FEBS Letters 389 (1996) 32–34 FEBS 17191

Minireview

The *Escherichia coli* trigger factor

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Received 13 May 1996

Abstract ***E. coli*** **trigger factor is an abundant cytosolic protein originally identified by its ability to maintain the precursor of a secretory protein in a translocation competent form. Recent studies shed new light on the function of this protein. Trigger factor was found to be a peptidyl-prolyl-*cis/trans*-isomerase capable of catalysing protein folding in vitro, to associate with nascent cytosolic and secretory polypeptide chains, and to cooperate with the GroEL chaperone in promoting proteolysis of an unstable polypeptide in vivo. These findings suggest roles for trigger factor in various folding processes of secretory as well as cytosolic proteins.**

Key words: Ribosome; Prolyl isomerase; Chaperone; Protein folding; Translocation; Proteolysis

1. Discovery

Trigger factor was discovered in a biochemical screen for cytosolic components of the secretion machinery of *E. coli*. Analysing the translocation of the outer membrane protein A (OmpA) across inner membrane vesicles in vitro, Wickner and coworkers identified an activity that stabilized the precursor (proOmpA) in a loosely folded conformation thereby stimulating its membrane translocation. This activity was shown to reside in a monomeric protein with an apparent molecular weight of 60 kDa, termed trigger factor [1–3]. Trigger factor formed stable 1:1 complexes with chemically denatured proOmpA that was diluted from denaturant. When proOmpA was allowed to fold in the absence of trigger factor, however, translocation of proOmpA could not be restored, indicating that trigger factor could not actively unfold the substrate [2].

2. Genetic analysis

The *tig* gene encoding trigger factor was cloned [4] and its expression found to be growth phase controlled and thus co-regulated with genes encoding ribosomal components (F. Neidhardt, personal communication cited in [4]). Genetic analysis of the in vivo function of trigger factor using a conditional depletion strain failed to provide new insights. At conditions depleting trigger factor to less than 5% of the normal levels, cells remain fully viable but form filaments indicative of cell division defects. No defects in the translocation of proOmpA, even in a trigger factor depletion strain with an additional deficiency for the secretion specific SecB chaperone, were observed [4]. These results suggest that trigger factor is dispensable for growth of *E. coli*, although analysis of a *tig* knockout mutant is required for a proof. It is not excluded either that trigger factor does play an important role in metabolism but that backup systems exist replacing missing trigger factor functions under depletion conditions. An important physiological role of trigger factor is indicated by the finding that *tig* homologs exist in other bacterial genomes [5–7] including that of *Mycoplasma genitalium* which is believed to contain the minimum set of genes required for life [7].

3. Trigger factor associates with nascent polypeptide chains and has prolyl isomerase activity

Work in three laboratories, aimed at investigating different processes related to protein folding in vivo, converged in the discovery of novel features of trigger factor that changed our view of its biological function. A search by Fischer and coworkers for a ribosome-bound peptidyl-prolyl-*cis/trans*-isomerase (PPIase) activity led to the identification of trigger factor [8]. In an in vitro refolding assay using a RNaseT1 mutant protein as substrate trigger factor accelerated the rate-limiting prolyl isomerisation step more efficiently than any other known PPIase. Using oligopeptide substrates the specificity of trigger factor was determined to resemble that of immunophilins of the FK506 binding protein (FKBP) family. However, the failure of the FK506 immunosuppressant to inhibit the PPIase activity of trigger factor led these authors to propose that trigger factor constitutes a novel family of PPIases [8].

Luirink and coworkers used an in vitro translation system to investigate the interactions of the *E. coli* signal recognition particle (SRP) with a variety of nascent secretory polypeptide chains by crosslinking [9]. In addition to crosslinks of nascent polypeptide chains with the P48 protein component of SRP, they observed prominent crosslinked products originating from interaction of trigger factor with nascent chains. P48 and trigger factor competed for crosslinking to prePhoE suggesting overlapping substrate specificities of both proteins. In contrast to P48, however, trigger factor was also efficiently crosslinked to non-secretory nascent chains [9].

Searching for *E. coli* proteins associated with nascent polypeptide chains of β-galactosidase in an in vitro transcription–translation system, Bukau and coworkers observed a salt-resistant association of trigger factor with translating ribosomes [10]. This association was sensitive to puromycin treatment and thus dependent on the presence of nascent β-galactosidase. A physical association of trigger factor with nascent chains of preprolactin and a non-secretory preprolactin mutant was demonstrated by crosslinking. For the non-secretory substrate, trigger factor was the single major crosslinking component of the *E. coli* cytosol. These authors noted a homology of trigger factor with PPIases of the FKBP family

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PII S0014-5793(96)00582-0

and demonstrated PPIase activity for purified trigger factor. Together, a role for trigger factor as a co-translationally acting folding catalyst was proposed [10].

