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STRUCTURE NOTE

Interactions between the archaeal transcription repressor FL11 and its coregulators lysine and arginine

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INTRODUCTION

Homologues of the *E. coli* leucine-responsive regulatory protein (Lrp) are widely distributed in eubacteria and archaea.[1–4] Lrp regulates *E. coli* metabolism by sensing the presence of rich nutrition by a high concentration of leucine.[5] Calvo and Matthews[5] called this the feast/famine regulation, and so Lrp homologues are referred to as feast/famine regulatory proteins (FFRPs).[1,3,4]

Among FFRPs so far crystallized, that is, LrpA,[6] FL11,[7,8] AsnC,[9] LrpC,[9] TTHA0845,[10] DM1,[11] Lrp,[12] NMB0573,[13] MtbLrp (Rv3291c),[14,15] ST1022,[16] and STS042,[17] Lrp was crystallized without its coregulator leucine.[12] Some others were crystallized with amino acids, but their roles in transcription regulation remain unclear. Asparagine does not change the association state of AsnC, and so it is not a coregulator, although it was cocrystallized with AsnC.[9] In this article, the crystal structures of the FFRP FL11 in complexes with its coregulators lysine and arginine are reported.

Lysine changes the association state of FL11 from the dimer to an octamer for regulating metabolism of the archaeon *Pyrococcus* OT3, which grows on amino acids.[8] In the presence of lysine in the feast mode, four FL11 dimers combine to form an octamer and selectively bind to the *fl11* promoter by covering ~110 bps in order to terminate transcription.[8] As the concentration of FL11 decreases, ~200 transcription units are derepressed, and metabolism is activated. In the absence of lysine in the famine mode, the FL11 octamer dissociates to dimers, so that repression of *fl11* transcription is relaxed. As the number of FL11 dimers increases to ~6000 per cell, transcription of many genes is repressed, and growth is arrested.[8]

In the presence of arginine, ~110 bps in the *fl11* promoter are covered by FL11 in a way similar to that in the presence of lysine (Yokoyama K and Suzuki M, unpublished). Along the ~110 bps an FL11 octamer is formed by combining two dimers at two specific sites separated by ~60 bps, the third dimer binding between the two sites, and the fourth dimer ~30 bps upstream of the first specific site. In the absence of lysine, if other amino acids are available, metabolic shift into the famine mode is unnecessary, because *P.* OT3 can synthesize lysine. Arginine functions as the second indicator of the nutritional condition, and in its presence, transcription of the *fl11* gene is repressed.

Abbreviations: FFRP, feast/famine regulatory protein; Lrp, leucine-responsive regulatory protein.

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The octamer formed along the *fl11* promoter in the presence of arginine is not stable in solution, because with arginine without DNA, the average MW of FL11 assemblies increases only to around MW of a hexamer.[8] In contrast, the octamer formed in the presence of lysine is stable in solution.[8] In fact, when FL11 was crystallized in complexes with lysine and arginine, slightly different octamers were formed. The difference between the two types of octamers is a focus of this article.

MATERIALS AND METHODS

Protein purification, crystallization, and data collection

FL11 was purified as has been previously described,[7] with a modification that a His-tag introduced at the N-terminus was cleaved by adding thrombin (Novagen), 1 unit per 1 mg FL11, and incubating at 20°C for 16 h. The His-tag free FL11 was flown through Ni-Sepharose HP (GE healthcare) and applied to gel filtration using Sephacryl S-300 (GE Healthcare) as has been described.[7]

Using Amicon Ultra (Millipore), FL11 was concentrated to 10 mg/mL in 10 m*M* Tris-HCl buffer (pH 8.0) containing 200 m*M* NaCl and 20 m*M* lysine or arginine. The solution, 3 μL, was mixed with the same volume of 22.5–25% PEG3350 containing 300 m*M* $CaCl_2$. Crystals of (0.2–0.3 mm) × (0.2–0.3 mm) × (0.05–0.1 mm) were obtained in a week by vapor diffusion using the sitting drop method.

