The functioning of the SRP receptor FtsY in protein-targeting in *E. coli* is correlated with its ability to bind and hydrolyse GTP

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Abstract In this study, we have established that FtsY, the *E. coli* homolog of the mammalian signal recognition particle (SRP) receptor, is a GTP-binding protein which displays intrinsic GTPase activity. GTP was found to influence the protease sensitivity of FtsY indicative of a conformational change. FtsY mutated in the 4th GTP-binding consensus element displayed reduced GTP-binding and -hydrolysis which correlated with a reduced ability to interact with SRP. Overexpression of the mutant proteins had a stronger inhibitory effect on protein translocation than overexpression of wild-type FtsY. These observations suggest that in *E. coli* GTP is important for proper functioning of FtsY in protein-targeting.

Key words: Escherichia coli; FtsY; Protein-targeting; Signal recognition particle

1. Introduction

Extensive study of the general secretory pathway (GSP) has given insight into the complexity of the mechanisms involved in secretion of proteins across the cytoplasmic membrane of *E. coli* [1]. In the GSP, preproteins interact with various cytosolic and membrane-associated Sec proteins for transfer to and through the membrane.

Until recently, homology with the eukaryotic protein translocation route to the endoplasmic reticulum (ER) was thought to be restricted to comparable functions in both pathways which were fulfilled by different factors. A key component in the ER route is the signal recognition particle (SRP) which consists of 7S RNA and six different proteins of 9, 14, 19, 54, 68 and 72 kDa [2]. This ribonucleoprotein (RNP) complex binds to the signal sequence of nascent preproteins as they emerge from the ribosome and targets the nascent chain–ribosome complex to a membrane receptor (called 'docking protein'). The interaction with the precursor retards further translation which is resumed when the SRP dissociates from the membrane-bound SRP-nascent chain–ribosome complex upon binding of GTP.

In the past few years, evidence for the existence of an SRP-mediated protein-targeting route in *E. coli* has accumulated. Through genetic and biochemical approaches, it has become clear that an RNP consisting of P48 (SRP54 homolog) and 4.5S RNA (7S RNA homolog) functions in an alternative protein-targeting route. It was found that depletion of the 4.5S RNA and P48 lead to accumulation of a subset of precursor proteins [3–5]. P48 binds specifically to functional signal sequences [6] which strongly suggests that the RNP acts as an SRP.

In addition, the *E. coli* FtsY protein was postulated to be the homolog of the α-subunit of the docking protein (SRα) [7,8]. Comparative sequence analysis revealed that the proteins are relatively conserved and can be divided into 3 domains [9]. The N-terminal domain which is relatively variable and the highly conserved X- and G-domains which are also similar to domains in the signal sequence-binding proteins SRP54 and P48. The G-domain contains the four consensus GTPase elements of the GTPase superfamily.

Since in the eukaryotic SRP route GTP-binding modulates the dissociation of the signal sequence of the precursor from the SRP [10] and subsequent GTP hydrolysis induces the release of the SRP from its receptor [11], it is conceivable that GTP has a comparable regulatory function in the bacterial system. In this study, we focused on the GTP-binding and -hydrolyzing properties of FtsY and analysed their effects on structure and functioning of FtsY as an SRP receptor in protein transport.

2. Experimental

2.1. Strains, plasmids and media

E. coli HMS 174 F⁻ *hsdR recA* Rif^r and BL21 F⁻ *hsdS gal*(DE3) harboring pLysE or pLysS [12] were used for initial subcloning and for expression of *ftsY*, respectively. Plasmids pET9-FtsY and pACYC177Δ*Hae*II were used for overexpression of FtsY and expression of β-lactamase, respectively, and have been described previously [13]. Cells were routinely grown in LB supplemented with 0.4% glucose and the appropriate antibiotics.

