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X-ray Structure of the *T. Aquaticus* FtsY:GDP Complex Suggests Functional Roles for the C-Terminal Helix of the SRP GTPases

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Abstract

FtsY and Ffh are structurally similar prokaryotic Signal Recognition Particle GTPases that play an essential role in the Signal Recognition Particle (SRP)-mediated cotranslational targeting of proteins to the membrane. The two GTPases assemble in a GTP-dependent manner to form a heterodimeric SRP targeting complex. We report here the 2.1 Å X-ray structure of FtsY from *T. aquaticus* bound to GDP. The structure of the monomeric protein reveals, unexpectedly, canonical binding interactions for GDP. A comparison of the structures of the monomeric and complexed FtsY NG GTPase domain suggests that it undergoes a conformational change similar to that of Ffh NG during the assembly of the symmetric heterodimeric complex. However, in contrast to Ffh, in which the C-terminal helix shifts independently of the other subdomains, the C-terminal helix and N domain of *T. aquaticus* FtsY together behave as a rigid body during assembly, suggesting distinct mechanisms by which the interactions of the NG domain “module” are regulated in the context of the two SRP GTPases.

Keywords

SRP; Ffh; FtsY; SR; GTPase; GDP; X-ray crystallography

INTRODUCTION

FtsY, the GTPase receptor for the Signal Recognition Particle (SRP) in prokaryotes,^{1,2} forms a GTP-dependent targeting complex with Ffh, the structurally similar GTPase of the SRP, that plays an essential role in co-translational targeting of proteins to the membrane.^{2–4} The SRP is a highly conserved ribonucleoprotein, and homologs of FtsY and Ffh in higher organisms are known as SR α and SRP54, respectively.^{1,5} The GTPase domains of FtsY and Ffh comprise two structural subdomains, termed N and G (or “NG”).⁶ The NG GTPase is a modular functional unit that occurs at the N-terminus in the sequence of Ffh, and at the C-terminus of FtsY.^{7,8} In some prokaryotes, such as *T. aquaticus*, however, FtsY comprises only the NG GTPase,⁹ and the FtsY NG GTPase alone has been shown to be sufficient for function.^{10–12}

Previous X-ray structures of the SRP NG GTPases from Ffh and FtsY^{6,13} have revealed a common three-dimensional fold [Fig. 1(A)], similar to that of other members of the GTPase superfamily.^{15,16} Interestingly, the N- and C-termini of the NG domains are closely apposed in their three-dimensional structures; therefore, although the position of the modular GTPase domain in the primary structure of each protein is different, its spatial relationship to the

auxiliary domains of each protein—the N-terminal A-domain of FtsY and the C-terminal M domain of Ffh—is similar. In Ffh, the M domain, C-terminal to the GTPase domain, mediates interaction with the signal sequence of the nascent polypeptide and also binds to the RNA component of SRP.^{17–19} However, little is known about its interaction with NG—a dynamic relationship between the M- and NG-domains has been demonstrated by fluorescence resonance energy transfer,²⁰ susceptibility to proteolysis of the linker polypeptide between the M and NG domains,¹⁷ and disorder between the two domains in the crystal structure of the intact *T. aquaticus* Ffh (“NG-M”),²¹ but modification of one domain of Ffh (e.g. the NG GTPase) affects the function of the other (e.g. the M domain),²² and there are specific interactions between the two domains in the presence and the absence of RNA.^{23,24} The relationship between the FtsY NG GTPase domain and its N-terminal A-domain is also poorly understood.^{11,25–27} The A domain may function in interaction with the plasma membrane,^{26,27} and proteolysis between the A and NG domains, possibly coupled to the targeting mechanism, has been reported.¹² However, the A domain of *E. coli* FtsY is not essential for SRP-mediated targeting,¹¹ and the A domain is absent in *T. aquaticus*.⁹ In eukaryotic SR α the role of the N-terminal “SRX” domain is more clearly defined, as it provides the specific interaction between SR α and its membrane anchor SR β .^{28,29}

Coordinate structural changes of the FtsY and Ffh NG GTPase domains occur during the assembly of their cotranslational targeting complex^{30,31} that may act to signal assembly of the complex³⁰ and provide a mechanism for regulating its assembly by recruitment of other elements of the cotranslational targeting machinery (i.e. the ribosome, the nascent polypeptide, and the translocon^{32–35}). In the GMPPCP-stabilized heterodimeric complex between Ffh and FtsY these changes generate a “latched” configuration of the two GTPase domains that exhibits a striking two-fold pseudo-symmetry.^{30,31} In the complex, the C-terminal helices of both proteins pack symmetrically at the interface of their respective N and G subdomains, displacing their N-termini such that, in Ffh NG, the first five residues become disordered,^{30,31} and in FtsY NG, the N-terminal ~20 residues become susceptible to proteolysis.⁹ That the linker peptides to the N-terminus (in FtsY) and the C-terminus (in Ffh) of the NG domain are located similarly adjacent to the N/G interface means that their interactions may modulate common structural features of the two GTPases. Neither linker is visualized in structures of the NG GTPase domain alone. But the modular structure of the paired NG GTPase domains, their two different primary sequence contexts, and their symmetric reorientation on formation of the targeting complex,³⁰ suggests that structural rearrangements common to the architecture of both the Ffh and FtsY GTPases may be exploited in different ways by the two proteins to signal their distinct partners.

Here, we report the structure of the GDP-bound *T. aquaticus* FtsY, allowing direct comparison with its structure in the heterodimeric SRP GTPase targeting complex.^{30,31} In contrast to *T. aquaticus* Ffh,⁶ the surface loops of FtsY that contribute to assembly of the heterodimeric interface are, in general, poorly ordered in the monomeric protein. Furthermore, GDP is bound such that the specificity-determining hydrogen bonds with motif IV are fully formed, in contradiction to the proposal that they might be absent prior to assembly.³⁶ Comparison of the conformational rearrangements that occur in Ffh and FtsY during assembly into their heterodimeric complex suggests that while they adopt symmetric configurations in the complex, the conformational path taken by each protein is different. These data support the hypothesis that the C-terminal helix, which is positioned distinctly in the SRP subfamily relative to its position in other small GTPases,^{15,16} plays a specific role in regulating the structural state of the targeting complex.

