From structure and dynamics of protein L7/L12 to molecular switching in ribosome.

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RUNNING TITLE:
Structure of L7 dimer in solution
SUMMARY

Based on the $^1$H-$^{15}$N NMR spectroscopy data the three-dimensional structure and internal dynamic properties of ribosomal protein L7 from *E. coli* were derived. The structure of L7 dimer in solution can be described as a set of three distinct domains, tumbling rather independently and linked via flexible hinge regions. The dimeric N-terminal domain (residues 1-32) consists of two antiparallel $\alpha-\alpha$-hairpins forming a symmetrical four-helical bundle, whereas the two identical C-terminal domains (residues 52-120) adopt a compact $\alpha/\beta$ fold. There is an indirect evidence of the existence of transitory helical structures at least in the first part (residues 33-43) of the hinge region. Combining structural data for the ribosomal protein L7/L12 from NMR spectroscopy and x-ray crystallography it was suggested that its hinge region acts as a molecular switch, initiating ratchet-like motions of the L7/L12 stalk with respect to the ribosomal surface in response to elongation factor binding and GTP hydrolysis. This hypothesis allows explaining events observed during the translation cycle and provides useful insights into the role of protein L7/L12 in the functioning of the ribosome.
INTRODUCTION

Ribosomes are complex and dynamic ribonucleoprotein assemblies, which provide the framework for protein biosynthesis in all organisms (see for review 1-4). One of the most remarkable features of the large subunit of the ribosome is the presence of a highly flexible protuberance called the stalk (see for review 5-8). Various conformations of the stalk are thought to reflect different functional states of the ribosome, which are essential for tRNA binding and translocation of peptidyl-tRNA from A- to P-site. In prokaryotes the stalk includes a highly conserved acidic 12-kDa protein L7/L12 (L7 is the N-terminal acetylated form of L12), which is present in four copies as two dimers associated with other ribosomal components via protein L10. Both L7/L12 dimers are necessary for optimal rates of protein synthesis and proper function of elongation factors, but a single dimer is sufficient for ribosomal activity (9). Spatial contacts of the L7/L12 stalk with elongation factors’ G and Tu (EF-G and EF-Tu) were observed by electron cryomicroscopy (10, 11), in keeping with the importance of L7/L12 for either factor binding to ribosome or stimulation of factor-dependent GTPase activity (12). Moreover, conformational changes in L7/L12 were revealed by limited proteolysis upon EF-G and EF-Tu binding with GTP or GDP to ribosome (13). Current X-ray crystallographic analysis failed to resolve the fine structural state of L7/L12 within the ribosome, since electron density for L7/L12 stalk was not apparent even in the recent 2.4 Å resolution structure of the large ribosomal subunit (14). Thus, high resolution X-ray crystallographic and NMR studies of the isolated protein L7/L12 and its complex with the protein L10 may still provide valuable insights into the atomic structures of the L7/L12 stalk.

Based on structural and biochemical investigations, the protein L7/L12 is composed of two distinct, organized domains connected by an extensive linker, so-called “hinge” region (5-8, 13, 15). The N-terminal domain (NTD) is responsible for L7/L12 dimerization and for anchoring the protein to the ribosome, while the C-terminal domain (CTD) is involved in translation factor interaction (6). In solution the hinge regions of L7/L12 dimer have an unordered flexible structure that enables independent movement of both CTDs relative to each other and to the dimeric N-terminal domain (dNTD) (15-19). The removal of one, although not two, CTD from L7/L12 dimer gives no marked effect on the ribosomal translation activity (20), while the deletions of the hinge residues significantly reduce the mobility of the CTDs and decrease activity of the modified ribosome (21, 22). Earlier, the crystal structure of Escherichia coli L7/L12 C-terminal fragment consisting of three β-strands and three α-helices has been solved at 1.7 Å resolution (23). NMR study of the Escherichia coli L7

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*The abbreviations used are: EF-G and EF-Tu, elongation factors G and Tu, respectively; NTD and CTD, N- and C-terminal domains of L7/L12; dNTD, dimeric NTD; RDC, residual dipolar coupling; NOE, nuclear Overhauser enhancement; CPMG, Carr-Purcell-Meiboom-Gill; r.m.s.d., root mean square deviation.*
dimer in solution has shown α-helical hairpin conformation of NTD (16, 19). Recently the crystal structure of isolated L12 from the hyperthermophilic bacterium *Thermotoga maritima* with two full-length molecules and two proteolysed N-terminal fragments associated into tetramer in the asymmetric unit was reported (24). The *T. maritima* protein L7/L12 is highly homologous to the *E. coli* counterpart with 64.7% sequence identity. In contrast to the parallel arrangement of the subunits in the dNTD assumed earlier (16), this crystal structure reveals antiparallel arrangement. Another important finding is that in crystal the hinge region of the full-length molecules is contracted into long α-helix which folds back on the NTD forming a compact overall protein structure, whereas the restricted hinge regions of the L12 fragments adopted elongated, unstructured conformations (24). Moreover, the hinge regions from the full-length molecules bind to each other, which results in additional intermolecular contacts between the N- and C-terminal domains in the crystalline L12 tetramer. Thus the protein L7/L12 in crystal has a very compact structure different from the solution model in which the N- and C-terminal domains are separated by a stretched and flexible hinge region (19). However, the high-resolution structure of protein L7/L12 in solution was not obtained to date and the molecular details of function of this protein during the translation process are still not fully understood. In the present study, we have determined the spatial structure and characterized the internal dynamics of protein L7 from *Escherichia coli* in solution by heteronuclear NMR spectroscopy. Based on this information along with the available structural and biochemical data a model of L7/L12 molecular switching during the translation process is proposed. This provides useful insights into the role of protein L7/L12 in the functioning of the ribosome.

**EXPERIMENTAL PROCEDURES**

*NMR Spectroscopy* – NMR experiments were performed on 600 MHz (1H) Varian *Unity* spectrometer equipped with pulsed-field-gradient unit and triple resonance probe. NMR spectra were acquired at 30°C using 1 mM samples of uniformly 15N-labeled L7 dissolved at pH 6.9 in 600 μl of buffer solution containing 0.05 M sodium phosphate, 0.1 M KCl and either 90% H2O / 10% D2O or 99.9% D2O. Details of the protein preparation and NMR experiments used to assign 1H/15N resonances (BMRB accession number 4429) and determine the L7 secondary structure are documented elsewhere (16). During the course of NMR data collection, there were no detectable changes in NMR spectra due to spontaneous proteolysis of L7 dimer (25). The interproton distance restraints used in this work for L7 structure calculation were obtained from three-dimensional 1H-15N NOESY-HSQC (60 ms mixing time) experiment featuring pulsed-field gradient coherence selection, sensitivity enhancement and flip-back pulse for minimizing saturation of water (26). Two-dimensional NOESY (60 ms mixing time) spectrum acquired for the D2O sample was also used as an additional source to the structure
information concerning aromatic rings of phenylalanine residues. Dihedral angle restraints were estimated from homonuclear $^3J_{\alpha\text{HN}}$ and heteronuclear $^3J_{\beta\text{N}}$ coupling constants obtained quantitatively from two-dimensional $^1H$-$^15N$ HMQCJ (27) and qualitatively from three-dimensional $^1H$-$^15N$ HNHB (28) experiments, respectively. The preliminary stereospecific assignments of methylene $\beta$ protons were deduced by inspection of the relative cross-peak intensities in three-dimensional $^1H$-$^15N$ HNHB, $^1H$-$^15N$ NOESY-HSQC (60 ms mixing time) and $^1H$-$^15N$ TOCSY-HSQC (26) (50 ms mixing time) spectra. The slowly exchanging amide protons were identified by reconstituting lyophilized L7 sample in D$_2$O and immediately recording a series of $^1H$-$^15N$ HSQC spectra (30 minutes duration each) over 4 h at 23°C.

