Identification of Nascent Chain Interaction Sites on Trigger Factor*

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The role of ribosome-binding molecular chaperones in protein folding is not yet well understood. Trigger factor (TF) is the first chaperone to interact with nascent polypeptides as they emerge from the bacterial ribosome. It binds to the ribosome as a monomer but forms dimers in free solution. Based on recent crystal structures, TF has an elongated shape, with the peptidyl-prolyl-cis/trans-isomerase (PPIase) domain and the N-terminal ribosome binding domain positioned at opposite ends of the molecule and the C-terminal domain, which forms two arms, positioned in between. By using site specifically labeled TF proteins, we have demonstrated that all three domains of TF interact with nascent chains during translation. Interactions with the PPIase domain were length-dependent but independent of PPIase activity. Interestingly, with free TF, these same sites were found to be involved in forming the dimer interface, suggesting that dimerization partially occludes TF-nascent chain binding sites. Our data indicate the existence of two regions on TF along which nascent chains can interact, the NC-domains as the main site and the PPIase domain as an auxiliary site.

Trigger factor (TF)³ was first identified based on its ability to maintain the precursor of the outer membrane protein A pro-OmpA in a non-aggregated form, competent for translocation across the Escherichia coli inner membrane in vitro (1). Subsequent studies have demonstrated that TF has a general role in cytosolic protein folding that overlaps partially with that of the Hsp70 chaperone system, DnaK, DnaJ, and GrpE (2, 3). Although the tig and dnaK genes are not individually essential, their combined deletion at temperatures >30 °C results in synthetic lethality, apparently because of large scale protein aggregation (4). However, the combined deletion of both systems is possible at lower temperature, and the resulting ΔtigΔdnaK cells are adapted to growth at up to 30 °C (4, 5). Other chaperones, such as SecB and GroEL may partially compensate for the loss of TF and DnaK under these conditions (4, 5). The absence of TF causes the flux of newly synthesized polypeptides through DnaK to increase (3).

TF is a ribosome-associated protein (6–8) that has been shown to increase the folding efficiency of certain multidomain proteins concomitant with delaying their folding relative to translation (9). Ribosome binding is mediated by the N-terminal 118 residues of TF (7), specifically by a loop region consisting of amino acids Phe-44, Arg-45, and Lys-46 that contacts the L23 protein in the 50 S ribosomal subunit (10). Recently, the crystal structure of full-length TF and co-crystal structures of the archaeal and the eu-bacterial 50 S ribosomal subunit with the N-domain of TF were reported (11–14). In these structures, the TF N-domain interacts with proteins L23, L29, and the 23 S rRNA near the peptide exit tunnel. Upon binding to eu-bacterial ribosomes, a conformational change in the structure of the N-domain was observed (13), resulting in increased solvent exposure of hydrophobic surface area compared with the structure of free TF (11, 13). Consistent with such a conformational change, a recent study has demonstrated that fluorescently labeled full-length TF undergoes a structural expansion upon ribosome binding. The rates with which TF relaxed to its compact conformation upon ribosome departure varied depending on the characteristics of the nascent chain (15). A marked delay in the relaxation rate was correlated with the presence of nascent chain segments with high mean hydrophobicity. The model protein firefly luciferase was shown to have two such preferred TF binding sites (15).

Previous analysis of the primary structure of TF identified three distinct domains (10, 12, 16). In the recent crystal structures, TF displays an elongated shape (12, 14) in which the PPIase domain (residues 150–247) is connected with the N-domain (residues 1–150) via a long extension, so that it is positioned at the other end of the molecule, whereas the C-domain lies between the N-domain and the PPIase domain (Fig. 1A). The function of the PPIase domain remains unclear. Although prolyl-cis/trans-isomerase activity has been detected in vitro, the domain is dispensable for TF function in vivo (4, 17). Moreover, the PPIase domain binds preferentially to peptide segments of at least eight amino acids that are enriched in

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3 The abbreviations used are: TF, trigger factor; IPTG, isopropyl β-D-galactopyranoside; Kan, kanamycin; Tet, tetracycline; pBpa, para-benzoyle-L-phenylalanine.
basic and aromatic amino acids but do not necessarily contain proline residues, suggesting a more general chaperone function (18, 19). The C-domain shows structural similarity to the periplasmic chaperone SurA (20) and to MPN555 of \textit{Methanococcus jannaschii}, a predicted protein of unknown function (21). This domain can be divided into two subdomains consisting of arm 1 and arm 2. The TF structure (12) suggests that these arms and a segment of the N-domain constitute a crevice that may interact with polypeptides emerging from the exit tunnel (22).