MATERIALS AND METHODS

Expression and Purification of FtsY NGd20

The vector pJGS3 expressing the protein FtsY NGd20, in which the N-terminal residues 2–20 of *T. aquaticus* FtsY are deleted, was constructed by PCR-based site-directed mutagenesis of pTP88.^{37,38} Initial expression trials in the BL21(DE3)/RP *E. coli* expression strain (Stratagene) resulted in low levels of protein expression, and the construct was further modified to optimize the sequence and position of the Shine-Dalgarno sequence. All sequences were verified by sequencing at the Northwestern University Biotechnology Laboratory. The FtsY NGd20 deletion of the *T. aquaticus* FtsY NG domain (comprising residues 21–304) was expressed from pJGS3 using the Rosetta-2(DE3)/pLysS strain of *E. coli*. (Novagen). Cells were pelleted by centrifugation and resuspended in 20 mL of buffer containing 20 mM EPES, pH 8.0, 2 mM EDTA, 250 mM NaCl, 10% glycerol, 1 mM DTT, and 0.1 mg/mL lysozyme. Following three freeze/thaw cycles, the cell paste was disrupted by sonication and then incubated at 70°C for 20 min. The resultant supernatant was filtered through 0.8 and 0.2 µm cellulose acetate membranes and passed over a 75 mL DE53 column equilibrated with 50 mM Tris, pH 8.0, 250 mM NaCl, and 1.0 mM DTT, then dialyzed against 10 mM HEPES, pH 7.5, and 1.0 mM DTT overnight at 4°C. The FtsY NGd20 protein was purified over a HiTrap 5 mL Blue column eluted using a linear gradient to 1.0 M NaCl, desalted and then passed over a HiTrap 5 mL SP column (Amersham Pharmacia) equilibrated with 10 mM HEPES, pH 7.5, 1.0 mM DTT, and eluted using a linear gradient to 1.0 M NaCl. The desalted fraction was, finally, passed over HiTrap 5 mL Q Sepharose HP column (Amersham Pharmacia) equilibrated with 50 mM Tris, pH 8.0, 1.0 mM DTT, and the protein eluted using a linear gradient to 1.0 M NaCl. Approximately 12 mg of protein were obtained from 2 L of culture.

Crystallization and Data Collection

Crystals of the FtsY:GDP complex were obtained during screens for crystallization of the GDP:AlF₄-stabilized Ffh:FtsY heterodimer. The proteins were each at ~5.5 mg/mL in 2 mM AlCl₃, 20 mM NaF, 1 mM GDP, 50 mM Hepes, 30 mM Tris, 250 mM NaCl, and 2 mM MgCl₂, pH 7.5. Crystals grew from 0.2 M ammonium acetate and 2.2 M ammonium sulfate, and were frozen using a nylon loop from a cryo-mother liquor supplemented with 10% ethylene glycol.³⁹ The crystal used for data collection was annealed by thawing in cryo-mother liquor three times, during which the diffraction limit increased from ~3.0 to ~2.0 Å. Data were measured at APS Bio-CARS beamline 14BMC using a wavelength of 0.900 Å and an exposure time of 10 s for 0.7° rotation over a total rotation angle of 196°. Data were integrated with MOSFLM,⁴⁰ and scaled using the CCP4 suite.⁴¹ The diffraction symmetry indicated space group I4 (or I4₁) and assuming one NG domain in the asymmetric unit, the crystal contained 45.8% solvent. Molecular replacement using the NG domains of both Ffh and FtsY (1OKK.pdb) as probes in PHASER⁴² yielded clearly distinguishable results (LLGs of –5 and 522 for Ffh and FtsY, respectively).

Refinement

The structure was built and refined using Arp/wARP⁴³ and REFMAC,⁴⁴ and the model inspected and manually corrected using O.⁴⁵ Both the N- and C-terminal residues (Ala²¹ and Asp³⁰⁴) are clearly located in the electron density map. Residues 80–82 and 95–96 of the linker peptide between the N and G domains cannot be located, but an intervening segment (83–94) is well ordered across a crystal packing contact (see text). In the G domain, residues 141–146 of motif II and residues 235–236 of the DAGQ motif cannot be placed in electron density. The two adjacent loops, residues 165–167 of the IBD and residues 195–201 of motif III, are both poorly ordered (B factors of 60–68 Å²) but can be located in the map. Electron density for the bound GDP is well defined, but there is some evidence for partial

occupancy of the nucleotide in the site—stronger density at the β -phosphate than at the α -phosphate is consistent with full occupancy at that position either by GDP or by SO_4 from the crystallization mother liquor. Following refinement to consistent B factors, we estimate that the occupancy of GDP is ~65%.

Analysis

Coordinates were superimposed using LSQMAN.⁴⁶ Superposition over the nucleotide was carried out using all GDP atoms, yielding an rmsd over 28 atoms of 0.85 and 0.37 Å, respectively, when compared with structures of the Mg^{2+} -GDP bound Ffh, 1NG1, and 1O87.^{14,47} The RMS deviations of the residues that pack against the nucleotide—the motif I loop (FtsY residues 109–118, Ffh residues 105–114), motif IV loop (FtsY residues 255–258, Ffh 245–248), and the closing loop (FtsY 281–286, Ffh 271–276)—were evaluated using this superposition [Fig. 2(A)]. The rmsd is 0.80 Å for all 134 atoms, and 0.54 Å for the 21 C α atoms only. Also, the *T. aquaticus* FtsY G domain was superimposed over 173 C α atoms of the G domain of *T. maritima* FtsY with a 0.97 Å rmsd, *E. coli* FtsY (165 atoms with rmsd 1.26 Å), and *M. mycoides* FtsY (165 atoms, rmsd of 1.63 Å).

GTPase assay

GTP hydrolysis was measured using a colorimetric assay under saturating nucleotide conditions.^{48–50} Protein was at a final concentration of 2 μM in 50 mM Hepes, pH 7.5, 10 mM MgCl_2 , and 50 mM NaCl; the reaction was started by adding 20 μL of a 1 mM GTP (or ATP) solution in ddH_2O to a 20 μL premix at 2 \times protein and buffer concentration. Reactions were incubated at 42°C for 60 min and stopped by placing in liquid nitrogen. The samples were thawed in a water bath and assayed by addition of 80 μL of freshly prepared filtered Malachite Green reagent.⁴⁹ After 10 min, the colorimetric reaction was stopped by adding 5.2 μL of 34% w/v NaCitrate. Samples (100 μL) were assayed in 96-well plates using a Molecular Devices Spectramax absorbance reader at 620 nm. A standard curve generated by twofold dilution was linear over the range 50–1600 pmol free phosphate. Each measurement is the average of duplicates.

Coordinates

Coordinates and structure factors have been submitted to the Protein Data Bank and assigned codes 2iy1 and r2iy1sf, respectively.

RESULTS

We describe here the structure of *T. aquaticus* FtsY from a crystal obtained during a screen for the heterodimeric complex of *T. aquaticus* Ffh and FtsY that was found to contain only the FtsY monomer, with GDP bound. The crystallization screen was carried out using an FtsY protein construct in which the N-terminal 20 amino acids were truncated, mimicking the proteolysis observed on assembly of the heterodimeric SRP GTPase complex.^{9,30,37} The 2.1 Å resolution X-ray structure was determined by molecular replacement and refined to an R_{cryst} of 0.21 and R_{free} 0.29 (Table I). The model comprises the FtsY NGd20 monomer (residues 21–304), one bound GDP, one sulfate ion, and 144 water molecules [Fig. 1(A)]. The electron density map clearly defines the position of the bound nucleotide [Fig. 1(B)]. Although magnesium was present in the crystallization buffer, there is no evidence for Mg^{2+} ion in the active site.