Residual $^1H$-$^15N$ dipolar couplings (RDCs) were extracted from $^1J_{\text{NH}}$ modulated HSQC experiments (29) collected on isotropic L7 sample and on partially aligned L7 samples containing the liquid crystalline phase of Tobacco mosaic virus (30) or dihexanoyl phosphatidylcholine/dimyristoyl phosphatidylcholine (DHPC/DMPC) mixture (1:2.9 molar ratio, 6.5% lipid (w/v)) (31). Alignments of both media were confirmed by observation of the quadrupole splitting of the D$_2$O signal. The splitting of 16 or 8 Hz was achieved for a sample containing virus particles or lipid bicelles, respectively. Sample homogeneity and stability were controlled before and after RDC measurements by arrayed deuterium experiments with slice selection gradients. In both lipid and virus media the legitimate dipolar couplings were observed only for the residues from the CTD (see Supplementary material).

$^15N$ relaxation measurements were performed using the pulse sequences by Farrow et al. (32) at 14.1 T and 30°C. The values of $^15N$ longitudinal ($R_1$) and transverse ($R_2$) relaxation rates were obtained by exponential fitting of the intensities of cross peaks in sets of two-dimensional $^1H$-$^15N$ correlation spectra recorded with relaxation delays ranging from 10 to 1000 ms and from 0 to 200 ms in $R_1$ and $R_2$ experiments, respectively. The $R_2$ data were numerically corrected to account for off-resonance effects associated with the CPMG refocusing pulses (33). The values of heteronuclear $^15N{^1H}$ steady state NOE were measured as a ratio of signal intensities in two-dimensional $^1H$-$^15N$ correlation spectra recorded with and without prior saturation of amide protons achieved by a sequence of 120° $^1H$ pulses spaced by 1.0 ms. The delay between scans in $^15N$ $R_1$, $R_2$ and in $^15N{^1H}$ NOE experiments was 2.5 and 5.0 s, respectively. The $R_1$, $R_2$, and NOE measurements were repeated several times and resulting values were averaged over all recorded data sets. The relaxation data analysis and hydrodynamic calculations were performed using DASHA program (34) (see Supplementary material).
Structure calculations – Spatial structure calculations were performed using the programs DYANA 1.5 (35) and CYANA 1.01 (http://www.guentert.com), which employ simulated annealing combined with molecular dynamics in torsion angle space. Meaningful upper distance restraints were derived using CALIBA (36) function of DYANA from the volumes of NOE cross-peaks integrated in the NOESY spectra by the program XEASY (37). The semiautomatic assignments of NOE cross-peaks were obtained with the program XEASY and NOAH (38) subroutine of DYANA package. Stereospecific assignments and torsion angle restraints for $\phi$, $\psi$ and $\chi^1$ were obtained by the analysis of local conformation in GRIDSEARCH (39) and GLOMSA (36) subroutines of DYANA using the available homonuclear $^3$J_HN and heteronuclear $^3$J_NP spin-spin coupling constants and sequential NOE data. The slowly exchanging amide protons were assigned as hydrogen bond donors with related hydrogen-acceptor partners on the basis of preliminary structure calculations. Corresponding hydrogen bond restraints were employed in subsequent calculations for $d(O,N)$, $d(O,N')$, $d(C,N')$ distances in accordance with angle and distance criteria for different types of hydrogen bonds (40).

During preliminary rounds of structure calculations (obtained without account for RDCs), typically 100-200 structures were generated using 5,000 simulated annealing steps followed by 1,200 steps of conjugate gradient minimization, with 10-20 structures being retained according to their standard DYANA target function values for relaxation matrix back-calculations of the NOEs and for analysis of hydrogen bonds, stereospecific assignments and ambiguous NOEs. In the final cycle of calculations, the standard CYANA simulated annealing protocol (modified to incorporate refinement against RDC restraints after employing distance and dihedral angle restraints) was applied to 200 random structures, and resulting 20 structures with the lowest target function were selected. Constrained energy minimization of the side-chains of the 20 best CYANA structures was performed in the program FANTOM (41) using ECEPP/2 potential with available distance and dihedral angle restraints. The mean structure calculation of the CYANA family and the analyses of root mean square deviations (r.m.s.d.), secondary structure, and hydrogen bonds were performed with MOLMOL (42). Spatial distribution of electrostatic potential on the protein solvent accessible surfaces and figures of the structures were generated by MOLMOL (42). The molecular hydrophobicity potential created by protein atoms on the protein solvent accessible surface was calculated as described earlier (43). The calculation and visualization of molecular hydrophobicity potential was done using the homemade software.
RESULTS

N- and C-terminal domains of L7 dimer tumble at different rates – The $^{15}$N{1H} NOE, $^{15}$N $R_1$ and $R_2$ values measured for the backbone $^{15}$N nuclei of the L7 dimer (Fig. 1, a-c) exhibit significant variations along the protein sequence. Moreover, it is clearly seen from the relaxation data (Fig. 1, a-c) that L7 consists of two relatively stable N- and C-terminal domains separated by mobile hinge region spanning residues 33-51. A more rigid part of the NTD comprises the residues from 3 to 31 with mean $^{15}$N $R_1$ and $R_2$ values of 1.53±0.05 s$^{-1}$ and 14.1±1.7 s$^{-1}$, respectively. The CTD spreads over the residues 52 to 120 with mean $^{15}$N $R_1$ and $R_2$ values of 1.72±0.14 s$^{-1}$ and 10.7±1.1 s$^{-1}$, respectively. The $^{15}$N nuclei of both N- and C-terminal domains have high positive $^{15}$N{1H} NOEs (Fig. 1a) which suggest restricted internal mobility for the NH vectors in pico-nanosecond time scale. Additionally, residues Phe 30 and Asn 64 from these domains have pronounced enhanced $R_2$, pointing to the micro-millisecond conformational exchange (see Supplementary material). In contrast, the residues 1-2 and 33-51 from N-terminus and hinge region, respectively, have nearly unrestricted mobility, resulting in low and negative $^{15}$N{1H} NOE’s and decreased $^{15}$N $R_1$ and $R_2$ rates (Fig. 1, a-c). The amides of Ser 1 and the hinge region residues, except for Val 38 and Val 40, have intensive water-exchange cross-peaks in the $^1$H-$^{15}$N NOESY-HSQC spectrum. Besides, the residues within the hinge region display only trivial sequential NOE connectivities. Hence, interdomain linker of L7 exhibits all the hallmarks of a mobile and unstructured region that is exposed to the aqueous environment.

Different mean values of $R_1$ and $R_2$ for $^{15}$N nuclei of the L7 N- and C-terminal domains strongly suggest that rotational diffusion of the domains occurs at different rates. This becomes evident from the local rotational correlation times, calculated from $R_2/R_1$ ratios of the individual $^{15}$N nuclei of L7 (Fig. 1d). The overall rotation correlation times, $\tau_R$, calculated from $R_2/R_1$ ratio averaged over $^{15}$N nuclei with $^1$H-$^{15}$N NOE higher than 0.6 (47) are 9.45±0.25 and 7.28±0.76 ns for the N- and C-terminal domains, respectively. Using the empirical dependence of the overall correlation time on the number of residues in globular proteins (44) one can estimate that the dNTD and each of the CTDs of the L7 dimer, having a total of 240 residues, tumble at the same rates as compact proteins of 152 and 115 residues, respectively. These values exceed the actual molecular sizes of the dNTD and the CTD by about 88 and 46 residues, respectively. Hence, the interaction of CTD-dNTD-CTD over long flexible hinge regions gives almost equal “overweight” by 45 residues to the size of the NTD (residues 1-32) and the CTD (residues 52-120). This proves our choice of the domain boundaries to within a few amino acids. Thus, according to the overall structure of L7/L12 in solution (19) where it forms a stable dimer with subunits interacting by the NTDs, one can suggest a model for its rotational diffusion assuming that L7/L12
dimer behaves as a set of three almost independent domains linked by flexible hinges. Namely, the dNTD of L7/L12 dimer behaves as a core unit, whereas two mutually independent CTDs diffuse as wings weakly interacting with the core. That is in agreement with the previous results of NMR and fluorescence polarization measurements suggesting that a hierarchy of motions exists in the L7/L12 molecule including facile motions of the dNTD and the two CTDs, in addition to the overall tumbling of the protein (17, 19).

