14. Rotin, D. WW (WWP) domains: from structure to function. *Curr. Top. Microbiol. Immunol.* **228**, 115–133 (1998).
15. Fainsod, A., Steinbeisser, H. & De Robertis, E. M. On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. *EMBO J.* **13**, 5015–5025 (1994).
16. Hemmati-Brivanlou, A. & Thomsen, G. H. Ventral mesodermal patterning in *Xenopus* embryos: expression patterns and activities of BMP-2 and BMP-4. *Dev. Genet.* **17**, 78–89 (1995).
17. Nishimatsu, S. & Thomsen, G. H. Ventral mesoderm induction and patterning by BMP heterodimers in *Xenopus* embryos. *Mech. Dev.* **74**, 75–88 (1997).
18. Thomsen, G. H. *Xenopus* mothers against decapentaplegic is an embryonic ventralizing agent that acts downstream of the BMP-2/4 receptor. *Development* **122**, 2359–2366 (1996).
19. Fenteany, G. *et al.* Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science* **268**, 726–731 (1995).
20. Treier, M., Staszewski, L. M. & Bohmann, D. Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. *Cell* **78**, 787–798 (1994).
21. Macias-Silva, M., Hoodless, P. A., Tang, S. J., Buchwald, M. & Wrana, J. L. Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. *J. Biol. Chem.* **273**, 25628–25636 (1998).
22. Hoodless, P. A. *et al.* Dominant-negative Smad2 mutants inhibit activin/Vg1 signaling and disrupt axis formation in *Xenopus*. *Dev. Biol.* **207**, 364–379 (1999).
23. Hemmati-Brivanlou, A. & Melton, D. Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell* **88**, 13–17 (1997).
24. Wilson, P. A., Lagna, G., Suzuki, A. & Hemmati-Brivanlou, A. Concentration-dependent patterning of the *Xenopus* ectoderm by BMP4 and its signal transducer Smad1. *Development* **124**, 3177–3184 (1997).
25. Suzuki, A., Chang, C., Yingling, J. M., Wang, X. F. & Hemmati-Brivanlou, A. Smad5 induces ventral fates in *Xenopus* embryos. *Dev. Biol.* **184**, 402–405 (1997).
26. Lagna, G., Hata, A., Hemmati-Brivanlou, A. & Massague, J. Partnership between DPC4 and SMAD proteins in TGF- β signalling pathways. *Nature* **383**, 832–836 (1996).
27. Candia, A. F. *et al.* Cellular interpretation of multiple TGF- β signals: intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* **124**, 4467–4480 (1997).
28. Eppert, K. *et al.* MADR2 maps to 18q21 and encodes a TGF β regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell* **86**, 543–552 (1996).
29. Ward, C. L., Omura, S. & Kopito, R. R. Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell* **83**, 121–127 (1995).
30. Nieuwkoop, P. D. & Faber, J. *Normal Table of Xenopus laevis (Daudin)* (North Holland, Amsterdam, 1967).

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Trigger factor and DnaK cooperate in folding of newly synthesized proteins

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The role of molecular chaperones in assisting the folding of newly synthesized proteins in the cytosol is poorly understood. In *Escherichia coli*, GroEL assists folding of only a minority of proteins¹ and the Hsp70 homologue DnaK is not essential for protein folding or cell viability at intermediate growth temperatures². The major protein associated with nascent polypeptides is ribosome-bound trigger factor^{3,4}, which displays chaperone and prolyl isomerase activities *in vitro*^{3,5,6}. Here we show that Δ tig::kan mutants lacking trigger factor have no defects in growth or protein folding. However, combined Δ tig::kan and Δ dnaK mutations cause synthetic lethality. Depletion of DnaK in the Δ tig::kan mutant results in massive aggregation of cytosolic proteins. In Δ tig::kan cells, an increased amount of newly synthesized proteins associated transiently with DnaK. These findings show *in vivo* activity for a ribosome-associated chaperone, trigger factor, in general protein folding, and functional cooperation of this protein with a cytosolic Hsp70. Trigger factor and DnaK cooperate to promote proper folding of a variety of *E. coli* proteins, but neither

is essential for folding and viability at intermediate growth temperatures.

