Chaperonins Fight Aminoglycoside-induced Protein Misfolding and Promote Short-term Tolerance in Escherichia coli*§

Lise Goltermann 1, Liam Good §, and Thomas Bentin ‡ 1

From the ‡ Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, 200 Copenhagen, Denmark and the § Department of Pathology and Infectious Diseases, Royal Veterinary College, University of London, NW1 0TU London, United Kingdom

Background: Aminoglycoside antibiotics cause misreading during mRNA translation.
Results: Aminoglycoside action resulted in misfolding of newly synthesized proteins, and chaperonins helped Escherichia coli cope with this insult.
Conclusion: Chaperonins fight aminoglycoside-induced protein misfolding.
Significance: The study shows how chaperones can increase bacterial tolerance to aminoglycoside antibiotics.

Antibiotics are essential to modern healthcare, and understanding their mechanism(s) of action is important to optimize clinical efficacy, battle microbial resistance, and develop new antibiotics. Aminoglycoside antibiotics are cationic aminomodified sugars that bind bacterial ribosomes and corrupt translation (1). Aminoglycosides are the only class of antibiotics known to cause translational misincorporation, which results in heterogeneous polypeptide synthesis (1). Since these early discoveries, it has been speculated that aminoglycoside-induced misreading causes misfolding of impacted proteins (2, 3), yet direct evidence for this hypothesis was lacking. Perhaps the strongest evidence for the “aminoglycoside-induced protein misfolding hypothesis” came from investigations of how streptomycin alters the synthesis and distribution of pulse-labeled protein in Escherichia coli (4). Using subcellular fractionation, Davis et al. (4) observed increased protein association with a low solubility “residual” fraction. The authors speculated that misread secretory and membrane proteins get stuck in the membrane due to abnormal folding and that such “poorly fit” protein could form nonspecific membrane channels, allowing increased uptake of aminoglycosides. This model could explain why aminoglycosides, through misreading, permeabilize the membrane to a range of low molecular weight compounds, a hypothesis that remains virtually undisputed to date (reviewed in Ref. 1).

Global mRNA analyses have revealed that the aminoglycosides gentamicin (5) and tobramycin (6) induce certain heat shock genes in Bacillus subtilis and Pseudomonas aeruginosa, respectively. Similar findings have been reported for E. coli, implying a response to misfolded proteins following aminoglycoside exposure (3). The aminoglycoside antibiotic kanamycin has been reported to induce several heat shock proteins, including GroEL, GroES, and DnaK in E. coli (7), and streptomycin-increased expression of heat shock proteins DnaK (Hsp70) and GroEL (Hsp60) in the opportunistic human pathogen Acinetobacter baumannii (8), consistent with aminoglycoside damage triggering protein misfolding. Whether these chaperones provide protection against exposure to aminoglycosides has not previously been investigated.

Here, we investigated the fundamental features of the aminoglycoside-induced protein misfolding hypothesis. We provide proof of misfolding of newly synthesized proteins following aminoglycoside exposure and found strong evidence that misfolding is a component of the aminoglycoside bactericidal mechanism and that chaperone activity is key to the early bacterial defense against these antibiotics.
Chaperonins Fight Aminoglycoside Corruption

EXPERIMENTAL PROCEDURES

All experiments were initiated by diluting overnight cultures transformed with the appropriate plasmid into fresh LB medium supplemented with the antibiotic required for plasmid maintenance or strain selection (see below) to $A_{595} = 0.03$. MG1655 cultures were grown at 37 °C to $A_{595} = 0.5$, at which time they were aliquoted into a 96-well microtiter plate (catalog no. W1555, Genetix) containing arabinose and antibiotic (290 $\mu$l of cell culture, 10 $\mu$l of aminoglycoside or aminocyclohexyl antibiotic, and 3 $\mu$l of arabinose). Experiments involving MGM100 were treated similarly, except at $A_{595} = 0.5$, the medium was exchanged by centrifugation (8000 $\times$ g) and resuspension in fresh 37 °C LB medium supplemented with the desired antibiotic and arabinose concentrations. Only cultures in the $A_{595} = 0.48–0.52$ range were used. Measurements were carried out in a Tecan GENios microtiter plate reader on living cells at 37 °C every 20 min for a period of 4 h with intermediate shake cycles in linear mode as the cells grew from mid-log phase into stationary phase. Cell cultures were allowed a lag phase of 200 s after each shake cycle before measurement. Absorbance was measured at 595 nm. GFP was excited at 480 nm, and fluorescence (relative fluorescence units) per well was recorded at 520 nm. In the figures, error bars indicate S.D. of biological replicates, where $n = 2–4$, as indicated in the figure legends. $p$ values were determined by Student’s $t$ test using Prism version 5.0.