Numerous ribosome-associated polypeptide chains, including membrane proteins and cytosolic proteins, have been chemically or site-specifically cross-linked to TF (22, 24–28). These interactions are abolished with a ribosome binding-deficient mutant TF F44A/R45A/K46A and thus depend on the ability of TF to dock onto the ribosome in close proximity to the opening of the polypeptide exit tunnel (25). TF displays a 30-fold increase in affinity for translating \textit{versus} non-translating ribosomes (15, 29, 30), which may be mediated by the exposure of hydrophobic sequence motifs in elongating nascent chains (15).

To determine which domain(s) of TF interact with elongating nascent polypeptides, we placed photoreactive probes at specific sites in each domain of TF and added the purified probe containing proteins to a reconstituted \textit{in vitro} translation system essentially free of chaperones (15, 31). We have demonstrated here that probes in the N- and C-domains lining the proposed binding crevice are adjacent to the nascent chain. The PPlase domain also participates in nascent chain interaction in a manner dependent on chain length and the specific polypeptide analyzed. Probe sites in the N- and C-domains that cross-linked to nascent chains (but not sites in the PPlase domain) proved to be involved in forming the TF dimer interface for free TF in solution.

**EXPERIMENTAL PROCEDURES**

\textbf{Plasmids—}The pYC-JYCUA and pBKpBpa plasmids were a generous gift from laboratory of Dr. Peter Schultz (32). The wild-type TF or the triple mutant F44A/R45A/K46A genes were subcloned into the pyc-JYCUA vector, resulting in the addition of C-terminal Myc and His\textsubscript{6} tags. This vector also encoded the mutRNA\textsubscript{\textit{C}}RNA, which encodes the triple mutant F44A/R45A/K46A genes and grown in LB medium supplemented with 30 \textmu g/ml kanamycin (Kan) and 25 \textmu g/ml tetracycline (Tet), and 1 mM para-benzoyl-L-phenylalanine (pBpa) to an \textit{A}_{600} = 0.6 at 30 °C. Protein expression was induced with 0.2% D-arabinose for 4 h. His\textsubscript{6}-tagged TF proteins were isolated from the soluble fraction of cell extracts on Ni\textsuperscript{2+}-nitrilotriacetic acid-agarose (Qiagen) followed by anion exchange chromatography on a Resource Q column (Amersham Biosciences). Proteins were stored at −80 °C in amber-colored tubes. TF and TF-NC (a version composed of the N- and C-domains only), which each had a cleavable N-terminal His\textsubscript{6} tag, were purified according to Ref. (15). The linker sequence between the N- and C-domains was GTSSAAAG (15).

\textbf{In Vivo Activity Test of TF and TF Variants—}\textit{E. coli} MG1655Δ\textit{dnaK}:Cm\textsuperscript{}/Δ\textit{tig} cells\textsuperscript{4} were co-transformed as above and grown at 23 °C in LB agar supplemented with 30 \mu g/ml Kan and 25 \mu g/ml Tet. After 36 h, colonies were inoculated in LB medium supplemented with Kan/Tet, as above, and grown at 23 °C overnight. The growth of cells serially diluted on LB Kan/Tet plates containing either 0.2% arabinose or 1 mM pBpa or both and incubated at 23, 30, and 37 °C was examined. Only the TF constructs that rescued growth at 37 °C were used in subsequent cross-linking experiments. The F44A/R45A/K46A construct, defective in ribosome binding, did not support growth at 37 °C and served as a negative control in subsequent cross-linking experiments.

