Interplay between the Hsp90 Chaperone and the HslVU Protease To Regulate the Level of an Essential Protein in Shewanella oneidensis

Flora Honore, Nathanael Jean Maillot, Vincent Méjean, Olivier Genest

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

OBSERVATION
Molecular Biology and Physiology

Interplay between the Hsp90 Chaperone and the HslVU Protease To Regulate the Level of an Essential Protein in *Shewanella oneidensis*

Flora Ambre Honoré,[a] Nathanael Jean Maillot,[a] Vincent Méjean,[a] Olivier Genest[a]

[a]Aix Marseille Université, CNRS, BIP UMR 7281, IMM, Marseille, France

ABSTRACT Protein synthesis, folding, and degradation are an accurately regulated process occurring in every organism and called proteostasis. This process is essential to maintain a healthy proteome since proteostasis dysregulation is responsible for devastating cellular issues. Proteostasis is controlled by a complex network of molecular chaperones and proteases. Among them, eukaryotic Hsp90, assisted by many cochaperones and the Hsp70 chaperone system, plays a major role in activating hundreds of client proteins, and Hsp90 inhibition usually leads to proteasomal degradation of these clients. In bacteria, however, the precise function of Hsp90 remains quite unclear, and only a few clients are known. Recently, we have shown that Hsp90 is essential at elevated temperature in the aquatic model bacterium *Shewanella oneidensis*, and we have identified a client of Hsp90, TilS, involved in tRNA modification. Here we found that two members of the proteostasis network with antagonist activities, the Hsp90 chaperone and the HslVU protease, which is considered the proteasome ancestor, together regulate the level of TilS. In particular, we show that deletion of the genes coding for the HslVU protease suppresses the growth defect of an *S. oneidensis* strain with *hsp90* deleted, by increasing the cellular level of the essential TilS protein. These results open up new avenues for understanding how proteostasis is controlled in bacteria, and new Hsp90 clients are much needed now to confirm the interplay between Hsp90 and proteases.

IMPORTANCE Maintaining a healthy proteome is essential in every living cell from bacteria to humans. For example, proteostasis (protein homeostasis) imbalance in humans leads to devastating diseases, including neurodegenerative diseases and cancers. Therefore, proteins need to be assisted from their synthesis to their native folding and ultimately to their degradation. To ensure efficient protein turnover, cells possess an intricate network of molecular chaperones and proteases for protein folding and degradation. However, these networks need to be better defined and understood. Here, using the aquatic bacterium *Shewanella oneidensis* as a model organism, we demonstrate interplay between two proteins with antagonist activities, the Hsp90 chaperone and the HslVU protease, to finely regulate the level of an essential client of Hsp90. Therefore, this work provides a new bacterial model to better study protein regulation and turnover, and it sheds light on how proteostasis by Hsp90 and proteases could be controlled in bacteria.

KEYWORDS heat shock, proteases, protein chaperone, protein folding, proteostasis, stress adaptation

Proteostasis is controlled in every organism by a complex network of chaperones and proteases (1, 2). Among them, the eukaryotic 90-kDa heat shock protein (Hsp90) chaperone, assisted by many cochaperones and the Hsp70 chaperone system, remodels and activates hundreds of client proteins, including kinases and receptors

Citation Honoré FA, Maillot NJ, Méjean V, Genest O. 2019. Interplay between the Hsp90 chaperone and the HslVU protease to regulate the level of an essential protein in *Shewanella oneidensis*. mBio 10:e00269-19. https://doi.org/10.1128/mBio.00269-19.

Editor Susan Gottesman, National Cancer Institute

Address correspondence to Olivier Genest, ogenest@imm.cnrs.fr.