Crystals were immersed into 22.5–25% PEG3350 containing 300 m*M* $CaCl_2$ and 20% (w/v) glycerol and flash frozen at 95 K using liquid nitrogen. Diffraction was recorded by covering ranges to 2.39 Å for the FL11-lysine complex and 2.59 Å for the FL11-arginine complex (Table I), using a synchrotron X-ray (1.0 Å) source (Photon Factory BL17A, Tsukuba) and an area detector (Quantum 270, ADSC). The data were processed using the HKL2000 package.[18]

Crystal structure determination

The crystal systems of the two complexes were monoclinic. The space group of the lysine complex was identified as *P*2 (a = 98.23 Å, b = 102.34 Å, c = 104.05 Å, and β = 118.05°), and that of the arginine complex was $P2_1$ (a = 102.31 Å, b = 73.50 Å, c = 103.06 Å, and β = 98.47°) (Table I). The initial phases were obtained by molecular replacement using the structure of the FL11 monomer crystallized with no ligand amino acid (PDB code 1RI7) and the MOLREP program[19] in the CCP4 package.[20] For both complexes, eight FL11 monomers were identified as forming four dimers in the asymmetric units.

Models were built on the 2Fo-Fc map using the COOT[21] and XtalView/Xfit[22] programs. All the monomer models were refined independently using the Refmac[23] and CNS[24] programs, monitoring *R*-factors, without imposing a restraint from noncrystallographic symmetry. To each density of 3σ or higher in the Fo-Fc map, when an oxygen or nitrogen atom was present within the hydrogen bonding distance, a water molecule was added using CNS. Altogether 459 water molecules were added to the lysine complex, and 248 water molecules to the arginine complex. After several rounds of refinements, eight lysine and six arginine molecules were modeled to the densities, which had characteristic shapes in the respective complexes, and refined.

The resolution of the arginine complex was lower, and B factors were higher (Table I). The model of the arginine complex was finalized when R_{cryst} became small enough, 25.4%, and R_{free} became the smallest, 25.8%, without further decreasing R_{cryst} by increasing R_{free}. In this way, overfitting of the model to unclear electron densities was avoided. The lysine complex was modeled using a clearer electron density map, and its R_{cryst}, 20.6%, and R_{free}, 20.9%, were smaller. Because of the different qualities of the electron density maps, the number of water molecules identified in the asymmetric unit of the arginine complex was smaller than that of the lysine complex. This difference also seems to have relatively increased R_{cryst} and R_{free} of the arginine complex. For calculation of R_{free}, 10% of the reflections were used.

The final model of the arginine complex included Leu5-Ile150 in the eight polypeptides. In that of the lysine complex, Asp7-Ile150 were included in polypeptide C, and Leu5-Ile150 in the other polypeptides. When Ramachandran plots[25] were made using the PROCHECK program,[26] all the dihedral angles were accepted. For FL11 in complex with lysine, 94.5% of the dihedral angles were found in the most favored regions, and for FL11 in complex with arginine, 90.0% dihedral angles were found. The coordinates of the lysine and arginine complexes have been deposited into the Protein Data Bank with the accession codes, 2ZNZ and 2ZNY, respectively.

Hydrogen bonds were identified using the HBPLUS program.[27] Atom pairs identified as not forming hydrogen bonds were analyzed by the method of Barlow and Thornton,[28] and ionic interactions were identified using the standard criterion of 4.0 Å. Pairs of C or S atoms distanced within 5.0 Å were identified as forming hydrophobic interactions. Figures of this article were made using the Pymol program.[29]

RESULTS AND DISCUSSION

Open and closed octamers of FL11

In the asymmetric unit of the FL11-lysine complex, four dimers were formed by monomers A and B, C and D, E and F, and G and H. Related by the crystallographic symmetry, two essentially the same octamers were closed,