Because trigger factor can associate with ribosomes and nascent polypeptide chains, it is a prime candidate for a chaperone that is dedicated to assist folding of newly synthesized proteins. To elucidate its *in vivo* role genetically, we replaced the complete coding sequence of the *tig* gene (encoding trigger factor) with a kanamycin cassette (Fig. 1a). The Δ tig::kan mutant lacking trigger factor was viable and showed no growth defects between 15 and 42 °C in rich (Fig. 1b) and minimal media (not shown). Furthermore, Δ tig::kan mutants showed no defects in protein folding at 30 and 37 °C, as judged by unaltered specific activities of a reporter enzyme (firefly luciferase) and solubility of cellular proteins. Trigger factor is thus not essential for viability and protein folding in *E. coli*.

We investigated whether other chaperones compensate for the missing activity of trigger factor in Δ tig::kan cells by determining the phenotypes resulting from combining the Δ tig::kan allele with additional chaperone-gene mutations. The Δ tig::kan allele could be transduced with normal efficiency into the chromosomes of Δ hspG::lacZ (ref. 7), *secB::Tn5* (ref. 8), Δ ibpAB::kan (unpublished results) and temperature-sensitive *groEL* mutants (L140, L673 and L44)⁹ without apparent alteration of the existing growth phenotypes. However, it could not be introduced into Δ dnaK52 mutant cells¹⁰, which lack DnaK and have low levels of the DnaJ co-chaperone. This finding was further substantiated by co-transduction experiments in which a *Tn10* selective marker (*zba-3054::Tn10*) placed close to the Δ tig::kan allele was transduced into Δ dnaK52 and *dnaK*⁺ cells. The co-transduction frequency of the Δ tig::kan allele was 83% in *dnaK*⁺ cells and 0% in Δ dnaK52 mutant cells. Combination of the Δ tig::kan and Δ dnaK52 mutations thus causes synthetic lethality.

To investigate the cause of the synthetic lethality, we constructed Δ tig::kan and *tig*⁺ strains in which expression of the chromosomal *dnaK dnaJ* operon is under the control of the IPTG-regulatable promoter *P*_{A1/lacO-1} (*P*_{IPTG}*dnaKJ*, Fig. 1a)¹¹. On plates containing 1 mM IPTG, the *P*_{IPTG}*dnaKJ* Δ tig::kan and *P*_{IPTG}*dnaKJ* *tig*⁺ strains formed colonies at all temperatures tested (15, 30, 37 and 42 °C). On plates lacking IPTG, the *P*_{IPTG}*dnaKJ* *tig*⁺ cells did not form colonies at 15 and 42 °C (Fig. 1b), consistent with the cold-sensitive and heat-sensitive phenotype of Δ dnaK52 mutants¹². In the absence of IPTG, *P*_{IPTG}*dnaKJ* Δ tig::kan cells did not form colonies at any temperature tested (Fig. 1b); growth of these mutants at 30 and 37 °C was restored by expression of the *tig* gene from plasmids (not shown). The onset of synthetic lethality could also be monitored in liquid medium (Fig. 1c). After overnight growth in medium with IPTG, *P*_{IPTG}*dnaKJ* Δ tig::kan and *P*_{IPTG}*dnaKJ* *tig*⁺ cells were diluted into medium lacking IPTG. DnaK and DnaJ levels decreased from about twice wild-type levels to undetectable amounts after 8 h growth without IPTG (time point 3, Fig. 1c). *P*_{IPTG}*dnaKJ* Δ tig::kan cells depleted for DnaK and DnaJ started to show slower growth after 8 h (time point 3). At 8 and 10 h (time points 3 and 4), viability (determined as plating efficiency) decreased by 3 and 5 orders of magnitude, respectively, below the *P*_{IPTG}*dnaKJ* Δ tig::kan control grown with IPTG.