Received 31 January 2019
Accepted 8 April 2019
Published 14 May 2019

(3–7). In bacteria, Hsp90 also collaborates with the DnaK chaperone system, but cochaperones are absent, and its function needs to be clarified (8–13). In addition, only a few bacterial Hsp90 client proteins are known (8, 14–19). Using the aquatic proteobacteria *Shewanella oneidensis*, we have recently found that Hsp90 is necessary under heat stress conditions to protect and activate the TilS protein, leading to bacterial growth (17). TilS is an essential enzyme that modifies the specificity of the only tRNA that translates the AUG initiator codon in methionine into a tRNA that translates the AUA rare codon in isoleucine (20, 21). Given the major importance for whole proteome synthesis to correctly translate the AUG initiator codon, the level of TilS has to be finely regulated in the cell, since an excess of TilS could lead to depletion of the tRNA-AUG in favor of tRNA-AUA. On the other side, the absence of TilS prevents translation of proteins containing the AUA codon. Here we show that there is interplay between two components of the proteostasis network to regulate the level of the TilS protein: (i) the Hsp90 chaperone for protection and activation and (ii) the HslVU protease for degradation. Therefore, the level of TilS is precisely adjusted in the cell to allow correct protein translation and bacterial growth.

The absence of HslVU suppresses the phenotype of the Δ*hsp90*$_{So}$ strain. To get insight into how proteostasis is controlled in *S. oneidensis* during heat stress, we wanted to identify new components of the proteostasis network that could work with *S. oneidensis* Hsp90 (Hsp90$_{So}$). As previously observed, we found that a strain with *hsp90*$_{So}$ deleted did not grow under heat stress conditions in liquid cultures and on solid media compare to the wild type (WT), whereas it grew well at the permissive temperature of 28°C (Fig. 1A and B) (17). We hypothesized that in the absence of Hsp90 at high temperature, one or several essential Hsp90$_{So}$ clients are not correctly folded and are therefore degraded. Consequently, inactivation of the protease responsible for this degradation could restore a sufficient level of the client protein and could allow growth of the Δ*hsp90*$_{So}$ strain. We thus deleted the genes coding for the HslVU protease and for the ClpP subunit of the ClpAP and ClpXP proteases. These major proteolytic machines are composed of an ATP-dependent chaperone subunit belonging to the AAA+ family (i.e., HslU, ClpA and ClpX) that unfolds substrates and directs them into the catalytic chamber of the peptidase subunit (i.e., HslV and ClpP) (22). Strikingly, we observed that the absence of the HslVU protease did rescue the growth of the Δ*hsp90*$_{So}$ strain at high temperature in liquid and solid media (Fig. 1A and B, compare the Δ*hsp90*$_{So}$ Δ*hslVU* strain with the Δ*hsp90*$_{So}$ strain). In contrast, the ClpP protease did not seem to be involved in this pathway since no growth improvement was observed in the Δ*hsp90*$_{So}$ Δ*clpP* strain compared to the Δ*hsp90*$_{So}$ strain. At 28°C, all strains grew as wild type (Fig. 1A and B).

We then confirmed the growth phenotypes by producing HslVU or Hsp90$_{So}$ from plasmids (Fig. 1C). We found that at high temperature, production of HslVU strongly reduced the growth of the Δ*hsp90*$_{So}$Δ*hslVU* strain as expected, whereas production of Hsp90$_{So}$ complemented the phenotype of the Δ*hsp90*$_{So}$ strain.

Altogether, these results support the idea that some essential Hsp90$_{So}$ clients that are degraded in the Δ*hsp90*$_{So}$ strain are stabilized in the absence of HslVU. They therefore strongly suggest that these clients are degraded by the HslVU protease.

The essential Hsp90$_{So}$ client TilS is degraded by HslVU. Since we have previously shown that the Hsp90$_{So}$ client TilS is responsible for the growth defect of the Δ*hsp90*$_{So}$ strain at high temperature (17), we looked at its level under stress conditions with or without Hsp90$_{So}$ and/or HslVU. To do that, a plasmid coding for TilS with a 6× His tag was introduced in the different genetic backgrounds of *S. oneidensis*. The strains were grown at sublethal high temperature, TilS expression was induced, and its amount was determined by Western blot analysis.