\textbf{In Vitro Cross-linking Reactions with TF and TF Single Site Mutants—}\textit{In vitro} runoff transcription/translation of Luc constructs lacking stop codons was performed in the PURE SYSTEM (Post Genome Institute Co. Ltd., Tokyo, Japan) (31) at 30 °C for 55 min in the presence of 0.8 \mu Ci/\mu l[\textsuperscript{35}S]Met. When indicated, TF variants labeled with pBpa were added to a final concentration of 1 \mu M. Reactions were stopped by the addition of 0.15 \mu l of chloramphenicol (34 mg/ml) and incubated for 10 min on ice. Next, the samples were placed under a 500 W Mercury arc lamp (LOT-Oriel, Darmstadt, Germany) for 2–60 min on ice. A 20-\mu l aliquot of a 25-\mu l reaction was layered over a 100-\mu l sucrose cushion (0.5M sucrose, 15 mM MgCl\textsubscript{2}, 100 mM KOAc, and 200 mM Hepes, pH 7.5) and centrifuged in a TLA 100 rotor (Beckman) at 100,000 × \textit{g} for 20 min at 4 °C. Pelleted ribosome-nascent chain complexes were resuspended in 100 \mu l of water, and then RNase A (protease-free) (Roche Applied Science) and EDTA were added to final concentrations of 100 \mu g/ml and 10 \mu M, respectively, followed by incubation at 37 °C for 10 min. After trichloroacetic acid precipitation, the reactions were resolved on 4–10% SDS-PAGE followed by autoradiography. The identities of the photoadducts were confirmed by immunoprecipitation with anti-TF antibodies.

\textbf{Western Blots of TF and TF Single Site Mutants—}5 \mu M purified TF and TF single site mutants were photolyzed for 15 min, loaded on 10% SDS-PAGE, and transferred to nitrocellulose membranes (Whatman). The membranes were blocked with 5% (w/v) skim milk powder in TBST (20 mM Tris-HCl, pH 7.6, 136 mM NaCl, and 0.1% (w/v) Tween 20), washed three times with TBST, and then a primary antibody against the Myc tag (American Type Culture Collection) was used to probe the membrane at a dilution of 1:5000 followed by three washes and detection with a secondary anti-rabbit horseradish peroxidase.

\textsuperscript{4} P. Genevaxa, unpublished data.
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FIGURE 1. Function of TF and TF single site mutants. A, the crystal structure of E. coli TF (12) shown in two views, with the domain arrangement labeled as follows: N-domain (red), PPIase domain (yellow), C-domain, arm 1 (green), and arm 2 (blue). Amino acids replaced by the photoreactive probe pBpa and used in further experiments are shown in lighter blue, circled in black with the corresponding amino acid number indicated. All other sites tested are shown in gray. B, Coomassie Blue gel of equivalent amounts of purified TF variants. C, serial dilutions of MG1655ΔdnaK:CamΔtig cells expressing TF or TF single site mutants (indicated on the left of the panels) were spotted onto LB agar plates containing 0.2% arabinose, 1 mM pBpa, 25 μg/ml Tet, and 30 μg/ml Kan and grown overnight at the selective temperature of 37 °C. Wild-type TF was cloned into the pOFX plasmid under an IPTG-inducible promoter and grown as above in the presence of 250 μM IPTG instead of arabinose.
conjugate (Sigma) used at 1:5000 in TBST. The signal was developed using the ECL Plus Western blotting detection system (Amersham Biosciences) and visualized using Imagereader LAS-3000 (Fuji).

**In Vitro Cross-linking Reaction with the Probe in the Nascent Chain**—In *in vitro* runoffs transcription/translation reactions were preformed with Luc-nascent chains of various lengths from 77 to 280 amino acids (as above). Nascent chains contained a single amber stop codon at position 5 from the N terminus, as previously described (25). TF or TF-NC was present at 1 μM.

