Alternate Recruitment of Signal Recognition Particle and Trigger Factor to the Signal Sequence of a Growing Nascent Polypeptide*

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Accordingly, nascent secretory proteins are not found cross-linked to Ffh but, like most nascent polypeptides, to the ribosome-associated chaperone Trigger factor (TF) (5–8).

From the results of a number of experiments it has been concluded that it is the low overall hydrophobicity characteristic of cleavable signal sequences of secretory proteins that excludes an interaction with SRP (5, 9–12). Surprisingly, however, nascent secretory proteins exposing only the signal sequence from the ribosome have been found cross-linked to Ffh (13, 14). Only upon further growth of the nascent chain did the signal sequence move from a close contact with Ffh to one with TF. For the outer membrane protein OmpA, this transition was determined to occur, after 37–53 amino acids downstream of the signal sequence have exited the ribosome (13). A length-dependent change in the contact of a nascent signal sequence from Ffh to TF relates to the controversial issue if Ffh and TF can bind to the same ribosome at the same time. This question was raised by the disclosure of partially overlapping binding sites for both proteins at the ribosomal proteins L23 and L29 (15–21) and by studies directly addressing a competitive binding behavior (22–24).

The finding that the vicinity of a signal sequence changes from Ffh to TF upon growth of the nascent chain might be explained by a change in the overall affinity of the nascent chain for the two ligands. In the case of a secretory protein like OmpA, high affinity binding sites for TF following downstream of the signal sequence would recruit TF, which thereby sequesters the adjacent signal sequence from contacts with Ffh. We therefore asked if by the same token, a downstream recognition site for Ffh being exposed upon continued growth of the nascent chain would have an influence on the liganding behavior of a signal sequence. Our results show that distal sequence elements of the nascent chain in fact determine the molecular vicinity of a signal sequence and that Ffh and TF compete for binding to nascent secretory and membrane proteins.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—The following E. coli strains were used, MRE 600 (25), SL119 (26), C600 (Δtag) (27), and LGS322 (ΔmtlA) (28). Plasmids used were vector pkSM717 (29), p17MtlA-B expressing mnt-mtipepsin (MtlA) (6), and p717OmpA-V11 encoding pOmpA with a TAG stop codon at residue Val-11 (13).

The gene encoding the hybrid protein Omtl was constructed from plasmids p717OmpA (6, 13) and p717MtlA-B. Plasmid p717OmpA was linearized at an EcoRI site located at the 3’-end of ompA and subsequently cut with SpeI to remove the ompA sequence except for the nucleotides encoding the first 26 residues including the signal sequence. This fragment was ligated onto a Spel-EcoRI fragment of p717MtlA- Spe2 (12) encoding MtlA except for the first 46 residues. The resulting plasmid is p717Omtl. Likewise, p717Omtl-V11 harboring a stop codon in the signal sequence was constructed using p717OmpA-V11 and p717MtlA-Spe2. To construct plasmid p717Omtl-136 carrying a stop...
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![Image](126x398 to 486x733)

**FIGURE 1.** Replacing the first signal anchor sequence of MtlA by a cleavable signal sequence does not interfere with membrane localization. A, topography model and construction of pOmtl. The 22 amino acid long signal sequence of pOmpA (bold black line) plus the four first residues of mature OmpA were fused to an N-terminally truncated version of MtlA lacking 46 amino acids including the first TM (dotted lines). The five remaining TMs of MtlA are represented by gray boxes with the numbers indicating the first and last residue of each predicted TM. A recently proposed topography model of MtlA differs from the one shown by predicting two additional TMs between TM4 and TM5 (34). The about 300-residue-long soluble C-terminal part of MtlA is cytosolic. Numbering starts with the first amino acid of pOmtl. B, MtlA and pOmtl were expressed in E. coli strain LG5322 lacking the mtlA-gene (ΔmtlA) from plasmids p717MtlA-B or p717Omtl. Control cells were transformed with vector pKSM717. Soluble and membrane fractions were each subjected to SDS-PAGE in a 1:1 protein ratio (corresponding to 0.4:1 equivalent volumes). They were analyzed by Western blotting using antibodies raised against MtlA (αMtlA) and SecG (αSecG) as a marker protein for the inner membrane. C, full-length MtlA and pOmtl were synthesized by a reconstituted E. coli transcription/translation system in the presence or absence of inside-out inner membrane vesicles (INV). Radiolabeled translation products were separated by SDS-PAGE and visualized by phosphorimaging. The molecular masses of marker proteins are indicated. Half of each reaction was digested with protease K (PK). Addition of INV leads to the generation of membrane protected fragments of MtlA and pOmtl that are resistant toward protease K (lanes 4 and 8). In the case of pOmtl, addition of INV also causes signal sequence cleavage (open arrowhead). D, plasmid-encoded pOmtl and MtlA were expressed in E. coli strain LG5322 (ΔmtlA). At zero time, protein synthesis was blocked by the addition of spectinomycin to a final concentration of 100 μg/ml. Aliquots were removed at the indicated time points, precipitated with 5% trichloroacetic acid, resuspended in gel loading buffer, and analyzed by SDS-PAGE and Western blotting using antibodies raised against MtlA (upper panel). Scanned blots were quantified using Imagequant software (lower panel). The data shown are representative of two parallel experiments.