As already seen, we found that about 85% of TilS was degraded in the Δ*hsp90*$_{So}$ strain compared to the wild type (Fig. 2A and B) (17). Interestingly, the TilS level was dramatically increased in the Δ*hsp90*$_{So}$ Δ*hslVU* strain, reaching more than 15 times the level observed in the absence of Hsp90$_{So}$. These results strongly support the idea that

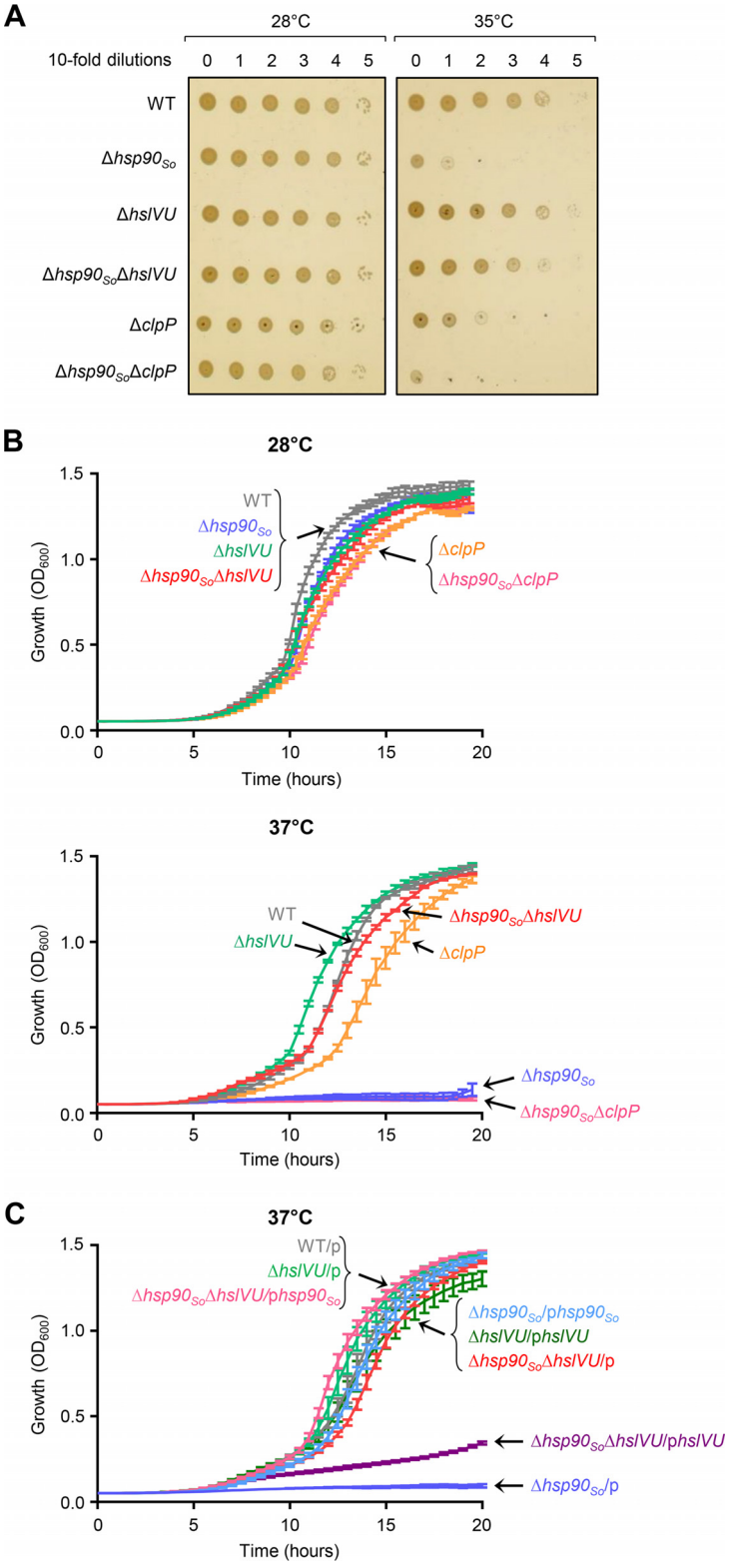

FIG 1 *hslVU* deletion suppresses the growth phenotype of the Δ*hsp90*$_{So}$ strain at high temperature. (A) Strains grown at 28°C to late exponential phase were diluted to an optical density at 600 nm (OD_{600}) of 1, and 2 μl of 10-time serial dilutions was spotted onto LB agar plates. The plates were incubated at 28 or 35°C. (B) Strains grown at 28°C to late exponential phase were diluted to an OD_{600} of 0.0005 and incubated with shaking in a microplate reader at 28 or 37°C. (C) Strains were treated as in panel B, except that LB rich medium was supplemented with 0.015% arabinose to induce protein production from the plasmids. In panels B and C, data from at least three replicates are shown as mean ± standard error of the mean (SEM).