2.2. Site-directed mutagenesis of ftsY

To generate mutations in the 4th putative GTP-binding consensus element of FtsY, site-directed mutagenesis was performed by successive PCR reactions essentially as described [14], using pET9-FtsY as template. The following mutagenic oligonucleotides were employed as primers in the PCR reaction: 5′-CCGTCCAGTTTGTTTAGCGTGATG-3′ and 5′-CATCACGCTAAACAACTGGACGG-3′ to convert Thr^{446} to Asn^{446}; 5′-AACGAAACTGGCTGGCACGGCGAA-3′ and 5′-TTCGCCGTGCCAGCCAGTTTC GTT-3′ to convert Asp^{449} to Ala^{449}.

2.3. General methods

Recombinant DNA techniques were carried out as described [15]. Radiolabeled protein bands on dried polyacrylamide gels as well as phosphate and GTP spots on thin-layer plates were visualized by autoradiography or by phosphor-imaging using a Molecular Dynamics

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Abbreviations: IPTG, isopropyl-1-thio-β-D-galactopyranoside; SDS-PAGE, sodium dodecylsulfate polyacrylamide-gel electrophoresis; SRP, signal recognition particle; GTP, guanosine-5′-phosphate.

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Phosphor Imager 473 and quantified using the Imagequant quantification software from Molecular Dynamics.

2.4. Overexpression and purification of (mutant) FtsY, 4.5S RNA and P48

Strain BL21 (DE3) carrying pLysS and either pET9-FtsY or pET9-N446 or pET9-A449 was grown and induced for overexpression as described [13]. FtsY wild-type and mutant proteins were purified as described previously [13]. 4.5S RNA was purified as described previously [16] and purified P48 was a gift of I. Sinning (EMBL, Heidelberg).

2.5. GTP photoaffinity cross-linking

The ability of FtsY proteins to bind to GTP was determined by photoaffinity cross-linking of [γ-^{32}P]GTP [17]. Purified FtsY protein samples (1 μM final concentration) were incubated in 20 μl 20 mM Tris-acetate, pH 7.5, 100 mM K^+-acetate, 1 mM EDTA, 10 mM Mg^{2+}-acetate, 0.5 mM dithiothreitol and 0.1 μM [γ-^{32}P]GTP (5000 Ci/mmol; Amersham) for 5 min at 0°C. Cross-linking was induced by irradiation with UV light (30W; 254 nm) at a distance of 1 cm for 15 min at 0°C. Proteins were TCA-precipitated and subjected to 10% SDS-PAGE. After Coomassie brilliant blue staining and drying, the gels were analysed by phosphor-imaging.

2.6. Determination of GTPase activity

GTPase activity was assayed by incubation of FtsY proteins at a final concentration of 1 μM in 50 μl incubation buffer containing 50 mM Tris-Cl, pH 7.0, 30 mM KCl, 30 mM NH_4Cl, 4 mM Mg^{2+}-acetate, 1 mM dithiothreitol and 10 μM [γ-^{32}P]GTP (10 Ci/mmol) at 37°C. At various time points, 5-μl samples were taken from the incubation mixture and the reaction was stopped by quick freezing the samples in liquid nitrogen. After thawing on ice, 1 μl of each sample was applied to a PEI-cellulose thin-layer chromatography plate (Merck) and developed in 0.75 M KH_2PO_4/H_3PO_4, pH 3.3. The radioactive spots of GTP and Pi were quantified (see Section 2.3.) and the amount of hydrolysis was determined by dividing the integrated volume derived from the radioactive P_i spot by the total volume (derived from P_i + GTP). As a control for aspecific GTP-hydrolysis, ovalbumin was applied instead of FtsY.

2.7. Protease-protection assay

The sensitivity of FtsY proteins to degradation by proteinase K (Boehringer) was determined by incubating 4 μg protein in 40 μl of 20 mM Tris-acetate, pH 7.5, 10 mM Mg^{2+}-acetate and 8 ng proteinase K for 20 min at 37°C. The reaction was stopped by the addition of trichloroacetic acid to a final concentration of 10% (w/v). The samples were analysed by SDS-PAGE and Coomassie staining. The effect of GTP, GDP and GMP on the degradation was determined by including these nucleotides in the reaction mixture at a concentration of 2 mM.