codon in TM2 of pOmtl at residue Ile-36, plasmid p717Omtl was cut with SpeI and HincII and ligated onto a SpeI-HincII fragment which was obtained using p717MtlA-I56 (30) as template and the primers 5′-CCGAGCAGACTAGTGAGAAGTGGTTAATGGCGTTGTT-3′ (the SpeI-l cleavage site is underlined) and 5′-ACCGTGTTAATGGCGTTGTT-3′. Addition of INV leads to the generation of membrane protected fragments of MtlA and pOmtl that are resistant toward protease K (lanes 4 and 8). Chemical cross-linking using disuccinimidyl suberate (DSS), introduction of TAG stop codons by site-directed mutagenesis, incorporation of the site-specific cross-linker Tmd-Phe, and immunoprecipitation on 4-fold scaled-up reactions were performed as described (6, 13). Translation products were resolved by SDS-PAGE using either linear 10% (Fig. 1D) and 15% (Fig. 1, B and C) acrylamide gels or, in the case of cross-linking reactions, 9–20% (Fig. 3) and 7–20% (Figs. 4 and 5) gradient gels, and radiolabeled proteins were visualized by phosphorimaging using an Amersham Biosciences PhosphorImager and quantified using Imagequant software (Amersham Biosciences). The preparation of inside-out inner membrane vesicles (INV) as well as the protease protection assay have been described previously (31, 32).

Miscellaneous—To express MtlA and pOmtl, the E. coli strain LG5322 (ΔmtlA) was transformed with plasmids p717MtlA-B or p717Omtl, respectively. Control cells were transformed with the vector plasmid pKSM717. Cells were grown to mid logarithmic phase in LB medium containing 50 μg/ml ampicillin and expression from plasmids was induced by adding isopropyl thio-β-D-galactopyranoside at a final...
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Concentration of 1 mM. After an additional hour of growth, cells were harvested, and a crude protein extract was obtained by sonication and low speed centrifugation (15 min, 5,000 × g) to remove debris. Extracts were further separated into soluble supernatant and membrane pellet by high speed centrifugation (1 h, 70,000 rpm, Beckman rotor TLA100.2).

Ffh (13) and TF (14) were purified, and polyclonal antibodies were raised against the purified proteins (14, 32) as described. Western blots were developed using horseradish peroxidase-conjugated goat anti-rabbit antibody, followed by enhanced chemiluminescence (Amersham Biosciences).

RESULTS

Correct Localization of a Polytopic Membrane Protein Harboring a Cleavable Signal Sequence at Its N Terminus—To construct a polypeptide in which a signal sequence is followed by a Ffh recognition site, i.e. a signal anchor sequence, we replaced the first 46 amino acid residues of the inner membrane protein mannitol permease (MtlA) by the signal sequence plus the four subsequent residues of PompA creating the fusion protein pOmtl (Fig. 1A). MtlA has six to eight predicted transmembrane spans (TMs) with the N and the C termini located in the cytoplasm (33, 34). Despite the substitution of the first TM of MtlA for the signal sequence of PompA, the fusion protein pOmtl was recovered from the membrane fraction of pOmtl overexpressing E. coli cells to about the same extent as MtlA (Fig. 1B); even in wild type E. coli strains up to 20% of MtlA are found soluble following ultracentrifugation of cell extracts (35). Furthermore, when expressed in vitro by a cell-free transcription/translation system, pOmtl integrated into INV as indicated by the accumulation of proteinase K-resistant fragments when INV were present (Fig. 1C, compare lanes 6 and 8). A 30-kDa protease-resistant fragment (lane 4) is diagnostic for membrane integration of MtlA (36).