**RESULTS**

Characterization of TF with Photoreactive Probes at Different Locations—To determine which regions of TF interact with the nascent chain as it emerges from the ribosome, we made use of a recently developed technology (32) allowing for *in vivo* expression of a protein with a benzophenone photoreactive probe incorporated at a specific site through amber codon suppression. Benzophenone, a phenylalanine analog, consists of two phenol rings with the photoreactive moiety residing in between the two rings (supplemental Fig. 1). Purified TF proteins with single probes incorporated were then added to a fully reconstituted bacterial translation system lacking chaperones. Following runoffs translation in the presence of TF and upon exposure to UV light, covalent bonds formed between TF and nascent chains adjacent to the photoreactive probe in TF. Several sites in each domain of TF (Fig. 1 and supplemental Fig. 2A), including residues 34, 88, 118, 185, 233, 320, 373, and 419, were chosen for probe incorporation. We confirmed that the amber codon was suppressed and the TF variants were translated, resulting in full-length proteins with C-terminal Myc and His6 tags (Fig. 1B). The benzophenone-carrying proteins were expressed in MG1655 cells from an arabinose promoter at a level ~2-fold above that of endogenous TF (data not shown). In contrast, under the same conditions, recombinant wild-type TF was expressed at substantially higher levels, because it does not contain an amber site and does not rely on suppression for completion of translation. Truncated TF products that were not suppressed and therefore did not contain probes or C-terminal tags were not observed after purification.

**FIGURE 2. Site-specific photocross-linking of TF to Luc-nascent chains.** In *in vitro* translation reactions were performed in the PURE system to generate [35S]Met-labeled Luc-nascent chains of different lengths in the presence of TF containing the photocross-linker pBpa at the positions indicated. Light-dependent photoaducts between Luc-nascent chains and TF were observed (open arrowheads). Non-cross-linked nascent chains are indicated with black arrows. A, Luc-nascent chains of 60 amino acids cross-linked for 15 min, Luc-nascent chains of 77 amino acids cross-linked for 15 min (B), and Luc-nascent chains of 164 amino acids cross-linked for 2 min (C). Photoaduct formation was dependent on ribosome binding, as no photoaducts were observed with TF320 FRK/AAA (lanes 11 versus 12). Length-dependent photoaducts were observed with TF233 and TF185, whereas TF34, TF320, and TF373 showed length-independent cross-linking to Luc-nascent chains of 60 residues and longer. Positions of probe sites are indicated in crystal structures of *E. coli* TF to the left of each panel. Sites that resulted in photoaduct formation are shown in green, and sites that failed to form photoaducts are in red; other colors are as in Fig. 1A.
In the probe-containing mutant proteins compared with wild-type TF (supplemental Fig. 3 and supplemental Table 1).

**Photocross-linking of TF Single Site Mutants to Ribosome-nascent Chain Complexes** — Using the TF single site mutants with probes in each of its domains, *in vitro* translation reactions were performed to determine which probes were adjacent to the nascent chain as it emerged from the ribosome. Luc was chosen as a model substrate, because it efficiently interacts with TF upon translation (9, 15, 25). Luc-nascent chains of 60, 77, and 164 amino acids in length were stalled on the ribosome and analyzed. Cross-links to each domain of TF were observed for the 60- and 164-mer (Fig. 2, A and C). N-domain interactions were observed with TF34 to Luc-nascent chains of 60, 77, and 164 amino acids in length (Fig. 2).

TF320 and TF373, in which probes are in the tip of arm 1 and arm 2 of the C-domain, respectively, cross-linked to all lengths of Luc-nascent chains tested (Fig. 2). Photoadducts with TF320 were the most intense and hence were used as a reference in all panels of Fig. 2. Interestingly, TF320 resulted in two photoadducts to the Luc 60- and 77-mers differing in size, presumably because of TF binding to two independent sites on the nascent chain (Fig. 2, A and B). Importantly, no cross-links were observed when the probes were placed on the back surface of the TF molecule at positions 419 (Fig. 2) or 118 (supplemental Fig. 2D), thus suggesting that the nascent chain moves through or spans the proposed binding crevice accessible from the front side of the molecule. No cross-links were observed with the TF320 version of the F44A/R45A/K46A mutant, confirming that ribosome binding is a prerequisite for nascent chain interaction (Fig. 2).