2.8. FtsY–SRP complex formation

Complex formation of either wild-type or mutant FtsY proteins with reconstituted *E. coli* SRP was induced by a 20-min incubation at 20°C of 0.5 μM P48, 0.5 μM 4.5S RNA and 1 μM FtsY and 1 mM GMP-PNP in a buffer consisting of 20 mM HEPES/KOH pH 7.5, 500 mM K^+-acetate, 100 mM NH_4Cl, 10 mM $MgCl_2$, 0.5 mM EDTA and 0.1 mM dithiothreitol. After addition of Ficoll to 1.25% (w/v), the reaction mixture was subjected to non-denaturing 7% PAGE [16].

3. Results

3.1. FtsY is a GTP-binding protein

To test whether FtsY is able to bind GTP, a photo cross-linking approach was taken. Purified wild-type FtsY protein was incubated in the presence of radiolabeled GTP and irradiated with UV light. The results presented in Fig. 1A show that FtsY is a GTP-binding protein. In the absence of UV irradiation, hardly any FtsY protein was labeled by [^{32}P]GTP (lane 1) whereas labeling was observed when the sample was irradiated with UV light (lane 2). The preference to bind the triphosphate guanoside is demonstrated in lanes 3–5. The addition of unlabeled GTP reduced the specific activity of the GTP present in

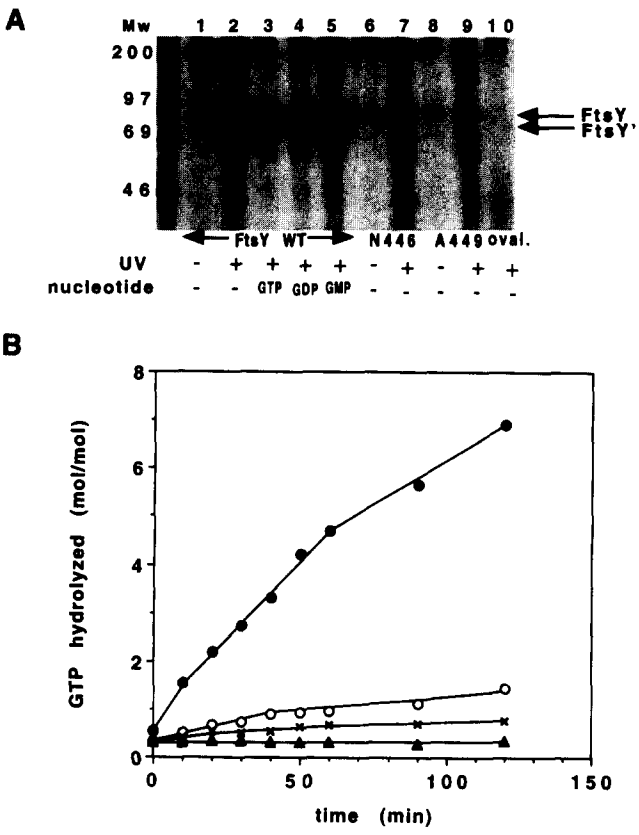

Fig. 1. (A) GTP photoaffinity cross-linking of FtsY, analysed by SDS-PAGE and visualized by phosphor-imaging. FtsY wild-type (lanes 1–5), FtsY N446 (lanes 6 and 7), FtsY A449 (lanes 8 and 9) and egg ovalbumin (lane 10) were incubated with radiolabeled GTP at 0 °C for 20 min. The samples shown in lanes 2–5, 7, 9 and 10 were irradiated with UV 258 nm. Samples 3–5 contained the indicated unlabeled nucleotides at a final concentration of 100 μM. The positions of FtsY and FtsY′ (a co-purified degradation product of FtsY) are indicated by arrows. (B) GTP-hydrolysis by FtsY. FtsY wild-type (closed circles), N446 (open circles) and A449 (crosses) and egg ovalbumin (triangles) were incubated in the presence of [γ-^{32}P]-GTP and at the indicated time points samples were drawn and analysed by thin-layer chromatography on PEI cellulose plates. The amounts of radiolabeled phosphate and remaining GTP were determined by phosphor-imaging. Hydrolysis is expressed as mol GTP hydrolyzed/mol protein. The data are the mean of five separate experiments.