In the case of pOmtl, the appearance of more proteinase K fragments smaller than 30 kDa (compare lanes 4 and 8) are suggestive of a less stable membrane association when compared with MtlA, which was also seen in an in vivo time course experiment (Fig. 1D). Consistent with membrane integration, the addition of INV to the in vitro synthesis reaction also generated a smaller species of Omtl most likely due to signal sequence cleavage (Fig. 1C, lane 7, arrowhead). Although as expected pOmtl lacking the first TM of MtlA did not assemble into a functional permease (data not shown), it obviously was recognized and at least transiently localized to the membrane by the targeting machinery of the cell.

A Downstream Signal Anchor Sequence Mediates Contact of the N-terminal Signal Sequence with Ffh—Next a series of elongation-arrested chains of pOmtl (Fig. 2) were synthesized in vitro. These nascent pOmtl chains carried a photoactivatable derivative of phenylalanine (Tmd-Phe; 1-4-[3-[(trifluoromethyl)-3H-diazirin-3-yl]phenylalanine) either within the hydrophobic core of the PompA signal sequence (position 11) or within TM2 of MtlA (position 36; arrows). Fig. 2 also illustrates the various sequence portions of PompA being exposed from the ribosomal exit tunnel. Following synthesis in the presence of Ffh and TF, the various radioactively labeled pOmtl nascent chains were irradiated with UV light and the obtained cross-links were visualized by SDS-PAGE and phosphorimaging (Fig. 3). For comparison two short nascent chains of PompA carrying Tmd-Phe at position 11 in the signal sequence were also included (A). In the latter cases a prominent photoduct of the PompA signal sequence was obtained (X) that was immunoprecipitated by anti-Ffh antibodies (Fig. 3A, lanes 3 and 6). When the shortest nascent chain of PompA (pOmtl-65) was UV-irradiated (Fig. 3A, lanes 7–10) a more discrete Ffh adduct (X), which was masked by an

non-further characterized translation product (○), became visible after immunoprecipitation with anti-Ffh antibodies (lane 9). Thus the signal sequence of PompA contacts Ffh also when present in short nascent chains of pOmtl. As expected, pOmtl-65 nascent chains carrying the cross-linker at position 36 within TM2 of MtlA did not show this adduct (Fig. 3B, lanes 1–3), since this cross-linking site is predicted to be buried within the ribosomal exit tunnel (cf. Fig. 2). In pOmtl-62 exposing the entire TM2 of MtlA (cf. Fig. 2) the Ffh adduct of the signal sequence became more prominent (Fig. 3A, lanes 11–13, upper panel), whereas immunoprecipitation revealed also some faint contact with TF (asterisk, lane 14). The same ligands were picked up when the cross-linker was placed into TM2 of MtlA, now with a relative increase in number of TF adducts (Fig. 3B, lanes 4–7). Irrespective of the position of Tmd-Phe, nascent chains of pOmtl-110 exposing about 30 amino acids downstream of TM2 of MtlA (cf. Fig. 2) overwhelmingly cross-linked to TF (Fig. 3A, lanes 15–18, upper panel, and B, lanes 8–11). Hence in pOmtl, the signal sequence of PompA and the TM2 of MtlA share the same molecular environment as observed previously for the signal sequence of PompA (13): they loose contact to Ffh in favor of one with TF when the nascent chain increases in length.

Extension of the pOmtl nascent chain by another 30 amino acids (pOmtl-140) did not change the predominant liganding of TM2 of MtlA.

FIGURE 2. Structure of the ribosome-associated nascent chains of PompA and pOmtl used. The length of the various nascent chains is indicated by the numbers of amino acid residues. The signal sequence and downstream mature sequence sections of PompA are represented by hatched and stippled boxes, respectively. TMs and interspersed loops of MtlA are drawn as light and dark gray boxes, respectively. TM2 and TM4 are indicated. TM3 is denominated SA (signal anchor sequence). The black clamp symbolizes the ribosomal exit tunnel assumed to bury 30 amino acid residues of each nascent chain (49). Arrows indicate the two positions of the cross-linker Tmd-Phe at amino acid positions 11 and 36. The bottom diagram depicts full size pOmtl for comparison.