**Structure Determination** – Taking into account that the domains of the L7 dimer are almost independent in solution and remain in the separated states most of the time, we calculated their spatial structures individually to optimize the computational task.

The dNTD was modeled as a dimer of two L7 N-terminal fragments, Ser 1-Ala 37 and Ser 1’-Ala 37’, linked by twenty pseudo-residues (L-Gly) to give the monomers enough mutual arrangement. Structure calculation protocol for the dNTD is described in Supplementary material. The full set of input data for structure calculation of the dimer included 686 NOE distance restraints, upper and lower distance restraints for 30 hydrogen bonds, 198 backbone, \( \phi \) and \( \varphi \), and side chain, \( \chi^1 \), dihedral angle restraints. Representative ensemble of the 20 structures of the dNTD with small violations and low molecular energies is shown in Fig. 2a. The backbone r.m.s.d. of the dimer is 0.56 Å for well-defined residues 3-31 and is 0.41 Å when comparing the monomers only.

[Fig. 2 here]

The structure of the CTD was calculated as the L7 C-terminal fragment, Ala 47-Lys 120, based on NMR-derived constraints including 670 NOE distance restraints, upper and lower distance restraints for 46 hydrogen bonds, 196 backbone, \( \phi \) and \( \varphi \), and side chain, \( \chi^1 \), dihedral angle restraints. Two final sets of 20 best CTD structures were obtained using separately 66 or 58 \(^1\)H\(^{15}\)N RDC measured in the alignment media containing the virus particles (Fig. 2b) or the lipid bicelles, respectively. In both virus and lipid media the backbone conformations of the CTD comprising residues 52-120 are well defined, and the overall mean global r.m.s.d. from the average structure are, respectively, 0.36 Å and 0.47 Å for backbone atoms.

A survey of the structural statistics and residual violations of experimental restraints for the dNTD and CTD ensembles is provided in the Table I. The atomic coordinates and experimental restraints for the representative structures and the ensembles of 20 structures of the dNTD and CTD (in virus alignment medium) of L7 dimer in solution have been deposited in the Protein Data Bank under accession codes 1RQT and 1RQS, respectively. Additionally, two representative structures of whole L7 dimer (PDB accession code 1RQU and 1RQV) were generated using the NMR structures of the L7 domains mutually remote and linked by two hinge
regions, either both of which were unstructured or one of them had been turned in a helix with a fashion similar to the crystal structure (24).

**Tertiary fold of L7/L12 in solution** – The data presented in this paper indicate that overall structure of L7 dimer in solution can be described as a set of three distinct domains, tumbling almost independently and linked via flexible hinge regions. Fig. 3 shows the ribbon and surface representations of the L7 dimer structure. It is seen that the hydrophobic and hydrophilic areas are almost uniformly distributed over the entire surface of the L7 domains, but the dNTD and especially the hinge region are less polar than the CTDs (Fig. 3b). The dNTD responsible for anchoring into the ribosome (6) carries mainly negative surface charge, whereas the CTD has large clusters of both negatively and positively charged areas, which may be involved in elongation factor binding (Fig. 3c). Indeed it was found that the head cluster of basic amino acids located on the CTD helices α4 and α5 can be used by L7/L12 to interact with EF-Tu in a manner similar to elongation factor Ts (45). The point mutation L7/L12(K70A) in this cluster had a strong inhibitory effect on Pi release from EF-G·GDP·Pi complex on the ribosome (46). The hinge region have three negatively charged Glu residues in its C-terminal part (Fig. 3c). The importance of the negatively charged and hydrophobic residues in the hinge region is indicated by the fact that deletion of C-terminal part of the hinge region (residues 35-52, 38-52, 42-52 or 44-52) from the L7/L12 produces virtually inactive ribosomes, while the replacement of the region 39-51 by an arbitrary sequence mainly composed of hydrophobic and negatively charged residues does not affect the function of L7/L12 (21, 22).

[Fig. 3 here]

In solution the dNTD (Fig. 2a) is formed by two antiparallel V-shaped α–α-hairpins, resulting in a symmetrical four-helical bundle. The α–α-hairpin includes helices α1 and α2 spanning residues 4-11 and 16-30, respectively. The dNTD four-helical bundle of L7 from *E. coli* showed the expected similarity in global fold with the homologue from the hyperthermophilic bacterium *T. maritima*. Namely, the dNTD structure in solution is close to that of a dimer formed by two antiparallel α1-α2 hairpins which, in crystal, incorporates the hinge helix α3 forming a five-helix bundle joining together the N-terminal fragment and full-length molecule of *T. maritima* L12 (24). The superposition of these four-helix bundles composed of two α1-α2 hairpins from *E. coli* and *T. maritima* reveals an r.m.s.d. of 1.4 Å for backbone atoms of regular secondary structure elements. Differences between the two structures correspond primarily to a small translational displacement of the helices relative to one another.
The CTD adopts a compact fold made up of three antiparallel α-helices arranged in a curved layer over three β-strands joined to a twisted antiparallel β-sheet that permits a classification of the CTD as an α/β protein (Fig. 2b). The sequential arrangement of the secondary structure elements is β1, α4, α5, β2, α6 and β3 spanning residues 53-60, 65-76, 80-88, 92-98, 100-113 and 116-120, respectively. Overall, the backbone conformation of the CTD in solution is very similar to that of the crystalline CTD from *E. coli* (23) and *T. maritima* (24), as shown by the small r.m.s.d. of approximately 1.4 Å throughout most of the protein backbone in both cases.

**Anisotropic rotational diffusion of the L7 domains** – Variations in $^{15}$N $R_1$ and $R_2$ (Fig. 1b, c) as well as in local rotational correlation time (Fig. 1d) over the sequence of L7 domains are presumably caused by rotational diffusion anisotropy. Anisotropic diffusion of the molecule is described by rotational diffusion tensor $\mathbf{D}$, which can be estimated based on $^{15}$N $R_1$ and $R_2$ data for the set of NH vectors with restricted internal mobility (for review see 47) or, otherwise, predicted by hydrodynamic calculations based on spatial structure of the molecule (34, 47, 48). Comparison of $\mathbf{D}$, obtained from these two methods may provide useful information about mutual dependence of the rotational diffusion of the L7 domains.

Efforts have been made to calculate rotational diffusion tensor for the dNTD. However, the set of 11 residues of the regular secondary structure with well resolved NH resonances having $^1$H($^{15}$N) NOE’s higher than 0.6 (47) appears to be insufficient for characterization of anisotropic rotational diffusion of the dNTD based on the experimental data.

Rotation diffusion tensor $\mathbf{D}$, obtained from $^{15}$N $R_1$ and $R_2$ data for the CTD using 45 NH vector directions taken from NMR structure of L7 in virus alignment medium is nearly axially symmetric with the ratios of principal components $D_x/D_z = 0.45\pm0.02$ and $D_y/D_z = 0.49\pm0.02$ and with $\tau_R = 1/[2(D_x+D_y+D_z)] = 7.07\pm0.04$ ns (Fig. 4a). The longest axis of the tensor $\mathbf{D}$ runs approximately parallel to the line connecting the CTD center with the junction of the hinge region. $\mathbf{D}$ does not change much if the directions of NH vectors are taken from NMR structure of L7 in lipid alignment medium and X-ray structure (PDB code 1CTF) but the quality of fitting indicated by $\chi^2$ loss function degrades significantly (about 3.3 and 1.4 times, respectively). Hydrodynamic calculations based on NMR structure of the CTD also result in almost axially symmetric $\mathbf{D}$, with the ratios of principal components of 0.73 and 0.80. Directions of principal axes of $\mathbf{D}$, calculated from the relaxation data correspond well to those obtained from hydrodynamic calculations, with angle between symmetry axes of $\mathbf{D}$, calculated with two different methods of about 25°. Although both methods predict the same overall prolate shape for rotational diffusion ellipsoid of the CTD, significant difference in the predicted degree of rotational
anisotropy indicates that rotational diffusion of the CTD is not completely independent upon the rest of the protein.

