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

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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, TiiS, 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 TiiS. 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 TiiS 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

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(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 TiIS protein, leading to bacterial growth (17). TiIS 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 TiIS has to be finely regulated in the cell, since an excess of TiIS could lead to depletion of the tRNA-AUG in favor of tRNA-AUA. On the other side, the absence of TiIS 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 TiIS protein: (i) the Hsp90 chaperone for protection and activation and (ii) the HslVU protease for degradation. Therefore, the level of TiIS is precisely adjusted in the cell to allow correct protein translation and bacterial growth.

The absence of HslVU suppresses the phenotype of the $\Delta hsp90_{so}$ 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 $\Delta hsp90_{so}$ 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 $\Delta hsp90_{so}$ strain at high temperature in liquid and solid media (Fig. 1A and B, compare the $\Delta hsp90_{so} \Delta hslVU$ strain with the $\Delta hsp90_{so}$ strain). In contrast, the ClpP protease did not seem to be involved in this pathway since no growth improvement was observed in the $\Delta hsp90_{so} \Delta clpP$ strain compared to the $\Delta hsp90_{so}$ 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 $\Delta hsp90_{so} \Delta hslVU$ strain as expected, whereas production of Hsp90_{so} complemented the phenotype of the $\Delta hsp90_{so}$ strain.

Altogether, these results support the idea that some essential Hsp90_{so} clients that are degraded in the $\Delta hsp90_{so}$ 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 TiIS is degraded by HslVU. Since we have previously shown that the Hsp90_{so} client TiIS is responsible for the growth defect of the $\Delta hsp90_{so}$ 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 TiIS with a 6× His tag was introduced in the different genetic backgrounds of *S. oneidensis*. The strains were grown at sublethal high temperature, TiIS expression was induced, and its amount was determined by Western blot analysis.

As already seen, we found that about 85% of TiIS was degraded in the $\Delta hsp90_{so}$ strain compared to the wild type (Fig. 2A and B) (17). Interestingly, the TiIS level was dramatically increased in the $\Delta hsp90_{so} \Delta hslVU$ strain, reaching more than 15 times the level observed in the absence of Hsp90_{so}. These results strongly support the idea that