4. Trigger factor binds to ribosomes

The ability of trigger factor to associate with nascent polypeptide chains raises the question of the mechanism of its targeting to the substrate. Crosslinking of trigger factor with nascent chains was abolished after their puromycin-mediated release from the ribosome [9,10]. Thus, either misfolding of the puromycyl fragments or loss of the ribosomal environment prevented substrate association with trigger factor under these conditions. Trigger factor has indeed been shown to bind to the isolated large ribosomal subunit [11]. This subunit contains the exit site for nascent polypeptide chains and it is tempting to speculate that trigger factor binding to the ribosome in the vicinity of the nascent chain exit site allows its efficient association with the substrate.

It is important to note that the association of trigger factor with non-translating ribosomes is salt-sensitive, in contrast to its salt-resistant association with translating ribosomes in vitro [10]. There are different possible explanations for this finding. First, it is possible that the association of trigger factor with nascent chains stabilizes the trigger factor–ribosome complex. The PPIase activity, however, is unlikely to contribute to this stabilisation since PPIases generally [12] and the PPIase domain of trigger factor (T.H. and B.B., unpublished) do not form stable complexes with their substrates. Alternatively, the substantial conformational changes in ribosomes leading to their tightly coupled state during translation might increase the affinity for trigger factor. In this respect the observed puromycin-mediated release of trigger factor from translating ribosomes [10] may result from conformational changes uncoupling the ribosome. Consistent with this interpretation is our observation that trigger factor disappeared at physiological salt concentrations from ribosomes, isolated from *E. coli* cultures as run-off ribosomes, when the ribosomal subunits were uncoupled by magnesium chelation. In contrast, in the presence of magnesium this complex was resistant to treatment with 1 M potassium chloride (T.H. and B.B., unpublished). Taken together, it is conceivable that the coupling status of ribosomes during the translation process influences the binding of trigger factor.

5. Structure–function relationship of trigger factor's PPIase activity

Hesterkamp et al. and Callebaut and Mornon reported on a homology of trigger factor with PPIases of the FKBP family [10,13]. The region of homology is located within the central part of the trigger factor polypeptide chain, between residues 165 and 240, and is restricted to residues forming the substrate binding pocket in human FKBP12 [14–16]. The PPIase domain of trigger factor exhibits a higher degree of conservation in *Escherichia coli*, *Campylobacter jejuni*, *Haemophilus influenzae* and *Mycoplasma genitalium* than full-length trigger factor, indicating the functional importance of this domain [17].

These homology predictions were recently confirmed by functional analyses of trigger factor fragments obtained by limited proteolysis with endoproteinase Glu-C (V8) [17] and subtilisin [18]. Proteolytic fragments of 12.8 kDa (Val_{132} to Glu_{247}) and 11.8 kDa (Arg_{145} to Glu_{251}), respectively, displayed a specific PPIase activity similar to that of full-length trigger factor. An even smaller recombinant fragment comprising only 102 amino acids (Glu_{148} to Thr_{249}) also displayed full PPIase activity [18]. The size of this PPIase domain is in good agreement with the size of the prototype FKBP12. A number of non-conservative amino acid exchanges between the trigger factor FKBP-domain and FKBP12 may account for the observed resistance of the PPIase activity of trigger factor to the immunosuppressive drugs FK506 and rapamycin [8].

Assuming a modular structure of trigger factor, the central PPIase domain would be flanked by two almost equally sized N- and C-terminal domains. While a proteolytic fragment of 14 kDa apparent molecular weight starting with the authentic N-terminus of trigger factor has been reported [18], the polypeptide chain C-terminal to the PPIase domain is highly susceptible to proteolysis [17,18]. The functional roles of these domains remain to be elucidated.

6. Trigger factor interacts with GroEL in the degradation of abnormal proteins

A different function for trigger factor has been proposed by work from the Goldberg laboratory aimed at understanding the role of chaperones in protein degradation. The fusion protein CRAG, composed of protein segments of λ cro, protein A and β-galactosidase, is degraded in vivo by the ClpP protease in a process stimulated by the GroEL chaperonin [19]. Using conditional trigger factor depletion or overproduction strains it has now been shown that trigger factor also accelerates the degradation of CRAG. Furthermore, a physical interaction of GroEL and trigger factor with this substrate has been demonstrated on a CRAG affinity column [20]. Addition of ATP to elute GroEL from the affinity column resulted in co-elution of trigger factor and both proteins remained associated in a subsequent gel filtration step. This finding was interpreted to indicate that pre-existing GroEL–trigger factor complexes bound to CRAG [20]. Even in the absence of the artificial substrate, GroEL and trigger factor co-purified from *E. coli* lysates [20]. These observations raise the possibility that trigger factor plays a role in targeting substrates to the GroEL chaperonin. This might be required for the efficient folding to the native state of a subset of newly synthesized proteins as well as for the assisted folding or degradation of misfolded proteins.