Rotational diffusion tensor $D_r$ for the CTD has been calculated from $^{15}$N $R_1$ and $R_2$ data for a limited set of residues with $^1$H($^{15}$N) NOE higher than 0.6 (47), which belong to the regular secondary structure elements. Fig. 4b, however, shows that the effective $\tau_R$ predicted from obtained $D_r$ correspond well to those calculated from $R_2/R_1$ ratio for majority of the CTD residues. Hence, variations of $R_1$ and $R_2$ within the CTD are mostly due to its anisotropic rotation diffusion rather than ms/µs conformational exchange or ps/ns internal motions. The only exception is the residue Asn 64 where the effective $\tau_R$ calculated from the experimental $R_2/R_1$ is considerably higher than the value predicted from $D_r$, possibly resulting from small exchange contribution to $^{15}$N $R_2$ of this residue, which can arise from structural interconversion (see Supplementary material).

Thus, the domain tumbling of L7 is slower than expected for the single modules, suggesting motional restrictions imposed by other domains via hinge linker. In a model comprising three non-interacting domains joined by a long linking segments, reorientation of the individual domains in not quite isotropic and is the most rapid around the axis that connect the center of each module with the linker. This is consistent with the rotational diffusion tensor we derived. Overall, the NMR data indicate that the relative orientations of the domains in the L7 dimer is not fixed in solution, that the domains do not stably interact with one another, and that each domain constitutes an autonomous structural unit.

**DISCUSSION**

*Dimerization mode of L7/L12 in solution* – A recent crystal structure of L12 from the *T. maritima* shows two alternative dimerization modes (24) of the protein associated into tetramer in the asymmetric unit. In the first mode, the two full-length L12 monomers form a tight symmetric and parallel ‘core dimer’ held together by a four-helix bundle that encompasses two $\alpha_2$ helices and two hinge regions in $\alpha$-helical conformation. In the second dimerization mode both full-length molecules are furthermore associated in antiparallel configuration with an N-terminal L12 fragments via a five-helix bundle that incorporates two $\alpha_1$, two $\alpha_2$ helices, and the hinge $\alpha_3$-helix of only one partner, forming two so-called ‘peripheral dimers’. The presented NMR structure of L7 dimer in solution supports conclusively the second mode of dimerization, namely the antiparallel configuration of the N-terminus $\alpha$-hairpins in the dNTD. Then the first dimerization mode of crystalline L7/L12 may result from the crystal packing and/or the conditions used for crystallizing the protein.
As seen in Fig. 5a, the NTD α-helices are amphipathic, that results in a continuous and almost perfectly aligned surface of hydrophobic residues conserved throughout the L7/L12 family, which cover the inside concave face of the NTD α-α-hairpin. Such a position of the hydrophobic residues is well suited for a nearly antiparallel interaction of two α-α-hairpins through the hydrophobic surfaces, giving rise to a tight four-helical bundle. The interhelical angle in each α-α-hairpin is large, which gives rise to two deep hydrophobic clefts (Fig. 5b) between the pairs of helices α1-α1' and α2-α2' from both monomers. In the crystalline L7/L12 structure the hinge helix α3 is engaged in the hydrophobic α2-α2' cleft elongated through the entire top surface of the dNTD. The remaining hydrophobic α1-α1' cleft including the L7 N-terminus is possibly essential for the dNTD anchoring into ribosome via protein L10. Thus, NMR and x-ray crystallography shows two alternative conformations for the hinge region, an extended coil or a long α-helix that folds back on the dNTD forming a compact overall protein structure.

[Fig. 5 here]

Transitory structures of the L7/L12 hinge region – Although the hinge interdomain linker of L7/L12 dimer is α-helical in the crystalline state, our NMR data conclusively show that this region is highly flexible through the entire length. The flexible hinge conformation agrees better with the body of evidence indicating that the protein L7/L12 has a great deal of overall flexibility in its complex with the protein L10 (19) and in the ribosome body (7, 49). Clearly, the flexibility of the hinge region is a key property allowing the diversity of L7/L12 structure in the ribosome and permitting rearrangement of the relative positions of the L7/L12 domains to achieve optimal intermolecular interaction during translation process.

The structure of the hinge region in solution is likely to represent an average over an ensemble of interconverting extended and bent conformations. The L7/L12 hinge region has a large number of hydrophobic residues of valine and alanine having high helix propensities (50). As stated above, the amide protons of hinge valine residues exhibit slower exchange with the bulk water, which suggests that any dynamic disorder is correlated to protect the hydrophobic residues from contact with the aqueous solvent. Perhaps, the hinge residues arrange themselves in such a way as to exclude solvent, but these structures are transitory. The fact that the $^{15}$N{$^1$H} NOE values fall down by steps of 3-4 residues (Fig. 1a) is an additional hint to the existence of molten helical elements at least in the first part (residues 33-43) of the hinge region. Indeed, such stepwise decrease of $^{15}$N{$^1$H} NOEs, in turn arising from the stepwise increase of backbone mobility, coincides precisely with a sequence of three first turns of α3 helix observed in the crystal structure of L12. The presence of strong helix-destabilizing residues such as Gly 43 and Pro 44 in the middle of the hinge region can promote disruptions of its helical structure in solution. In solution, the hinge region is involved in slow (millisecond-second) process...
revealed by disproportional quadrupling (at least) and/or tailing of the amide cross peaks in the NMR spectra (see Supplementary Fig. 2S). The cis-trans isomerization of the Gly 43-Pro 44 peptide bond was earlier proved by our NMR study (16). The slow conformational exchange between the four species of hinge region can arise through interference of Pro-mediated cis and trans isomers from each monomer of the dimer. This may also indicate that two hinge regions of the L7 dimer are curled into transient structures, which experience random interaction between themselves resulting in the signal quadrupling especially pronounced in the first half (residues 33-43) of the hinge region.

Overall, these findings suggest that the hinge region can undergo a helix-coil transition in aqueous solution, whereas in a nonpolar environment it prefers to fold into a long \( \alpha \)-helix, which closes up the hydrophobic cleft of the dNTD. Therefore, certain protein properties should be very sensitive to mild variations of local environment, which might be coupled with binding and release of elongation factors to/from the ribosome. This is supported by earlier observations of the variable helical content of L7/L12 dependant upon the external conditions (51). Another interesting finding is that the hinge-binding hydrophobic cleft of the dNTD allows only one of the hinge regions of the dimer to adopt a helical conformation in this position (24). Hence, the L7/L12 dimer can have one retracted CTD due to the presence of a helical hinge region and one remote CTD (52) (Fig. 6). This is consistent with the chemical cross-linking data and cryo-electron microscopy data obtained with radio- and Nanogold-labeled L7/L12, respectively, indicating a ribosomal site for the CTD adjacent to the dNTD and remote sites at the distances up to the combined length of the CTD and extended hinge region (53, 54). Thus, although the dNTD remains tied with the protein L10, the CTDs can visit different locations on the ribosome depending on the conformation of the hinge region (52). The association of the elongation factors with the ribosome could change the environment from polar to more hydrophobic and cause helical contraction of the hinge region, while dissociation of the elongation factors after GTP hydrolysis and Pi release (3) leads to extended hinge. The monomers in the L7/L12 dimer could alternate between the helical and extended states of the hinge region in response to changes of the local environment conditions. As a result, the CTDs would be able to close up and go backwards to/from the elongation factors and the ribosomal body.