FtsY Binds GDP in a Canonical Configuration

Two solvent exposed loops, comprising much of GTPase motif II (residues 141–146)⁵¹ and the “DAGQ” motif (235–236)³⁰ adjacent to the binding site, cannot be located in the

electron density map, and the adjacent loops of GTPase motif III (196–201) and the connecting loop between β 2a and α 2b of the IBD (165–166) are poorly ordered [Fig. 1(A)]. The disorder of these regions contrasts with the well-ordered conformations adopted in structures of the *T. aquaticus* Ffh,⁶ which are stabilized by a network of interactions that couple them to each other.⁶ Only motif II exhibits substantial disorder¹⁴ or conformational variability⁴⁷ in structures of the GDP complexes of *T. aquaticus* Ffh. Motifs II and III correspond approximately to the GTPase “switch 1” and “switch 2” loops that are often disordered in the GDP-bound state of other GTPases,^{16,52} and three of the motifs—II, III, and DAGQ—undergo concerted rearrangement on formation of the heterodimeric SRP GTPase targeting complex to generate an intricate set of hydrogen bonding and van der Waals packing interactions across the interface.³⁰ That the residues of these loops are disordered presumably reflects their propensity to adopt different structural configurations in the monomeric and complexed states; however, why more flexibility is seen in the context of FtsY than in the structurally similar Ffh (35% identity, 52% similarity over the “NG” GTPase domain) is unclear.

The binding configuration of the GDP molecule and its interactions with the conserved GTPase motifs I–IV¹⁵ are similar to that for other GTPases¹⁶ and for the binding of GDP to *T. aquaticus* Ffh¹⁴ [Fig. 1(A)]. These interactions include the specificity-determining H-bonding interaction, provided by the aspartate sidechain (Asp²⁵⁸) of motif IV, that would have to be displaced if a noncognate nucleotide (i.e. ATP, XTP) were to be accommodated within the site [Fig. 1(B)]. Indeed, when the magnesium-free binding configuration for GDP in the *T. aquaticus* FtsY structure is superimposed over the nucleotide alone with the GDP configuration in the Mg²⁺GDP complex of *T. aquaticus* Ffh, the relative positions of the three polypeptide segments that mediate all interactions with the nucleotide in the two structures—motif I, motif IV, and the closing loop—are essentially identical [Fig. 2(A)]. These observations appear to be inconsistent with a requirement for assembly into its targeting complex with Ffh prior to acquisition of nucleotide specificity by the *T. aquaticus* FtsY,³⁶ and are consistent with other biochemical studies of FtsY from different species.^{53,54} However, in solution, both the full length *T. aquaticus* FtsY (which comprises the NG domain) and the FtsY NGd20 construct used in the crystal structure determination retain some activity towards the noncognate nucleotide, ATP [Fig. 2(B)].

A Common Arrangement of the N and C Terminus Prior to Assembly

In addition to the structure of the *T. aquaticus* FtsY described here, there are three other published crystal structures of the NG domain of FtsY, all in the apo state—from *E. coli* (1FTS),¹³ *T. maritima* (1VMA),⁵⁵ and *M. mycoides* (1ZU4, 1ZU5).⁵⁶ Their different sequences are ~35% identical,⁵⁶ and the G domains share a common core structure that allows them to be directly superimposed with an rmsd of 1.0–1.6 Å [Fig. 3(A)]. The relative orientations of the N- and G-domains of the proteins vary, as has been noted previously for different structures of Ffh¹⁴; when superimposed over the 18 atoms of the G domain motif I P-loop (rmsd ranges from 0.28 to 0.46 Å for the three pairs), the N domains of *E. coli* FtsY and *T. maritima* FtsY are superimposable in a configuration that is similar in orientation, but not in detail (see below), to that found when FtsY assembles with Ffh in its GTP-dependent targeting complex,^{30,31} and the N domains of *T. aquaticus* and *M. mycoides* FtsY are rotated away from this position to different extents around the interface helix α 4.⁵⁷ However, all four structures share two commonalities—the α 4 interface helix, which couples the N and G domains, is superimposable in a position ~2.5 Å away from that adopted by the helix in the *T. aquaticus* Ffh:FtsY complex, and despite the disparate disposition of each N domain, in each case the C-terminal helix that packs against it adopts a common orientation that is similar between structures and that is distinct from that adopted in the Ffh:FtsY complex [Fig. 3(A)].

The configuration of the C-terminal helix in each of the structures is stabilized by an N-terminal interposition that packs between the C-terminal helix and the adjacent motif IV of the G domain.^{13,56} In the *M. mycoides* and *T. maritima* FtsY crystal structures,^{55,56} a short α -helix at the N-terminus of the N domain helical bundle (“N1”) packs against the C-terminus [Fig. 3(A)], and in the crystal structure of the *E. coli* FtsY NG domain a cleaved and relatively unstructured N-terminus extends around the C-terminal helix.¹³ In the former cases the helix packing is mediated by a highly conserved heptad repeat at the N-terminus of FtsY NG domain sequences⁵⁶ that nestles into a conserved hydrophobic surface at the N/G domain interface.⁵⁸ The structural and functional implications of these interactions have recently been discussed⁵⁶—in the intact FtsY they may couple the putative targeting function of the N-terminal A-domain (or perhaps, the N1 helix itself) to the NG GTPase domain.^{12,26,59}

That a similar orientation of the C-terminal helix is maintained in the structure of *T. aquaticus* FtsY, despite the first 20 amino acids of the NG domain being absent (see above), is explained by a crystal packing interaction that compensates—although much of the linker peptide between the N- and G-domains is disordered, residues 83–94 can be located unambiguously in the electron density map, splayed across a crystal contact to pack at the N/G interface of an adjacent monomer [Fig. 3(B)]. The linker adopts an extended conformation that allows three hydrophobic sidechains (Leu⁸⁸, Phe⁹⁰, and Pro⁹²) to pack between the C-terminal helix and the motif IV/DARGG loop. The packing interactions of the linker peptide in this structure closely mimic the hydrophobic interactions of the N-terminal helix (in *T. maritima* and *M. mycoides* FtsY) or unstructured peptide (in *E. coli* FtsY) against the hydrophobic face of the N/G interface [Fig. 3(A)].

Specific Structural Transitions Between the Free and Complexed FtsY

The structural changes that occur on assembly of the heterodimer can be inferred from comparison of the monomeric FtsY:GDP and assembled FtsY:GMPPCP complex structures. The G domain of *T. aquaticus* FtsY in its GDP-bound state can be superimposed on its structure in the GMPPCP-stabilized heterodimer complex with an rmsd of only 0.97 Å for 171 C α s, as the conformational changes occurring within the G domain (but not between the N and G domains) are largely limited to the loops at the interface. GTP binding itself does not functionally activate SRP receptor prior to its interaction with SRP,^{60,61} and a noncanonical binding mode is observed in the *T. aquaticus* Ffh NG:GMPPNP complex,⁶² suggesting that while GTP binding to Ffh and FtsY serves to prime interaction between the SRP GTPases, nucleotide binding alone is not accompanied by activating protein structural changes. Therefore, in the following, we can describe the structural changes between the GDP-bound monomeric protein and its GMPPCP-bound state in the heterodimeric complex as representative either of assembly or of disassembly of the complex. The structural changes of each loop are coupled across the protein interface—thus, for example, a \sim 2.0 Å shift between the DAGQ motif and the N-terminus of helix α 3 that follows it accommodates rearrangement of the adjacent motif III loop (corresponding to the GTPase “switch 2”) as it adopts its “latched” configuration.³⁰ The motif III shifts are large (Fig. 4)—switch 2 residue His¹⁹⁷ moves by 8.0 Å across the protein surface, and motif III residue Leu¹⁹⁶ by 2.3 Å, to contribute to a conserved and symmetric packing interaction at the heterodimer interface.³⁰ In conjunction with this rearrangement a peptide flip between motif III residues Gly¹⁹⁴ and Arg¹⁹⁵ allows the arginine sidechain to interact with Glu²⁰⁵ along helix α 2 and reorients the carbonyl oxygen of the conserved motif III glycine away from the active site.³⁰ The latter releases a backbone hydrogen bond coupling motif III to motif I in the monomeric protein and allows motif I residue Asn¹¹¹ to shift by 1.3 Å at the C α , which opens up the motif I P-loop to engage the γ -phosphate oxygens of the GTP. Interestingly, it appears in this respect that FtsY mimics Ffh, as its structure in the monomeric state can serve to regulate the access

of GTP to its canonical binding configuration in FtsY, allowing GTP binding to prime the GTPase, but not activate it.⁶²