Table I
Data Collection and Structure Determination

	FL11-lysine	FL11-arginine
Data collection		
Temperature (K)	95	95
Wavelength (Å)	1.00	1.00
Spacegroup	*P*2	$P2_1$
a (Å)	98.23	102.31
b (Å)	102.34	73.50
c (Å)	104.05	103.06
β (°)	118.05	98.47
Resolution (Å)	50.00–2.39 (2.48–2.39)[a]	50.00–2.59 (2.68–2.59)[a]
No. of measured reflections	311,277	142,948
No. of unique reflections	71,303	47,467
R_{merge}	0.087 (0.230)[a]	0.080 (0.233)[a]
Completeness (%)	99.6 (99.7)[a]	95.2 (98.1)[a]
Average $I/\sigma(I)$	17.47 (4.83)[a]	19.10 (4.80)[a]
Structure determination		
R_{cryst}/R_{free}	0.206/0.209	0.254/0.258
Rms deviation from ideality		
Bond length (Å)	0.009	0.014
Bond angle (°)	1.20	1.90
Ramachandran plot		
Most favored (%)	94.5	89.5
Allowed (%)	5.5	9.9
Generously allowed (%)	0.0	0.6
Average B factor		
Main-chains	42.13	51.04
Side-chains	43.01	51.53
Water molecules	41.61	41.98
Ligands	31.00	40.57

[a]Value calculated for the highest resolution shell in parentheses.

one by two copies of the AB and CD dimers [Fig. 1(a,b)], and the other by two copies of the EF and GH dimers [Fig. 1(c,d)]. In each interface between neighboring dimers, two lysine molecules were present so that two copies of lysine molecules I–L were binding to the ABCD octamer [Fig. 1(a,b)], and two copies of lysine molecules M–P to the EFGH octamer [Fig. 1(c,d)].

In the asymmetric unit of the FL11-arginine complex, dimers AB, CD, EF, and GH were arranged with a right-handed helicity [Fig. 1(e,f)]. This helicity was too slight to form a helical turn, but a gap was created between GH and AB similarly to the crystal structure of Lrp.[12] Two arginine molecules were present in each dimer interface so that six arginine molecules, I–N, were binding to the open octamer. The 3D structures of FL11 dimers in the lysine and arginine complexes were essentially the same as those of dimers crystallized with no amino acid.[7,8]

The octamer formed with arginine is unstable without DNA, but the octamer formed with lysine is stable in solution.[8] As described later, the different stabilities of the two octamers can be explained by comparing the crystal structures of the open and closed octamers. And so it is likely that the open and closed octamers correspond to the octamers formed along the *fl11* promoter in the presence of arginine and lysine, respectively.

Stabilization of the closed octamer by interaction with lysine

In each dimer interface in the lysine complex, hydrophobic interactions were formed between the dimers by 10 pairs of residues; Leu121 in dimer A-Leu139 in dimer B, Leu139-Leu121, Ile137-Thr100, Thr100-Ile137, Thr100-Leu117, Leu117-Thr100, Leu117-Leu139, Leu139-Leu117, Met67-Met67, and Ile137-Ile137. On average 2.0 hydrogen bonds were formed; in five of eight interfaces in the two octamers, two sets of Tyr103 O_ηH-Asp102 O_δ, and in two interfaces one set of Lys74 N_ζH-Glu131 CO and two sets of Asn118 N_δH-Glu98 O_ε. Interactions with two lysine molecules stabilized each dimer-interface by

