Low temperature or GroEL/ES overproduction permits growth of *Escherichia coli* cells lacking trigger factor and DnaK

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Abstract ***Escherichia coli*** **trigger factor (TF) and DnaK cooperate in the folding of newly synthesized proteins. The combined deletion of the TF-encoding *tig* gene and the *dnaK* gene causes protein aggregation and synthetic lethality at 30°C. Here we show that the synthetic lethality of Δ*tig*Δ*dnaK52* cells is abrogated either by growth below 30°C or by overproduction of GroEL/GroES. At 23°C Δ*tig*Δ*dnaK52* cells were viable and showed only minor protein aggregation. Overproduction of GroEL/GroES, but not of other chaperones, restored growth of Δ*tig*Δ*dnaK52* cells at 30°C and suppressed protein aggregation including proteins ≥60 kDa, which normally require TF and DnaK for folding. GroEL/GroES thus influences the folding of proteins previously identified as DnaK/TF substrates.**

Key words: Chaperone; De novo folding; 2D gel electrophoresis; Nascent polypeptide; Protein aggregation

1. Introduction

In the *Escherichia coli* cytosol, ribosome-associated trigger factor (TF) is the first chaperone interacting with nascent polypeptide chains [1,2]. Beyond their interaction with TF, a subpopulation of newly synthesized proteins requires further chaperone assistance for folding. The DnaK chaperone, acting in concert with its co-chaperones, DnaJ and GrpE, associates co- or posttranslationally with 9–18% of newly synthesized proteins [3,4], while GroEL and its GroES co-chaperone associate posttranslationally with 10–15% of newly synthesized polypeptides in vivo [5]. The majority of the GroEL-interacting proteins have molecular weights between 20 and 60 kDa, with a minority (≤20%) having higher molecular weights [5,6].

GroEL is essential for *E. coli* at all temperatures, while DnaK is only essential at temperatures above 37°C and below 15°C. TF is not essential for growth at any temperature [3,4,7,8]. However, the combined deletion of the *tig* gene encoding TF and the *dnaK* gene is synthetically lethal at 30°C and 37°C [3,4]. In Δ*tig* cells depleted of DnaK several hundred cytosolic proteins misfold and aggregate at 37°C. These proteins comprise a broad range of molecular weights, with a strong enrichment in proteins of ≥60 kDa. The amount of aggregated proteins increases with increasing temperature, from approx. 1% of total soluble protein at 30°C to over 10% at 37°C [3,9].

Assuming that the misfolding of proteins is the cause of synthetic lethality in Δ*tig*Δ*dnaK52* cells at 30°C, we speculated that there are two ways to override lethality. First, temperatures below 30°C may cause less aggregation and thus allow growth of Δ*tig*Δ*dnaK* cells. Second, the overproduction of cytosolic chaperones, such as HtpG, ClpB, GroEL/ES, DnaJ and IbpA/IbpB, may prevent protein aggregation and suppress Δ*tig*Δ*dnaK52* lethality. In this study we set out to test both assumptions by screening for the viability of Δ*tig*Δ*dnaK52* cells.

2. Materials and methods

2.1. Strains, culture conditions and preparation of aggregates

E. coli strains were derivatives of MC4100. Growth media contained isopropyl-β-D-thiogalactose (IPTG) as indicated, ampicillin (100 μg/ml), tetracycline (5 μg/ml) or kanamycin (40 μg/ml) when appropriate. Aggregated material was isolated as reported [9,10].

2.2. In vivo complementation and quantification of chaperone levels

The *E. coli* Δ*tig*Δ*dnaK52* strain was constructed by P1 transduction using a P1 lysate prepared from *E. coli* Δ*tig::kan zba-3054*::Tn*10* [3]. Δ*dnaK52* cells were transformed using plasmids pDS56, pDS56-*tig* [2] or pDS56-groESL [11] and plasmid (pZA4) expressing the repressor LacI [11]. To quantify TF and GroEL levels in different strains, the same amounts of total lysate (1–20 μg/lane) prepared from those strains were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). GroEL and TF levels were directly quantified from the Coomassie-stained SDS gel and additionally detected by immunoblotting using chaperone-specific antibodies. Quantification of signals was performed using the MacBasV2.5 program.

2.3. 2D gel electrophoresis and identification of proteins

2D gel electrophoresis was performed as described [11]. For protein identification, spots were analyzed by mass spectrometry and/or protein spot matching with reference gels using visual inspection and the ImageMaster software (Pharmacia).