the incubation mixture and, therefore, less FtsY was labeled (lane 3); GDP also appears to have affinity for FtsY since this nucleotide also inhibited the association of GTP albeit to a lower extent (lane 4). The presence of GMP did not affect the GTP-binding capacity of FtsY (lane 5). As a control for aspecific binding of GTP, egg ovalbumin, which is not a GTP-binding protein, was added to the reaction mixture in stead of FtsY at the same concentration (lane 10).

3.2. Effects of mutations within the G4-domain of FtsY on GTP-binding and -hydrolysis

To study the effects of GTP-binding and -hydrolysis on the functioning of FtsY we have chosen to mutagenize the G4 consensus sequence of the predicted GTP-binding region in FtsY (Fig. 2). The analog sequence in H-*ras* p21 was shown to interact with the guanine base of GTP [18]. As has been described for other GTP-binding proteins involved in the SRP-

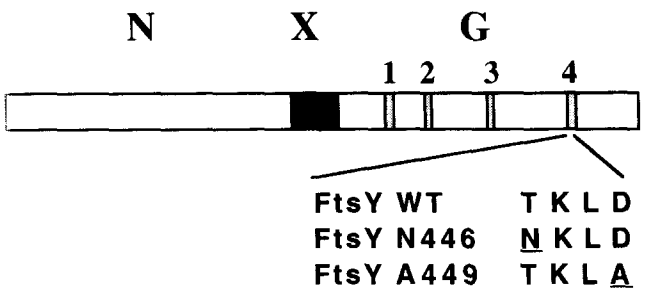

Fig. 2. Schematic presentation of FtsY. N, amino terminal domain; X, X-domain; G1–4, putative GTP-binding domains. The amino-acid sequences of the G4-domain of wild-type FtsY and mutant proteins FtsY N446 and A449 are indicated.

mediated transport pathway, the G4 sequence of FtsY differs from the consensus NKXD found in most GTPases [19]. Two *FtsY* alleles were constructed. In FtsY N446, the atypical Thr at position 446 was converted to Asn by analogy with the same mutation at position 588 in SRα which resulted in decreased protein translocation and SRP-binding [20]. In FtsY A449, the Asp residue at position 449 was converted to Ala by analogy with the same substitution at position 591 in SRα which resulted in completely defective protein translocation and SRP-binding [20]. This position is expected to be most important for binding to the guanine ring of GTP [18].

FtsY N446 and FtsY A449 were purified and the effects of the mutations with respect to GTP-binding were determined by photo cross-linking as described above. Both mutants were shown to have reduced GTP-binding capacity (Fig. 1A, lanes 7 and 9). The Asp to Ala substitution at position 449 had the most severe effect on GTP-binding (reduction of GTP-binding by 83% compared with wild-type FtsY) (lane 9) whereas the Thr to Asn conversion at positon 446 had an intermediate effect (reduced by 45%) (lane 7). This is in agreement with the observation of Rapiejko and Gilmore that, compared with wild-type SRα, the Thr to Asn convertant requires a much higher concentration of GTP to function in in vitro protein import into microsomes [20]. Inhibition of the residual binding of labeled GTP to FtsY N446 by cold GTP, GDP and GMP did not differ qualitatively from wild-type FtsY (not shown).

To study GTP-hydrolysis by FtsY, purified FtsY was incubated in the presence of [γ-^{32}P]GTP and the reaction products were analysed by thin-layer chromatography. As shown in Fig. 1B, FtsY displayed a stable GTPase activity which hardly declined during 120 min of incubation. Furthermore, we investigated whether the reduction in GTP-binding capacity found for the 2 FtsY mutant proteins correlates with a diminished GTPase activity. Indeed, both mutant FtsY proteins appeared to have a reduced activity, FtsY A449 being most strongly affected (Fig. 1B).