Unique flexible structure of L7/L12 dimer is a key feature for its biological function – The ribosome is a conveying molecular machine using a functional macromolecular mimicry of translation factors (4, 55). The L7/L12 stalk is shown to be a moveable ribosomal module, which participates in EF-Tu- and EF-G-induced events, especially in the EF-G-catalyzed translocation (4). The binding sites of both elongation factors on the ribosome were directly visualized in the immediate vicinity of the L7/L12 stalk (56, 57) and spatial contacts of
the L7/L12 stalk with GTP-holding elongation factors, EF-G and EF-Tu, were observed (10, 11). Recently a bifurcation of the L7/L12 stalk after EF-G·GMPPCP binding to the ribosome and its reversal to a single, elongated form after GTP hydrolysis was detected (58). Moreover, it was found that the changes in the L7/L12 stalk upon EF-G·GTP binding and then during GTP cleavage are accompanied by conformational alterations in both ribosomal subunits (3, 4), which suggests long-range conformational shifts within ribosome during translocation process. In particular, the binding of the EF-G to the pretranslocational ribosome leads to a small counter-clockwise rotational movement of the small subunit relative to the large subunit in the direction of the mRNA movement (59, 60). The EF-G dissociation induces the backward rotation (59, 60). These “ratchet-like” rotational motions of the ribosomal subunits during the interaction with EF-G were suggested to be fundamental for the translocation process (59). Overall, the conformation of L7/L12 is modulated by interaction of the elongation factors with the ribosome and depends on hydrolysis of GTP (3-8), but the details of these processes are yet unclear.

The L7/L12 dimers are associated with the ribosome by their dNTDs via protein L10 (6, 19, 61). Although the spatial structure of the protein L10 is not known at present, it is established that hydrophobic interactions are responsible for binding the L7/L12 dNTD to the protein L10 (62) and that the dNTD is mobile in the complex with L10 (19). Our present results and literature data suggest that the hinge regions within ribosomal L7/L12 stalk may act as a molecular switch (Fig. 6, 7) alternating ‘closed’ and ‘open’ conformations of the dNTD hinge-binding cleft depending on elongation factor association with ribosome during different steps of elongation cycle. According to the NMR structure of the L7 dimer, in the open conformation there are two highly hydrophobic surfaces (H1 and H2 on the Fig. 5b) on the exposed sides of the dNTD hinge-binding cleft. In order for L7 to form stable complex with L10, these hydrophobic surfaces must contact hydrophobic regions of the protein L10 (H1’ and H2’, Fig. 7). There are also two smaller hydrophobic patches (h1 and h2 on the Fig. 5b) located on the back and front surfaces of the dNTD, formed by the exposed side chains of the highly conserved residues Ile 8 and Ala 25, that also should have hydrophobic counterparts on the surface of L10 (h1’ and h2’ on the Fig. 7). In the vicinity of each of the four hydrophobic regions of L7 there is a positively charged side chain of Lys residue (Fig. 5c), located nearly identically with respect to the hydrophobic region. According to crystal structure (24), when one of the two hinge regions folds into helix, the helix associates with the hinge-binding cleft in such a way, that one of the exposed sides of the cleft (e.g. H1) is almost closed by this helix (Fig. 7b). Since the C-terminal part of the hinge region and the CTD are strongly charged (Fig. 2c), in the ‘closed’ conformation the environment around H1 changes from nonpolar to strongly polar, weakening or destroying the hydrophobic contact between the closed edge of the cleft and its counterpart on the L10 surface.
This makes the initial configuration of dNTD-L10 complex unfavorable and can cause a subsequent conformational change. In the ‘closed’ conformation the surface of the closed H1 mimics the surfaces around h1 and h2, namely, it consists of a small hydrophobic spot with adjacent positive charge of Lys residue surrounded by negatively charged area. Thus, if the dNTD rotates around its symmetry axis, the closed H1 can superpose over the region on the L10 surface, originally corresponding to one of the two hydrophobic patches, h1 or h2. At that, it takes a clockwise rotation by about 60° to combine it with h1’ and by about 120° in the opposite direction to combine with h2’.

[Fig. 7 here]

This suggests a hypothesis that after the L7/L12 hinge region folds upon binding the elongation factors, the dNTD rotates to move H1 with the charged CTD away from the highly hydrophobic region H1’ of L10. As depicted in the Fig. 7b, there are two allowable (preferable) positions for the L7/L12, in which H1 would face the surface of L10, initially occupied by h1 or h2. The clockwise rotation (H1 to h1) seems more preferable for several reasons. First of all, h1 is spatially closer to H1 and the rotation transposing H1 to h1 does not require a Lys residue to transverse the highly hydrophobic region H1’. Besides, the clockwise rotation of the L7/L12 complex with the elongation factors is consistent with the counter-clockwise rotational movement of the small ribosomal subunit relative to the large subunit upon EF-G binding (59, 60). The rotation would result in the transition of the whole system to a “spring-loaded” state (Fig. 7c). Subsequent events (e.g. dissociation of elongation factors from the ribosome) cause unfolding of the L7/L12 hinge helix (Fig. 7d) and backwards rotation of the dNTD to the initial position (Fig. 7a). Thus, the conformational switching of L7/L12 between open and closed states should result in “ratchet-like” rotational movements around the symmetry axis of the dNTD.

Concluding remarks – High resolution NMR structure of the ribosomal protein L7/L12 in the solution along with its dynamics properties and the bulk of available literature data suggest hypothesis that the L7/L12 acts as a molecular switch in the course of translation process. Namely, binding of elongation factors to the ribosome causes folding of the L7/L12 hinge region to the base of L7/L12 stalk that induces reversible rotation of L7/L12 relative to the ribosome surface. The hypothesis itself and how it might relate to other motions within functioning ribosome (e.g. with “ratched-like” motions of ribosome subunits (59, 60) and translocational mechanism) remains to be tested.
REFERENCES

SUPPLEMENTARY MATERIALS

Supplementary materials are available containing the detailed descriptions of the RDCs measured, the structure calculation protocol for the L7 dNTD, the secondary structure elements of the L7 domains, and slow conformational exchange of Phe 30 and Asn 64 with three illustrating figures.

ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. **15N relaxation data for the L7 dimer.** Experimental relaxation data for the backbone 15N nuclei of the L7 dimer, measured at 14.1 T: **a**, steady-state 15N{1H} NOE; **b**, 15N longitudinal relaxation rate $R_1$; **c**, 15N transverse relaxation rate $R_2$; **d**, effective $\tau_R$ calculated from the experimental $R_2/R_1$. The relaxation data are presented for the major conformation of the L7 hinge region (see Discussion). The uncertainties are shown by bars. The secondary structure elements of the L7 dimer in solution as well the putative hinge helix $\alpha_3$ are indicated at the bottom.

Figure 2. **NMR structure of the L7 domains.** **a**, Ensemble of 20 dNTD NMR conformers after alignment of the backbone atoms of residues 3-31, 3'-31' is shown in comparison with the ribbon diagrams of representative NMR structure. The dimeric N-terminal domain, comprising residues 1-32, is presented as a dimer of two L7 N-terminal fragments, Ser 1-Ala 37 and Ser 1’-Ala 37’. **b**, Ensemble of 20 CTD NMR conformers (in virus alignment medium) superimposed for the best fit over all backbone atoms of residues 52-120 is shown in comparison with the ribbon diagrams of a representative NMR structure. The structure of the C-terminal domain, comprising residues 52-120, is presented as the L7 C-terminal fragment, Ala 47-Lys 120. Secondary structure elements and chain termini are labeled.

Figure 3. **Solution structure of the L7 dimer.** **a**, Ribbon diagram of NMR structure of the L7 dimer. The N- and C-terminal domains and hinge interdomain linker are marked. Distance scale is shown. **b** and **c**, Solvent-accessible surface of the L7 dimer colored according to hydrophobicity and electrostatic potential, respectively. Hydrophobic and hydrophilic surfaces are depicted in yellow and green with color intensity increasing with the module of positive and negative values of molecular hydrophobicity potential, respectively. Similarly, areas of negative, positive, and neutral electrostatic potential are depicted in red, blue, and white, respectively. Scale bars are shown. The values of molecular hydrophobicity potential are given in conventional units (43). The values of electrostatic potential are given in units of $e/\text{Å}$ ($e$ is electron charge).

