Erschienen in: Science ; 344 (2014), 6184. - S. 590-591
https://dx.doi.org/10.1126/science.1254064

Trigger Factor Flexibility

A nuclear magnetic resonance study shows how the bacterial chaperone trigger factor can dynamically bind and release many different substrates.

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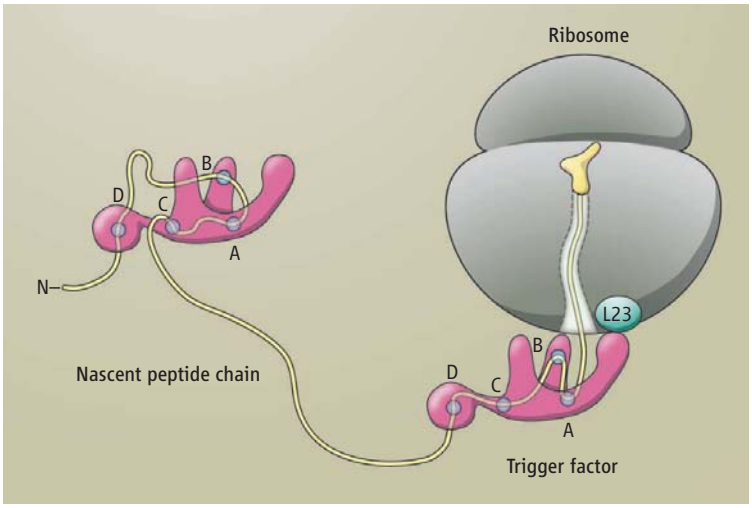
Molecular chaperones are found in all cells and are essential for maintaining a functional proteome. The main function of chaperones is to promote correct protein folding by protecting nonnative proteins from folding along pathways that lead to protein misfolding and aggregation. To fulfill this task, chaperones must recognize a nonnative protein, transiently bind to it, and then release it at precisely the right time to allow the substrate to proceed with its folding course. Many but not all chaperones use adenosine 5′ triphosphate (ATP) to control the dynamic substrate binding and release cycle (*1*). On page 597 of this issue, Saio *et al*. (*2*) unravel the structural basis and underlying mechanism of action of the ATP independent chaperone trigger factor (TF).



Plasticity in substrate binding. TF contacts the ribosome via ribosomal protein L23 and interacts with the nascent polypeptide that exits the ribosomal tunnel (dashed lines). As synthesis proceeds, TF may depart from the ribosome, remaining bound to the nascent polypeptide. A second TF molecule can then dock to the ribosome and interact with the same nascent chain. Saio *et al*. now show that TF interacts with the unfolded polypeptides through four different hydrophobic binding sites (blue, A to D) in its inner surface. The four binding sites show a high flexibility in their local architecture that allows binding to a large and diverse pool of substrates. Also, the order of engagement of these four sites may vary, as indicated in the two TF molecules.

TF is an abundant bacterial chaperone that associates transiently with ribosomes and binds to nascent polypeptide chains. It has an extended, dragon-like structure with a central body, two protruding arms, a head, and a tail region. Bound to the ribosome as a monomer by its amino terminal tail, TF leans over the ribosomal exit tunnel, thereby exposing its large interior surface speckled with multiple hydrophobic patches to the exiting nascent peptide chain (*3*) (see the figure). Eukaryotes use structurally different ribosome-associated chaperones, but bacterial TF is by far the best studied one and is regarded as a paradigm for ribosome-bound chaperones that support the folding of newly synthesized proteins (*4*).

The first evidence that TF acts as a chaperone came from experiments showing that combined loss of TF and another chaperone led to synergistic defects in protein folding, resulting in global protein aggregation and decreased viability (*5*, *6*). Ribosome-bound TF interacts with most newly synthesized polypeptides (*7*) and prevents their premature folding, thereby laying the groundwork for subsequent productive folding that involves additional chaperones (*8*). Recent data suggest that TF is much more versatile than initially thought. It can reshape and improve the folding pathway of a protein by protecting partially folded intermediates (*9*). Even more intriguing, TF can reverse premature folding by facilitating unfolding of preformed structures in nascent polypeptides, allowing the nascent peptide to reenter the productive folding path (*10*).

Despite comprehensive analyses of TF over many years, the molecular details of its versatile chaperone activity were not understood. Using sophisticated nuclear magnetic resonance techniques, Saio *et al*. have now determined the structure and dynamics of purified TF as it interacts with unfolded model substrates in solution, thereby disclosing its mechanism of action in great detail.

The authors show that TF forms a binding scaffold with four distinct substrate binding sites that are distributed along its inner surface (see the figure). The four binding sites can be used in a variable order, and not all sites are always occupied by substrates. The binding sites contain nonpolar residues forming hydrophobic pockets that bind to hydrophobic peptide stretches of 6 to 10 residues in substrate proteins. The binding sites have a flexible local architecture that allows interaction with a large and diverse population of peptide stretches with unrelated primary sequences. Furthermore, polar residues next to the hydrophobic binding sites can form hydrogen bonds with the substrate, probably to enhance affinity and navigate binding. This high degree of plasticity of its binding surfaces explains how TF can serve a large pool of nascent substrates.

How does TF affect folding of substrates using this highly flexible binding scaffold? Using all of its four binding sites, TF can directly bind up to 50 substrate residues. The hydrophobic peptide stretches bound by TF are separated by linker regions that remain unbound and may even loop outward (see the figure). Moreover, supporting earlier findings (*11*), Saio *et al*. show that multiple TF molecules can bind simultaneously to a substrate. This multistage chaperone mechanism enables TF to retain large polypeptides in an unfolded state and protect them from aggregation by shielding their exposed hydrophobic regions. The multivalent binding of TF to its substrate may even provide enough binding energy to unfold misfolded peptide structures of low thermodynamic stability.

TF binds with low affinity to individual substrate binding sites, but the affinity gradually increases with the number of bound segments. Substrate binding to the binding sites is highly dynamic and the residence time is short (1 ms at 25°C). Even when TF fully engages its unfolded protein substrate by using all of its available binding sites, the complex remains dynamic, with fast association and dissociation rates. This

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highly dynamic substrate engagement is very important during protein synthesis, allowing TF to accommodate the permanently changing composition of binding sites in the nascent polypeptide that grows by up to 20 amino acid residues per second.

Saio *et al*.'s study provides a milestone in understanding how TF performs its dynamic and multifaceted functions in the cell. The work will pave the way for further exciting advances in understanding the plasticity of this and other ATP independent chaperones. However, several important questions remain to be answered about the activity of the TF chaperone. For example, Saio *et al*. investigated purified TF, which was not in complex with the ribosome, and it thus remains to be shown whether the binding sites of ribosome tethered TF are similar. Three binding sites are located in the central body and one in the head domain, but no binding site is found in the amino terminal tail that tethers TF to the ribosome (see the figure). TF undergoes some structural changes upon binding to ribosomes (*11*) and may thus expose other or additional binding sites.

Another open question is how the dynamic association of one or perhaps more TF molecules with nascent polypeptides is coordinated with the activity of other chaperones that bind to nascent proteins in later stages of protein synthesis (*3*). And finally, TF that is not bound to ribosomes can form dimers that can encapsulate small native like proteins using primarily polar contacts. This TF activity may promote the assembly of substrates into large complexes (*12*). Further analyses are required to understand how TF can switch between hydrophobic and hydrophilic binding modes for substrates.

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