Like other described GTPases, FtsY-mediated hydrolysis of GTP required the presence of Mg^{2+} (not shown). No GTP was hydrolyzed when FtsY in the reaction mixture was substituted by egg ovalbumin.

3.3. *Effects of reduced GTP-binding and -hydrolysis of FtsY on protein export*

We have previously shown that the depletion of FtsY results in the inhibition of translocation of a subset of precursor proteins [13]. The overproduction of FtsY was also shown to cause the intracellular accumulation of pre-β-lactamase although Pre-OmpA translocation was unaffected [13]. We now assessed whether the overproduction of the FtsY GTP-binding mutants influenced protein secretion. BL21 (DE3) cells harboring pACYC177*ΔHae*II, encoding β-lactamase and either pET9-FtsY or pET9-N446 or pET9-A449 were grown for 1 h in the absence or presence of IPTG, which induces the overproduction of (mutant) FtsY, and the cell contents were analysed by immunoblotting (Fig. 3). At this time point, the overproduction of (mutant) FtsY had no detectable effect on cell growth and morphology (not shown). BL21 cells, which only contained pACYC177*ΔHae*II, served as a control to monitor the effects of wild-type FtsY expression.

In Fig. 3, immunoblot panels are shown which were developed with antisera directed against the indicated proteins. Lanes 1 and 2 demonstrate that at wild-type level of FtsY no accumulation of the model precursor proteins could be detected. When the expression of wild-type FtsY was slightly elevated, again no precursor proteins accumulated (lanes 3, uninduced background expression from the T7 promoter). Overproduction of the wild-type protein to a high level (lanes 4, IPTG-induced expression) led to an inhibition of the export of pre-β-lactamase and pre-OmpF but not of the precursor to OmpA or OmpC (lanes 3 and 4) as shown previously [13]. In contrast, the uninduced expression of the mutant FtsY proteins resulted in a strong accumulation of pre-β-lactamase and, to a lesser extent, of pre-OmpF (lanes 5 and 7). The inhibitory effect was even more pronounced when the production of these pro-

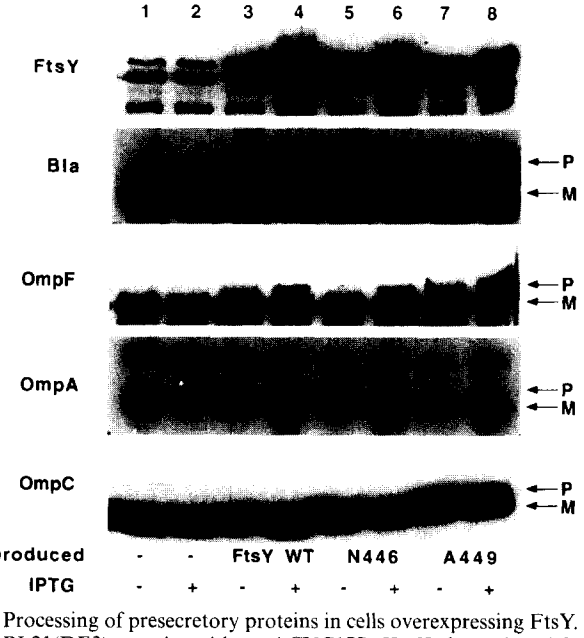

Fig. 3. Processing of presecretory proteins in cells overexpressing FtsY. Strain BL21(DE3) carrying either pACYC177*ΔHae*II (lanes 1 and 2) or a combination of pACYC177*ΔHae*II and either pET9-FtsY (lanes 3 and 4), pET9-N446 (lanes 5 and 6) or pET9-A449 (lanes 7 and 8) were grown in LB medium with 0.4 mM IPTG (lanes 2, 4, 6 and 8) or without induction (lanes 1, 3, 5 and 7). Samples were taken 1 h after induction and analysed by immunoblotting using antisera against FtsY, β-lactamase (Bla), OmpF, OmpA and OmpC as indicated at the left side of the blot panels. The positions of the precursor and mature forms of the secreted proteins are marked with 'P' and 'M', respectively, at the right side of the panels. The position of the precursor form of OmpA was identified in a *SecA*(Ts) strain grown at the non-permissive temperature (not shown).