Figure 4. **Anisotropic rotational diffusion of the L7 C-terminal domain.** **a**, Comparison of the rotational diffusion tensors obtained from experimental $^{15}$N $R_1$ and $R_2$ data (solid arrows) and hydrodynamic calculations (dotted arrows) for the CTD NMR structure shown by ribbon. **b**, Comparison of the effective $\tau_R$ predicted from rotational diffusion tensor $D$, (solid line) and calculated from $R_2/R_1$ ratio (open circle) for the backbone $^{15}$N nuclei of the CTD. The uncertainties are shown by bars.

20
Figure 5. Distribution of molecular hydrophobicity and electrostatic potential on the L7 N-terminal domain surface. a, The interaction of two L7 N-terminal α-α-hairpins forming the dNTD. Ribbon diagrams and molecular surface colored according to hydrophobicity potential are shown. The negatively charged, positively charged and hydrophobic side chains of the NTD are shown in red, blue, and yellow, respectively. b and c, Solvent accessible surface of the dNTD colored according to hydrophobicity potential and electrostatic potential, respectively. The front view orientation of the dNTD is similar to that on the Fig. 2a ribbon representation. Hydrophobic and hydrophilic surfaces are depicted in yellow and green with color intensity increasing with the module of positive and negative values of molecular hydrophobicity potential, respectively. Similarly, areas of negative, positive, and neutral electrostatic potential are depicted in red, blue, and white, respectively. Scale bars are shown. The values of molecular hydrophobicity potential are given in conventional units (43). The values of electrostatic potential are given in units of e/Å (e is electron charge). Putative hinge-binding hydrophobic cleft and exposed hydrophobic surfaces H1, H2 and h1, h2 of the dNTD are marked in the top view. Putative L10 binding hydrophobic cleft and the positively charged side chains of four Lys residues are also labeled in the bottom view.

Figure 6. Molecular switching of the hinge region of L7/L12 dimer suggested based on the x-ray crystallography and NMR data. The side ribbon diagrams of the L7/L12 dimer having one hinge region in extended random conformation observed in solution and the other turned in a helix in manner similar to the crystal structure (24). The monomers of the L7/L12 dimer can alternate between the helical hinge and extended hinge states in response to changes of local environment conditions coupled with factor binding and release during ribosome translation process.

Figure 7. Model of “ratchet-like” motions of L7/L12 dimer during ribosomal translation cycle. See text for the explanation of details of the presented model. H1’, H2’ and h1’, h2’ are the putative hydrophobic region of the surface of L10, which are counterparts to the exposed hydrophobic surfaces H1, H2 and h1, h2 of the dNTD, respectively. The L7/L12 hinge region is shown as unordered or as folded in a helix with C-terminal negative charge colored in black.
Table I. Structural statistics for the ensembles of 20 lowest CYANA target function structures of the L7 dimer domains.

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<tr>
<th>Parameter / Constraints</th>
<th>Quantity</th>
<th>dNTD</th>
<th>CTD</th>
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<td></td>
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<td>no. of &lt;sup&gt;1&lt;/sup&gt;H-&lt;sup&gt;15&lt;/sup&gt;N RDC constraint violations</td>
<td>&gt; 0.2 Hz</td>
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<td>RDC alignment tensor</td>
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<td></td>
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<td>RDC Q-factor</td>
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<td>Rmsd (Å) of residues (3-31) of dNTD and 52-120 of CTD</td>
<td>Backbone</td>
<td>0.56±0.19</td>
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<td>all heavy atoms</td>
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<td>0.99±0.13</td>
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<tr>
<td>Ramachandran analysis&lt;sup&gt;b&lt;/sup&gt; of residues (1-32) of dNTD and 52-120 of CTD</td>
<td>% residues in most favored regions</td>
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<td>% residues in additional allowed regions</td>
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<tr>
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<td>% residues in disallowed regions</td>
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</table>

Table note:

<sup>a</sup> The co-monomer NOE is a NOE connectivity which has both inter- and intramonomer contribution in a symmetric oligomer (63).

<sup>b</sup> Ramachandran statistics were determined using PROCHECK_NMR (64).
Figure 2

(a) Left and front views of a protein structure with labeled residues:
- Ala37
- Ser1
- Ala37'
- Thr52
- Lys120

(b) Left and front views of another protein structure with labeled residues:
- Ala47
- Val32
- Val32'
- β2
- β3
- β1
- α2
- α1
Figure 3

a) CTD and dNTD regions with flexible hinge regions.

b) Hydrophobic potential distribution with values ranging from -0.5 to 0.5.

c) Electrostatic potential distribution with values ranging from -0.7 to 0.7.
Figure 4
Figure 5

(a) L10 binding hydrophobic cleft
(b) bottom, front, top, right
(c) Lys 29', Lys 29, Lys 4, Lys 4'

Hydrophobic potential:
-0.5 0.5
Hydrophilic  Hydrophobic

Electrostatic potential:
-0.7 0.7
Negative  Positive
Figure 6

elongation factors
binding
release

elongation factors
binding
release
Figure 7

(a) tRNA:EF-Tu-GTP, EF-G-GTP
(b) h2', h1', h2', h1', hinge
(c) h2', h1', hinge
(d) h2', h1', h2', h1', EF-Tu-GDP, EF-G-GDP, Pi

CTD

H2'

H1'

h1'

h2'

h2'

h1'

-60°
Supplementary material

Residual dipolar couplings – The legitimate dipolar couplings were observed only for residues from the CTD in both lipid and virus media. They are presented graphically on the Supplementary Fig. 1S. Both positive and negative values are observed ranging from –13.3 to 16.1 Hz for the lipid medium and from –5.1 to 4.1 Hz for the virus medium. Thus, only the dipolar couplings obtained for the CTD residues (except for Asn 64, that revealed slow conformational exchange) were used in the spatial structure calculation. Also, no dipolar couplings were exploited for the residues whose peaks were obscured by spectral overlap preventing precise determination of coupling. Protocol of structure refinement exploiting RDCs consisted of determination of axial and rhombic components of the molecule alignment tensor using homemade program ORIENT, and conjugate gradient minimization of the target function consisted of distance, angle, and RDC restraints, using program CYANA.

The RDC measurement for the dNTD in the lipid alignment medium failed due to anchoring of the dNTD to the bicelles. In spite of the high signal-to-noise ratio (approximately 100:1) for most of the cross-peaks in the HSQC spectrum of the L7 dimer in the lipid medium, no cross-peaks of the L7 N-terminal residues from Ser1 to Ala39 are observable (like in the (L7)2(L7)2.L10 complex (Bocharov et al. (1998) FEBS Lett. 423, 347–350), i.e. they are broadened beyond detection due to binding of the dNTD to hydrophobic bicelles comprising the liquid crystal matrix. On the contrary, the CTD and C-terminal part of hinge region residues are not associated with bicelles. This implies that the dNTD has surface-exposed hydrophobic clusters, whereas the CTD does not have them. Therefore, due to the high degree of bilayer order in the magnetic field (Bax (2003) Protein Sci. 12, 1-16), the anchored dNTD became strongly oriented, resulting in very large dipolar couplings and signal broadening. In turn, a reasonably good alignment of the CTD in lipid medium is expected (Mesleh et al. (2003) J. Biomol. NMR 25, 55-61), since the structurally independent L7 domains are tethered and strong restriction of dNTD should lead to some restriction of the CTD. The signs of RDCs for the CTD in lipid solution demonstrate that the principal axis of the alignment tensor is aligned approximately parallel to its helices α4 and α6, and perpendicular to helix α5 (Supplementary Fig. 1Sa). The variations in the sign of RDCs in the β-sheet residues are consistent with intermediate orientations.