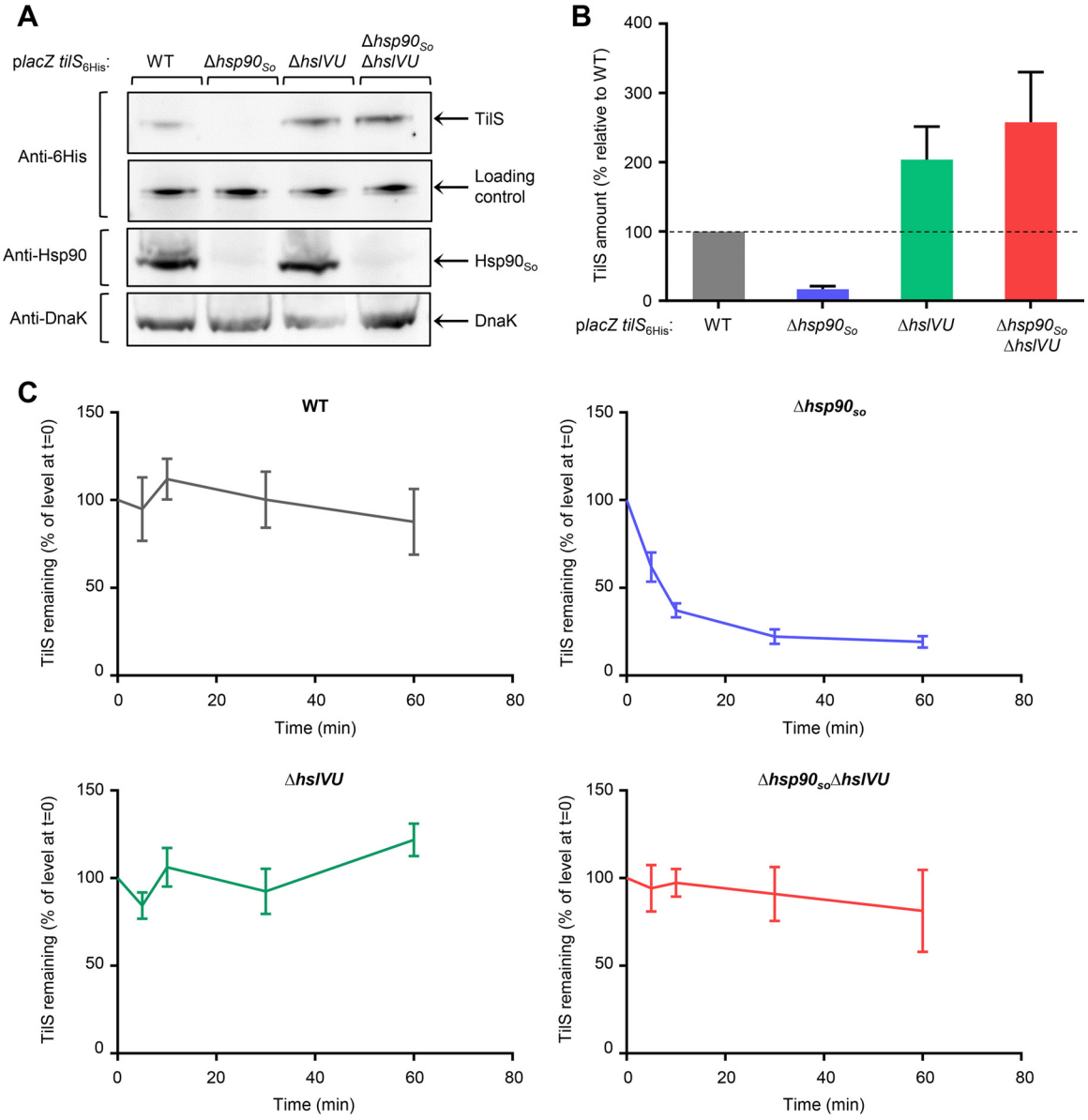

FIG 2 The HslVU protease degrades the $Hsp90_{So}$ client TilS. (A) Strains containing the p*lacZ* $tilS_{6His}$ plasmid, in which *lacZ* and $tilS_{6His}$ are two independent genes under the control of the P_{BAD} promoter, were grown at 35°C, a sublethal temperature. At an OD_{600} of 0.6, 0.02% arabinose was added. After 2 h at 35°C, the same amounts of total protein extract from each strain were loaded for SDS-PAGE, transferred by Western blotting, and revealed with anti-6× His antibody to detect the TilS protein, anti-Hsp90 antibody, or anti-DnaK antibody. The loading control corresponds to a contaminating band revealed with the anti-6× His antibody, indicating that the same amount of cellular extracts was loaded. (B) Quantification of the amount of TilS was performed from 3 independent Western blots described in panel A, revealed with the anti-6× His antibody using ImageJ software. The amount of TilS measured in the wild-type strain was set to 100%. Data are shown as mean ± SEM. (C) Chase experiments. Strains containing the p*lacZ* $tilS_{6His}$ plasmid were grown as in panel A, except that 0.2% arabinose was added to increase the level of the TilS protein, in particular in the Δ$hsp90_{So}$ strain. After 2 h of induction, 200 μg/ml chloramphenicol was added to block protein translation ($t = 0$). Samples were taken at several times after chloramphenicol addition, and proteins were precipitated with trichloroacetic acid (TCA), loaded for SDS-PAGE, and quantified on a Western blot, revealed with anti-6× His antibody using the ImageJ software. The amount of TilS measured in each strain at $t = 0$ (chloramphenicol addition) was set to 100%. Data are shown as mean ± SEM.