3. Results

3.1. Screening for viable Δtig*Δ*dnaK52 cells

To test whether Δ*tig*Δ*dnaK* cells are viable below 30°C, we performed co-transduction experiments at 23, 30 and 34°C employing either a Δ*dnaK52* [12] or a wild type strain as

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A

genetic background	# of Tc^R clones	# of Kan^R clones	co-transduction frequency	
wild type	50	44	88%	34°C
Δ*dnaK52*	35	0	0%	
wild type	49	42	85%	30°C
Δ*dnaK52*	36	0	0%	
wild type	50	40	80%	23°C
Δ*dnaK52*	50	20	40%	

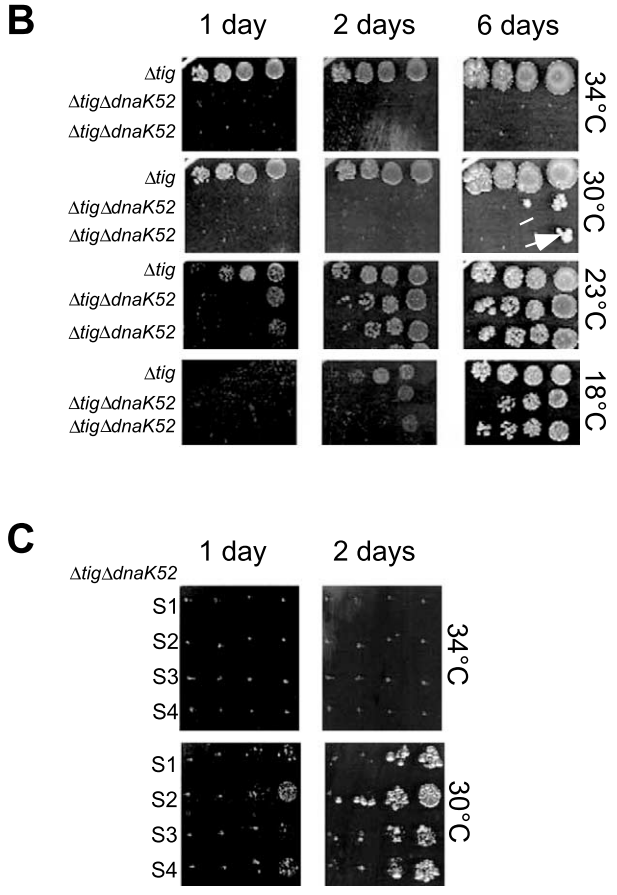

Fig. 1. Conditional lethality of Δ*tig*Δ*dnaK52* cells. A: Co-transduction frequencies as an indicator of synthetic lethality at various temperatures. The gray background emphasizes the condition where Δ*tig*Δ*dnaK52* co-transductants were obtained. B: Cells grown overnight at 23°C were diluted (corresponding to 10, 10^2, 10^3, 10^4 cfu/5 μl), spotted on LB plates and incubated at the indicated temperature. Note that two Δ*tig*Δ*dnaK52* clones independently isolated at 23°C were tested. After 6 days several single colonies arose (white arrows) in spots of Δ*tig*Δ*dnaK52* cells with the lowest dilution. These potential suppressors were tested in C. C: Growth of four independent potential suppressor colonies (S1–S4) at 30 and 34°C.

recipient. To screen for co-transduction, we used a Tn*10*::Tet-selective marker (zba-3054::Tn*10*) placed close to the Δ*tig::kan* allele. Transductants were first selected on LB plates with tetracycline and subsequently screened for kanamycin resistance, and hence deletion of the *tig* gene. Co-transduction frequency in wild type cells was around 80% at all temperatures tested (Fig. 1A). In agreement with earlier reports, no co-transduction was observed for Δ*dnaK52* cells at 30 and 34°C [3,4]. However, at 23°C we observed co-transduction in Δ*dnaK52* cells with a frequency of 40%. By Western blot analysis, we confirmed the loss of TF for several individual Δ*tig*Δ*dnaK52* co-transductants (data not shown). These results show that cells can tolerate the simultaneous loss of DnaK and TF at 23°C.