Cross-linking to the PPIase domain displayed a nascent chain length dependence. Short nascent chains of 60 amino acids in length cross-linked to positions 185 and 233 in the PPIase domain, whereas nascent chains of 77 amino acids did not (Fig. 2) (15). Thus, between the lengths of 60 and 77 amino acids, a portion of the Luc-nascent chain changes position relative to the probes in the PPIase domain. Taking the dimensions of TF in the crystal structure into account, cross-linking of the short 60-mer chain to the PPIase domain suggests that the nascent polypeptide must be in a rather extended conformation. Interestingly, longer nascent chains of 164 amino acids again cross-linked to TF233 but not to TF185 (even after 30 min of irradiation; data not shown), suggesting that different portions of the Luc nascent chain are adjacent to the probe at position 233 as the chain elongates from 60 to 164 amino acids (Fig. 2C). Luc 125-mers were also shown to cross-link to TF233 (Fig. 3B). The differential cross-linking to TF233 and/or TF185 suggests that the interaction with the PPIase domain does not merely result from collision with a flexible nascent chain but rather reflects a more specific participation of this domain in nascent chain binding.

The yield of Luc folding is known to be improved by the presence of TF *in vitro* and *in vivo*, and Luc-nascent chains have been shown to be relatively protected by TF against proteinase K (9, 25). To determine whether TF interacts in a similar way with different nascent chains, we analyzed its interaction with nascent chains of the natively unfolded protein α-synuclein. In contrast to Luc, α-synuclein was previously shown to be in close

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**FIGURE 3. Site-specific photocross-linking of TF to α-synuclein- and Luc-nascent chains.** *In vitro* translation reactions and photocross-linking were performed as in Fig. 2A. α-synuclein-nascent chains of 140 amino acids cross-linked for 15 min. B, Luc-nascent chains of 125 amino acids or α-synuclein-nascent chains of 140 amino acids were cross-linked for 15 min. Light-dependent photocrosslinks between Luc- or α-synuclein-nascent chains and TF are indicated by open arrowheads. Non-cross-linked nascent chains are indicated by black arrows. Photoadduct formation was dependent on ribosome binding, as no photoadducts were observed with TF320 FRK/AAA.

To test whether the probe-containing TF proteins were functional, we used an *in vivo* complementation assay. ΔdnaKΔtig cells are not viable at 37 °C unless TF is expressed from a plasmid (4, 17). TF14, TF34, TF73, TF88, TF118, TF185, TF233, TF320, TF373, and TF419, carrying the probe at the indicated residue, rescued growth at 37 °C (Fig. 1C and supplemental Fig. 2C). Because overexpression of wild-type TF in MG1655 ΔdnaKΔtig cells is toxic (4), an IPTG-inducible promoter was used to express wild-type TF at lower levels. However, when probes were placed at positions 377, 378, or 387 in arm 2 of TF, no *E. coli* growth was observed. Because the mutant proteins were produced (data not shown), incorporation of the probe at these positions likely interferes with TF chaperone activity or folding of the protein. These sites were therefore not further analyzed. Notably, probe incorporation at positions 233 and 185 in the PPIase domain resulted in a partial loss of PPIase activity measured with RNase T1 as a substrate, consistent with previous mutational studies (34) (and data not shown). Tryptophan fluorescence and circular dichroism measurements demonstrated the absence of significant conformational differences...
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**Figure 4. Dimerization interface of TF**. 5 μM purified TF variants containing photocross-linker Bpa at the positions indicated were photolyzed, and the resulting samples were subjected to Western blotting using primary antibodies against the Myc tag. A, samples photolyzed or 15 min; B, samples receiving no light. The purified monomers are indicated with a black arrow, and the cross-linked dimers are indicated with open arrowheads. TF34, TF320, TF320 F44A/R45A/K46A, and TF373 showed light-dependent photoadducts. C, TF structure with domains colored as in Fig. 1A and probe positions marked. Probe position sites in green indicate photoadduct formation; sites in red indicate sites that did not result in photoadducts.

proximity to TF by cross-linking but was not found to be shielded by TF against added protease (25). α-Synuclein also does not contain any hydrophobic stretches that cross the threshold for mean hydrophobicity that were shown to be the case for Luc to increase TF binding (supplemental Fig. 4) (15).