To account for synthetic lethality, we first ruled out that it resulted from DnaK's regulatory role as a negative modulator of σ ³², the transcriptional activator of chaperone and protease genes of the heat-shock regulon¹³. Accordingly, overproduction of chaperones and proteases resulting from DnaK and DnaJ depletion may be poisonous for Δ tig::kan cells. We artificially induced the heat-shock response in Δ tig::kan cells using a plasmid expressing the *rpoH* gene (encoding σ ³²) under the control of an IPTG-inducible promoter¹⁴. IPTG-induced chaperone overproduction was at least as high as chaperone overproduction caused by DnaK and DnaJ depletion (monitored, for example, for GroEL, Fig. 1d) but did not affect the viability of Δ tig::kan cells at 30 and 37 °C (Fig. 1d, lower panel).

To investigate whether synthetic lethality is caused by protein-

folding defects, we first tested the *in vivo* folding efficiency of firefly luciferase. At 5 h of growth without IPTG (time point 2, Fig. 1c) at 30 °C, DnaK and DnaJ levels were about 6-times lower than wild-type levels. Growth, viability and protein synthesis were not yet affected (data not shown). At this time point, synthesis of plasmid-encoded luciferase was induced for 30 min. Luciferase activity was reduced by 60–70% in $P_{IPTG}dnaKJ \Delta tig::kan$ cells as compared to $P_{IPTG}dnaKJ tig^+$ cells, although the amounts of luciferase present in both strains were similar (Fig. 2a). This decrease in specific activity was accompanied by increased luciferase aggregation (Fig. 2b).

We then tested the folding efficiency of *E. coli* bulk proteins. After 5 h of growth without IPTG (time point 2), cells were pulse-labelled with ^{35}S -methionine for 15 s and analysed for protein aggregation. Aggregated proteins and some membrane proteins were recovered by centrifugation and separated by two-dimensional-gel electrophoresis, followed by staining of pre-existing proteins and autoradiographic detection of newly synthesized proteins (Fig. 3). There was a large increase in the number (~40 proteins) and total amount (2.5-fold) of pelleted pre-existing proteins in $P_{IPTG}dnaKJ \Delta tig::kan$ cells as compared to $P_{IPTG}dnaKJ tig^+$ cells (Fig. 3a) and cells of both strains grown in IPTG (not

shown). Most spots that increased in intensity correspond to cytosolic proteins and thus represent aggregated proteins (for example, MetE and RpoB showed 4- and 25-fold increased aggregation, respectively; Fig. 3a, right panel). The amounts of membrane proteins present in the pellet fraction remained similar in the two strains tested (for example, inner and outer membrane proteins AtpD and OmpA; Fig. 3a), indicating that protein-folding defects affected predominantly non-membrane proteins. The autoradiogram revealed quantitatively (2.5-fold overall increase) and qualitatively similar changes in the solubility of newly synthesized proteins (Fig. 3b). Exposure of the gel was extended to allow detection of minor spots, but this also increased background staining, including that by nascent polypeptide chains.

The extent to which proteins misfold in DnaK- and DnaJ-depleted $\Delta tig::kan$ cells may be larger than detected, because proteases may degrade misfolded proteins and other chaperones may partially prevent aggregation. Furthermore, folding problems probably become more severe at later time points after IPTG withdrawal, conditions that were difficult to investigate because of general defects in, for example, protein synthesis.

To determine the functional relationship between DnaK and

Figure 1 Synthetic lethality. **a**, Relevant genotypes of used strains. WT, wild type. **b**, Growth of wild-type (1), $P_{IPTG}dnaKJ \Delta tig::kan$ (2), $P_{IPTG}dnaKJ tig^+$ (3) and $\Delta tig::kan$ (4) cells on LB plates with or without IPTG at indicated temperatures for 24 or 96 h (15 °C). 10 μ l of diluted cultures were spotted. **c**, Growth of $P_{IPTG}dnaKJ tig^+$ and $P_{IPTG}dnaKJ \Delta tig::kan$ cells in LB liquid medium without IPTG at 37 °C. Top: at an OD₆₀₀ of 0.6, cells were diluted to maintain logarithmic growth. Arrows indicate time points of sample withdrawals. Similar growth behaviour was found for cells grown in M9 medium at 37 °C. Bottom: to detect DnaK and trigger factor levels during depletion, identical OD₆₀₀ equivalents were harvested at time points 1 to 4

