, 67 and 92 % of the residues from the N, M and C-domains correspondingly were identified in the NMR spectra of the full-size human eRF1. Complete set of the ^{1}H, ^{13}C and ^{15}N backbone resonances were assigned in 163 (37 %) protein residues. We were unable to assign resonances in NMR spectra for 67 of 437 residues. Most of the missing residues are from the M-domain of eRF1 (Fig. 3). In total, chemical shifts assignments were made for 71 % of $^{1}H^{N}$ and non-prolyl ^{15}N resonances, 25 % of $^{13}C^{\alpha}$, 31 % of $^{13}C^{\beta}$ and 65 % of $^{13}C'$ resonances.

Figure 3 illustrates completeness of the signal assignments and the chemical shift differences between the full size human eRF1 and its individual domains. Such residues with large chemical shift differences reveal the functionally important parts of the protein. Thus, residues 58–68 belong to the universally conserved NIKS motif, responsible for the recognition of the first stop codon nucleotide (Kryuchkova et al. 2013). Changes of the chemical shifts reflect structural plasticity of this fragment. Residues A135, A138, S144, L275 and K279 are situated on the border of the domains. Residues V12, I14, W15 of the N-domain and L389, I391 of the C-domain are likely to form an interface between two domains. Detailed analysis of the observed differences and their relationship with the structure and dynamics of human eRF1 will be published elsewhere.

The backbone ^{1}H, ^{15}N and ^{13}C chemical shifts have been deposited in the BioMagResbank database (http://www.bmrb.wisc.edu) under the accession number BMRB-19506.

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