Using α-synuclein 140-mer nascent chains we observed cross-linking to the TF N-domain (TF34), the C-domain (TF320, TF373), and the PPIase domain (TF233) (Fig. 3A). However, even when the additional methionine in Luc 125-mer chains relative to α-synuclein 140-mer chains was taken into account for the incorporation of radioisotope labeling, the photoadduct between α-synuclein and the PPIase domain was found to be substantially weaker than that of Luc (Fig. 3B). This would be consistent with the absence of contiguous hydrophobic segments in α-synuclein, which is thought to mediate a strong interaction with TF (supplemental Fig. 4) (15).

**Dimerization Interface of TF**—Non-ribosome-bound TF forms dimers with a Kd of 1–2 μM (15). It has been proposed that the dimer interface involves regions of TF that interact with the nascent chains. This hypothesis is supported by fluorescence resonance energy transfer measurements from which models were built to suggest a topology in which the binding regions of TF for the nascent chain face each other with the N-domains at opposite ends of the dimer interface (15). To determine which probe sites in TF are involved in forming the dimer interface, cross-linking experiments were performed with free TF at a final concentration of 5 μM. We observed efficient light- and probe-dependent dimer cross-linking with TF34, TF320, TF320 F44A/R45A/K46A, and TF373 but not with the other purified TF variants (Fig. 4, A and B). This is in agreement with a dimer model in which the binding crevice for the nascent chain of at least one of the protomers is at the dimer interface (Fig. 4C). Interestingly, two photoadducts were observed in the case of the TF34 protein, suggesting that this region of TF is flexible and may be adjacent to more than one site on its partner monomer.

To examine whether any of the sites involved in the dimer interface are adjacent to the ribosome when TF binds, we performed similar experiments to that of Fig. 4 but in the presence of 250 nM non-translating ribosomes and 1 μM TF to favor TF binding to ribosomes. No additional photoadduct formation to ribosomal proteins was detected (supplemental Fig. 5). Note that the TF dimer cross-links are weaker in this experiment, because ribosome binding shifts the dimer equilibrium toward the monomer (15).

**Major Role of the N- and C-terminal Domains in Nascent Chain Interactions**—It was previously shown that TF relatively protects nascent polypeptides against digestion by proteinase K (25, 26). To determine whether the N- and C-terminal domains played the major role in nascent chain shielding of Luc, a construct of TF was used in which the PPIase domain was deleted (TF-NC) (Fig. 5A) (15). In vitro translations were performed in the presence or absence of 1 μM TF or TF-NC, as previously described (Fig. 2). The protease accessibility of the nascent chains in the presence and absence of TF or TF-NC was measured. Notably, no significant difference in shielding efficiency was observed between TF and TF-NC for Luc-nascent chains of 92 and 280 amino acids in length, and only a minor difference was observed with the 125-mer nascent chains (Fig. 5B). To determine whether the nascent chains were in close proximity to TF and TF-NC, in vitro translations were performed in which an amber stop codon was introduced at either position 5 or 42 of the luc gene, allowing for incorporation of a photoreactive cross-linker at these positions in the nascent chain. Following translation in the presence of TF or TF-NC, the samples were exposed to UV light and analyzed by SDS-PAGE (Fig. 5C and supplemental Fig. 6). Based on the presence of photoadducts including TF, we can conclude that the N- and C-domains of TF are indeed adjacent to the N-terminal region of the Luc-nascent chain when it is between 60 and 280 amino acids in length (Fig. 5C and data not shown). These results support the view that the N- and C-domains provide the primary binding region for the nascent chain substrate, resulting in shielding against protease, whereas the PPIase domain appears to represent a secondary binding site, consistent with previous observations utilizing fluorescence spectroscopy (15). This idea is also supported by recent work that shows that an NC-domain construct of TF is sufficient for protein refolding in vitro (22).

**DISCUSSION**

In this study, we determined which regions of TF interact specifically with nascent chains. Our results indicate that the N- and C-domains of TF provide a primary binding site for nascent chains used by different polypeptides (Luc and α-synuclein), with arm 2 of the C-domain having a prominent role. These regions are also mainly responsible for the protection of Luc-nascent chains from protease digestion. In contrast, the PPIase domain interacts with nascent chains in a length-dependent
manner that is also dependent on the presence of appropriate hydrophobic segments in the nascent chain. Such hydrophobic regions are exposed by Luc but not by α-synuclein-nascent chains. These findings are consistent with a model in which nascent chains, dependent on their length and conformational state, may span a binding crevice formed by the N- and C-domains of TF, reaching the PPIase domain. Furthermore, cross-linking data indicated that the dimer interface of non-ribosome-bound TF overlaps with sites that were adjacent to the nascent chain during translation, in support of the view that dimer formation partially occludes the chaperone sites of TF (15).