TilS is degraded by the HslVU protease. In the Δ*hslVU* strain, we observed that the TilS level is higher than in the wild-type strain, suggesting that in the presence of $Hsp90_{So}$, part of the pool of TilS is degraded by the HslVU protease. In addition, we found that most of TilS protein was present in the soluble fraction of the different strains (see Fig. S1A and B in the supplemental material).

As a control, we showed that transcription from this plasmid did not vary in the different strains grown at sublethal temperature (see Fig. S2 in the supplemental

material). We also checked that deletion of *hsp90*$_{So}$ and/or *hslVU* did not modify the heat shock response by quantifying the level of DnaK, whose gene is under the control of the RpoH sigma factor. Indeed, no significant variation was observed in the four strains (Fig. 2A).

Finally, chase experiments were performed to measure kinetic of TilS degradation in the different genetic backgrounds. Strains containing the plasmid coding for TilS with a 6× His tag were grown as described previously, and after induction, a high concentration of chloramphenicol was added to block translation. The amount of TilS was quantified at several time points after chloramphenicol addition and was expressed relative to the level observed at time zero (Fig. 2C; see Fig. S3 in the supplemental material). In the absence of Hsp90$_{So}$, TilS was degraded with time, and its level reached a plateau after about 30 min, whereas low or no degradation was found in the wild-type, Δ*hslVU*, and Δ*hsp90*$_{So}$ Δ*hslVU* strains (Fig. 2C; Fig. S3). These experiments demonstrate that TilS is degraded by the HslVU protease in the absence of Hsp90.