3.2. *Viability of* ΔtigΔdnaK52 *cells*

To investigate their growth behavior, co-transductants were grown overnight at 23°C, spotted in serial dilutions on LB plates and incubated at different temperatures (Fig. 1B). Δ*tig*Δ*dnaK52* cells formed colonies exclusively at 18 and 23°C, even though the colonies appeared only after 48 h and thus grew more slowly compared to colonies of a Δ*tig* (Fig. 1B) or Δ*dnaK52* strain (data not shown). We conclude

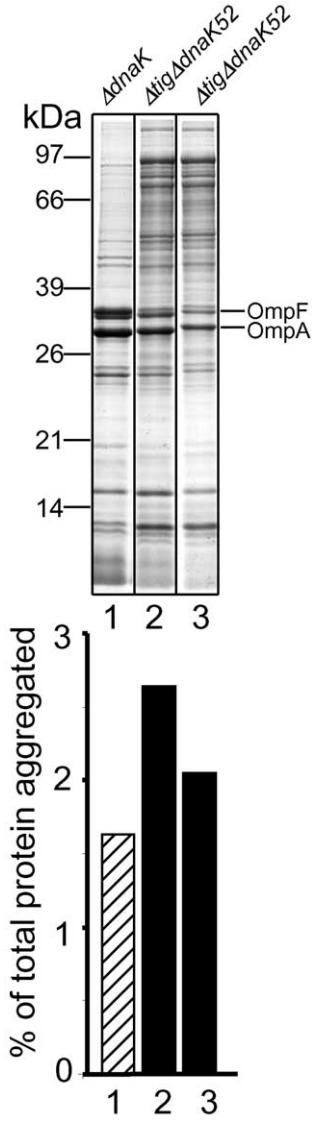

Fig. 2. Protein aggregation in Δ*tig*Δ*dnaK52* cells. Aggregates were isolated from cells grown at 23°C and analyzed by SDS–PAGE and Coomassie staining. Two individual Δ*tig*Δ*dnaK52* clones were tested. Bars show quantification of aggregated proteins by Bradford assay. The outer membrane proteins OmpF and OmpA co-purify with aggregated cytosolic proteins. The reason for their partial disappearance in Δ*tig*Δ*dnaK52* cells is unknown.

that simultaneous deletion of the *tig* and *dnaK* gene is conditionally lethal depending on the growth temperature.

Strikingly, when Δ*tig*Δ*dnaK52* cells were incubated for 6 days at 30°C, several single colonies appeared in spots with the lowest dilution factor (Fig. 1B) indicating that spontaneous suppressor mutations have accumulated in these cells. In order to test whether these clones represent suppressors, four independent colonies (S1–S4) were isolated and tested for growth at different temperatures (Fig. 1C). The growth behavior of these clones was in sharp contrast to the growth of freshly generated Δ*tig*Δ*dnaK52* cells. All four clones grew at 30°C after 24 h. We conclude that spontaneous suppressor mutations arise in Δ*tig*Δ*dnaK52* cells, which extend the growth temperature spectrum. The nature of these suppressors is unknown and will be investigated in an independent study.

3.3. Analysis of protein aggregation in ΔtigΔdnaK52 cells

Next, we analyzed protein aggregation in Δ*tig*Δ*dnaK52* cells. We grew cells at 23°C to logarithmic phase and isolated insoluble proteins. In Δ*dnaK52* cells only minor folding defects were detectable at 23°C, with approximately 1.6% of total cellular protein recovered as aggregates (Fig. 2). The extent of protein aggregation in Δ*dnaK52* cells grown at 23°C was very similar to the folding defects of these cells at 30°C ([9] and Fig. 3C). Protein aggregation at 23°C in Δ*tig*Δ*dnaK52* cells (2.5% of total protein) was only slightly higher compared to Δ*dnaK52* cells (Fig. 2, lanes 2 and 3). We conclude that the viability of Δ*tig*Δ*dnaK52* at low temperature is accompanied by a rather mild protein folding defect.

3.4. Overexpression of the GroEL/GroES complements synthetic lethality of ΔtigΔdnaK52 cells at 30°C

Next, we analyzed whether overproduction of chaperones (ClpB, HtpG, GroEL/GroES, DnaJ and IbpA/IbpB) can complement the synthetic lethality of Δ*tig*Δ*dnaK52* cells at 30°C. We introduced plasmids expressing the different chaperone genes under IPTG control into Δ*dnaK52* cells. Subsequently, we performed co-transduction experiments as described above in the presence of 50 μM IPTG. Exclusively the overproduction of the GroEL/GroES gave rise to Δ*tig*Δ*dnaK52* co-transductants at 30°C (Fig. 3A). No co-transductants could be observed in the absence of IPTG, and no other chaperone tested could compensate for the loss of DnaK and TF even when experiments were performed in presence of lower and higher IPTG concentrations (data not shown). Taken together the results show that overproduction of GroEL/GroES compensates the loss of DnaK and TF at 30°C.

