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Review

Structure and function of the molecular chaperone Trigger Factor

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ABSTRACT

Newly synthesized proteins often require the assistance of molecular chaperones to efficiently fold into functional three-dimensional structures. At first, ribosome-associated chaperones guide the initial folding steps and protect growing polypeptide chains from misfolding and aggregation. After that folding into the native structure may occur spontaneously or require support by additional chaperones which do not bind to the ribosome such as DnaK and GroEL. Here we review the current knowledge on the best-characterized ribosome-associated chaperone at present, the *Escherichia coli* Trigger Factor. We describe recent progress on structural and dynamic aspects of Trigger Factor's interactions with the ribosome and substrates and discuss how these interactions affect co-translational protein folding. In addition, we discuss the newly proposed ribosome-independent function of Trigger Factor as assembly factor of multi-subunit protein complexes. Finally, we cover the functional cooperation between Trigger Factor, DnaK and GroEL in folding of cytosolic proteins and the interplay between Trigger Factor and other ribosome-associated factors acting in enzymatic processing and translocation of nascent polypeptide chains.

1. Protein biosynthesis and de novo folding

A vital principle in all living cells is the conversion of genetic information into functional proteins. It involves biosynthesis of linear polypeptide chains by ribosomes, and, for the majority of proteins, folding of the linear chain into a specific three-dimensional conformation, the native protein structure. How the thousands of different proteins fold within cells is still one of the most fascinating research areas of today.

Over the last 50 years, numerous in vitro studies have established basic principles of protein folding. Early on it was shown that the information for the native fold is encoded in the linear amino acid sequence of each protein [1]; and it became clear that protein folding cannot resemble a random search of all possible conformations, which would require an immense amount of time. In fact, protein folding proceeds along certain pathways within funnel-shaped energy landscapes from energetically higher unfolded states via folding transitions and energetically favored, partially structured intermediates to the thermodynamically most stable conformations [2,3]. One major driving force of protein folding is the burial of hydrophobic side chains from the aqueous, hydrophilic environment [4]. Accordingly, native conformations of many soluble proteins are defined by a hydrophobic core and a largely hydrophilic surface.

Depending on the particular protein and the folding environment, folding can occur spontaneously and unassisted with kinetics in the range of milliseconds to seconds [5]. However, in particular in the crowded environment of a cell with protein concentrations of ~300–400 mg/ml [6], spontaneous folding is frequently error-prone, inefficient and time-consuming. The persistence of folding intermediates and misfolded proteins, which expose "sticky" hydrophobic regions and unstructured chain segments, drives unproductive intermolecular interactions leading to protein aggregation [7]. This challenges cells not only with the loss of protein function, but can also lead to the accumulation of toxic protein species as evident in Alzheimer's, Parkinson's or Huntington's disease [8]. Protein folding within cells therefore depends on the action of many protein folding-helpers, the molecular chaperones (see Section 2).

Folding of newly synthesized proteins is coupled to translation. Nascent polypeptide chains, which are generated in a vectorial manner from the N- to the C-terminus, commence the folding process before synthesis is terminated and the folding information is complete. They leave the ribosome through a rather narrow exit tunnel [9–11], which accommodates about 30 residues in an extended or about 60 residues in an α-helical conformation [12–15,144]. Nascent chains may already build minimalist tertiary structure within the broader, funnel-shaped parts of the tunnel close to the ribosomal exit site [16]. Outside of the ribosome, they can form native-like structural elements and even completely folded domains while still connected to the peptidyl transferase center [17–25,145].

Co-translational folding can improve the folding efficiency of newly synthesized proteins, but whether it occurs productively depends on the synthesized protein itself, the speed of translation

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and on chaperone action. For instance, reduced elongation rates during translation of rare codons were reported to improve the folding efficiency of several newly synthesized proteins [26–30]; and a co-translational, domain-wise folding of certain multi-domain proteins was shown for eukaryotes, but not for prokaryotes, which have a higher translation speed and a different chaperone machinery [19,31,32]. In fact, reduced polypeptide elongation rates have recently been demonstrated to improve the folding efficiencies of diverse eukaryotic proteins in bacteria [146]. When C-terminal sequences are required for the formation of stable structures, productive folding must occur essentially post-translationally, after the newly synthesized polypeptide has been released from the ribosome [33,34]. Moreover, premature co-translational folding events can even be detrimental for the productive folding process especially in the case of proteins that build long-range interactions in the native state. Hence, nascent polypeptide chains are exposed in partially folded, aggregation-prone states during synthesis and often require co-translational folding-assistance to reach the native fold efficiently.

2. Molecular chaperones assist protein folding in vivo

A multitude of molecular chaperones works in protein quality control and supports protein folding in all living organisms. Chaperones act in various cellular processes: they assist de novo folding, refolding of stress-denatured or aggregated proteins, assembly of oligomeric proteins, protein transport, proteolytic degradation, and, in some cases, control the activity of folded client proteins. Depending on their respective functions, some chaperones are constitutively expressed, whereas others are stress-induced, e.g. at higher temperatures or other conditions perturbing protein homeostasis (hence the generic term “heat shock proteins” (Hsps) for many chaperones).