Cell-free Extracts—Cell-free extracts were as described (9), except that 100 $\mu$g/ml lysozyme was included in the B-PER/DNase I cell lysis buffer.

Western Blot Analyses—SDS-PAGE was performed using 4–12% NuPAGE gels (Invitrogen) and Page Blue staining (Fermentas). GFP detection by Western blotting was done using an anti-GFP primary antibody (catalog no. A-11122, Invitrogen) at 10,000-fold dilution. GroEL detection was performed using a rabbit anti-GroEL primary antibody (catalog no. G6532, Sigma) at 50,000-fold dilution. An HRP-conjugated goat anti-rabbit IgG antibody (catalog no. 65–6120, Zymed Laboratories Inc.) was used as the secondary antibody at 160,000–320,000-fold dilution. We used an ECL Plus kit (Amersham Biosciences) for band visualization. Purified GroEL was from Abcam.

Membrane Potential Disruption Measurements—For measurement of membrane potential disruption, the commercial probe bis(1,3-dibutylbarbituric acid)trimethine oxonol (DiBAC$_{3}(3)$)2 from Invitrogen was used. After 4 h of growth with antibiotics, the cells were harvested and resuspended in PBS. Approximately 10$^8$ cells were transferred to 5 $\mu$g/ml DiBAC$_{3}(3)$ in 100 $\mu$l of PBS, incubated for 15 min in the dark, and profiled on an Accuri flow cytometer counting 50,000 cells/profile. To eliminate non-bacterial particulate material, we used 0.22-$\mu$m filtered PBS and gated the instrument using an FSC-H threshold of 10,000. Data were processed using FlowExpress 4.0 software.

Cell Survival Assays—Cell survival was measured by plating aliquots of a culture every hour during a 4-h antibiotic treatment. Cells were plated in 10–10,000-fold dilutions on LB agar plates containing the antibiotic needed for plasmid maintenance or strain selection and incubated overnight at 37 °C, and the cfu/ml was determined by counting.

Minimum Inhibitory Concentration Determination—All strains were grown overnight in LB medium supplemented with 0.2% arabinose and 40 $\mu$g/ml chloramphenicol (MG1655 transformants) or 50 $\mu$g/ml kanamycin (MGM100). The cultures were diluted to a density of 10$^3$ cfu/ml in fresh medium. The MG1655 samples were diluted in LB medium with chloramphenicol and 0.02% arabinose, and the MGM100 samples were diluted in LB medium with kanamycin. The cultures were aliquoted into a microtiter plate, after which antibiotics and, for MGM100, arabinose were added. The plate was incubated for 18 h at 37 °C, and the minimum inhibitory concentration was determined as the minimum antibiotic concentration preventing visible growth in the wells. See supplemental “Experimental Procedures” for additional methods.