Oppositely, the weakness of the alignment of the dNTD in the virus media prevents usage of its dipolar couplings dispersed around 0 Hz in structure calculation (Supplementary Fig. 1Sb). The magnitude of the L7 RDCs measured in the virus alignment medium is highly different for the L7 domains. This indicates that the CTD is aligned much stronger than dNTD. Therefore, a mechanism whereby the CTD becomes more ordered that the dNTD must be proposed. One such mechanism is a transient electrostatic interaction of the CTD with...
the virus itself. The small fraction of time during which the protein diffuses to within a distance where the alignment particles exerts a force on the protein (either electrostatically or sterically) contributes the nonzero dipolar coupling (Bax (2003) *Protei Sci.* 12, 1-16). The particles of *Tobacco mosaic* virus carry negative surface charge. As a result, electrostatic interaction between the virus surface and the solute protein usually dominates the alignment. Solution structure analysis revealed (see “*Tertiary fold of L7/L12 in solution*”) that the dNTD has much more negative surface potential than the CTD. Hence, the L7 alignment in the virus medium is dominated by the strong repellent interaction between the negatively charged dNTD and virus particles, essentially preventing direct contacts between them, whereas the CTD weakly interact with the virus particles giving rise to legitimate RDCs. Thus, these significant differences in the dNTD and CTD alignments suggest that the two domains are dynamically independent and that they experience different ordering forces as manifested by the measured RDC values.

*The relaxation data analysis and hydrodynamic calculations* - The primary focus of our $^{15}$N relaxation data analysis was characterization of the rotational diffusion for the N- and C-terminal domains of L7. An attempt has been made to estimate rotational diffusion tensor $D_i$ for each of the domains by least-square fitting of the experimental $^{15}$N $R_1$ and $R_2$ data to the theoretical values calculated using spectral density function for a vector attached to anisotropic molecule (Woessner (1962) *J. Chem. Phys.* 37, 647-654). The calculations for the N- and C-terminal domains were performed, respectively, for 11 and 45 NH groups of the regular secondary structure ($\alpha$-helices and $\beta$-strands) having well resolved resonances in $^1$H,$^{15}$N HSQC spectra and $^1$H{$^{15}$N} NOE higher than 0.6 (Korzhnev et al. (2001) *Prog. Nucl. Magn. Reson. Spectrosc.* 38, 197-266). Neither of the $^{15}$N nuclei selected show pronounced dependence of $R_2$ on the pulse repetition rate of CPMG sequence ensuring that contributions $R_{ex}$ to $R_2$ due to millisecond conformational exchange have little effect on $D_i$ calculation. The directions of NH vectors in the molecular frame were derived from NMR structure of the L7 dimer and from X-ray structure of the *E. coli* L7/L12 C-terminal fragment (PDB code 1CTF). The fitting was performed in DASHA program (Orekhov et al. (1995) *Appl. Magn. Reson.* 9, 581-588).

The rotational diffusion tensors $D_i$ for the domains of the L7 dimer were theoretically predicted by hydrodynamic calculations using beads model approximation (de la Torre and Bloomfield (1981) *Q. Rev. Biophys.* 14, 81-139) assuming that the domains diffuse independently from each other. Each of the mentioned subunits was represented by a set of 3.5 Å beads centered at $C^\alpha$ positions with hydrodynamic interaction between beads accounted for by modified Oseen tensor (Barbato et al. (1992) *Biochemistry* 31, 5269-5278). The calculations were performed using DIFFC function of DASHA program.
The $^{15}$N relaxation data for the L7 dimer suggests that the dNTD and the globular CTDs diffuse at different rates (see Results). Thus, overall rotation of L7 in solution cannot be described as motion of a single subunit, but represents a complex mutual diffusion of weakly interacting domains connected by flexible linkers. Therefore, we have chosen not to analyze the relaxation data in terms of order parameters and correlation times of internal motions using conventional model-free analysis (Lipari and Szabo (1982) *J. Am. Chem. Soc.* **104**, 4546-4570), which assumes that rotational diffusion of the molecule can be effectively separated from its internal motions. Thus, intradomain dynamics of the L7 dimer were assessed only qualitatively based on the measured $^{15}$N $R_1$, $R_2$ and $^{15}$N-{H} NOE values.

**Structure calculation of the dimeric N-terminal domain of L7** – The dNTD was modeled as the dimer of two L7 N-terminal fragments, Ser 1-Ala 37 and Ser 1’-Ala 37’, linked by twenty pseudo-residues (L-Gly) to give the monomers enough mutual arrangement. Since the dNTD of the L7 homodimer is symmetric (Bushuev et al. (1984) *FEBS Lett.* **178**, 101–104; Bocharov et al. (1996) *FEBS Lett.* **379**, 291–294) on the chemical shifts time scale (microsecond to millisecond), its two monomer chains display indistinguishable 1H and 15N chemical shifts. As a consequence of this, the NOEs arising from intermonomer NOE contacts are indistinguishable from those arising from intramonomer contacts (Nilges and O’Donoghue (1998) *Prog. Nucl. Magn. Reson. Spectrosc.* **32**, 107-139). Typically, this problem is circumvented by preparing an isotopic heterodimer and by carrying out experiments, which select NOEs arising between isotopically bound and non isotopically bound protons (Weiss (1990) *J. Magn. Reson.* **86**, 626-632). Unfortunately, asymmetric $^{15}$N-labeling experiment failed both due to lower signal-to-noise ratio in this differential method, and weak signal from the NTD in the NMR spectra (Supplementary Fig. 2S) (Bocharov et al. (1996) *FEBS Lett.* **379**, 291–294). An alternative computational solution to the symmetry degeneracy problem is the ambiguous distance restraints method (Nilges and O’Donoghue (1998) *Prog. Nucl. Magn. Reson. Spectrosc.* **32**, 107-139). Therefore, spatial structure of the dNTD was calculated in two stages. Our computational strategy involved an initial stage of refinement of the monomer subunit before proceeding to the dimer. The monomer structure of the NTD was solved with the distance restraints converted from the NOEs unambiguously assigned as intramonomeric in the three-dimensional 1H-$^{15}$N NOESY-HSQC spectrum. All NOEs were interpreted in a very conservative manner, most of them could be unambiguously assigned as intramonomeric and only those that were clearly inconsistent with the global fold of the monomer were assigned as possible intermolecular contacts. The full set of input data for structure calculation of the L7 N-terminal fragment 1-37 included 279 NOE distance restraints, upper and lower distance restraints for 15 hydrogen bonds, 99 backbone, $\phi$ and $\varphi$, and side chain, $\chi_1$, dihedral angle restraints.
This resulted in a high-resolution monomer structure of the NTD comprising residues 1-32 (Supplementary Fig. 3S) that resembled that of N-terminal α1-α2 hairpin of T. maritima L12 in crystal (approximately 0.5 Å r.m.s.d. for backbone atoms of regular secondary structure elements).