(upper panel) and immunoblot analysis was performed using specific antisera. As controls, different OD₆₀₀ equivalents of wild-type cells were applied. **d**, Protein patterns of σ^{32} and *dnaK*, *dnaJ*-regulatable *tig*⁺ and $\Delta tig::kan$ cells. Top: Coomassie-stained SDS-PAGE of total lysates (identical OD₆₀₀ equivalents) of cultures grown in LB at 37 °C with IPTG or depleted from IPTG for 5 h. EF-Tu, for unknown reasons, is reduced in σ^{32} -overproducing cells. Bottom: growth of wild-type and $\Delta tig::kan$ cells with and without IPTG-induced σ^{32} overproduction on LB agar.

Figure 2 Specific activity and aggregation of firefly luciferase. **a**, *In vivo* activity and cellular levels of firefly luciferase in $P_{IPTG}dnaKJ\ tig^+$ (light-grey bar) and $P_{IPTG}dnaKJ\ \Delta tig::kan$ cells (dark-grey bar). DnaK- and DnaJ-depleted cells were induced for 30 min to produce luciferase and assayed for luciferase activity² (set 100% for $P_{IPTG}dnaKJ\ tig^+$ cells). Upper panel: identical OD₆₀₀ culture equivalents of the cells were subjected to immunoblot analysis with luciferase-specific antiserum. **b**, Solubility of luciferase. Lysates of cells treated as for **a** were prepared, divided into soluble and insoluble fractions by centrifugation and analysed by immunoblotting with luciferase-specific antiserum. Total lysate (T), supernatant (S) and aggregated material recovered in the pellet (P; double amount loaded).

trigger factor, we tested whether, in the absence of trigger factor, DnaK shows altered association with newly synthesized proteins tig^+ and $\Delta tig::kan$ cells were labelled for 15 s with ³⁵S-methionine and DnaK-bound substrates were rapidly recovered by co-immunoprecipitation with DnaK-specific antisera (Fig. 4a). About 2–4% of total labelled protein co-immunoprecipitated with DnaK from tig^+ cells, whereas 2–3-fold more labelled material was initially co-immunoprecipitated from $\Delta tig::kan$ cells (Fig. 4a, lower panel). The labelled material consisted of various proteins appearing as a smear (Fig. 4a), probably because nascent polypeptides were among these substrates. Chasing with non-radioactive methionine for 1–10 min before co-immunoprecipitation converted the smear to distinct bands, some of which associated with DnaK to similar extents in tig^+ and Δtig cells (Fig. 4a). Some nascent chains showed a rapid flux through DnaK (<1 min half-life), the extent, or perhaps the kinetics, of their association with DnaK being affected by trigger factor. Other proteins showed prolonged interaction with DnaK (>10 min half life), to an extent that is apparently not affected by trigger factor. The mechanistic basis for these protein classes remains unclear. Taking into account the efficiency of co-immunoprecipitation and the amount of recovered labelled protein, we estimate that 9–18% and 26–39% of newly synthesized proteins interact transiently with DnaK in wild-type and $\Delta tig::kan$ cells, respectively.

We also found that, in $\Delta tig::kan$ cells, a slightly increased amount (1.3-fold) of newly synthesized protein associates with GroEL (Fig. 4b). The most notable difference between the DnaK and GroEL substrates is an upper size limitation for GroEL substrates (relative molecular mass (M_r) of ~65K). This size exclusion by GroEL of large proteins, which are particularly prone to aggregation in $\Delta tig::kan$ mutants depleted for DnaK and DnaJ, explains the importance of the DnaK chaperone system for protein folding in $\Delta tig::kan$ cells.