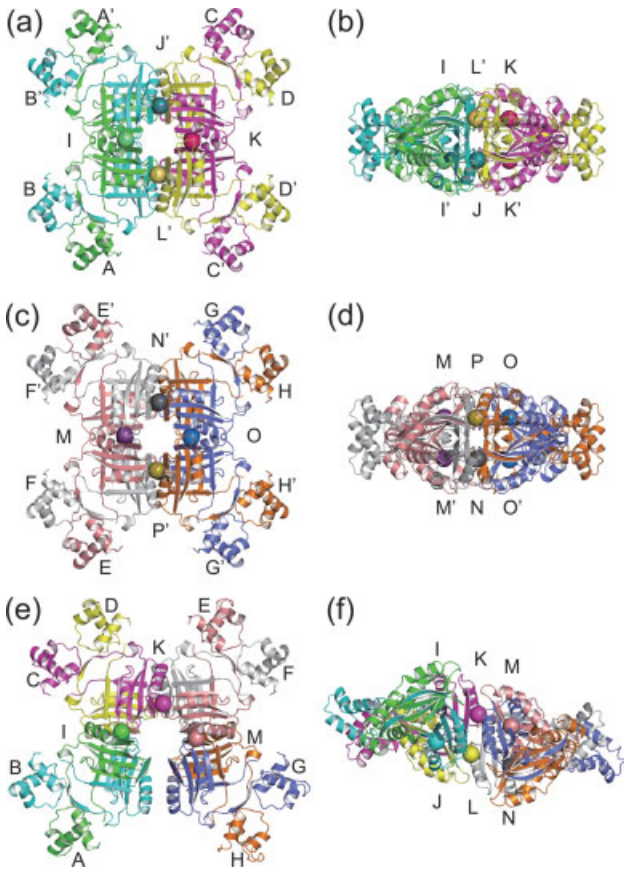

Figure 1
Two closed octamers of FL11 formed in complex with lysine (**a**–**d**), and the open octamer of FL11 formed in complex with arginine (**e**, **f**). (a, b) The ABCD octamer formed by two copies of dimers AB and CD, and bound by two copies of lysine molecules I–L. The second copy of, for example, monomer A is labeled A′. The positions of the lysine molecules are indicated by balls. In (a) lysine I is positioned on top of lysine I′, J′ on top of L, K on top of K′, and L′ on top of J. (c, d) The EFGH octamer bound by two copies of lysine molecules M–P shown in the same ways as the ABCD octamer (a, b). (e, f) The octamer formed by dimers, AB, CD, EF, and GH, and bound by arginine molecules I–N (indicated by balls), with a gap formed between GH and AB. In (e) I is positioned on top of J, K on top of L, and M on top of N.

hydrophobic contacts between four pairs of side-chains and as many as 50.26 hydrogen bonds on average.

Each lysine donated hydrogen bonds from its N-terminus to Asp104 O_δ, and the main-chain CO of Thr99 and Gly101, all in the same monomer, that is, monomer I, in FL11 dimer A [Fig. 2(a)]. Each lysine accepted hydrogen bonds to O_A at the C-terminus from Thr133 $O_\gamma H$ and Thr135 $O_\gamma H$ in another monomer, that is, monomer II, in dimer B, and to O_B from the main-chain NH of Thr135 in monomer II and Gly101 in monomer I. The lysine side-chain donated hydrogen bonds to Asp122 O_δ and Asn118 O_δ in monomer II, and Glu98 O_ε in monomer I. In addition, lysine molecule P donated a hydrogen bond from its main-chain NH to the main-chain CO of Tyr103 in monomer I. Lysine side-chain C atoms approached C atoms in Tyr80 and Leu121 side-chains, thereby forming on average 17.50 hydrophobic contacts with Tyr80 and 7.63 contacts with Leu121.

In this way, each pair of neighboring FL11 dimers were bridged by interactions with two lysine molecules, and four sets of such interactions closed each octamer. These interactions were similar to those we had predicted[11] and explain the higher stability of the octamer formed in the presence of lysine.

When another FFRP, DM1, was crystallized in complexes with isoleucine and Se-methionine, octamers were formed.[11] Direct interaction between each pair of neighboring DM1 dimers in the octamers does not seem to be weaker than that between FL11 dimers. In each interface of DM1 dimers hydrophobic contacts were formed between 12 pairs of residues, and on average 9.5 hydrogen bonds were formed. But in solution, in the absence of amino acids, DM1 remains as the dimer similarly to FL11.[11] In the crystal structures, altogether four isoleucine or Se-methionine molecules were binding to two of the four interfaces in order to stabilize each octamer.[11]

Stability and roles of the open octamer

The FL11-arginine interaction was similar to the FL11-lysine interaction as a whole, but not all the contacts were formed with the same arginine molecule. On average 7.67 hydrogen bonds, 0.5 ionic interactions, and 11.0 hydrophobic contacts were formed with each arginine, in comparison with 10.13 hydrogen bonds and 25.13 hydrophobic contacts formed with each lysine. In short, arginine fitted into the dimer interface less ideally than lysine. The weaker FL11-arginine interaction and the lack of such interaction in the gap can explain the weaker stability of the open octamer in solution.