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to TF (Fig. 3B, cf. lanes 8–15), whereas the signal sequence of pOml-140 showed about as many contacts with Ffh as with TF (Fig. 3A, lanes 19–22). Remarkably, however, as soon as TM3 of MtlA, which by its topogenic information is a signal anchor sequence, became fully exposed in pOml-169 nascent chains (cf. Fig. 2), the preceding signal sequence almost exclusively cross-linked to Ffh (Fig. 3A, lanes 23–26, upper panel) and also TM2 of MtlA showed relatively many more Ffh adducts than in the shorter pOml-140 and pOml-110. Obviously the signal sequence regained contact to Ffh upon exposure of a downstream signal anchor sequence. Incidentally, incorporation of Tmd-Phe into TM3 of MtlA had revealed multiple Ffh binding sites (Ref. 30 and data not shown). The cross-linking data illustrated in Fig. 3 therefore suggest that it is this late signal anchor TM3 that recruits SRP to the nascent chain of pOml-169 and that the signal sequence and TM2 must be so close that both resume contact to Ffh. The formation of such a helix bundle-type structure of nascent pOml-169 at the ribosomal exit site is supported by data shown in Fig. 3A (lanes 23–26); in addition to Ffh, the cross-linker placed in the signal sequence yields a strong adduct of pOml-169 with a 8 kDa protein (black star), which is immunoprecipitated with antibodies against ribosomal protein L29 (lower panel). Cross-linking to L29 was not observed for TM2 (Fig. 3B, lanes 16 and 17). On the other hand, also the shorter chains pOml-82 and pOml-110 were found to cross-link to ribosomal proteins L29 and L23 (Fig. 3A, lanes 11–18, black and white stars, respectively). These findings indicate

**FIGURE 3.** The molecular vicinity of a nascent signal sequence depends on emerging downstream sequence sections. Ribosome-associated pOmpA and pOml nascent chains of the indicated chain lengths were synthesized by a reconstituted transcription/translation system prepared from E. coli. Truncation of the mRNAs was achieved by the addition of complementary oligodeoxynucleotides and RNaseH cleavage (31). Stop (UAG) codons at the indicated positions were suppressed by tRNA<sup>sup</sup> charged with Tmd-Phe. In vitro reactions were supplemented with 80–100 nM Ffh. UV irradiation (UV) resulted in adducts with Ffh (X, 50 kDa) and TF (asterisk, migrating on SDS-PAGE at 58 kDa) and with the ribosomal proteins L23 (white star, 11 kDa) and L29 (black star, 8 kDa) as identified by co-immunoprecipitation with specific antibodies (αFfh, αTF, αL23, αL29). TF adducts (brackets) often resolve into several species (13). Labels a UV-independent transcription/translation product of the Omtl-DNAs. A, UV-induced cross-links obtained with Tmd-Phe replacing Val at position 11 within the hydrophobic core of the signal sequence of pOmpA. B, UV-induced cross-links obtained with Tmd-Phe replacing Ile at position 36 within TM2 of MtlA.
that even after exposure of more than 130 amino acids the signal sequence of nascent pOmtl is not freely mobile but remains closely apposed to the ribosomal exit site.

**Ffh and TF Compete for the Same Nascent Chains**—Among the various pOmtl nascent chains analyzed thus far, pOmtl-110 and pOmtl-169 display the most distinct liganding behavior. When the two species are synthesized in the presence of both Ffh and TF, the signal sequence present in pOmtl-110 picks TF as interaction partner (“TF binder”) and that of pOmtl-169 Ffh (“SRP binder”). Thus the molecular vicinity of the signal sequence in the folding pOmtl nascent chain appears to be determined by the nature of downstream sequence sections. These findings do, however, not allow for predicting the fate of the disfavored ligand; while it is virtually not found in contact with the nascent pOmtl chain, it might still remain bound to the synthesizing ribosome via L23/L29.

In the experiments summarized in Fig. 3, cross-links of a given nascent chain obtained with either of the two positions of Tmd-Phe were not always entirely identical. Thus by comparison with the signal sequence, TM2 showed relatively more contacts with TF than with SRP (cf. pOmtl-82 and pOmtl-140 in A and B). While this finding could reflect a closer location of TM2 to the ribosome-bound TF, it raised the general question as to the influence of the sidedness of the cross-linker on the liganding pattern. In theory, the changing cross-linking partners of a growing pOmtl nascent chain could also be the result of a change in conformation of a folded nascent chain at the ribosomal exit site. To render the results less dependent on the orientation of a single cross-linker, we employed the NH2-group-specific cross-linker DSS, as the N-terminal part of pOmtl including TM2 contains five Iysyl residues. Likewise it seemed to be important to address the influence of various concentrations of Ffh and TF on the cross-linking behavior of pOmtl nascent chains. In the particular cell-free transcription/translation system used here, the endogenous concentration of Ffh is so low that Ffh cross-links to MtlA and its SRP-dependent interaction into membrane vesicles are only observed if purified Ffh is added (6, 32). On the contrary, sufficient depletion of the cell-free system of TF requires preparing its components from a TF knock-out mutant strain.