7. A chaperone function of trigger factor?

The original observation that trigger factor forms stable 1:1 complexes with unfolded proOmpA is reminiscent of a chaperone-like activity. This is further supported by the finding that the activity of trigger factor in preventing misfolding of the substrate in vitro can be replaced by the activities of the SecB and GroEL chaperones [21]. It is also remarkable in this context that trigger factor outscores by far all other known PPIases in the efficiency of catalysis of the isomerisation of the single *cis*-proline residue during RNaseT1 refolding [8]. Trigger factor had lower activity in catalysing the isomerisation of a *cis*-proline residue in a tetrapeptide of similar local sequence [8]. It is thus conceivable that trigger factor recognizes and modulates the polypeptide chain surrounding the

critical proline residue, eventually as part of a chaperone activity, to increase the efficiency of prolyl isomerisation catalysis. This hypothesis is further supported by the intriguing finding that trigger factor-mediated refolding of RNaseT1 can be competitively inhibited by addition of the chaperone substrate reduced carboxymethylated lactalbumin (F.X. Schmid, personal communication). Differences, however, exist between trigger factor and the well known DnaK and GroEL chaperone systems in that trigger factor fails to prevent the aggregation of refolding firefly luciferase (T.H. and B.B., unpublished).

Although quite speculative at this point, the idea of trigger factor being a ribosome-associated folding factor which accelerates prolyl isomerisation and stabilizes the folding chain until it is completed or handed over to other chaperones is highly attractive and will trigger future experiments.

Acknowledgements: Work in the laboratory was supported by grants from the Forschungsschwerpunkt des Landes Baden-Württemberg, the DFG, and the Fonds der Chemischen Industrie to B.B. and a fellowship from the Boehringer Ingelheim Fonds to T.H.

References

[1] Crooke, E. and Wickner, W. (1987) Proc. Natl. Acad. Sci. USA 84, 5216–5220.
[2] Crooke, E., Guthrie, B., Lecker, S., Lill, R. and Wickner, W. (1988) Cell 54, 1003–1011.
[3] Crooke, E., Brundage, L., Rice, M. and Wickner, W. (1988) EMBO J. 7, 1831–1835.
[4] Guthrie, B. and Wickner, W. (1990) J. Bacteriol. 172, 5555–5562.
[5] Griffiths, P.L., Park, R.W.A. and Connerton, I.F. (1995) Microbiology 141, 1359–1367.
[6] Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F. et al. (1995) Science 269, 496–512.
[7] Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A. et al. (1995) Science 270, 397–403.
[8] Stoller, G., Rücknagel, K.P., Nierhaus, K.H., Schmid, F.X., Fischer, G. and Rahfeld, J.-U. (1995) EMBO J. 14, 4939–4948.
[9] Valent, Q.A., Kendall, D.A., High, S., Kusters, R., Oudega, B. and Luirink, J. (1995) EMBO J. 14, 5494–5505.
[10] Hesterkamp, T., Hauser, S., Lütcke, H. and Bukau, B. (1996) Proc. Natl. Acad. Sci. USA 93, 4437–4441.
[11] Lill, R., Crooke, E., Guthrie, B. and Wickner, W. (1988) Cell 54, 1013–1018.
[12] Fischer, G. (1994) Angew. Chem. Int. Ed. Engl. 33, 1415–1436.
[13] Callebaut, I. and Mornon, J.-P. (1995) FEBS Lett. 374, 211–215.
[14] Michnik, S.W., Rosen, M.K., Wandless, T.J., Karplus, M. and Schreiber, S.L. (1991) Science 252, 836–839.
[15] Van Duyne, G.D., Standaert, R.F., Karplus, P.A., Schreiber, S.L. and Clardy, J. (1991) Science 252, 839–842.
[16] Moore, J.M., Peattie, D.A., Fitzgibbon, M.J. and Thomson, J.A. (1991) Nature 351, 248–250.
[17] Hesterkamp, T. and Bukau, B. (1996) FEBS Lett. 385, 67–71.
[18] Stoller, G., Tradler, T., Rücknagel, K.P., Rahfeld, J.-U. and Fischer, G. (1996) FEBS Lett. 384, 117–122.
[19] Kandror, O., Busconi, L., Sherman, M. and Goldberg, A.L. (1994) J. Biol. Chem. 269, 23575–23582.
[20] Kandror, O., Sherman, M., Rhode, M. and Goldberg, A.L. (1995) EMBO J. 14, 6021–6027.
[21] Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P.J., Kumamoto, C.A. and Wickner, W. (1989) EMBO J. 8, 2703–2709.