Figure 3 Aggregation of pre-existing and newly synthesized *E. coli* proteins. **a**, Coomassie-blue-stained gels of the pellet fractions of $P_{IPTG}dnaKJ\ tig^+$ and $P_{IPTG}dnaKJ\ \Delta tig::kan$ cells grown for 5 h in the absence of IPTG. Arrows indicate cytosolic (MetE, RpoB) and membrane (AtpD, OmpA) proteins identified by mass

spectrometry. Right: spot quantification of the Coomassie-stained gels. $P_{IPTG}dnaKJ\ tig^+$ cells, light-grey bars; $P_{IPTG}dnaKJ\ \Delta tig::kan$ cells, dark-grey bars. Quantification was performed using ImageMaster 2D program (Amersham/Pharmacia). **b**, Autoradiography of the Coomassie-stained gels shown in **a**.

Our findings indicate cooperative functions for trigger factor and DnaK in folding of newly synthesized proteins, although neither function is essential for folding. Trigger factor probably acts co-translationally, as it associates with ribosomes and nascent polypeptide chains^{3,15}. DnaK associates co- and/or post-translationally with a subset of newly synthesized proteins even in the presence of trigger factor, indicating that it regularly participates in protein folding even in wild-type cells. The nature of the functional cooperation between trigger factor and DnaK remains unclear. □

Figure 4 Association of DnaK with nascent polypeptide chains. **a**, Co-immunoprecipitation with DnaK-specific antisera. The lower panel shows quantification of radioactivity of the immunoprecipitated material above; *tig*⁺, light-grey bars; *Δtig::kan*, dark-grey bars. Radioactivity immunoprecipitated in the *tig*⁺ lysate after 15 s pulse is set 100%. **b**, Co-immunoprecipitation with GroEL-specific antibodies. The lower panel shows quantification of radioactivity of the immunoprecipitated material. Radioactivity immunoprecipitated in the *tig*⁺ lysate after 15 s pulse is set 100%.

Methods

Growth of P_{IPTG}*dnaKJ* cells. The strains used for depletion experiments are in the C600 background. Cells were grown overnight with 1 mM IPTG (isopropyl-β-D-thiogalactoside), then washed three times in saline to remove IPTG, inoculated in fresh medium to an absorbance at 600 nm (OD₆₀₀) of 0.015–0.03 and grown for analyses without IPTG at 37 °C for ~5 h to OD₆₀₀ = 0.6–0.8.

Luciferase activity. Cultures of P_{IPTG}*dnaKJ* *tig*⁺ and *Δtig::kan* cells containing plasmid pBB516 (ref. 11) were grown overnight at 30 °C in LB medium with ampicillin (100 μg ml^{–1}) containing 1 mM IPTG, washed three times in saline (0.8% NaCl) and inoculated in LB medium lacking IPTG to an OD₆₀₀ of 0.030. At mid-log phase, luciferase production was induced by the addition of 0.4% arabinose and *in vivo* activity was determined as described².

Isolation of insoluble proteins. Cultures were grown at 37 °C in M9 medium containing 0.2% glucose and all L-amino acids except methionine to an OD₆₀₀ of 0.6. Cells were pulsed with ³⁵S-methionine (15 μCi ml^{–1} final concentration) for 15 s, rapidly chilled on ice, lysed as described² and the protein content was determined (Bradford assay, Biorad). We subjected 2.4 mg protein of each lysate to a two-step centrifugation protocol where the first low-speed centrifugation removes most membrane proteins². 2D-gel electrophoresis of pellet fractions was performed according to the distributor (Amersham/Pharmacia). Experiments were reproduced at least three times.