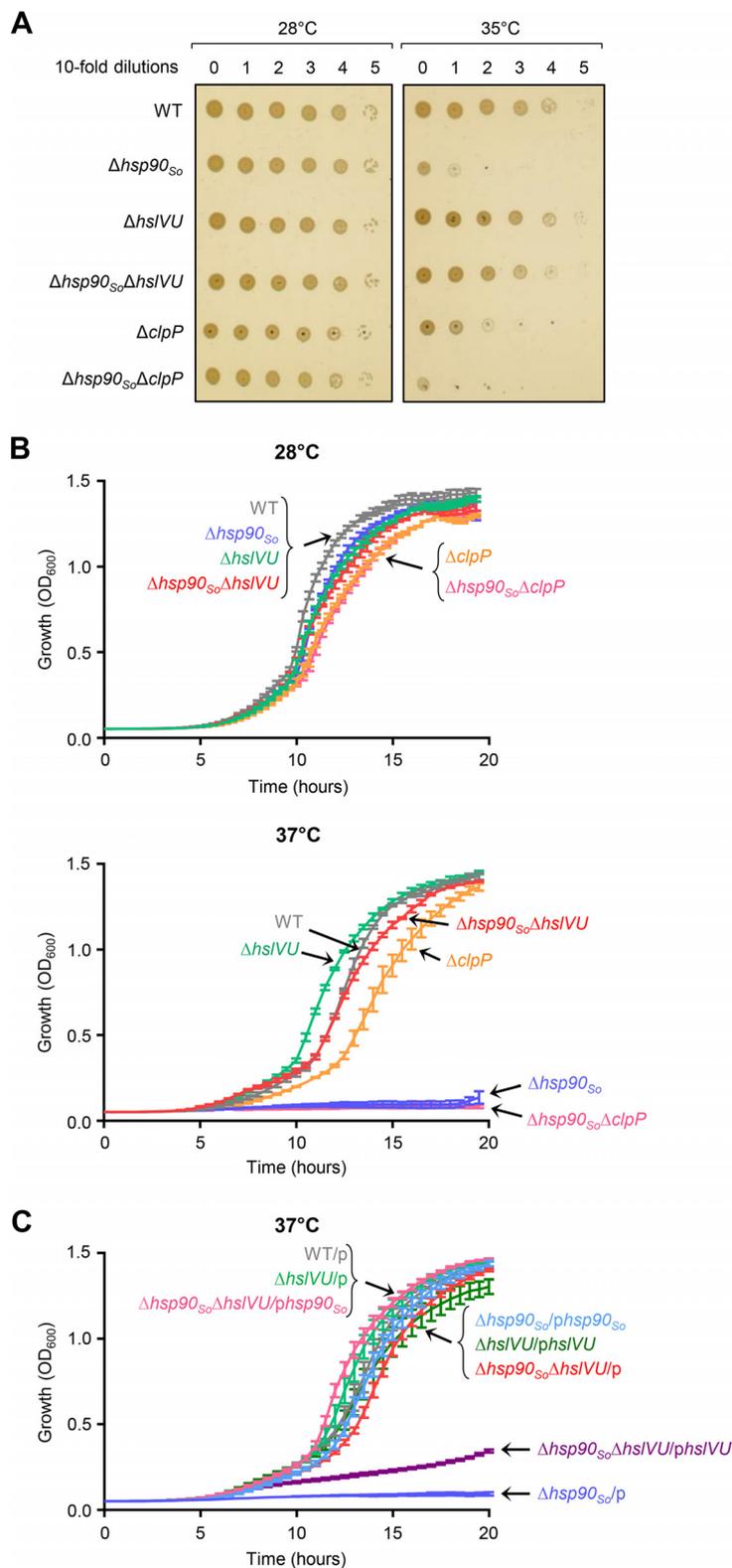


FIG 1 *hslVU* deletion suppresses the growth phenotype of the $\Delta hsp90_{so}$ strain at high temperature. (A) Strains grown at 28°C to late exponential phase were diluted to an optical density at 600 nm (OD_{600}) of 1, and 2 μ l of 10-time serial dilutions was spotted onto LB agar plates. The plates 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 \pm standard error of the mean (SEM).