RESULTS AND DISCUSSION

Aminoglycosides Trigger Misfolding of Newly Synthesized Proteins—To provide an example of aminoglycoside-induced protein misfolding, we used the wild type-like strain MG1655 and a well-established protein folding assay involving GFP expressed as a soluble cytosolic protein (Ref. 9; see also Refs. 10 and 11). If unperturbed, this system produces correctly folded GFP that is fully soluble and fluorescent, but point mutations can severely alter GFP folding and fluorescence in a position- and amino acid-dependent manner (9). We speculated that misreading induced by aminoglycosides could similarly impact the folding and fluorescence of newly synthesized GFP. To measure GFP protein folding status under antibiotic growth-limiting conditions, MG1655 cultures were grown to mid-log phase, followed by induction of GFP expression while simultaneously titrating with aminoglycoside antibiotic, and then grown to stationary phase (Fig. 1). This approach ensured selective expression of the reporter under misreading conditions. As expected, aminoglycoside antibiotics reduced bacterial growth in a concentration-dependent manner (Fig. 1A and supplemental Fig. S1, A and C). Reduced growth was mirrored by a corresponding decrease in GFP fluorescence (Fig. 1B and supplemental Fig. S1, B and D). We used Western blotting to analyze soluble and insoluble fractions of total GFP in extracts made from aminoglycoside-treated cultures. Gentamicin, streptomycin, and kanamycin at concentrations ranging from benign to highly growth-inhibitory all caused a dose-dependent formation of up to ~50% insoluble GFP without significantly reducing overall GFP synthesis in MG1655 (Fig. 1C). We did not observe reduced band intensities or truncated products, suggesting the absence of translation frameshifting, blockage, premature termination, or peptidyl-tRNA drop-off. Spectinomycin, a closely related translation inhibitor that does not cause misreading (12) and that has previously been used as a non-misreading control (3), reduced both growth and GFP fluorescence in a dose-dependent manner (supplemental Fig. S1, E and F). In contrast, spectinomycin reduced GFP yield without giving rise to detectable insoluble GFP, consistent with translation blockage without misreading (Fig. 1C).

2 The abbreviation used is: DiBAC$_{3}(3)$, bis(1,3-dibutylbarbituric acid)trimethine oxonol.
When challenging MG1655 with gentamicin (Fig. 1, compare A and C) or kanamycin (compare Fig. 1C and supplemental Fig. S1C), GFP protein misfolding and growth inhibition could not be kinetically separated. However, streptomycin titration revealed only very limited growth inhibition up to 5 \mu g/ml (supplemental Fig. S1A) but caused abundant insoluble GFP formation at 4 \mu g/ml (Fig. 1C). These observations reveal extensive GFP misfolding associated with only marginal growth inhibition, suggesting that misfolding precedes growth arrest with streptomycin.

We next analyzed the effects of gentamicin and streptomycin on the folding status of endogenous cytosolic \beta-galactosidase expressed from the native chromosomal locus in MG1655 (Fig. 1D). The lacZ gene was tightly repressed without inducer but highly isopropyl \beta-D-thiogalactopranoside-inducible (supplemental Fig. S2), enabling interrogation of aminoglycoside effects on newly synthesized \beta-galactosidase. Gentamicin and streptomycin caused a dose-dependent increase in insoluble \beta-galactosidase, with little reduction of total yield. In contrast, spectinomycin reduced \beta-galactosidase yield without formation of an insoluble fraction. These results mirror those obtained with recombinant GFP, providing proof that misreading antibiotics also cause misfolding of an endogenous newly synthesized cytosolic protein.

### Chaperonins Fight Aminoglycoside Corruption

**Chaperonin Overexpression Reduces Aminoglycoside-induced Protein Misfolding and Ameliorates Growth Inhibition**—We asked if chaperone overexpression counters protein misfolding and bacterial growth retardation in aminoglycoside-treated cultures. In *E. coli*, GroEL/GroES overexpression suppresses mutations in many genes (13) and buffers mutations in recombinant proteins, allowing folding of proteins with destabilizing mutations, enabling adaptive evolution (14). GroEL/GroES overexpression also partly complements folding of wild-type GFP (15), as well as other GFP mutants (9). Like GroEL/GroES (16), DnaK/DnaJ/GrpE shows a broad substrate spectrum (17) and forms part of the heat shock response that is induced upon aminoglycoside treatment (3, 8). We cotransformed MG1655 with compatible GFP and GroEL/GroES or DnaK/DnaJ/GrpE expression plasmids and measured GFP folding as described above. In MG1655 cells, GroEL/GroES provided partial remediation of GFP misfolding compared with MG1655 transformants overexpressing DnaK/DnaJ/GrpE (Fig. 2D), consistent with GFP being a GroEL/GroES substrate in bacteria (15). Importantly, GroEL/GroES overexpression almost fully restored growth (Fig. 2, A–C), consistent with chaperonins playing a key role in the defense against aminoglycosides. Similar overexpression of DnaK/DnaJ/GrpE failed to provide strong bacterial growth remediation (Fig. 2, A–C). As shown above (Fig. 1 and supplemental Fig. S1), GFP fluorescence paralleled the growth and solubility data (Fig. 2 and supplemental Fig. S3). Cultures overexpressing GroEL/GroES showed increased fluorescence compared with cells harboring recombinant DnaK/DnaJ/GrpE (supplemental Fig. S3, A–D). This phenotypic difference was due, at least in part, to reduced GFP folding capacity in DnaK/DnaJ/GrpE-overexpressing cells compared with GroEL/GroES-overexpressing cells (Fig. 2D).