The comparison also reveals that the conformation of the guanine nucleotide twists between the GDP-bound monomeric and GMPPCP-bound complexed states of FtsY, as the ribose and guanine base of the bound nucleotides slide approximately 0.7–1.0 Å from one structure to the other (Fig. 5) while the α - and β -phosphate groups in common between the two structures remain fixed relative to the motif I P-loop. Because motif IV Asp²⁵⁸ tracks the shift of the N1 and 2-amino group nitrogens, the hydrogen bonding interactions with the base are likely maintained throughout. Such flexibility in nucleotide binding configuration, also observed in ultrahigh resolution structures of GDP and GMPPNP-bound Ffh (Ramirez et al., in preparation), belies the functional significance of deviation of active site elements from their canonical spatial relationship.^{13,31} It suggests instead that these binding interactions are maintained during assembly and disassembly of the GTPase heterodimer and accommodate large rearrangements, such as that of the DARGG motif loop adjacent to motif IV. There, a peptide flip between Lys²⁶² and Gly²⁶³ allows the loop and the following α 4 helix to maintain packing interactions during the acquisition or release of the “latched” configurations of the DAGQ motif, on one side, and the ALLEADV motif of the N domain, on the other (Figs. 1A and 4).

The ALLEADV motif at the N/G interface is shifted \sim 5.2 Å relative to the G domain between the two states as the G domain pivots relative to the N domain on assembly (Fig. 4). This shift, and the rearrangement of the DARGG motif loop that accompanies it, regulates the accessibility of a hydrophobic pocket at the N/G interface to the C-terminal helix. Four hydrophobic sidechains of the FtsY C-terminal helix (Phe²⁹⁷, Val²⁹⁸, Leu³⁰¹, and Leu³⁰²) mediate its packing against the N domain. The hydrophobic character at each of these positions is conserved in the sequences of the NG GTPases.⁶³ Interestingly, position 297 is phenylalanine or tyrosine in all sequences of FtsY,⁶³ but is less conserved, and smaller, in sequences of Ffh. In FtsY, the sidechain of Phe²⁹⁷ is inserted between the α 4 helix and the universally conserved Phe²⁹² of the loop that precedes the C-terminal helix, and it maintains this packing relationship in both the monomeric and complexed states. This causes the C-terminal helix of *T. aquaticus* FtsY to maintain a rigid body relationship with the N domain between the two states (rmsd 0.59 Å), such that, in the N domain frame, the structural transition that occurs on assembly can be considered to be a rotation of the G domain against the N domain and C-terminal helix (Fig. 4). Further, the configuration adopted in the heterodimer complex with Ffh requires that the “N1” extension, when present (Fig. 3), must be displaced during assembly (Fig. 4). The heptad signature characteristic of the “N1” extension (Phe⁴, Leu⁷, and Leu¹¹) occurs in the sequence of *T. aquaticus* FtsY,⁵⁶ and a requirement for its displacement in the full length protein may explain the observation that cleavage of the N-terminal 20 residues of *T. aquaticus* FtsY must occur prior to formation of the stable heterodimeric *T. aquaticus* NG domain complex.⁹

Ffh and FtsY Adopt a Common Structure Through Different Paths

The heterodimer of the Ffh and FtsY NG domains exhibits pseudo-twofold symmetry, and seven loops contributed by each protein to the interface between them, as well as the C-helix of each protein, can be almost exactly superimposed on that of the other by a simple \sim 180° rotation.³⁰ Five of these loops—motifs I, III, DARGG, IV, and ALLEADV—contribute to the “latch” interface. Although the DARGG loop, which also contributes directly to the N/G interface, is quite flexible in both Ffh and FtsY [Fig. 6(A)], it reorients on assembly to adopt a common structure that in both proteins directs the polar sidechains of the motif symmetrically across the heterodimer interface, and allows the respective C-terminal helix to pack against the glycine backbone atoms of the motif. In Ffh, as for FtsY, a spine of four hydrophobic residues along the C-terminal helix mediates packing against the hydrophobic

face at the N/G interface. However, unlike in FtsY, the packing relationship with the N domain changes during the transition, as the sidechains of Leu²⁸⁷ and Ile²⁹¹ shift away from the α N1 helix to pack against the glycine backbone of the DARGG motif and the C-terminal helix undergoes a rotation of $\sim 25^\circ$ relative to the N domain. Sequence conservation in Ffh is consistent with increased mobility of the C-terminal helix, as the residue corresponding to conserved Phe²⁹⁷ in FtsY (see above) is present as the more flexible methionine or leucine in most sequences of Ffh.⁶³ Also, there is no “N1” extension in Ffh; instead, a phenylalanine residue is universally conserved near the N-terminus (Phe² in *T. aquaticus* Ffh) that packs against the hydrophobic pocket at the N/G interface in the monomeric protein, and is displaced on assembly of the heterodimeric complex.³⁰ It, like the “N1” helix of FtsY (see above), serves to protect the hydrophobic pocket of the monomeric protein prior to assembly of the heterodimer.

If the C-terminal helix is taken as the frame of reference, the cumulative effect of these differences between Ffh and FtsY can be distinguished as differences in the interdomain motion that occur during assembly and disassembly. In FtsY, the G domain rotates against the C-terminal helix frame [Figs. 4 and 6(A)], as rearrangements of the DARGG, DAGQ, and motif III loops generate the “latch” interactions that span the N/G subdomains at the heterodimer interface (Fig. 4). The relative shift of the G domain of FtsY is quite large, being ~ 6.2 Å for the motif I P-loop, for example (Fig. 4). A similar comparison of the structures of Ffh in its C-terminal helix frame reveals, as a consequence of the repacking of the C-terminal helix, quite different behavior [Fig. 6(A)]. Thus, the corresponding shift of the G domain is less than for FtsY (2.1 Å at P-loop), but the N domain helical bundle also shifts relative to this frame, by 5.9 Å at the ALLEADV motif [Fig. 6(A)]. Importantly, Ffh and FtsY adopt similar structural configurations at the interface of the heterodimer; therefore, the different behaviors of the N and G domain rearrangements in the two proteins provide two distinct paths between the structure of the monomeric protein and the same (or similar) structure adopted in the heterodimeric complex.