Fig. 4 illustrates the cross-linking results obtained for pOmtl-110 and pOmtl-169 synthesized under these adapted conditions. In the absence of Ffh and TF, DSS did not give rise to any specific adduct (lanes 1 and 2; the ones marked with • were present under all conditions tested). When synthesized in the presence of 0.5 μM TF, a pronounced cross-linking product of pOmtl-110 with a protein of the size of TF appeared (upper panel, lane 4, asterisk). This concentration of TF had previously been determined to be present in similar cell-free systems (37). Obviously 0.5 μM TF was saturating for the interaction with pOmtl-110, because raising the level of TF to 5 μM did not improve the yield of the cross-linking product (lane 6), even though this concentration of TF amounts to only 10% of its estimated intracellular concentration (38). Different from previous experiments (Fig. 3), in which the concomitant presence of Ffh and TF predominantly led to TF adducts of pOmtl-110, a clear interaction with Ffh was observed when pOmtl-110 nascent chains were synthesized in the complete absence of TF (lane 8, •). The amount of Ffh sufficient to reveal this interaction (0.1 μM) is the same as that required to see Ffh activity in vitro and furthermore compares fairly well with the estimated intracellular concentration of Ffh. Cross-linking of pOmtl-110 to Ffh was, however, completely overridden by 0.5 μM TF (lane 10).

Similarly, the SRP binder pOmtl-169 was also able to cross-link to TF, if this was the only ligand exogenously added during synthesis (Fig. 4, lower panel, compare lanes 2 and 4, asterisk). In this case, however, the number of TF adducts increased if the concentration of TF was raised 10-fold (lanes 4 and 6). Again in accordance with the results shown in Fig. 3, the Ffh cross-links of pOmtl-169 (Fig. 4, lane 8, •) largely persisted in the presence of 0.5 μM TF (lane 10). Interestingly at the higher concentration of TF, contacts of pOmtl-169 to both ligands were obtained (lane 12, asterisk and •). Hence the results presented in Fig. 4 clearly indicate that both nascent chains, pOmtl-110 and pOmtl-169, in principle can interact with each ligand but that there is an obvious, concentration-dependent competition between TF and SRP for their substrates.

**TF and Ffh Can Displace Each Other from a Nascent Chain**—The experiments presented thus far are consistent with the notion that a pOmtl nascent chain synthesized in the presence of the two ligands TF and SRP recruits the one for which it has exposed high affinity binding sites. These are for SRP the signal anchor-type TM3, nota bene not the stop-transfer-type TM2, and for TF obviously areas around TM2 and the subsequent loop of MtlA (cf. Fig. 1A). If in fact downstream domains of the nascent chain ultimately recruit the ligands of a signal sequence, it should be possible to displace a weak ligand by a post-translational addition of a strong one. This has been addressed by the experiments shown in Fig. 5. First we confirmed that pOmtl-110 and pOmtl-169 chains still cross-linked to their favored ligands even if those were absent during synthesis and only post-translationally added (lanes 1–4 and 15–18). As already shown in Fig. 4, synthesis of pOmtl-110 in the mere presence of Ffh led to the smaller Ffh adduct (Fig. 5, lane 6, •). Remarkably, post-translational incubation of these Ffh-pOmtl-110 complexes with 0.5 μM TF completely displaced the co-translationally bound Ffh resulting in a sole TF adduct (lane 8, asterisk). As pointed out above, displacement is again meant to indicate removal from nascent pOmtl but not necessarily from the ribosome. If this displacement of Ffh by TF from pOmtl-110 was the result of a higher overall affinity of this nascent chain for TF, allowing for more binding of Ffh during synthesis by raising its concentration 10-fold (compare lanes 6 and 10) should partially resist the displacement by TF. In fact, in these conditions pOmtl-110 was found cross-linked to both Ffh and TF (lanes 12–14). In much the same manner, TF, the disfavored signal sequence ligand of pOmtl-169, when bound co-translationally (lane 20) was virtually completely displaced by a post-translational addition of Ffh (lanes 22–24). If
these findings obtained in vitro using the model protein pOmtl can be extrapolated to the in vivo situation, our results collectively indicate that a bacterial nascent chain possessing an N-terminal signal sequence alternates between contacts to SRP and TF depending on the downstream exposure of high affinity binding sites for either ligand.