Without antibiotics, growth was slightly enhanced by GroEL/GroES overexpression plasmids and measured GFP folding capacity in DnaK/DnaJ/GrpE-overexpressing cells compared with GroEL/GroES-overexpressing cells (Fig. 2D). This phenotypic difference was due, at least in part, to reduced GFP folding capacity in DnaK/DnaJ/GrpE-overexpressing cells compared with GroEL/GroES-overexpressing cells (Fig. 2D). Without antibiotics, growth was slightly enhanced by GroEL/GroES overexpression, whereas DnaK/DnaJ/GrpE did not change growth compared with an empty control plasmid (supplemental Fig. S3E).

The chaperone-mediated growth complementation results were confirmed in a system devoid of GFP. In the presence of aminoglycoside, MG1655 growth was rescued (supplemental Fig. S4) by GroEL/GroES overexpressed at an estimated ~25-fold of non-stressed wild-type levels (supplemental Fig. S7, compare lanes 8 and 11). Induction of the DnaK/DnaJ/GrpE expression plasmid did not rescue growth (supplemental Fig. S4). Spectinomycin-treated cells showed no growth complementation by GroEL/GroES or DnaK/DnaJ/GrpE overexpression, consistent with chaperonin protection in aminoglycoside-treated cells resulting from a greater capacity for protein misfolding management and not simply a general growth augmentation effect.

**Chaperone Overexpression Increases Survival following Aminoglycoside Exposure**—We next measured MG1655 survival after aminoglycoside exposure using a plating assay (Fig. 3). With GroEL/GroES overexpression, the number of cfu remained virtually constant over 4 h of gentamicin treatment. In contrast, when the groES-groEL coding region was deleted from the expression construct (empty control), MG1655 cfu/ml dropped by 2 orders of magnitude (Fig. 3A). Similarly, MG1655 survival decreased with either of two other control
plasmids (supplemental Fig. S5). One control plasmid overexpressed GFP and did not promote survival. Hence, only chaperone overexpression provided early protection against the aminoglycoside onslaught ensuring survival. Given the inability of DnaK/DnaJ/GrpE to sustain growth in the presence of aminoglycoside (Fig. 2), we were surprised to find this chaperone system to be efficient in promoting cell survival, trailing GroEL/GroES by a modest 2-fold (Fig. 3B). We speculate that slowed bacterial growth allowed the DnaK/DnaJ/GrpE-overexpressing cells to survive within the investigated time period, whereas GroEL/GroES-overexpressing cells supported growth as well as survival because of reduced protein misfolding.

**Chaperonin Overexpression Counters Aminoglycoside-induced Membrane Disruption**—Because the membrane potential is impacted during aminoglycoside-mediated cell killing (4), we asked if chaperone overexpression could sustain the membrane potential. MG1655 cell cultures overexpressing GroEL/GroES or DnaK/DnaJ/GrpE and treated with gentamicin were stained with DiBAC4(3) (a fluorescent stain that enters membrane-depolarized bacteria) and profiled by flow cytometry (supplemental Fig. S6). A population of live bacteria showed a single peak profile with low fluorescence. Isopropyl alcohol-killed bacteria showed a single high fluorescence peak. Gentamicin-treated bacteria showed a double peak profile, consistent with membrane depolarization in a significant subpopulation due to membrane disruption. Interestingly, GroEL/GroES overexpression reduced the intensity of the high fluorescence peak (dead cells) compared with DnaK/DnaJ/GrpE overexpression, suggesting that the membrane potential (and hence membrane integrity of MG1655) can be partly rescued by supplying an increased cellular chaperonin protein folding capacity. A control plasmid without chaperone expression showed just a single high intensity peak profile, consistent with widespread membrane disruption.