A Charged Surface is Organized at the Ffh N/G Domain Interface

There is an additional distinction in the behavior of the C-terminal helix between the two proteins. In Ffh, but not FtsY, two arginine residues, Arg²⁸⁶ and Arg²⁹⁰, located along the surface of the C-terminal helix are completely conserved in all prokaryotic sequences.⁶³ In structures of the monomeric protein the sidechains are directed away from the protein surface and interact only with solvent.^{6,47,58} The two arginine sidechains track the shift of the C-terminal helix on assembly of the heterodimer and adopt well-ordered configurations that, with the sidechain of conserved Arg²⁵² of the DARGG loop, generate a positively charged surface across the heterodimer interface [Fig. 6(B)]. The resulting “ladder” of basic residues, precisely positioned by hydrophobic packing interactions of the C-terminal helix (e.g. Leu²⁹⁷), and by interactions of the Arg²⁹⁰ sidechain, is located where the signal sequence recognition subunit of Ffh, which comprises both the M domain and the 4.5S RNA, interacts with the surface of the assembled heterodimer.²⁴ In the intact Ffh, the peptide that links the C-terminal helix of the NG domain to the M domain has substantial conformational freedom.^{17,21,22} That the C-terminal helix of the NG GTPase moves independently of the N and G domains and exhibits highly conserved and well-defined charge interactions in the assembled state provides a mechanism by which the signal sequence recognition subunit of Ffh, the M domain/4.5S RNA complex, may modulate acquisition of a structural configuration competent for assembly with receptor.

DISCUSSION

The X-ray structure of the monomeric FtsY from *T. aquaticus* bound to GDP is similar to that of the apo FtsY (and Ffh) from other species, despite the deletion of 20 N-terminal

amino acids in the crystallized protein [Fig. 3(A)]. A number of conformational changes suggested by the comparison of the crystal structures of FtsY with the structure in the assembled heterodimeric complex provide insight into the mechanisms available for the acquisition and regulation of the “latched” configuration observed in the complex of the SRP GTPases. We argued previously based on the published structures of the Ffh NG domain in its monomeric and complexed states that it was likely that the C-terminal helices of *both* Ffh and FtsY rearranged independently during assembly of the heterodimer interface.³⁰ The observation of a rigid body rearrangement of the N domain/C-terminal helix in *T. aquaticus* FtsY contradicts this notion. Instead, interactions with the N/G domain interface of Ffh and FtsY are modulated by distinct structural mechanisms that exploit the different positions of the “NG” module within the context of each primary sequence (i.e. Ffh NG being N-terminal, and FtsY NG being C-terminal). Conservation of an N-terminal helix extension in the structures of the apo FtsY, and biochemical data that supports the notion that it plays a functional role in vivo both in targeting and as a substrate for cleavage,^{12,26,27} suggests that the N-terminal extension may provide a mechanism for regulation of the heterodimeric “latched” configuration of FtsY, specifically by modulating the conformational change of the N domain relative to the G domain.

The surface of the N/G interface adjacent to the C-terminal helix has conserved hydrophobic character in both Ffh and FtsY,⁵⁸ and it provides the packing context for the N-terminal extension of FtsY in the monomeric protein.^{55,56} Positioning of the DARGG loop and elements of the G domain against the hydrophobic face of the C-terminal helix is coupled to conformational changes that extend across the loops of the “latch” interface.^{30,31} In Ffh, the positioning of the helix contributes to formation of a conserved positively charged surface that in the targeting heterodimer is oriented towards the M domain/4.5S SRP RNA complex.²⁴ Interestingly, mutants that generate “half-of-sites” defects in the GTP hydrolysis activity of SRP:FtsY complexes locate to this region of the structure (the N/G interface),⁶⁴ and mutation of the DARGG motifs of Ffh and FtsY cause specific, and distinct, defects along the targeting pathway in Ffh and FtsY.^{65,66} Further, the region, broadly defined, has been implicated in interaction with the signal peptide bound to SRP.⁶⁷

That the *T. aquaticus* FtsY exhibits a canonical nucleotide binding configuration and minimal degeneracy of nucleotide hydrolysis (Fig. 2), apparently in contrast to the behavior of the *E. coli* FtsY,³⁶ may arise from differences in the two protein constructs. In the *E. coli* work, the FtsY construct comprised an N-terminal GST-fusion to a truncated A-domain. The N-terminal peptide deleted in the *T. aquaticus* FtsY NGd20 construct directly interacts with the C-terminal helix and the motif IV recognition region in other structures of FtsY,⁵⁶ and its deletion in the *T. aquaticus* protein allows rapid formation of a stable heterodimeric complex.^{9,37} Given the structural rearrangements we can infer for both proteins, this suggests a mechanism by which constraints on the packing of the C-terminal helix and the DARGG loop may inhibit adoption of a stable “latched” configuration at the interface. Such interactions might also modulate acquisition of a specific nucleotide binding conformation at motif IV, but the flexibility of the nucleotide binding conformation observed between the GDP and GMPPCP-bound complexed states (Fig. 5) suggests as well that it is the “protein-binding” specificity, the acquisition of the latched configuration of the heterodimer interface rather than the “nucleotide-binding” specificity of FtsY, that may be regulated by conformational change between the N and G subdomains of the SRP GTPases.

The SRP GTPases undergo a coordinate rearrangement during the transition from the quiescent monomeric state of each protein to the assembled heterodimeric SRP targeting complex. GTP binding primes the assembly of the complex, enabling it to interact with and direct the signal peptide to the membrane translocon, and GTP hydrolysis, activated by interactions subsequent to release of the signal peptide, then disengages SRP from its

receptor. Recent studies have mapped the 4.5S RNA in the assembled complex to extend across a face of the heterodimer²⁴ that is defined by highly conserved surface-exposed sequence elements contributed by both Ffh and FtsY.³⁷ The distinct conformational changes between the monomeric and complexed states of *T. aquaticus* Ffh and FtsY suggest that the interactions of the C-terminal helix can provide two different means by which assembly of the GTPase targeting complex can be regulated. In the case of FtsY, rotation of the G domain of FtsY relative to the C-terminal helix and N domain framework must be coupled to displacement of the “N1”-linked N-terminal extension, the likely site of membrane interaction²⁶; this is equivalent to the N-terminal extension inhibiting rotation of the G domain, and, thus, assembly of the targeting heterodimer. In the case of Ffh, the arginine sidechain interactions contributed by the C-terminal helix on assembly, some with the DARGG loop, provide a “handle” by which the M domain or the anionic 4.5S RNA could gain purchase to regulate the structure and assembly of the Ffh heterodimer interface. Thus, the symmetric interaction between the two SRP GTPases can be regulated asymmetrically by the other components of the SRP targeting complex that provide distinct contexts, or environments, for each.