Thr135 $O_\gamma H$ donated a hydrogen bond to arginine O_A [Fig. 2(b)] at five FL11-arginine interaction sites, but at the other site to O_B. At three sites, the main-chain NH of Thr135 donated a hydrogen bond to O_B, but at another site to O_A. At three sites, arginine $N_{\eta 1}H$ and Asp122 O_δ formed a hydrogen bond [Fig. 2(b)], but at

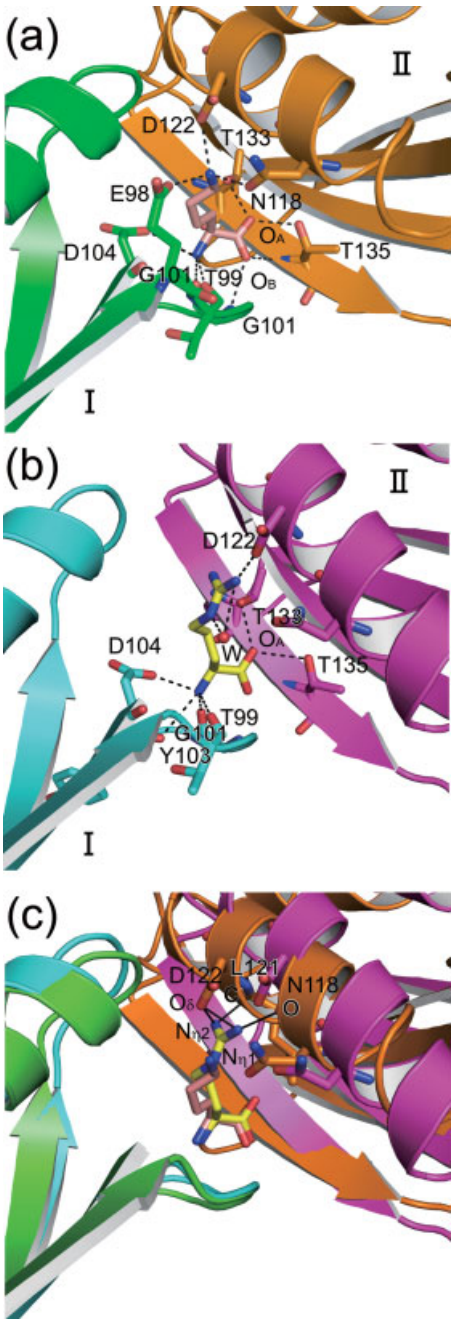

Figure 2

Dimer interfaces in the lysine (**a**) and arginine (**b**) complexes, and their comparison (**c**). (a) Hydrogen bonds formed between lysine molecule I (pink), and monomers I (green) and II (orange) of neighboring FL11 dimers are indicated by broken lines, with coloring O (red) and N (blue) atoms. (b) Hydrogen bonds formed between arginine molecule L (yellow), and monomers I (cyan) and II (magenta) of neighboring FL11 dimers are indicated by broken lines, with coloring O (red) and N (blue) atoms. A water molecule (W) accepts hydrogen bonds from $N_{\eta 1}H$ and $N_\varepsilon H$ of the arginine. (c) The two interfaces in (a) and (b) are overlaid by maximizing alignment of C_α atoms in monomers I. Conflicts between the arginine and the FL11 monomer II in the lysine complex are indicated by solid lines. They are found between arginine $N_{\eta 1}$ and Asp122 O_δ, arginine $N_{\eta 1}$ and O of Asn118 CO, arginine $N_{\eta 2}$ and Asp122 O_δ, and arginine $N_{\eta 2}$ and C of Leu121 CO.