**Inhibition of GroEL/GroES Expression Exacerbates Aminoglycoside Action**—Aminoglycosides are reported to induce GroEL/GroES expression (18). If chaperonin abundance is impor-
tant in fighting aminoglycoside corruption, disabling their induction should sensitize bacteria to these antibiotics. We used strain MGM100, a MG1655 derivative displaying arabinose-controlled chromosomal groE operon expression via the P<sub>BAD</sub> promoter (19). MGM100 will not grow without arabinose, but GroEL/GroES can be depleted by medium change during culture growth (see Refs. 17, 16, and 19).

We first examined the extent to which GroEL abundance was impacted in MGM100 upon arabinose omission in exponential cultures. After an initial small increase, GroEL abundance was reduced by ~2-fold in MGM100 over 4–6 h of incubation when growing cells from <i>A</i><sub>595</sub> = 0.5 to stationary phase (supplemental Fig. S7, A and C, compare lanes 6 and 7). This is as expected for a stable protein that dilutes with cell division in the absence of synthesis. After continued incubation for 18 h, followed by plating, no reduction in survival was observed (supplemental Fig. S7B). We also examined GroEL abundance after growth from a dilute inoculum. In this case, GroEL depletion was much more extensive, yet no reduction of survival was observed after continued incubation overnight under non-stress conditions (data not shown).

We next investigated if the omission of arabinose impacts the cytosolic folding capacity in MGM100 by overexpressing GFP using an isopropyl β-D-thiogalactopyranoside-inducible vector. We observed a small but consistent shift in newly synthesized GFP from soluble to insoluble fractions upon inhibition of GroEL/GroES expression in exponential cultures (supplemental Fig. S8). Hence, inhibition of chaperonin expression has functional consequences, decreasing the cytosolic protein folding capacity.

On the basis of the above findings that inhibition of chaperonin expression impacts cytosolic folding capacity without impacting survival, we grew MGM100 to mid-log phase and eliminated arabinose by exchanging the growth medium. As expected, arabinose omission showed no effect on continued growth for 4 h (supplemental Fig. S9). In contrast, inhibition of GroEL/GroES expression was strongly growth-inhibitory in bacteria challenged with low concentrations of gentamicin or streptomycin, whereas cells with sustained chaperonin expression showed no growth inhibition at the same aminoglycoside concentrations (supplemental Fig. S9). In contrast, we did not observe GroEL/GroES-dependent differential growth in MGM100 upon spectinomycin exposure (supplemental Fig. S9). Thus, chaperonin action promotes growth and survival only in aminoglycoside-treated bacteria, most likely because GroEL/GroES buffers the effects of translation corruption.

Inhibition of chaperonin expression in MGM100 caused a large increase in DiBAC<sub>4</sub>(3) fluorescence after 4 h of gentamicin treatment, indicating strong depolarization of the membrane, whereas MGM100 with arabinose and the MG1655 control showed no increase (Fig. 4A). Thus, chaperonin under-expression sensitizes bacteria to aminoglycoside disruption of the membrane potential. Moreover, the survival of gentamicin-treated MGM100 was reduced by 4 orders of magnitude compared with MGM100 with arabinose (Fig. 4B). Surprisingly, MG1655 treated with gentamicin suffered a 2-order of magnitude cfu/ml reduction upon arabinose omission (Fig. 4B), although no significant effect on the membrane potential was observed. We cannot fully explain this “sugar effect” but note that sugar/antibiotic combinations can alter antibiotic suscep-
tibilities in unexpected ways (20). Nevertheless, there remains a 100-fold “chaperonin” effect on survival in the presence of gentamicin and absence of arabinose in strains MGM1655 and MGM100 (Fig. 4B). This difference is likely due to insufficient protein folding capacity in MGM100. In contrast, spectinomycin did not reduce survival (or alter membrane potential) in MGM100 or MG1655 in the presence or absence of arabinose (Fig. 4, C and D).