Chaperones can be defined as proteins which monitor non-native conformations, stabilize proteins and assist folding processes, but are not part of the final native structures [35]. They optimize the folding efficiency or even facilitate folding of non-native intermediates that would otherwise be kinetically trapped, but they do not add structural information to the folding process.

A typical feature of chaperones is the stoichiometric and transient binding of non-native polypeptides mostly at exposed hydrophobic patches. Chaperone binding stabilizes productive folding intermediates, hinders non-native proteins from building incorrect intra- and intermolecular interactions and in this way reduces protein misfolding and aggregation. In some instances, chaperone binding additionally triggers transient local unfolding [36,37]. Chaperones may act as “holdases” stabilizing non-native protein conformations, as “foldases” assisting folding to the native state or as “unfoldases” unfolding misfolded protein species or extracting proteins from aggregates [38–41]. While substrate holding can be energy-independent, active assistance of productive folding (e.g. by Hsp70 or Hsp60 chaperone systems) often requires cycles of ATP-regulated binding and release.

Two different groups of chaperones support folding of newly synthesized polypeptides in all three domains of life (see also the reviews by J. Frydman, A. Horwich and S. Rospert in this issue) [42,43]: at first, ribosome-associated chaperones co-translationally interact with growing polypeptide chains and guide the initial steps of de novo folding. After that, downstream chaperones, which do not bind to ribosomes, may further assist de novo folding both during and after translation. Here, we review the current knowledge on the best-characterized ribosome-associated chaperone so far: *Escherichia coli* Trigger Factor.

3. *E. coli* Trigger Factor acts as a ribosome-associated chaperone

Trigger Factor (TF) represents the only ribosome-associated chaperone known in bacteria. It is found in bacteria and chloroplasts, whereas structurally different ribosome-associated factors exist in the archaeal and eukaryotic cytosol [44,45]. Despite of its absence in the eukaryotic cytosol, TF shares a certain functional similarity with eukaryotic ribosome-associated chaperones. When expressed in *S. cerevisiae*, *E. coli* TF binds to yeast ribosomes and partially complements the knockout phenotype of the yeast ribosomal chaperone triad consisting of Ssb, Ssz and Zuotin [46].

E. coli TF is a constitutively expressed and abundant cytosolic protein that exists in a two- to three-fold molar excess relative to ribosomes (~50 μM TF versus ~20 μM ribosomes) [47]. It transiently associates with ribosomes in a 1:1 stoichiometry using ribosomal protein L23 as major docking site [48–52]. In this way, TF is localized directly at the ribosomal exit site where growing polypeptide chains leave the ribosome and enter the crowded cytosol. TF potentially interacts with most polypeptides early during ongoing synthesis and, as the first chaperone associating with nascent chains, restricts the access of downstream factors such as the DnaK and GroEL chaperone machineries which cooperate with TF in de novo folding of cytosolic proteins (Fig. 1A) (see Section 10) [53–59].

Early on, the importance of TF's ribosome binding was addressed by mutating the ribosomal docking site of TF. Point mutations in L23 not only restricted ribosome binding of TF, but also severely impaired TF's abilities to associate with nascent chains and to assist protein folding in vivo [49]. Hence, ribosome binding was assumed to be crucial for the functionality of the TF in assisting de novo folding and led the field to concentrate on the ribosome-associated function of TF. Later on, TF mutants that are partly or completely deficient in ribosome binding were analyzed in more detail. Though they were less efficient than wild type TF (especially at temperatures above ~36 °C), they still maintained chaperone activity in counteracting protein aggregation in vivo [60–62]. This suggests that although ribosome binding is required for efficient TF action, TF may also act independently of its ribosome association and perhaps exert a chaperone function independent of its role in assisting initial de novo folding (see Section 4).

4. Trigger Factor might fulfill a second function as protein assembly factor

Just recently, Martinez-Hackert and Hendrickson have assigned a ribosome-independent function to TF [62] (Fig. 2). By means of a mass spectrometry analysis they identified 68 different full-length proteins from *E. coli* lysates which copurify and stably associate with TF. Copurified proteins, among them ribosomal protein S7, ranged in size from ~8 to ~120 kDa and were mostly components of protein complexes such as the ribosome or protein homo- or hetero-oligomers. The authors showed that purified *Thermotoga maritima* TF (*tm*TF) and *tm*S7 formed 1:1 and 2:2 complexes in vitro and determined the crystal structure of a 2*tm*TF–2*tm*S7 complex. Intriguingly, *tm*S7 adopts a native-like conformation in this complex, and TF binds with relatively low contact specificity large parts of the interface that S7 uses for ribosome incorporation. Based on these findings, the authors proposed that TF not only associates with nascent polypeptide chains, but also with full-length proteins in order to stabilize native-like substrates until they are assembled into protein complexes. In support of such an assembly function of TF they detected a mild ribosomal assembly defect under heat stress conditions in cells lacking Trigger Factor, which resembles the ribosomal assembly defects reported earlier for cells lacking functional DnaK [63,64]. The proposed assembly function of TF is in line with the concept that cytosolic chaperones facilitate ribosomal biogenesis [65] and, what has been neglected over a long time, formation of protein complexes [66,67]. The molar excess of TF relative to ribosomes may allow TF to indeed fulfill a dual function in the *E. coli* cytosol and to assist both co-translational protein folding and post-translational protein assembly, but this awaits further verification.