In this article, we found that the level of TilS is highly regulated at a posttranslational level by the Hsp90$_{So}$ chaperone and the HslVU protease, and we show that growth of the Δ*hsp90*$_{So}$ strain at elevated temperature strongly depends on the amount of the TilS protein. Interestingly, this result is reminiscent of our previous work, in which overproduction of TilS led to growth of the Δ*hsp90*$_{So}$ strain at high temperature (17). Therefore, increasing the level of TilS by two opposite mechanisms, overproduction or inactivation of the degradation, did result in the rescue of the phenotype of the Δ*hsp90*$_{So}$ strain. This finding reinforces the notion that the level of TilS needs to be tightly controlled.

Interestingly, interplay between Hsp90 and HslVU has already been proposed for the posttranslational regulation of an unknown protein involved in the synthesis of toxins in extraintestinal pathogenic *Escherichia coli* (23). In addition, the level of the Cas3 protein, a client of *E. coli* Hsp90, is reduced in the absence of Hsp90 in *E. coli*; however, the protease involved in the degradation has not yet been identified (15). This thus suggests that the antagonist activities of Hsp90 and HslVU could serve as a general mechanism to control the level of some proteins. In eukaryotes, connections between folding by the Hsp90 chaperone and degradation by the proteasome have been well established (24, 25). It therefore becomes essential to identify new clients of bacterial Hsp90 to confirm this model.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.00269-19.

TEXT S1, DOCX file, 0.1 MB.
FIG S1, PDF file, 0.1 MB.
FIG S2, PDF file, 0.1 MB.
FIG S3, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We thank Marianne Ilbert, Eric Durand, Aurélia Battesti, and members of our group for help and fruitful discussions.

This work was supported by the Centre National de la Recherche Scientifique and Aix Marseille Université (AMU). O.G. was supported by a grant from the Agence Nationale de la Recherche (ANR-16-CE11-0002-01).

REFERENCES

1. Balchin D, Hayer-Hartl M, Hartl FU. 2016. In vivo aspects of protein folding and quality control. Science 353:aac4354. https://doi.org/10.1126/science.aac4354.
2. Finka A, Mattoo RUH, Goloubinoff P. 2016. Experimental milestones in the discovery of molecular chaperones as polypeptide unfolding enzymes. Annu Rev Biochem 85:715–742. https://doi.org/10.1146/annurev-biochem-060815-014124.
3. Schopf FH, Biebl MM, Buchner J. 2017. The HSP90 chaperone machinery. Nat Rev Mol Cell Biol 18:345–360. https://doi.org/10.1038/nrm.2017.20.
4. Radli M, Rüdiger SGD. 2018. Dancing with the diva: Hsp90-client interactions. J Mol Biol 430:3029–3040. https://doi.org/10.1016/j.jmb.2018.05.026.
5. Mayer MP, Le Breton L. 2015. Hsp90: breaking the symmetry. Mol Cell 58:8–20. https://doi.org/10.1016/j.molcel.2015.02.022.