Previous reports showed that GroEL/GroES up-regulation during aminoglycoside induced stress in different bacteria (8, 18). Considering these observations, it seems likely that the MGM100 strain is violently impacted by aminoglycosides during arabinose omission, even if inhibition of GroEL/GroES expression leads to only limited depletion, because the natural response involving up-regulation of GroEL/GroES has been disabled.

The effect of the chaperonin expression level was confirmed by minimum inhibitory concentration measurements in which underexpression sensitized bacteria to gentamicin but not to spectinomycin (supplemental Table S1). In contrast, chaperonin overexpression did not provide protection in the minimum inhibitory concentration assay. This observation reveals that increased chaperonin abundance yields short-term protection only, similar to AsrR protease-mediated heat shock response activation in P. aeruginosa, leading to a temporary increase in tobramycin tolerance (6). It seems likely that chaperonins provide an efficient early defense but eventually become overwhelmed due to systemic accumulation of heterogeneous polypeptide products.

Here, we have demonstrated that aminoglycoside antibiotic action impacts cytosolic protein folding, leading to accumulation of insoluble protein. Misfolding was observed even when growth was not impacted, suggesting that misfolding precedes growth retardation. Overexpression of GroEL/GroES reduced protein misfolding and rescued the membrane potential, cell growth, and survival. Overexpression of DnaK/DnaJ/GrpE increased survival, however less efficiently compared with the chaperonins. Inhibition of GroEL/GroES expression increased aminoglycoside sensitivity and facilitated early collapse of the membrane potential, accelerating cell death. The present data therefore indicate that misfolding is a central and early event in aminoglycoside-mediated cell killing and that chaperonins, in particular, initially fight the antibiotic onslaught by providing folding assistance to casualty polypeptides, thus promoting growth and survival (Fig. 5). We found that chaperonin overexpression counters the collapse of the bacterial membrane potential, which occurs following aminoglycoside exposure. Whether this is a direct consequence of GroEL/GroES action involving membrane proteins or an indirect effect of chaperonin-assisted folding of cytosolic proteins remains unknown.

While this paper was in revision, a complementary study was published. Consistent with our results, the authors found that aminoglycoside induced protein misfolding (21). We propose that chaperonin-mediated buffering of aminoglycoside-induced protein misfolding provides a new mechanism for “phenotypic tolerance,” a term that covers various non-inherited antibiotic-resistant phenotypes (22). This is important because phenotypic tolerance is known to promote heritable resistance.

Acknowledgments—We thank Marianne Hvidtfeldt Frandsen for technical help, Stanley Brown (University of Copenhagen) for strain MG1655, and Millicent Masters (University of Edinburgh) for strain MGM100.

REFERENCES
1. Davis, B. D. (1987) Mechanism of bactericidal action of aminoglycosides. Microbiol. Rev. 51, 341–350
2. Kohanski, M. A., Dwyer, D. J., and Collins, J. I. (2010) How antibiotics kill bacteria: from targets to networks. Nat. Rev. Microbiol. 8, 423–435
3. Kohanski, M. A., Dwyer, D. J., Wierzbowski, J., Cottarel, G., and Collins, J. I. (2008) Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. Cell 135, 679–690
4. Davis, B. D., Chen, L. L., and Tai, P. C. (1986) Misread polypeptide is encountered co- or post-translationally by several chaperones acting in succession. Limiting chaperone capacity results in protein misfolding, promoting cell killing via membrane permeabilization and possibly depletion of essential cytosolic activities. In contrast, elevated chaperone capacity (GroEL/GroES in particular) shifts the folding trajectory of (essential) misread proteins toward productive folding, hence increasing bacterial survival. om, outer membrane; im, inner membrane; J and K, DnaK/DnaJ/GrpE chaperone system.