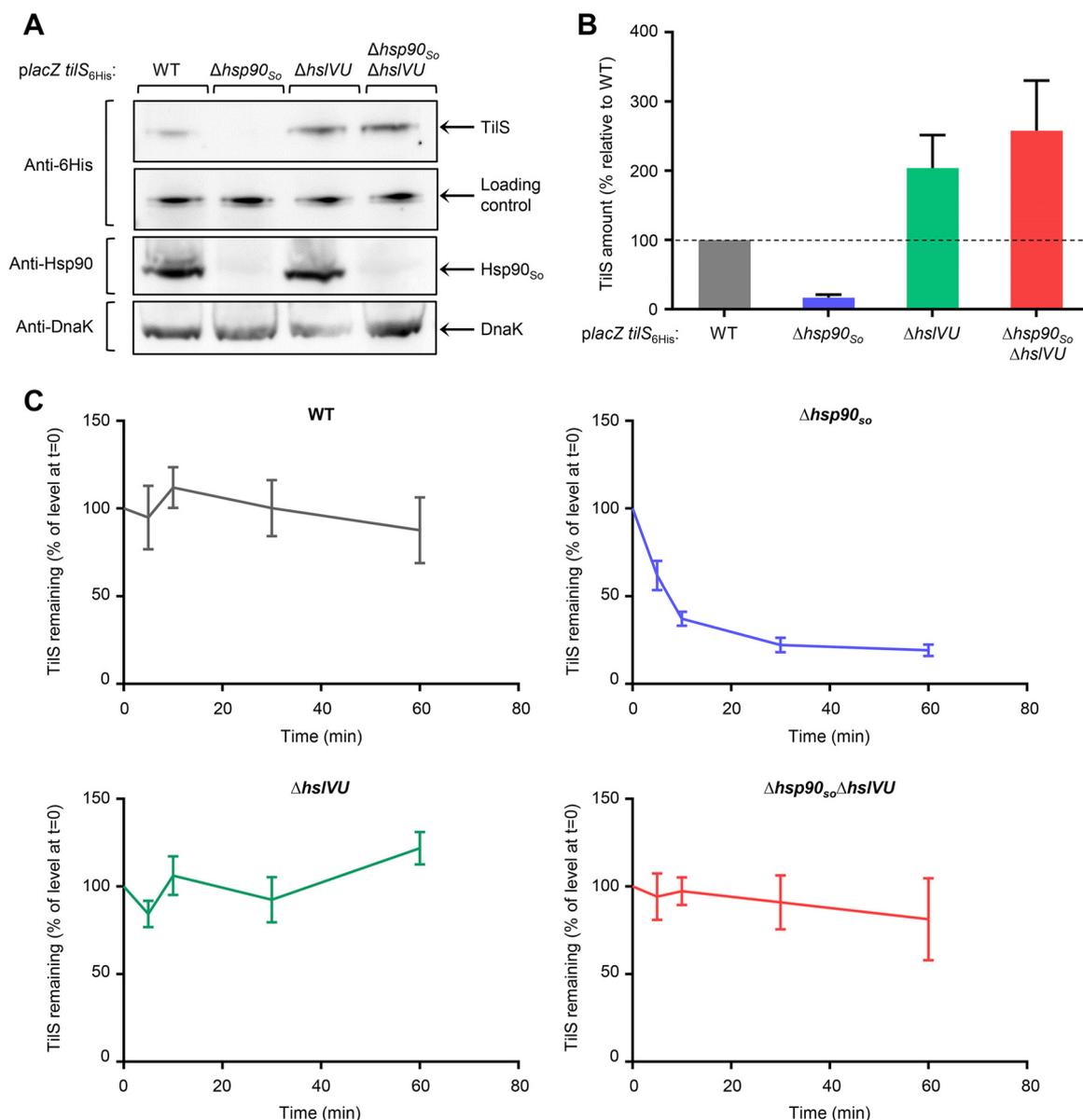


FIG 2 The HsIVU protease degrades the Hsp90_{So} client TiIS. (A) Strains containing the *placZ tilS_{6His}* plasmid, in which *lacZ* and *tilS_{6His}* are two independent genes under the control of the P_{BAD} promoter, were grown at 35°C, a sublethal temperature. At an OD₆₀₀ of 0.6, 0.02% arabinose 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 TiIS 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 TiIS was performed from 3 independent Western blots described in panel A, revealed with the anti-6× His antibody using ImageJ software. The amount of TiIS measured in the wild-type strain was set to 100%. Data are shown as mean ± SEM. (C) Chase experiments. Strains containing the *placZ tilS_{6His}* plasmid were grown as in panel A, except that 0.2% arabinose was added to increase the level of the TiIS protein, in particular in the Δhsp90_{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 TiIS measured in each strain at t = 0 (chloramphenicol addition) was set to 100%. Data are shown as mean ± SEM.

TiIS is degraded by the HsIVU protease. In the ΔhslVU strain, we observed that the TiIS level is higher than in the wild-type strain, suggesting that in the presence of Hsp90_{So}, part of the pool of TiIS is degraded by the HsIVU protease. In addition, we found that most of TiIS 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 TiIS degradation in the different genetic backgrounds. Strains containing the plasmid coding for TiIS 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 TiIS 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}, TiIS 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 TiIS is degraded by the HslVU protease in the absence of Hsp90.

In this article, we found that the level of TiIS 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 TiIS protein. Interestingly, this result is reminiscent of our previous work, in which overproduction of TiIS led to growth of the Δ *hsp90_{so}* strain at high temperature (17). Therefore, increasing the level of TiIS 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 TiIS 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.

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