Using both Luc- and α-synuclein-nascent chains, cross-links to multiple TF single site mutants with probes in the N- and C-domains were observed (Figs. 2 and 3). In the case of Luc chains of 60 residues and longer, cross-linking to these positions was found to be length-independent (Fig. 2). Probes at positions 34, 320, and 373 were adjacent to the nascent chains, identifying the tip of arms 1 and 2 of the C-domain and the ribosome-binding loop region of the N-domain as the main regions of TF for nascent chain association. It is interesting to note that cross-linking was not observed when the probe was placed at position 88 in the N-domain, although this position might have been expected to be in close proximity to the nascent chain. It is possible that conformational changes known to occur when TF binds to the ribosome (13, 15) increase the distance of this position to the nascent chain.

Luc-nascent chains of 60 residues were cross-linked to position 233 in the PPIase domain of TF. In the crystal structure of TF (12), the distance between position 34, next to the ribosomal exit site, and position 233 is 87 Å. The length of the ribosomal exit tunnel is 100 Å. Thus, a 60-amino-acid nascent chain, which maximally spans 210 Å, would be in a rather extended conformation for cross-linking to position 233 to occur. Our data indicate that the binding region for this length of Luc includes the tip of arms 1 and 2 of the C- and the N-domains around position 34, threading between arms 1 and 2 and spanning the entire length of TF. Of note is the PPIase domain cross-linking to Luc-nascent chains in a length-dependent manner (Fig. 2). Photoadducts were observed to TF233 when the nascent chain was 60, 125, and 164 residues in length but not when it was 77 residues in length (Figs. 2 and 3). This argues for the possibility that the nascent chain can move relative to TF, with the PPIase domain representing an auxiliary binding site that is used dependent on the length and conformational state of the nascent chain. Indeed, the PPIase domain has been shown to interact with peptides in a proline-independent manner (23).
Consistent with this finding, cross-linking of nascent chains to the PPIase domain was independent of PPIase activity, suggesting a more general chaperone function for this domain. Although the NC-domain segment of TF alone is sufficient for function in vivo (4), the PPIase domain may nevertheless contribute to fully efficient TF chaperone function. Consistent with this notion, recent experiments showed that the PPIase domain prolongs the duration of TF association with certain nascent chains (15).

TF binds to the ribosome as a monomer (12), but the high cytosolic concentration of TF (~50 μM) favors dimer formation in solution (8). Recent fluorescence resonance energy transfer experiments (15) support a crevice-to-crevice model for the dimer interface in which the monomers are positioned nearly perpendicular to one another. Our cross-linking experiments presented in this study are consistent with such a model. Cross-linking with the purified TF variants TF320, TF320 F44A/R45A/K46A, TF373, and TF34 (Fig. 4) showed that these residues, which are adjacent to the nascent chain when TF is bound to the ribosome, are involved in the dimer interface when TF is free in solution. Although a crevice-to-back surface topology in the dimer is not formally ruled out by these experiments, this possibility seems unlikely based on the recent fluorescence resonance energy transfer data and on the finding that no dimer cross-linking was observed with TF carrying a cross-linker at residue 419. This residue is exposed on the back surface of the molecule pointing away from the binding crevice for the nascent chain. The TF dimer was in rapid equilibrium with the monomer, thereby supplying monomer for ribosome binding (15). A partial occlusion of the nascent chain binding surface in the TF dimer could readily explain how binding to the ribosome activates TF for nascent chain association. In addition, the conformational expansion that is observed upon nascent chain binding (15) likely contributes to activating TF chaperone function.

In summary, our study sheds light on how nascent chains emerging from the ribosome interact with TF and how the dynamic monomer-dimer equilibrium of TF (15) contributes to the regulation of the accessibility of its chaperone sites. Whether and how TF and the signal recognition particle communicate when a signal sequence containing protein is translated remains to be addressed.

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