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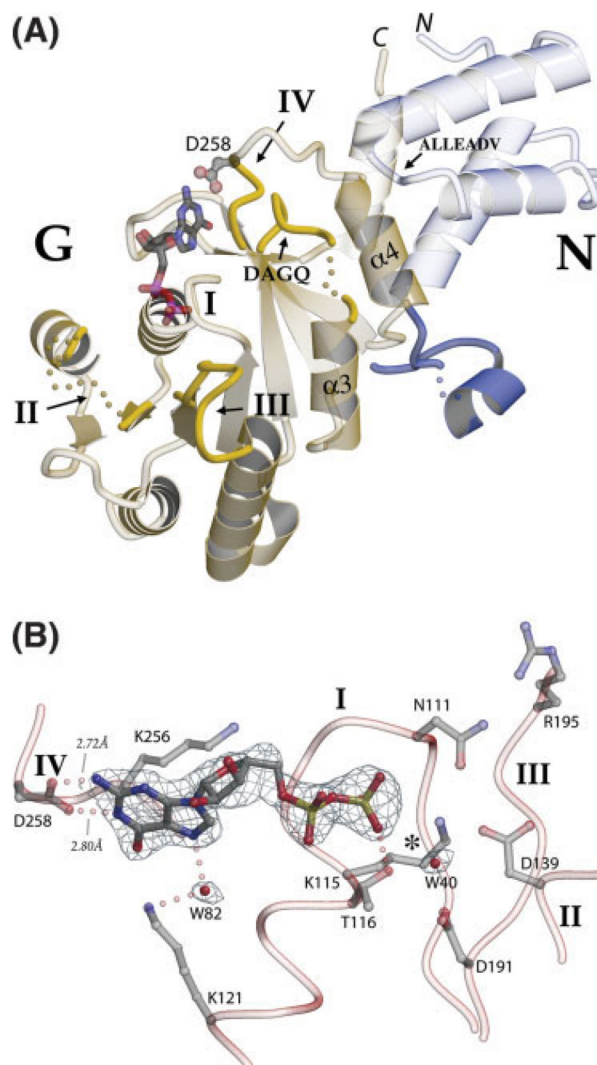


Fig. 1.
 A GDP-bound structure of *T. aquaticus* FtsY. **(A)** A ribbon diagram of the structure of the FtsY:GDP complex. The NG GTPase domain comprises the “N” subdomain (right), an α -helical bundle, and the “G” subdomain (left), which exhibits the fold, characterized by four conserved sequence motifs (I–IV), common to all members of the GTPase superfamily. Motif II occurs as part of an β - α - β “IBD” subdomain unique to the SRP GTPases (at left). The loops of the *T. aquaticus* FtsY G domain motifs II, III, IV, and DAGQ are highlighted in yellow; disordered regions of the polypeptide are shown as dots (yellow in the G domain, blue at the N-G linker). The polypeptide that links the N and G domains is highlighted in dark blue; it packs against a neighboring molecule across a crystal contact [Fig. 3(B)]. Note the N- and C-termini of the FtsY NG domain (“N,” “C,” top) are closely apposed in space. **(B)** A 2Fo-Fc electron density map for the bound GDP molecule and two water molecules within the FtsY active site contoured at 1.0σ . The positions of GTPase sequence motifs I–IV are indicated. The paired hydrogen bonds (2.72 and 2.80 Å) between motif IV Asp²⁵⁸ and the N1 and 2-amino nitrogens of guanine are canonical specificity-determining interactions, seen in all GTPases. A hydrogen bond with a buried water (W⁸²) mediates interaction between the guanine N7 and Lys¹²¹, as in the structures of GDP-bound Ffh.¹⁴ There is no

evidence for Mg^{2+} ion binding (the predicted position for Mg^{2+} ion is indicated by an asterisk).

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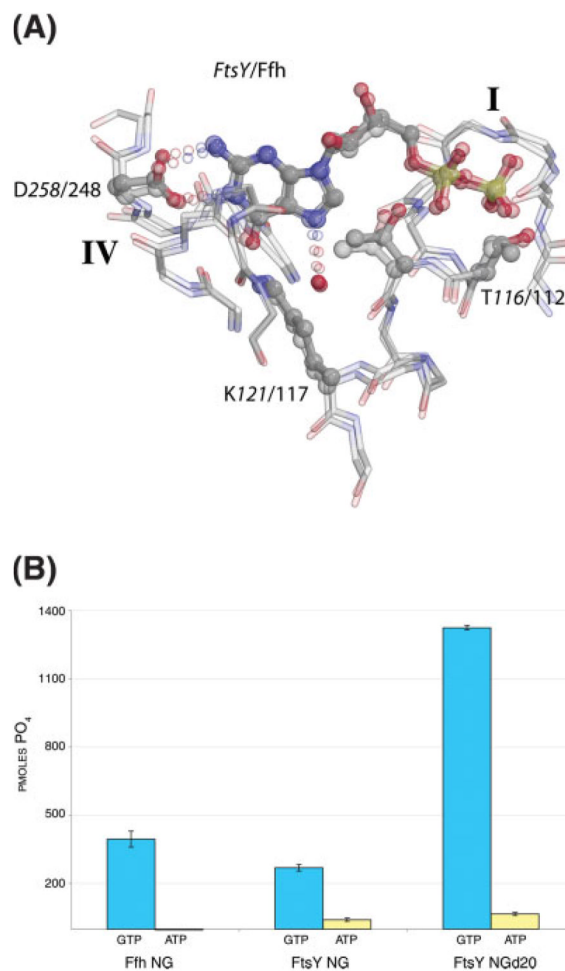


Fig. 2. *T. aquaticus* FtsY binds GDP and hydrolyzes GTP with specificity. (A) Comparison of the GDP-binding interactions of the FtsY:GDP complex with the Ffh:Mg²⁺:GDP complex (1O87).⁴⁷ The structures were superimposed over the nucleotide—shown are the residues of motifs I and IV and the closing loop (left foreground) that mediate all packing interactions with the nucleotide in the two proteins. The relative positions of the specificity determining residue Asp²⁵⁸ (Asp²⁴⁸ in Ffh) (left) and the motif I P-loop (right) are identical in the two structures. (B) GTP hydrolysis activity of *T. aquaticus* FtsY wt and FtsY NGd20 construct. Hydrolysis of GTP (pmoles phosphate released) is shown in blue and ATP in yellow. Under these experimental conditions (which are saturating for GTP, but not for ATP, see Methods) the activity with respect to ATP is less than 5–15% that for GTP. The activity of *T. aquaticus* Ffh NG under the same conditions is shown for comparison.