At the second stage, for the refinement of the dimer structure of the dNTD we have used together both the three-dimensional 1H-15N NOESY-HSQC spectrum and the aromatic part of the two-dimensional NOESY spectrum. The dNTD proved to be an auspicious case because many intermolecular contacts could be readily identified as a subset of NOEs that were inconsistent with the presence of monomeric hairpin of the two α-helices. Clear examples include the well-resolved intersubunit NOEs between the aromatic ring of Phe 30’ and the γ-methyls of Val 11 and Val 16. These NOEs were used as a starting point for the structure calculation of the dimer. The dihedral angle restraints and both intra- and inter-monomer distance restraints were symmetrically doubled for each dimer subunit that resulted in a dimer with 2-fold symmetrical structure averaged over the ensemble of calculated NMR structures. Once correct starting structure of the dNTD was obtained, the originally ambiguous NOEs that could not be readily assigned were included subsequently in the calculations. The structures were then improved in an interactive fashion as errors or ambiguities present in the restraints were removed or new restraints were added. The computational protocol consisted of several rounds of semiautomated analysis of NOE data by XEASY and NOAH, structure calculations with simulated annealing approach and relaxation matrix back-calculations of the NOEs for the ensemble of converged structures. During the semiautomatic refinement procedure, more intermonomer and a few (ten) co-monomer NOEs (which possibly have both inter- and intramonomer contribution) could be identified. The co-monomer NOEs anticipated near the 2-fold symmetry axis of the dimer were omitted throughout the initial structure determination process. Further, sum averaging (Nilges and O’Donoghue (1998) Prog. Nucl. Magn. Reson. Spectrosc. 32, 107-139) was used to include 40 distance restraints resulting from the co-monomer NOEs, which were treated in an ambiguous fashion during the final structure calculation round implemented in the program CYANA. In total, 34 intermolecular contacts per monomer of the dNTD were assigned unambiguously, corresponding to a total of 68 distance constraints for the dimer interface, and these were sufficiently distributed to establish the antiparallel nature of dimer symmetry. Thus, the proportion of inter- and co-monomer restraints was only a small fraction of the total number (686) of distance restraints. The obtained representative ensembles of the 20 structures of the dNTD with small violations and low molecular energies are shown the low backbone r.m.s.d. of 0.56 Å for well-defined residues 3-31 and is 0.41 Å when comparing the monomers only. It is important to note that symmetry restraints were not imposed during any phase of the dNTD structure
calculations, yet the pairwise r.m.s.d. between the mean subunit structures is low, reflecting the expected symmetry between the two subunits in solutions.

The secondary structure elements of the L7 domains in solution – The V-shaped $\alpha$–$\alpha$-hairpin of NTD helices $\alpha 1$ and $\alpha 2$ (residues 4-11 and 16-30, respectively) has a short connection Ala 12-Ala 13-Met 14-Ser 15 in the conformation $(\alpha_m) \gamma \gamma \beta \beta (\alpha_n)$ resulting in the interhelical angle of approximately 60° (here and below we use shorthand nomenclature for the polypeptide chain conformation (Efimov, A.V. (1993) Prog. Biophys. Molec. Biol. 60, 201-239)). The first helix $\alpha 1$ begins from a standard perpendicular entry formed by residues Ile 2-Thr 3 with the conformation $\delta \beta (\alpha_n)$. The helix $\alpha 2$ is terminated at the residues Phe 30-Gly 31-Val 32 which form a standard perpendicular exit with the conformation $(\alpha_n) \gamma \alpha_L \beta$.

The sequential arrangement of the CTD secondary structure elements is $\beta 1$, $\alpha 4$, $\alpha 5$, $\beta 2$, $\alpha 6$ and $\beta 3$ spanning residues 53-60, 65-76, 80-88, 92-98, 100-113 and 116-120, respectively. All the three $\beta$-strands have the “bulges” including residues 59-60, 94-97 and 116. The first CTD strand $\beta 1$ begins with an approximately perpendicular bend encompassing residues Lys 51-Thr 52-Glu 53 in the $\beta$-$\gamma$-half-turn conformation. The $\beta 1$ and $\alpha 4$ are joined together in a $\beta$–$\alpha$-arch by the protruding $\beta$-turn close to type II and formed by residues Ala 61-Gly 62-Ala 63-Asn 64 with the conformation $(\beta_m) \alpha \gamma \delta (\alpha_n)$. The $\alpha 4$ and $\alpha 5$ form a standard right-handed $\alpha$–$\alpha$-corner with connection Thr 76-Gly 77-Leu 78-Gly 79 which exhibited conformation $(\alpha_m) \gamma \alpha_L \beta (\alpha_n)$ frequently occurring in proteins. The short connection Ser 89-Ala 90-Pro 91 with conformation $(\alpha_n) \gamma \beta (\beta_n)$ between $\alpha 6$ and $\beta 2$ is a $\text{cis}$-proline type VI $\beta$-turn. A following right-handed $\beta$–$\alpha$–$\beta$-unit is comprised of $\beta 2$, $\alpha 6$ and $\beta 3$ with connections Gly 97-Val 98-Ser 99 and Ala 113-Gly 114-Ala 115 having conformations $(\beta_m) \alpha_L \beta (\alpha_n)$ and $(\alpha_m) \gamma \alpha_L \beta (\beta_n)$, respectively.

Slow conformational exchange of Phe 30 and Asn 64 – The partially solvent-exposed boundary location of Phe 30 preceding the flexible hinge region causes the residue to undergo micro-millisecond conformational exchange revealed by increasing of $^{15}$N $R_2$ value (Fig. 1c). The conserved Phe 30 probably plays an important role in the dimer formation, clasping two NTD monomers. In particular, the aromatic side chain of Phe 30 is directed into a very restricted space between helices $\alpha 1'$ and $\alpha 2$, which may partially explain for the upfield-shifted $H^\epsilon$ resonances and downfield-shifted $H^\delta$ resonances, which is not common.

The effective $\tau_\text{e}$ (Fig. 4b) calculated from the experimental $R_2/R_1$ for Asn 64 is considerably higher than the value predicted from $D_\text{e}$, possibly resulting from small exchange contribution to $^{15}$N $R_2$ of this residue, which
can arise from structural interconversions. Indeed, the cross peaks from $^{15}$NH$_2$ side chain group of Asn 64 are splitting with strong disproportion (of about 20/1; Supplementary Fig. 2S) in the peak intensities of major and minor conformations that also indicate slow conformational exchange in the millisecond time scale, which probably has arisen as a result of formation of transient hydrogen bonds. Evidence for possible additional hydrogen bonds between the side chain of Asn 64 and spatially adjacent backbone chain was observed in a subset of the family of CTD structures. Moreover, the backbone amide of Asn 64 exhibit fast exchange with the bulk water and the hydrogen bond, formed in crystal by the carbonyl of Ala 61 and backbone amide proton of Asn 64, are not observed in solution. As a result, the loop 61-64 adjacent to the putative EF-Tu and EF-G binding sites (Wieden et al. (2001) J. Mol. Evol. 52, 129–136; Savelsbergh et al. (2003) Mol. Cell 11, 1517–1523) has slightly different conformations in the solution and crystal structures.

**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1S.** Residual dipolar couplings of the L7 dimer in the two liquid crystal solutions. a, Experimental $^1$H-$^{15}$N RDC values measured in the lipid alignment medium. The approximate alignment of the CTD relative to the magnetic field direction in lipid medium is shown. b, Experimental $^1$H-$^{15}$N RDC values measured in the virus alignment medium. The RDC values are presented for the major conformation of the L7 hinge region. The uncertainties are shown by bars. The secondary structure and amino acid sequence of L7 are also presented. The domain boundaries are shown by a dotted line on the histograms.

**Supplementary Figure 2S.** $^{15}$N-$^1$H HSQC spectrum of the L7 dimer. The $^{15}$N-$^1$H HSQC spectrum was recorded for 1 mM uniformly $^{15}$N-enriched L7 in buffer solution containing 0.05 M sodium phosphate, 0.1 M KCl and 90% H$_2$O / 10% D$_2$O at 30°C and pH 6.9. The $^1$H-$^{15}$N backbone resonance assignments of L7 major conformation are indicated by numbers

**Supplementary Figure 3S.** NMR structure of the NTD monomer. Ensemble of 20 dNTD NMR conformers after alignment of the backbone atoms of residues 3-31 is shown in comparison with the ribbon diagrams of representative NMR structure. On the ribbon diagrams, the negatively charged, positively charged and hydrophobic side chains of the NTD are shown in red, blue, and yellow, respectively.
Supplementary Figure 1S

(a) 

(b)
Supplementary Figure 3S

[Diagram showing molecular structures with labeled α1 and α2]
From structure and dynamics of protein L7/L12 to molecular switching in ribosome
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