3.5. Viability of ΔtigΔdnaK52 cells overproducing GroEL/GroES

To investigate growth, overnight cultures of Δ*tig*Δ*dnaK52* cells overproducing GroEL/GroES were spotted in serial di-

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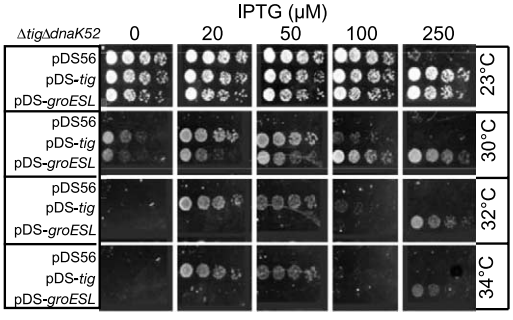
Δ*dnaK52*	# of Tc^R clones	# of Kan^R clones	co-transduction frequency
pDS56-*tig*	50	26	52%
pDS56-*dnaK*	54	26	48%
pDS56-*dnaJ*	23	0	0%
pDS56-*groESL*	42	21	50%
pDS56-*htpG*	25	0	0%
pDS56-*clpB*	17	0	0%
pDS56-*ibpAB*	22	0	0%
pDS56-*secB*	17	0	0%

B



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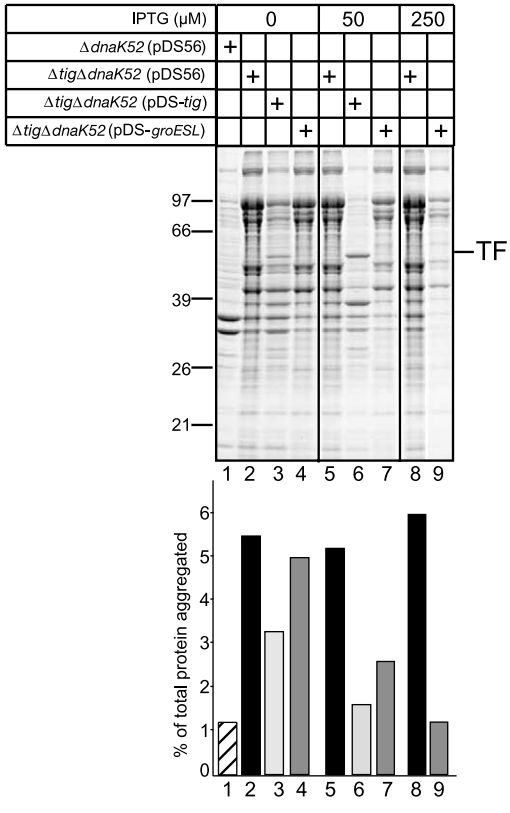

Fig. 3. GroEL/ES overproduction complements synthetic lethality of Δ*tig*Δ*dnaK52* cells. A: Co-transduction frequencies of Δ*dnaK52* cells overexpressing different chaperones. The gray background emphasizes the condition where Δ*tig*Δ*dnaK52* cells overproducing GroEL/ES were obtained. B: Growth analysis of Δ*tig*Δ*dnaK52* cells complemented with GroEL/GroES or TF at different temperatures. Δ*tig*Δ*dnaK52* cells carrying the vector pDS56 served as control. Dilutions of cells (see Fig. 1) were spotted on LB plates and incubated 24 h at different temperatures. C: Aggregates were isolated from cells grown at 30°C in the presence of different amounts of IPTG and analyzed by SDS–PAGE and Coomassie staining. B: Bars show quantification of aggregated proteins by Bradford assay.

lutions on LB plates containing different IPTG concentrations and incubated at different temperatures (Fig. 3B). For control Δ*tig*Δ*dnaK52* cells producing plasmid-encoded TF or containing the vector pDS56, which was used for cloning and expression of chaperone genes [13], were analyzed in parallel.