FIGURE 5. Model of chaperone effects on bacterial killing and survival during early aminoglycoside exposure. An initial limited uptake of aminoglycoside (pink stars) across the intact bacterial envelope and binding to ribosomes promotes translational misreading (orange asterisks). Misread polypeptides are encountered co- or post-translationally by several chaperones acting in succession. Limiting chaperone capacity results in protein misfolding, promoting cell killing via membrane permeabilization and possibly depletion of essential cytosolic activities. In contrast, elevated chaperone capacity (GroEL/GroES in particular) shifts the folding trajectory of (essential) misread proteins toward productive folding, hence increasing bacterial survival. om, outer membrane; im, inner membrane; J and K, DnaK/DnaJ/GrpE chaperone system.
9. Goltermann, L., Larsen, M. S., Banerjee, R., Joerger, A. C., Ibba, M., and Bentin, T. (2010) Protein evolution via amino acid and codon elimination. *PLoS ONE* 5, e10104

10. Mayer, S., Rüdiger, S., Ang, H. C., Joerger, A. C., and Fersht, A. R. (2007) Correlation of levels of folded recombinant p53 in *Escherichia coli* with thermodynamic stability in vitro. *J. Mol. Biol.* 372, 268–276

11. Waldo, G. S., Standish, B. M., Berendzen, J., and Terwilliger, T. C. (1999) Rapid protein-folding assay using green fluorescent protein. *Nat. Biotechnol.* 17, 691–695

12. Davies, J., Anderson, P., and Davis, B. D. (1965) Inhibition of protein synthesis by spectinomycin. *Science* 149, 1096–1098

13. Van Dyk, T. K., Gatenby, A. A., and LaRossa, R. A. (1989) Demonstration by genetic suppression of interaction of GroE products with many proteins. *Nature* 342, 451–453

14. Tokuriki, N., Tawfik, D. S. (2009) Chaperonin overexpression promotes genetic variation and enzyme evolution. *Nature* 459, 668–673

15. Wang, J. D., Herman, C., Tipton, K. A., Gross, C. A., and Weissman, J. S. (2002) Directed evolution of substrate-optimized GroEL/S chaperonins. *Cell* 111, 1027–1039

16. Kerner, M. J., Naylor, D. J., Ishihama, Y., Maier, T., Chang, H. C., Stines, A. P., Georgopoulos, C., Frishman, D., Hayer-Hartl, M., Mann, M., and Hartl, F. U. (2005) Proteome-wide analysis of chaperonin–dependent protein folding in *Escherichia coli*. *Cell* 122, 209–220

17. Calloni, G., Chen, T., Schermann, S. M., Chang, H. C., Genevaux, P., Agostini, F., Tartaglia, G. G., Hayer-Hartl, M., Hartl, F. U. (2012) DnaK Functions as a central hub in the *E. coli* chaperone network. *Cell Rep.* 1, 251–264

18. Dukan, S., Farewell, A., Ballesteros, M., Taddei, F., Radman, M., and Nystrom, T. (2000) Protein oxidation in response to increased transcriptional or translational errors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 5746–5749

19. McLennan, N., and Masters, M. (1998) GroE is vital for cell-wall synthesis. *Nature* 392, 139

20. Allison, K. R., Brynildsen, M. P., and Collins, J. J. (2011) Metabolite-enabled eradication of bacterial persisters by aminoglycosides. *Nature* 473, 216–220

21. Ling, J., Cho, C., Guo, L. T., Aerni, H. R., Rinehart, J., and Söll, D. (2012) Protein aggregation caused by aminoglycoside action is prevented by a hydrogen peroxide scavenger. *Mol. Cell* 48, 713–722

22. Nathan, C. (2012) Fresh approaches to anti-infective therapies. *Sci. Transl. Med.* 4, 140sr2

---

**Chaperonins Fight Aminoglycoside Corruption**

APRIL 12, 2013 • VOLUME 288 • NUMBER 15 • JOURNAL OF BIOLOGICAL CHEMISTRY