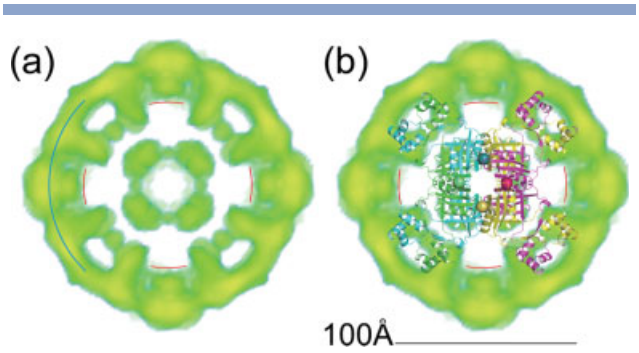

Figure 3

Comparison of the closed octamer of FL11 with the electron microscopic 3D reconstruction of FL11 in complex with ~100 bp DNA. The ABCD octamer of the FL11-lysine complex [Fig. 1(a)] is overlaid (**b**) onto the average 3D reconstruction imposed with 422 symmetry (**a**). Red circles are 92 Å in diameter. In (a) a quarter of a circle of diameter 131 Å is drawn in blue. Figure 3(a) without the blue quarter circle was originally published as Figure 6J from Structure 15(12), Yokoyama et al., Feast/famine regulation by transcription factor FL11 for the survival of the hyperthermophilic archaeon *Pyrococcus* OT3, 1542–1554, 2007, with permission from Elsevier.

two other sites an ionic interaction. At four sites, arginine $N_{\eta 1}H$ and Glu98 O_{ε} formed a hydrogen bond, but at another site an ionic interaction. At no site, Asn118 formed a chemical contact with the arginine side-chain. In addition to these arginine-mediated interactions, hydrophobic contacts between seven pairs of residues and on average 4.0 hydrogen bonds were formed directly between FL11 dimers in each dimer interface.

The arginine side-chain is longer than the lysine side-chain. When an FL11-arginine interaction site was overlaid onto an FL11-lysine interaction site by best aligning C_{α} atoms of monomers I [Fig. 2(c)], generally arginine $N_{\eta 1}$ and $N_{\eta 2}$ became too close to one of Asp122 O_{δ} in monomer II of the lysine complex. Arginine $N_{\eta 1}$ was too close also to O of Asn118 CO, and arginine $N_{\eta 2}$ to C of Leu121 CO. To avoid these conflicts while keeping contacts with the N and C termini of arginine, each interface opened on the side nearer the center of the octamer [Fig. 2(c)]. Accumulation of such opening resulted in formation of the gap between dimers GH and AB. Together with the weaker FL11-arginine interaction, lack of such interaction in the gap explains the weaker stability of the octamer formed in the presence of arginine.

In the absence of lysine and presence of other amino acids, shift into the famine mode is unnecessary, because *P.* OT3 can synthesize lysine. To prevent such shift by arginine, a concentration higher than that of lysine might be needed, as *P.* OT3 does not synthesize arginine (Yokoyama K and Suzuki M, unpublished). The weaker stability of the open octamer seems to be consistent with this idea.

Transcription regulation of the *fl11* gene is further complicated by another fact that FL11 and DM1 form a hetero-octamer in the presence of m*M* arginine and bind to the *fl11* promoter.[11] A mechanism of regulating the DM1 concentration in *P.* OT3 is not known.

Comparison of the closed octamer with the FL11-DNA complex

When FL11 was mixed with lysine and a 150 bp DNA from the *fl11* promoter, containing the ~110 bps, particles with diameters of ~150 Å were observed by electron microscopy.[8] In these particles, spokes protruded from internal rings to outer rings. Very similar particles were recorded in tilt series using an FL11 fraction, where *E. coli* DNA was contaminating. Using these series, 3D reconstructions were made and averaged by imposing 422 symmetry.[8]

The crystal structure of the closed octamer fitted well into the internal ring plus spokes of the average reconstruction [Fig. 3(b)]. Thus the outer ring corresponds to the DNA. Because of 422 symmetry imposed, the ring was closed, but the genomic DNA binding around the closed octamer will form a superhelix. Using topoisomerase I, the superhelix formed by a plasmid DNA around the closed octamer was identified as right-handed (Ishijima SA and Suzuki M, unpublished). So upon interaction with the *fl11* promoter the DNA-binding domains in the closed octamer will form a right-handed helix, similarly to the domains in the open octamer crystallized.

The outer ring in the average 3D reconstruction spanned ~100 Å from the center of a site bound by an FL11 dimer to the center of another site bound by the neighboring dimer [indicated by the blue quarter circle in Fig. 3(a)], and so ~30 bps are expected between the two centers. Two dimers in the closed octamer bind to specific sites separated by ~60 bps in the *fl11* promoter, and another dimer binds to a nonspecific site between the two sites.[8] In this way details of the FL11-lysine crystal complex fit well with the mechanism of regulating transcription by FL11, which we reported earlier.[8]

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