At 23°C growth of all strains was similar. Only growth of Δ*tig*Δ*dnaK52* (pDS56) cells was impaired at the highest IPTG concentration for unknown reasons (Fig. 3B). IPTG-induced overexpression of GroEL restored the growth of Δ*tig*Δ*dnaK52* cells in a temperature- and concentration-dependent manner (Fig. 3B). Induction with 50 μM IPTG was sufficient to restore growth at 30°C. However, at 32°C and 34°C growth was only observed at 250 μM IPTG indicating an enhanced requirement of GroEL/GroES at higher temperatures. In contrast, lower production levels of TF using 20 μM IPTG were sufficient to complement lethality of Δ*tig*Δ*dnaK52* cells at all temperatures tested. As reported earlier, strong overproduction of TF (100–250 μM IPTG) is lethal at 30°C in Δ*tig*Δ*dnaK52* cells and even in wild type cells ([14,15] and data not shown). The reason is unclear, but TF-overexpressing cells form filaments and are likely to have cell division defects [14].

3.6. Analysis of protein aggregation

We analyzed whether the overproduction of GroEL/GroES prevents protein aggregation in Δ*tig*Δ*dnaK52* cells. Therefore, Δ*tig*Δ*dnaK52* strains overproducing either GroEL/GroES or TF and Δ*dnaK52* cells for control were grown at 30°C in liquid media to logarithmic phase in the presence of different IPTG concentrations. It should be noted that the IPTG-regulated promoter which drives the expression of chaperone genes from pDS56 was leaky, leading to a production of the plasmid-encoded chaperones to approximately two-fold wild type levels even in the absence of IPTG (data not shown). Moreover, we noticed that Δ*tig*Δ*dnaK52* cells could grow for about four doublings at 30°C in liquid medium, perhaps because protein aggregation did not reach a critical level within this short period. Thus, we were also able to investigate Δ*tig*Δ*dnaK52* cells carrying the vector as control.

Aggregated proteins isolated from Δ*tig*Δ*dnaK52* cells amounted to over 5% of total protein (Fig. 3C, lanes 2, 5, 8). Overproduction of GroEL/GroES gradually suppressed aggregation in Δ*tig*Δ*dnaK52* cells (Fig. 3C, lanes 4, 7, 9). Induction of GroEL/ES production with 50 μM IPTG sup-