6. Taipale M, Jarosz DF, Lindquist S. 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11:515–528. https://doi.org/10.1038/nrm2918.
7. Johnson JL. 2012. Evolution and function of diverse Hsp90 homologs and cochaperone proteins. Biochim Biophys Acta 1823:607–613. https://doi.org/10.1016/j.bbamcr.2011.09.020.
8. Genest O, Wickner S, Doyle SM. 2019. Hsp90 and Hsp70 chaperones: collaborators in protein remodeling. J Biol Chem 294:2109–2120. https://doi.org/10.1074/jbc.REV118.002806.
9. Genest O, Hoskins JR, Camberg JL, Doyle SM, Wickner S. 2011. Heat shock protein 90 from *Escherichia coli* collaborates with the DnaK chaperone system in client protein remodeling. Proc Natl Acad Sci U S A 108:8206–8211. https://doi.org/10.1073/pnas.1104703108.
10. Nakamoto H, Fujita K, Ohtaki A, Watanabe S, Narumi S, Maruyama T, Suenaga E, Misono TS, Kumar PKR, Goloubinoff P, Yoshikawa H. 2014. Physical interaction between bacterial heat shock protein (Hsp) 90 and Hsp70 chaperones mediates their cooperative action to refold denatured proteins. J Biol Chem 289:6110–6119. https://doi.org/10.1074/jbc.M113.524801.
11. Morán Luengo T, Kityk R, Mayer MP, Rüdiger S. 2018. Hsp90 breaks the deadlock of the Hsp70 chaperone system. Mol Cell 70:545–552.e9. https://doi.org/10.1016/j.molcel.2018.03.028.
12. Bardwell JC, Craig EA. 1988. Ancient heat shock gene is dispensable. J Bacteriol 170:2977–2983. https://doi.org/10.1128/jb.170.7.2977-2983.1988.
13. Thomas JG, Baneyx F. 2000. ClpB and HtpG facilitate de novo protein folding in stressed *Escherichia coli* cells. Mol Microbiol 36:1360–1370.
14. Motojima-Miyazaki Y, Yoshida M, Motojima F. 2010. Ribosomal protein L2 associates with *E. coli* HtpG and activates its ATPase activity. Biochem Biophys Res Commun 400:241–245. https://doi.org/10.1016/j.bbrc.2010.08.047.
15. Yosef I, Goren MG, Kiro R, Edgar R, Qimron U. 2011. High-temperature protein G is essential for activity of the *Escherichia coli* clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system. Proc Natl Acad Sci U S A 108:20136–20141. https://doi.org/10.1073/pnas.1113519108.
16. Press MO, Li H, Creanza N, Kramer G, Queitsch C, Sourjik V, Borenstein E. 2013. Genome-scale co-evolutionary inference identifies functions and clients of bacterial Hsp90. PLoS Genet 9:e1003631. https://doi.org/10.1371/journal.pgen.1003631.
17. Honoré FA, Méjean V, Genest O. 2017. Hsp90 is essential under heat stress in the bacterium *Shewanella oneidensis*. Cell Rep 19:680–687. https://doi.org/10.1016/j.celrep.2017.03.082.
18. Sato T, Minagawa S, Kojima E, Okamoto N, Nakamoto H. 2010. HtpG, the prokaryotic homologue of Hsp90, stabilizes a phycobilisome protein in the cyanobacterium *Synechococcus elongatus* PCC 7942. Mol Microbiol 76:576–589. https://doi.org/10.1111/j.1365-2958.2010.07139.x.
19. Grudniak AM, Markowska K, Wolska KI. 2015. Interactions of Escherichia coli molecular chaperone HtpG with DnaA replication initiator DNA. Cell Stress Chaperones 20:951–957. https://doi.org/10.1007/s12192-015-0623-y.
20. Suzuki T, Miyauchi K. 2010. Discovery and characterization of tRNAIle lysidine synthetase (TilS). FEBS Lett 584:272–277. https://doi.org/10.1016/j.febslet.2009.11.085.
21. Nakanishi K, Bonnefond L, Kimura S, Suzuki T, Ishitani R, Nureki O. 2009. Structural basis for translational fidelity ensured by transfer RNA lysidine synthetase. Nature 461:1144–1148. https://doi.org/10.1038/nature08474.
22. Sauer RT, Baker TA. 2011. AAA+ proteases: ATP-fueled machines of protein destruction. Annu Rev Biochem 80:587–612. https://doi.org/10.1146/annurev-biochem-060408-172623.
23. Garcie C, Tronnet S, Garénaux A, McCarthy AJ, Brachmann AO, Pénary M, Houle S, Nougayrède J-P, Piel J, Taylor PW, Dozois CM, Genevaux P, Oswald E, Martin P. 2016. The bacterial stress-responsive Hsp90 chaperone (HtpG) is required for the production of the genotoxin colibactin and the siderophore yersiniabactin in *Escherichia coli*. J Infect Dis 214:916–924. https://doi.org/10.1093/infdis/jiw294.
24. Theodoraki MA, Caplan AJ. 2012. Quality control and fate determination of Hsp90 client proteins. Biochim Biophys Acta 1823:683–688. https://doi.org/10.1016/j.bbamcr.2011.08.006.
25. Edkins AL. 2015. CHIP: a co-chaperone for degradation by the proteasome. Subcell Biochem 78:219–242. https://doi.org/10.1007/978-3-319-11731-7_11.