**DISCUSSION**

We have performed a comprehensive analysis of the molecular environment of a bacterial signal sequence when it emerges on the ribosome during ongoing synthesis. The signal sequence of pOmpA, whose overall hydrophobicity falls short of those of classical SRP substrates (8), nevertheless interacts with Ffh when nascent chains are so short that they expose little more than the signal sequence from the ribosome (Figs. 2 and 3 and Ref. 13). Upon emergence of TF binding sites in longer nascent chains, TF becomes the predominant cross-linking partner of the pOmpA signal sequence. This is exemplified by pOmtl-110 in Fig. 6, A and C. Only when pOmtl-110 is synthesized in the complete absence of TF does Ffh remain a cross-linking partner. If Ffh and TF are concomitantly present during synthesis, potential cross-links of pOmtl-110 with Ffh are overcome by those with TF. The same result is obtained when TF is added to preformed Ffh-pOmtl-110 complexes, indicating that TF can in fact displace Ffh from the signal sequence upon growth of the nascent chain. Displacement could partially be prevented by raising the concentration of Ffh 10-fold during the initial binding period. These results are consistent with a competition of TF and SRP for their binding sites on an emerging nascent chain.

When the nascent chain of pOmtl has grown to a size of 169 amino acids (Fig. 6, B and D), it recruits again Ffh with a clear competitive advantage over TF. This is inferred from the predominant cross-linking of pOmtl-169 to Ffh when TF and SRP were simultaneously present during synthesis. The prevalence of Ffh cross-links could only be reduced by performing synthesis with 10 times more TF. The preferential binding of pOmtl-169 to Ffh is most clearly demonstrated by the fact that TF is displaced from pOmtl-169 by a post-translational incubation with Ffh.

A positive correlation between the extent of cross-linking and the binding affinity of both ligands for a given nascent chain is indicated by the mutual displacement of the ligands and the influence that the concentration of the ligands exerts on the cross-linking intensities. The validity of this cross-linking approach is underscored by the fact that, where comparable, the employment of the site-specific photoprobe and of the more global chemical cross-linker gave identical results (compare Fig. 3 with Figs. 4 and 5). Moreover, the carbene generated by UV irradiation from the diazirine group of Tmd-Phe covalently links only to ligands that come closer than 2 Å, which is unlikely to frequently happen by casual collisions with non-functional binding partners.

To evaluate the dependence of the cross-linking intensity on the
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ligand concentration, we have varied the ratio of the effective concentrations of TF and SRP in our in vitro conditions. The reason is that for both purified ligands it is difficult to estimate the fraction of active material that seems particularly relevant for the oxidation-sensitive Ffh (39). Furthermore, the functionally meaningful concentration of TF is probably that of the ribosome-bound chaperone, which again is difficult to determine accurately. Following estimations of intracellular concentration, probably every ribosome has at least one molecule of TF bound, whereas the level of Ffh would only be sufficient to saturate 1–10% of the ribosomes. If this assumption is correct, a simultaneous binding of Ffh and TF (23, 24) would be limited to only a fraction of ribosomes. In any event, our results addressing only the interactions of Ffh and TF with the nascent polypeptide do not exclude such a situation (cf. Fig. 6, C and D).

The model drawn in Fig. 6C suggests that TF, when recruited to the exposed nascent pOmtl-110 chain, sequesters the signal sequence from a direct association with Ffh. The possibility of an auxiliary function of TF in influencing the accessibility of the signal sequence of a secretory protein to SRP has previously been discussed (6, 13). While this has remained a point at issue (11), the finding of a loss of Ffh interaction upon growth of a nascent secretory protein strongly suggests that Ffh cross-links, when documented for short presecretory proteins, do not necessarily prove that the protein is a functional SRP substrate (13, 14, 40).

The cross-linking results presented here are consistent with a model in which high affinity binding sites for TF and Ffh, when emerging on the ribosome, determine the liganding of the N-terminal signal sequence. In contrast to the signal anchor-type TM3 of MtlA, the stop-transfer TM2 did not prove to be such a high affinity binding site for Ffh. One major difference between both topogenic sequences is the restriction of positively charged amino acid residues to either flanking end (41). Thus while positive charges usually precede a signal anchor sequence, they are based on the findings that the pOmpA signal sequence.

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Alternate Liganding of a Nascent Signal Sequence