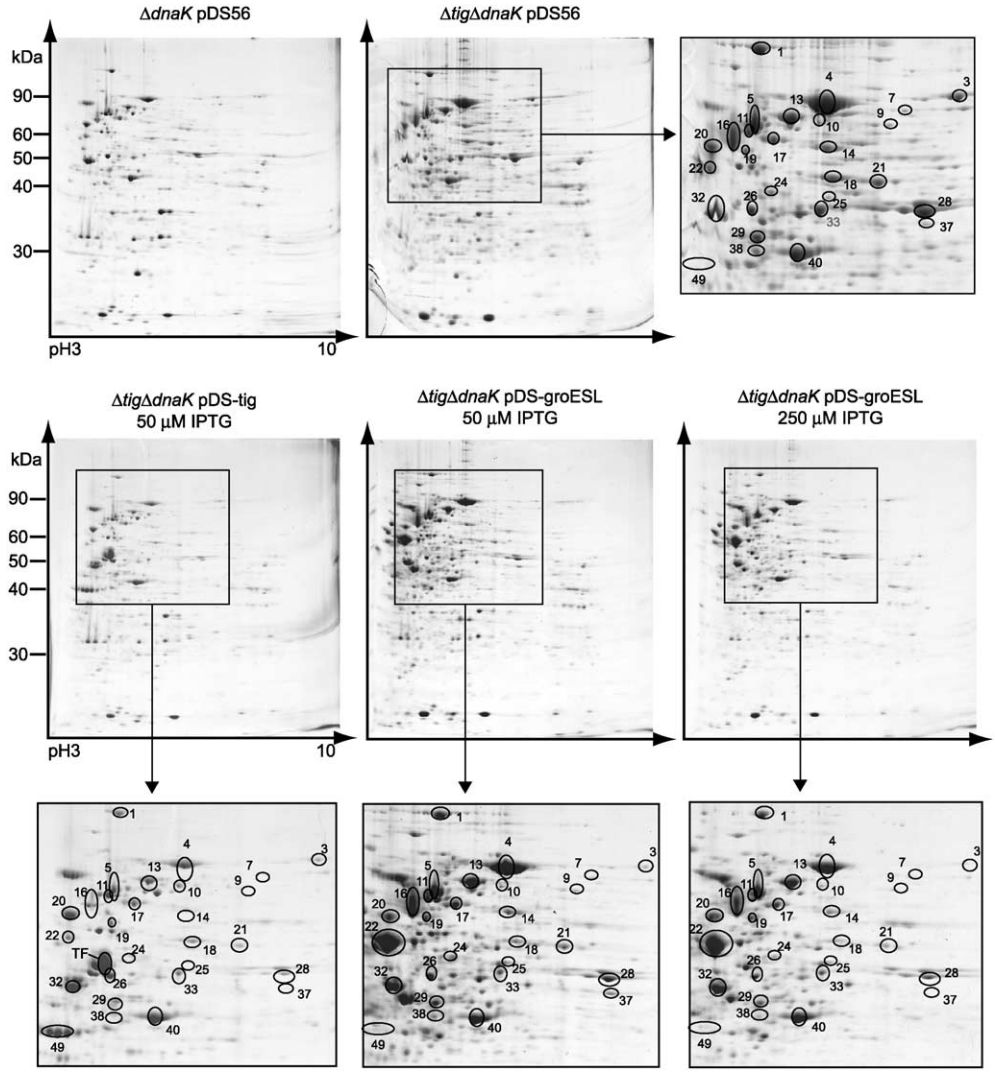

Fig. 4. Identification of aggregated proteins. Aggregated proteins isolated under conditions indicated in legend of Fig. 3 were separated by 2D gel electrophoresis and subsequently Coomassie-stained. Regions of interest in each gel showing most spots are shown magnified and protein spots correlating with proteins identified are indicated by numbers (see Table 1).

pressed protein aggregation only partially, whereas 8–10-fold overproduction of GroEL/GroES in the presence of 250 μM IPTG suppressed protein aggregation almost completely. Approximately 1.2% of total proteins were aggregation-prone in the presence of high GroEL/ES levels which is similar to the amount of insoluble proteins found in Δ*dnaK52* cells (Fig. 3C, lanes 1 and 9).

Production of TF using 50 μM IPTG (corresponding to approximately four- to six-fold enhanced TF levels) efficiently suppressed protein aggregation in the double knockout cells (Fig. 3C, lane 6). The leakiness of the IPTG-controlled promoter explains that Δ*tig*Δ*dnaK52* cells complemented with pDS56-*tig* already exhibited less aggregation even in the absence of inducer (Fig. 3C, lane 3).

Interestingly, the patterns of the aggregated proteins isolated from TF- or GroEL/GroES-producing Δ*tig*Δ*dnaK52* cells differed significantly (Fig. 3C, compare lanes 5, 6, 7 and 9). Whereas aggregation of proteins was suppressed by TF irrespective of the molecular size of the proteins, prevention of aggregation by GroEL system seemed less efficient for large-sized proteins >60 kDa.