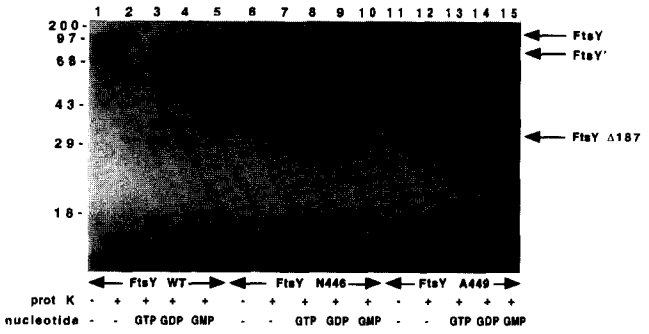

Fig. 4. Partial resistance of FtsY against proteolytic degradation analysed by SDS-PAGE and Coomassie staining. FtsY wild-type (lanes 1–5), N446 (lanes 6–10) and A449 (lanes 11–15) were incubated with our without proteinase K and nucleotides as indicated. Lanes 1, 6 and 11 show the initial amount of protein. The position of FtsY, FtsY′ and the 32.8-kDa degradation product (FtsYΔ187) are indicated at the right side of the gel.

teins was increased by the inducer IPTG (lanes 6 and 8). Thus, the effect of overproduction of the FtsY GTP-binding mutants was considerably more severe than that seen with the wild-type protein. This 'dominant negative' effect of the GTP-binding mutants on β-lactamase and OmpF transport, indicated that GTP-binding and -hydrolysis are important for functioning of FtsY in protein export.

The expression (both to low and high levels) of the mutant FtsY species did not affect the export of the precursors to OmpA and OmpC (lanes 5–8). This is in agreement with our earlier observation that FtsY is only involved in the membrane-targeting of a subset of precursor proteins [13].

3.4. Conformational consequences of GTP-binding

Binding of nucleotides can change the conformation of nucleotide-binding proteins as has for instance been observed for H-*ras* p21 [18] and for the *E. coli* SecA protein [21]. As a consequence, it may affect the functional properties of these proteins.

We studied whether GTP affects the conformation of FtsY by testing its sensitivity in vitro to limited proteolysis by proteinase K under different conditions. The proteolysis patterns, presented in Fig. 4, show that during 20 min of incubation wild-type FtsY was readily degraded by proteinase K, resulting in degradation products of low molecular weight (bottom of lane 2). When GTP was present during the protease treatment, the degradation was not completed and an intermediate product with an apparent molecular weight of 33 kDa appeared (lane 3). If the altered protease sensitivity is the direct result of a conformational change induced by GTP-binding, then it is to be expected that the FtsY mutant proteins N446 and A449 show less resistance to proteolysis in the presence of GTP. Consistent with this idea, less intermediate product remained after protease treatment of FtsY N446 in the presence of GTP (lane 8), compared with wild-type FtsY (lane 3). The digestion of FtsY A449, of which the GTP-binding capacity is most strongly reduced, is not affected at all by the presence of GTP (lanes 12 and 13). Remarkably, GDP, which has only a small protective effect on wild-type FtsY, has a strong protective effect on FtsY N446 and FtsY A449. Possibly, these mutant proteins bind GDP with high affinity or have a decreased ability to release GDP. By using photoaffinity cross-linking, we indeed observed that at high GDP concentrations the inhibitory effect of GDP on labeled GTP-binding to FtsY N446 was more pronounced than on GTP-binding to the wild-type FtsY species (not shown). GMP did not change the degradation pattern of wild-type and mutant FtsY (lanes 5, 10 and 15). The effect of a non-hydrolyzable GTP analog, GMP-PNP, closely resembled the effect of GTP (not shown), indicating that the protection by GTP can not be ascribed to GDP generated by FtsY-mediated hydrolysis of GTP.