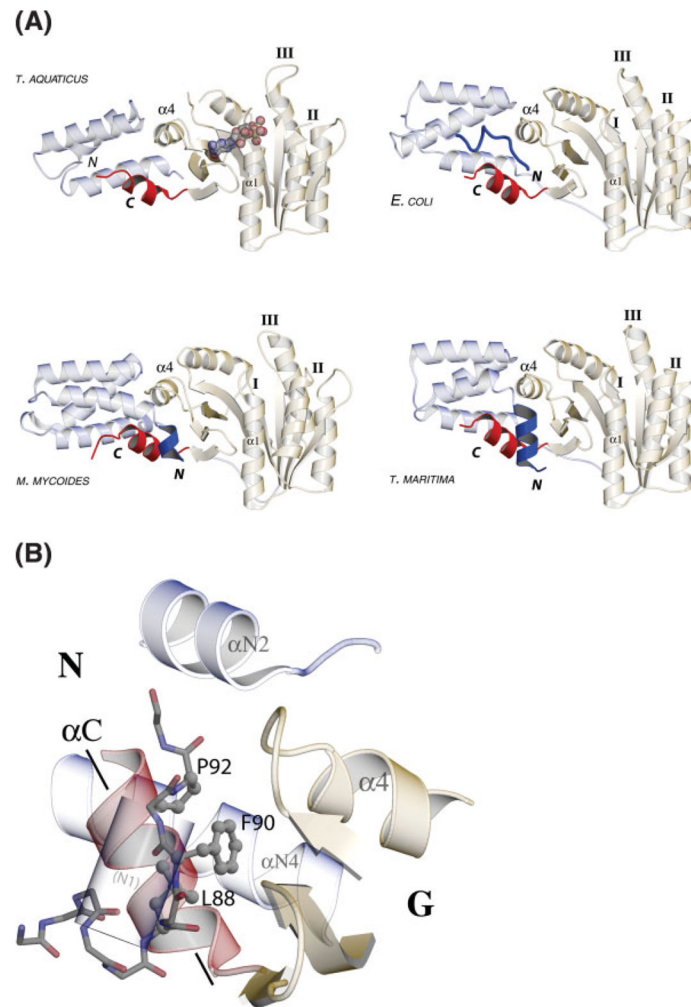


Fig. 3. Common features of the structures of FtsY from different species. **(A)** Structures of FtsY from *T. aquaticus*, *E. coli*, *M. mycooides*, and *T. maritima* are aligned using a superimposition based on the motif I P-loop; the $\alpha 1$ helix that follows the motif is vertical at the center of each image. The $\alpha 4$ helix (labeled) defines the interface between the N and G subdomains. The C-terminal helix (highlighted in red) adopts a similar configuration in each of the structures, despite the slightly different orientation of each N domain. In the structures of the apo *M. mycooides* and *T. maritima* FtsY an N-terminal helix extension (“N1”, highlighted in blue) packs between the C-terminal helix and the N/G interface. In the *E. coli* structure the N-terminal polypeptide is poorly structured (blue coil) but appears to play a similar role. The GDP in the *T. aquaticus* FtsY complex is indicated with a CPK model. In each structure of the apo or GDP-bound state of FtsY shown here, the motif II and motif III loops (labeled) adopt distinct (or disordered) conformations. **(B)** The N/G linker peptide packs across a crystal contact, occupying space that would accommodate the “N1” N-terminal helix seen in other structures of FtsY. The position of the N1 helix is indicated by a ghosted cylinder. The backbone atoms of the linker splayed across the crystal contact are shown as sticks; the hydrophobic sidechains of Leu⁸⁸, Phe⁹⁰, and Pro⁹² (ball-and-stick) pack into a conserved hydrophobic pocket at the interface. The orientation of the C-terminal helix, αC , is indicated by an axis line. The ribbon representation includes helices $\alpha N2$ and $\alpha N4$ of the N domain, and the $\alpha 4$ helix, the motif IV/DARGG loop that precedes it, and the

closing loop (bottom) of the G domain, which together contribute to the hydrophobic pocket at the N/G interface.

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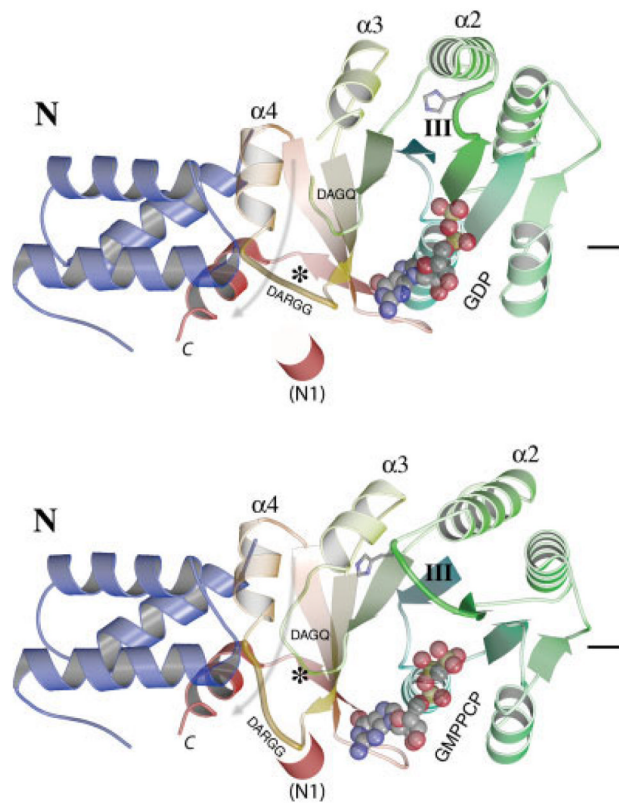


Fig. 4.

A rotation between the N and G domains of FtsY. The structures of the FtsY:GDP complex (top) and the FtsY:GMPPCP complex, the latter as observed in the assembled targeting heterodimer (1OKK, bottom), are compared following superposition over their N domains. The C-terminal helix and the $\alpha 4$ helix of FtsY remain fixed in the N domain frame, while the G domain undergoes a rotation relative to the N domain (ghosted arrow) that is accompanied by conformational changes of the DARGG loop (“*”), the motif III loop (labeled), and the DAGQ loop (between them), that generate the close-packed interactions of the “latched” configuration with Ffh at the heterodimer interface. The bound nucleotide is shown for each case, and the asterisk and the short line at right provide reference points. The shift of the DARGG loop necessarily displaces the N terminal helix extension (“N1”) if it were present in these structures.