We conclude that GroEL/GroES is a potent back-up sys-

Table 1
Identification of aggregated proteins

No.	Name	Size (kDa)	Accession number	Function	Δ*tig*Δ*dnaK52*			
					pDS56	pDS-*tig* (50)	pDS-*groESL* (50)	pDS-*groESL* (250)
1	RpoB	151	P00575	RNA polymerase	+	+	+	+
2	PutA	145	P09546	proline dehydrogenase (DH)	+			
3	SucA	106	P07015	E1 of 2-oxoglutarate DH	+	+*	+*	
4	AceE	100	P06958	E1 of pyruvate DH	+	+*	+	+
5	PepN	99	P04825	aminopeptidase N	+	+*	+	+
6	AlaS	96	P00957	Ala-tRNA synthetase	+			
7	GyrB	90	P06982	DNA gyrase	+		+*	
8	Lon	87	P08177	ATP-dependent protease	+	+	+	+
9	PflB	86	P08177	formate acetyltransferase	+		+*	
10	B2463	83	P76558	unknown	+	+	+	+
11	DeoD	80	P09743	purine nucleoside phosphatase	+	+*	+	+*
12	KatG	80	P13029	catalase	+	+	+	+
13	FusA	78	P02996	elongation factor EF-G	+	+*	+	+
14	TktA	72	P27302	transketolase 1	+		+*	+*
15	RpoD	70	P00579	sigma factor 70	+	+	+	+
16	AceF	66	P06959	E2 of pyruvate DH	+	+*	+	+*
17	TypA	66	P32132	EF-G homolog	+	+*	+	+*
18	TreC	64	P28904	trehalose phosphate hydrolase	+	+*	+	+*
19	ProS	64	P16659	proline tRNA synthetase	+	+	+	+
20	S1	61	P02349	ribosomal protein S1	+	+	+	+
21	PyrG	61	P08398	CTP synthetase	+	+*	+*	+*
22	GroEL	60	P06139	Hsp60 chaperone	+	+	+	+
23	PrfC	59	P33998	termination factor RF-3	+	+	+	+
24	GuaA	59	P04079	GMP synthetase	+		+	+*
25	LeuA	58	P09151	leucine biosynthesis	+		+	+
26	YdgA	55	P77804	unknown	+	+	+	+
27	PepA	55	P11648	aminopeptidase	+	+	+	+*
28	TnaA	54	P00913	tryptophanase	+	+*	+	+*
29	Gnd	52	P37754	6-phosphogluconate DH	+	+*	+	+*
30	Syn	52	P17242	asparagine tRNA synthetase	+	+	+	+
31	LpdA	51	P00391	dihydrolipoamide DH	+	+	+	+
32	AtpD	50	P00824	ATP synthetase	+	+	+	+
33	SerS	49	P09156	serine tRNA synthetase	+	+*	+*	+*
34	PurA	48	P12283	AMP biosynthesis	+	+	+	+
35	Rho	47	P03002	transcription terminator	+	+*	+*	+*
36	ClpX	46	P33138	chaperone	+	+	+	+
37	MurA	45	P28909	peptidoglycan biosynthesis	+		+	
38	DeoB	45	P07651	phosphopentomutase	+		+	+
39	FabF	43	P39435	fatty acid biosynthesis	+			
40	Tuf-BA	43	P02990	EF-Tu	+	+	+	+
41	Pgk	41	P11665	phosphoglycerate kinase	+	+	+	+
42	FtsZ	40	P06138	cell division	+			
43	RpoA	37	P00574	RNA polymerase	+	+	+	+
44	TrpS	37	P00954	tryptophan tRNA synthetase	+		+*	+*
45	CysB	36	P06613	transcription activator	+	+*	+	+
46	GapA	36	P06977	GAPDH	+	+	+	+
47	FdoH	34	P32175	formate DH	+	+	+	+
48	DhsB	27	P07014	succinate DH	+		+	
49	YadF	25	P36857	carbonic anhydrase	+			
50	IbpA	16	P29209	chaperone	+	+*	+	+*
51	IbpB	16	P29210	chaperone	+	+*	+	+

Protein spot identified in the aggregated fraction '+'; *protein spot that is significantly less aggregation-prone compared with its aggregation in a Δ*tig*Δ*dnaK52* strain.

tem for TF and DnaK. Large amounts of GroEL/GroES override the synthetic lethality of Δ*tig*Δ*dnaK* cells at 30°C and suppress the protein aggregation.

3.7. Identification of GroEL substrates

To identify aggregated proteins, we performed 2D gel analysis of aggregated fractions. In Δ*tig*Δ*dnaK52* cells approx. 325 aggregation-prone protein species were found, of which 51 were identified by mass spectrometry or spot matching with reference gels (Fig. 4, Table 1). The aggregated proteins identified in Δ*tig*Δ*dnaK52* cells in this study were almost identical (92%) to the insoluble proteins found earlier in DnaK-, DnaJ-depleted Δ*tig* cells at 37°C [9]. As reported [9], the aggregation-prone proteins found in cells lacking TF and DnaK do not possess any common feature regarding p*I* values or the content of α-helices or β-strands. Overexpression of TF using 50 μM IPTG efficiently suppressed aggregation in Δ*tig*-Δ*dnaK52* cells irrespective of the molecular weight of the aggregated species (Fig. 4, Table 1). Compared to Δ*tig*Δ*dnaK52* cells not only the amount but also the number of aggregated protein spots (176) was significantly reduced. Overproduction of GroEL/ES using 50 μM IPTG caused only a slight reduction in the amount of aggregated proteins (Fig. 4). In contrast, the high overproduction of GroEL/ES (250 μM IPTG) efficiently reduced the amount of protein aggregation in Δ*tig*Δ*dnaK52* (Fig. 4 and Table 1). Confirming the results above several large-sized proteins were less efficiently prevented from aggregation by GroEL/GroES as compared with the overproduction of TF in Δ*tig*Δ*dnaK52* cells, including RpoB (spot 1, 151 kDa) and FusA (spot 13, 78 kDa). However, 78 protein spots out of 325 aggregated proteins found in Δ*tig*Δ*dnaK52*, also including large-sized proteins such as GyrB (spot 7, 90 kDa) or PflB (spot 9, 86 kDa), were no longer discovered in the insoluble fraction or significantly less aggregation-prone, such as SucA (spot 3, 106 kDa), TktA (spot 14, 72 kDa) and TreC (spot 18, 64 kDa). This indicates that GroEL/GroES is in principle able to prevent aggregation of large-sized proteins.