Co-immunoprecipitation. Ten-millilitre aliquots of cells grown in M9 medium at 37 °C to mid-log phase were pulsed for 15 s with ³⁵S-methionine (15 μCi ml^{–1}; Amersham) and chased by addition of unlabelled methionine (160 μg ml^{–1}) when indicated. Cells were immediately transferred to 10 ml of frozen crushed solution (0.8% NaCl, 10 mM EDTA; pH 8.0), incubated for 5 min and then pelleted by low-speed centrifugation. Lysates were prepared as described², except that lysis buffer contained 100 mM EDTA to prevent ATP-dependent dissociation of DnaK-bound substrates, and that lysates were subjected to additional centrifugation for 5 min at 13,000g to remove insoluble material before immunoprecipitation. Eighty microlitres of lysate (2 mg ml^{–1}) was incubated with 80 μl protein A–Sepharose beads in PBS, 4 μl of appropriate antibodies and 1 ml of RIPA-buffer (50 mM Tris/HCl, 10 mM EDTA, 150 mM NaCl, 0.5% NP-40, pH 7.5) for 45 min at 8 °C. Beads were washed 3 times with RIPA-buffer, once with PBS/5 mM EDTA and finally boiled in 80 μl SDS-sample buffer. Incorporation of ³⁵S-methionine in TCA-precipitated lysates was determined to correct for differences in the labelling of the lysates.

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1. Ewalt, K. L., Hendrick, J. P., Houry, W. A. & Hartl, F. U. *In vivo* observation of polypeptide flux through the bacterial chaperonin system. *Cell* **90**, 491–500 (1997).
2. Hestekamp, T. & Bukau, B. Role of the DnaK and HscA homologs of Hsp70 chaperones in protein folding in *E. coli*. *EMBO J.* **17**, 4818–4828 (1998).
3. Hestekamp, T., Hauser, S., Lütcke, H. & Bukau, B. *Escherichia coli* trigger factor is a prolyl isomerase that associates with nascent polypeptide chains. *Proc. Natl Acad. Sci. USA* **93**, 4437–4441 (1996).
4. Valent, Q. A. *et al.* Early events in preprotein recognition in *E. coli*: interaction of SRP and trigger factor with nascent polypeptides. *EMBO J.* **14**, 5494–5505 (1995).
5. Scholz, C., Stoller, G., Zarnt, T., Fischer, G. & Schmid, F. X. Cooperation of enzymatic and chaperone functions of trigger factor in the catalysis of protein folding. *EMBO J.* **16**, 54–58 (1997).
6. Stoller, G. *et al.* A ribosome-associated peptidyl-prolyl *cis/trans* isomerase identified as the trigger factor. *EMBO J.* **14**, 4939–4948 (1995).
7. Bardwell, J. C. A. & Craig, E. A. Ancient heat shock gene is dispensable. *J. Bacteriol.* **170**, 2977–2983 (1988).
8. Kumamoto, C. A. & Beckwith, J. Mutations in a new gene, *secB*, cause defective protein localization in *Escherichia coli*. *J. Bacteriol.* **154**, 253–260 (1983).
9. Zeilstra-Ryalls, J., Fayet, O., Baird, L. & Georgopoulos, C. Sequence analysis and phenotypic characterization of *groEL* mutations that block λ and T4 bacteriophage growth. *J. Bacteriol.* **175**, 1134–1143 (1993).
10. Paek, K. H. & Walker, G. C. *Escherichia coli* *dnaK* null mutants are inviable at high temperature. *J. Bact.* **169**, 283–290 (1987).
11. Tomoyasu, T., Ogura, T., Tatsuta, T. & Bukau, B. Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *E. coli*. *Mol. Microbiol.* **30**, 567–581 (1998).
12. Bukau, B. & Walker, G. C. Cellular defects caused by deletion of the *Escherichia coli* *dnaK* gene indicates roles for heat shock protein in normal metabolism. *J. Bacteriol.* **171**, 2337–2346 (1989).
13. Gross, C. A. in *Escherichia coli and Salmonella* (ed. Neidhardt, F. C.) 1382–1399 (ASM, Washington, 1996).
14. Gamer, J. *et al.* A cycle of binding and release of the DnaK, DnaJ and GrpE chaperones regulates activity of the *E. coli* heat shock transcription factor σ³². *EMBO J.* **15**, 607–617 (1996).
15. Valent, Q. A. *et al.* Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and trigger factor. *Mol. Microbiol.* **25**, 53–64 (1997).

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