Amino-acid sequencing of the 6 N-terminal residues of the intermediate degradation product revealed that this fragment represented FtsY missing 187 N-terminal residues (designated FtsYΔ187). The protected C-terminal fragment corresponded almost exactly with the X- and G-domains predicted on the basis of comparative sequence analysis [9]. Possibly, these domains fold in a packed protease-resistant conformation upon GTP-binding with the less rigid N-terminal (protease accessible) part protruding.

3.5. Interaction between SRP and FtsY requires GTP and a functional G4-domain in FtsY

The binding of the *E. coli* SRP to its receptor FtsY is dependent on GTP as has been shown by a co-precipitation approach [22]. It is not clear whether for a proper interaction between SRP and FtsY GTP-binding by both components is required. We tested this by a gel-shift assay (Fig. 5). Wild-type FtsY and the GTP-binding mutants FtsY N446 and FtsY A449 were incubated with 4.5S RNA and P48 in the presence of either a GTP analog or GDP and subjected to non-denaturing PAGE followed by Western blotting and immunostaining using antiserum directed against P48 (Fig. 5). In the presence of 4.5S RNA and GDP, P48 migrated as a single band (lane 1), indicating that the complex of 4.5S RNA and P48 (SRP) is formed since, in the absence of 4.5S RNA, P48 does not enter the gel (not shown) which is probably due to its net positive charge. The same blot developed with an anti-FtsY serum showed that this band did not contain any FtsY (not shown; the position of uncomplexed FtsY on this blot is indicated by the asterisk). When, instead of GDP, a GTP analog was added to the incubation, the P48 band partly shifted to a higher position in the gel (lane 2), indicating that a complex between SRP and FtsY is formed. Immunostaining using anti-FtsY serum confirmed that

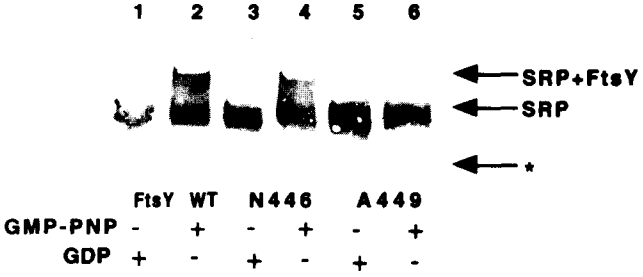

Fig. 5. Complex formation of FtsY and *E. coli* SRP. FtsY wild-type en mutant proteins were incubated with 4.5S RNA and P48 in the presence or absence of GMP-PNP or GDP as indicated and analysed by non-denaturing gel electrophoresis followed by immunoblotting using antiserum against P48. The position of the SRP and the SRP–FtsY complex in the non-denaturing gel are marked by arrows. The asterisk shows the position of uncomplexed FtsY proteins detected by immunostaining of the same blot with antiserum against FtsY (not shown).

the shifted band also contains FtsY (not shown). FtsY N446 is also capable of complex formation with P48 and 4.5S RNA in the presence of GTP analog albeit with a reduced efficiency (lane 4). The mutant protein with the lowest GTP-binding capacity, FtsY A449, is unable to interact with the SRP since no mobility shift was observed with this protein (lane 6). Taken together, the results demonstrate that GTP-binding by FtsY is essential for complex formation with the SRP.

4. Discussion

The involvement of FtsY in a novel protein-targeting route in *E. coli* has been demonstrated in 2 biochemical studies. In the first of these, Miller et al. [22] showed that, in the presence of a non-hydrolyzable GTP analog, reconstituted *E. coli* SRP forms a stable complex with glutathion transferase–FtsY fusion protein, suggesting that FtsY serves as a receptor for the SRP. In the second study, we found that perturbation of normal intracellular levels of FtsY severely affected the translocation of a subset of precursor proteins [13]. The translocation of pre-β-lactamase was most strongly affected which is consistent with its sensitivity to depletion of P48 [5] and 4.5S RNA [3,4], the constituents of the SRP, suggesting that FtsY and SRP function in the same targeting pathway.