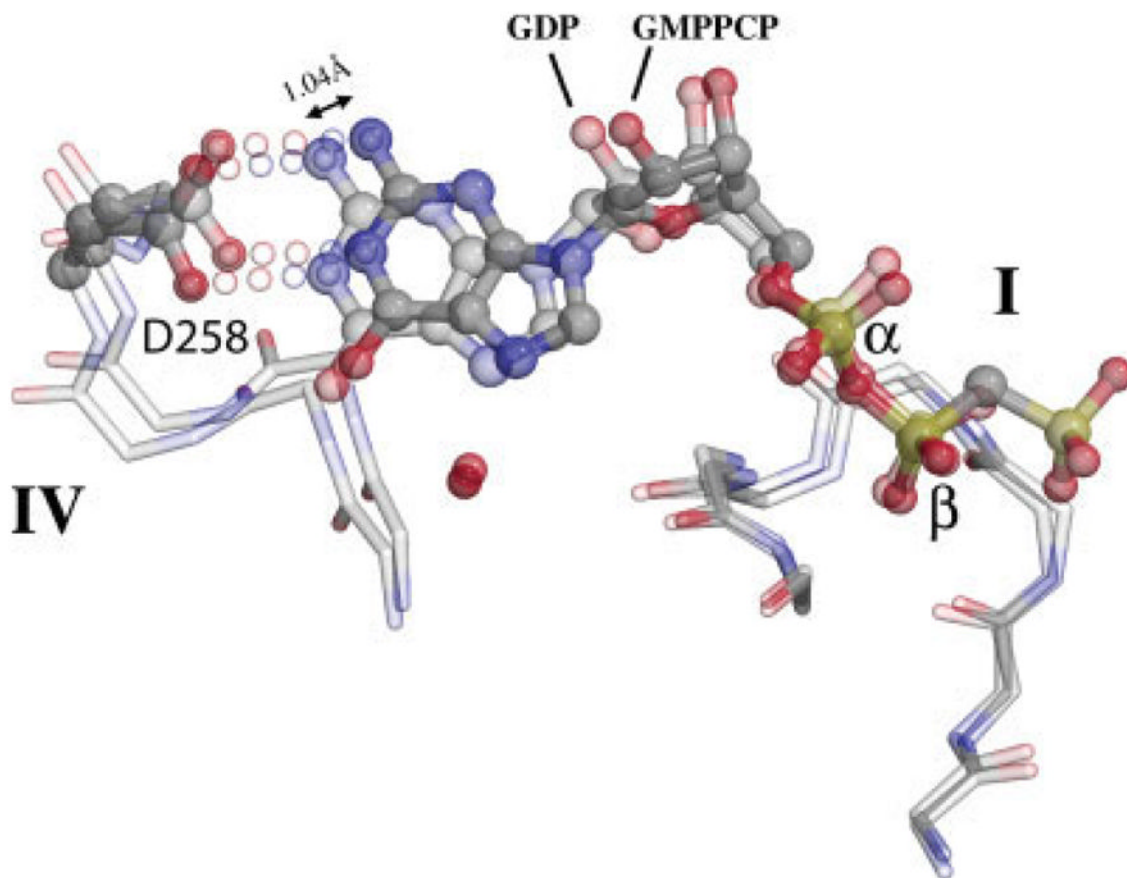


Fig. 5. The nucleotide position slips between the GDP and GMPPCP states. Following superposition of the FtsY:GDP monomeric and FtsY:GMPPCP complexed states of FtsY over the motif I P-loop (at right), the α - and β -phosphate groups of GDP and GMPPCP (labeled) remain fixed relative to each other, but the ribose and the guanine base shift by ~ 1 Å between the two structures (1.04 Å at the 2-amino nitrogen, as indicated). This slip is tracked by movement of the motif IV loop and by the specificity determining carboxylate sidechain of Asp²⁵⁸.

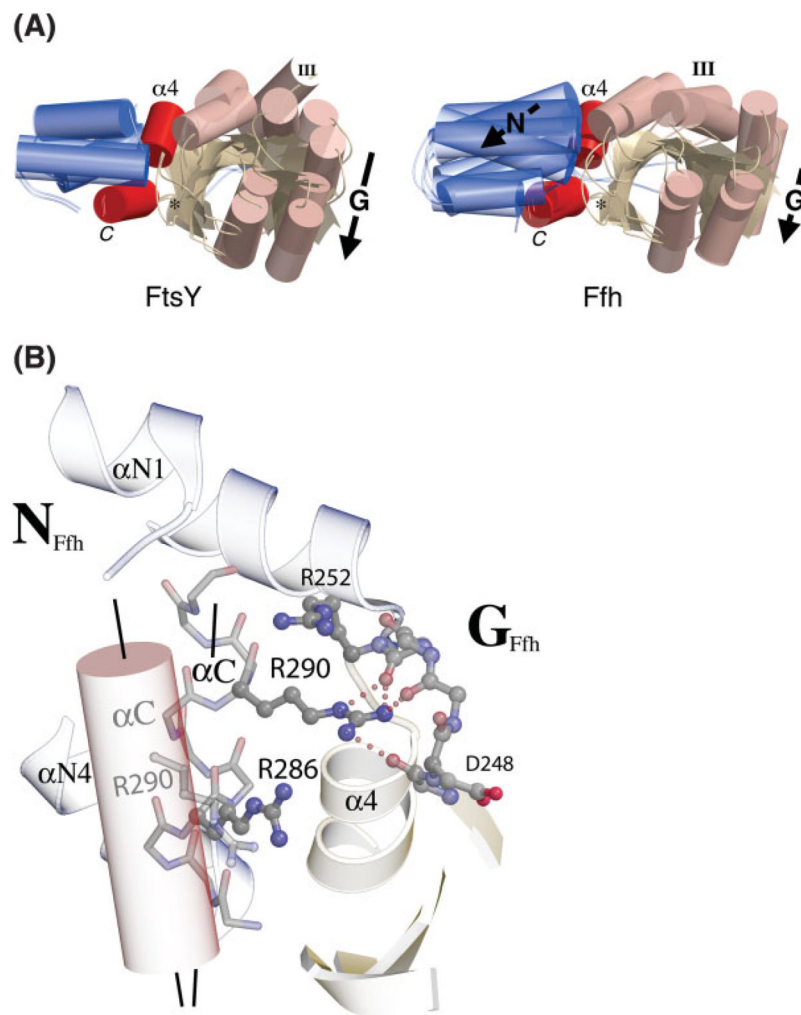


Fig. 6. Distinct behavior of Ffh and FtsY on assembly. **(A)** The domain shifts of *T. aquaticus* Ffh and FtsY that occur during acquisition of the heterodimeric complex interface structure are different. Here, the structures of the GDP and GMPPCP (heterodimer) complexes of each protein are superimposed on a common C-terminal helix frame, which is approximately constant between the two pairs of structures. In FtsY, this frame is constant with the N domain, and the G domain shifts relative to it (Fig. 4). In Ffh, both the N- and G-domains shift relative to the helix; this is equivalent to movement of the C-terminal helix relative to the N domain on assembly into the heterodimer. In the structure of the Ffh:FtsY heterodimer the interface loops at the N/G interface (DARGG, ALLEADV) and within the G domain (I, II, III, and DAGQ) adopt symmetric configurations; their paths to acquisition of that common structure are distinct. **(B)** In Ffh, rearrangement of the C-terminal helix (αC) is stabilized by an interaction between Arg²⁹⁰ and the DARGG loop that links the position of the C-terminal helix and the sidechain of Ffh motif IV Asp²⁴⁸. The positions of αC in the apo (ghosted cylinder) and heterodimeric complex (backbone representation) configurations are indicated and their relative orientations highlighted by axial lines. Both Arg²⁸⁶ and Arg²⁹⁰ are conserved in the sequences of Ffh and they contribute to the formation of a basic “ladder” likely to underlay the M domain/4.5S components of the SRP in the heterodimeric state.

TABLE I

Crystallographic Statistics (FtsY:GDP)

Spacegroup/cell	I4/105.20, 105.20, 54.50 Å
Resolution range (Å)	18.92–2.06 (2.17–2.06)
R_{sym}	0.080 (0.440)
I/σ	20.1 (3.9)
Completeness (%)	99.4 (100.0)
Redundancy	7.4 (7.7)
R_{cryst}	0.208 (0.225)
R_{free}	0.291 (0.305)
No. of atoms	2251
<Temperature factor>	45.18 Å ²
RMS bonds/angles	0.014 Å/1.527°

$$R_{\text{sym}} = \frac{\sum_{hk} \sum_i |I_o - \langle I_o \rangle|}{\sum_{hk} \sum_i I_o}$$

$$R_{\text{cryst}} = \frac{\sum_{hkl} |F_o - F_c|}{\sum_{hkl} F_o}$$

Values in parenthesis calculated for the high resolution bin.

R_{free} calculated for 5% reflections omitted from the refinement.