We conclude that TF, DnaK and GroEL have partially overlapping or complementing functions and act synergistically in the folding of newly synthesized polypeptides.

4. Discussion

In this study we showed that the synthetic lethality of Δ*tig*Δ*dnaK52* cells can be suppressed under two different conditions, either by growth at temperatures below 30°C or by overproduction of the GroEL/GroES at 30°C. In both situations the viability of Δ*tig*Δ*dnaK52* cells correlates with decreased intracellular protein aggregation indicating that the magnitude of protein misfolding governs lethality of cells lacking TF and DnaK. We cannot exclude that the survival of Δ*tig*Δ*dnaK52* cells is also due to a change in the level of certain key proteins, although we did not observe significant differences in the general protein expression pattern in these cells.

The finding that Δ*tig*Δ*dnaK52* cells are viable and show little protein aggregation at 23°C can best be explained with the lower speed of translation and a change in the folding kinetics or folding pathways of proteins. Such conditions may disfavor unproductive inter- and intramolecular interactions during co-translational as well as posttranslational folding steps and thus support folding of newly synthesized polypeptides [9,16]. Consequently, newly synthesized proteins may be less vulnerable to misfolding and aggregation during de novo folding at low temperature and hence be less dependent on TF and DnaK.

The second condition found to override the synthetic lethality of Δ*tig*Δ*dnaK52* cells is the overproduction of GroEL/GroES. In contrast to TF and DnaK, GroEL is known to associate exclusively in a posttranslational manner with newly synthesized proteins. It is therefore likely that GroEL substitutes for TF and DnaK by interaction with unfolded or misfolded proteins after their release from the ribosome, thereby preventing the aggregation and promoting the folding of these polypeptides. Interestingly, the spontaneous suppressors S1–S4 isolated from Δ*tig*Δ*dnaK52* cells at 30°C (Fig. 1) revealed a moderate 1.1–1.5-fold increase in the cellular GroEL level compared to Δ*tig* or Δ*dnaK52* cells (data not shown), indicating that in these suppressor strains GroEL may contribute to the ability of these cells to grow at 30°C.

In vivo GroEL preferentially associates with polypeptides ≤ 60 kDa because of size limitations of its substrate binding cavity [5,6,17,18]. In contrast, the substrates for TF and DnaK are enriched for large-sized proteins ≥ 60 kDa [9,11], demonstrating intriguing differences in the substrate pools of GroEL/ES and TF/DnaK. Here, we show that overproduction of GroEL/ES in Δ*tig*Δ*dnaK52* cells prevents the aggregation of many TF/DnaK substrates, including several large-sized proteins. We envision two explanations for this finding. GroEL may productively interact with the proteins that tend to aggregate in Δ*tig*Δ*dnaK52* cells, prevent their aggregation and promote their folding. It was shown that the GroEL system assists the folding of 82 kDa aconitase, which is too big to fit within the central cavity of the GroEL ring [19,20]. Alternatively, the increased solubility of large-sized proteins in the presence of high GroEL/ES levels may rather be an indirect effect. GroEL may promote the folding of proteins ≤ 60 kDa and thereby lower the concentration of aggregation-prone protein species which in turn may influence the aggregation tendency of large-sized proteins.

Interestingly, only GroEL/ES but no other chaperone tested substitutes for the missing function of TF and DnaK in Δ*tig*Δ*dnaK52* cells. One difference between the GroEL system and the other chaperones analyzed in this study is the ability of GroEL as a 'folder' chaperone to actively promote the folding of proteins to the native state in an the ATP-dependent mechanism [21,22]. The aggregation-prone proteins in Δ*tig*Δ*dnaK52* cells appear to require more than simple prevention of aggregation by 'holder' chaperones such as DnaJ and HtpG [23–25]. It is also interesting that GroEL assists the folding of aggregation prone proteins even in the complete absence of TF and DnaK in Δ*tig*Δ*dnaK52* cells. The functional network of chaperones, which acts in the folding of newly synthesized proteins is thus robust, allowing GroEL to substitute for TF and DnaK even in their complete absence.

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