In this study, we monitored nucleotide-binding to purified proteins by UV cross-linking to demonstrate that both FtsY and P48 are bona fide GTP-binding proteins (Fig. 1A and data not shown) as had been predicted from their respective amino-acid sequences [7,23]. Furthermore, we found that FtsY displayed a stable intrinsic GTPase activity (Fig. 1B).

To study the effects of GTP-binding on the functioning of FtsY in the reception of the SRP, 2 mutants of the 4th GTP-binding consensus element TKLD were constructed (Fig. 2). FtsY N446 (TKLD→NKLD) and FtsY A449 (TKLD→TKLA) were shown to have 45 and 83% reduced GTP-binding capacity, respectively (Fig. 1A), confirming the importance of this region for GTP-binding. Furthermore, both mutants appeared to have a strongly reduced GTPase activity (Fig. 1B). Considering the GTP-binding capacity of FtsY N446, the rate of GTP-hydrolysis by this mutant was more strongly reduced than expected. Possibly, the G4-domain also contributes to other features important for GTPase activity, e.g. the release of GDP.

When expressed moderately in vivo, both FtsY N446 and A449 but not the wild-type FtsY had a dominant inhibitory effect on the translocation of a subset of secretory proteins as evidenced by the accumulation of the precursor forms of these proteins (Fig. 3). In a previous study, we showed that translocation of the same subset of proteins was affected upon depletion and very strong overexpression of wild-type FtsY [13]. The accumulation of pre-OmpF was strongest upon expression of FtsY A449 which has the lowest GTP-binding capacity, suggesting that the functioning of FtsY in protein-targeting is correlated with its ability to bind and hydrolyse GTP. This is consistent with studies carried out in a mammalian in vitro system. Repopulation of SRα-depleted microsomes with in vitro synthesized SRα harboring identical substitutions in the G4-domain resulted in reduced protein translocation and membrane insertion [20].

The underlying mechanism for the observed translocation defect was studied by using different approaches. We monitored the interaction of the mutant FtsY with reconstituted *E. coli* SRP in the presence of a non-hydrolyzable GTP analog by using a gel-shift assay. Both mutants showed a reduced ability to bind SRP, again FtsY A449 being the most strongly affected. This would suggest that GTP-binding by FtsY is essential for the formation of a stable FtsY–SRP complex and, thus, for proper protein-targeting. These findings are consistent with those of Rapiejko and Gilmore in the mammalian system [20]. The importance of the GTP-binding site in P48 for the interaction with FtsY remains to be established. It has been proposed that SRα stimulates GTP-binding by SRP54 which reduces its affinity for bound signal sequence by inducing a conformational change [24].

GTP-binding and -hydrolysing proteins may adopt different conformations, GDP-bound, 'empty' and GTP-bound forms which differ in their affinity for macromolecules [19]. To study the conformational consequences of nucleotide-binding by FtsY more directly, alterations in the protease sensitivity were determined. Addition of GTP or a non-hydrolyzable GTP analog to the proteolysis reaction of wild-type FtsY resulted in the appearance of an intermediate protease 'insensitive' FtsY fragment that upon amino-acid sequencing proved to consist of the conserved X- and G-domains. Thus, the predicted domain structure of FtsY seems to have a structural basis [7,23]. The presence of GTP during proteolysis of the FtsY mutants was either less efficient in affording protection (N446) or gave no protection at all (A449). Taken together, the results suggested that upon nucleotide-binding the X- and G-domains fold into a more protease-resistant conformation. This conformational change is likely to modulate the interaction with the SRP and possibly the interaction with the cytoplasmic membrane. As a consequence, the SRP-mediated translocation of secretory proteins may be affected by the conformational state of FtsY. We intend to investigate this possibility in more detail by studying the role of GTP-binding and -hydrolysis in the consecutive steps in protein-targeting in an in vitro *E. coli* translocation reaction.

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