In vivo disassembly and reassembly of protein aggregates in

*Escherichia coli*

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Running title: PA assembly in *E. coli*
Abstract

Protein misfolding and aggregation are inevitable but detrimental cellular processes. Cells therefore possess protein quality control mechanisms based on chaperones and proteases that (re)fold or hydrolyze unfolded, misfolded and aggregated proteins. Besides these conserved quality control mechanisms, the spatial organization of protein aggregates (PAs) inside the cell has been proposed as an important additional strategy to deal with their cytotoxicity. In the bacterium *Escherichia coli*, however, it remained unclear how this spatial organization is established and how this process of assembling PAs in the cell poles affects cellular physiology.

In this report, high hydrostatic pressure was used to transiently reverse protein aggregation in living *E. coli* cells, allowing the subsequent (re)assembly of PAs to be studied in detail. This approach revealed PA assembly to be dependent on intracellular energy and metabolic activity, with the resulting PA structure being confined to the cell pole by nucleoid occlusion. Moreover, a correlation could be observed between the time needed for PA reassembly and the individual lag time of the cells, which might prevent symmetric segregation of cytotoxic PAs among siblings to occur and ensure a rapid spatial clearance of molecular damage throughout the emerging population.
Introduction

Protein misfolding is an inevitable process in cellular life, often aggravated by genetic defects and/or a number of stresses encountered in the environment (1-6). Since misfolded proteins typically expose hydrophobic residues that are normally buried within their native structure, they tend to aggregate with each other into larger insoluble structures termed protein aggregates (5). Misfolded and aggregated proteins not only reduce the concentration of functional proteins and squander the cellular time and energy invested in their translation (7), but have also been shown to be cytotoxic themselves by mediating aberrant interactions with other proteins (8), sequestering cellular folding aids away from other (essential) proteins (9), and possibly even affecting the integrity of lipid membranes (10). As such, this interference can (progressively) compromise cellular fitness (3, 11), and even extrapolate into debilitating neurodegenerative diseases in humans (12-14).

Due to the link with general cellular degeneracy, protein misfolding and aggregation are intensively studied processes in both prokaryotes and eukaryotes (15). Using a combination of biochemical, biophysical, structural and genetic approaches, this has led to an extensive knowledge of protein quality control mechanisms and aggregation in vitro, as well as the identification of a number of genes involved in these processes (16-19). These quality control mechanisms are highly conserved and essentially consist of chaperones and proteases that respectively (re)fold or hydrolyze unfolded, misfolded and aggregated proteins (17-22). In spite of this elaborate protein homeostasis network, however, the accumulation and aggregation of misfolded proteins to some extent remains inevitable (5, 23), and recent studies have forwarded
spatial organization as another important strategy of dealing with protein aggregates (PAs) inside the cell (24).

Recently, it became possible to monitor the emergence and fate of protein aggregates (PAs) inside living cells, using reporter systems based on fluorescently labeled heat shock proteins (e.g. IbpA of *E. coli*) or thermolabile proteins, which themselves interact or associate with PAs (2, 11, 25). These reporter systems have in turn revealed the specific sequestration and asymmetric inheritance of cellular PAs during growth and division in bacteria (2, 11, 25), yeast (4) and higher eukaryotes (26), altogether suggesting a general rejuvenation strategy through which cytotoxic PAs preferentially remain linked to the older compartment upon cell division (24). In the *Escherichia coli* model system, for example, the polar localization and subsequent asymmetric segregation of cellular PAs was shown to be at least partially responsible for the previously observed pattern of aging in clonal lineages of this bacterium. In fact, cells inheriting the older and PA bearing pole were shown to display a diminished growth rate, reduced offspring production and higher death rate (2, 11, 27). How *E. coli* manages to sequester PAs to one of the cell poles, however, still remains controversial, and active as well as passive mechanisms have been proposed. More specifically, one study claimed this process to be dependent on the actions of the proton motive force, cytoskeleton (MreB) and chaperones (DnaK and DnaJ) (25), while others provide evidence for nucleoid occlusion as a passive mechanism that is responsible and sufficient for the polar sequestration of PAs (2, 28).

In order to further elaborate on the intracellular organization and dynamics of PAs, we devised a new model system in which high hydrostatic pressure (HP) was used as a perturbant to reverse PA assembly in living *E. coli* cells, based on the ability of HP to thermodynamically interfere...
with the ionic and hydrophobic interactions that hold PAs together (29-32). Aside convincingly demonstrating that PA reassembly is an energy-dependent (i.e. active) process and that PAs are spatially constrained by nucleoid occlusion, this model system could also reveal the existence of a clear link between PA assembly and growth resumption, suggesting a novel cellular strategy that prevents growth as long as dispersed PAs have not been reassembled and positioned sufficiently.

Materials and methods

Strain construction and growth conditions

The ibpA-yfp locus of *E. coli* MGAY (11) was P1-transduced into *E. coli* MG1655 and its HP resistant derivative LMM1010 (33), resulting in MG1655 *ibpA-yfp* and LMM1010 *ibpA-yfp*, respectively. Different deletion strains of LMM1010 *ibpA-yfp* (**Δ** dnaK, **Δ** dnaJ, **Δ** clpB, **Δ** lon, **Δ** hslU, **Δ** hslV, **Δ** clpX and **Δ** recA) were constructed based on the protocol of Datsenko and Wanner (34), and using an amplicon prepared on pKD13 using the primers listed in Baba *et al.* (35). This procedure replaced the genes of interest with an *frt* flanked kanamycin resistance cassette, which could subsequently be excised by transiently equipping this strain with plasmid pCP20 expressing the Flp site-specific recombinase (36), resulting in the desired deletion strain.

For culturing bacteria, Lysogeny Broth (LB) medium (37, 38) was used either as a broth, or solid medium after the addition of 2 % agarose (for agar pads intended for microscopy). Stationary phase cultures were obtained by growing *E. coli* overnight for approximately 15 hours in LB broth at 37°C under well aerated conditions (200 rpm on an orbital shaker). When appropriate,
the following chemicals (Applichem, Darmstadt, Germany and Sigma-Aldrich) were added to the medium at the indicated final concentrations: chloramphenicol (30 µg/ml), DAPI (4',6-diamidino-2-phenylindole) (1 µg/ml) and CCCP (carbonyl cyanide m-chlorophenyl hydrazine) (1-20 µM).

**High hydrostatic pressure (HP) treatment**

HP treatment was performed essentially as described previously (39). Briefly, cells from a stationary phase culture were harvested by centrifugation (4000 ×g, 5 min) and resuspended in an equal volume of 0.85 % KCl. Subsequently, a 200 µl portion of resuspended cells was heat sealed in a sterile polyethylene bag after exclusion of the air bubbles and subjected to 300 MPa for 15 min in an 8-ml pressure vessel (HPIU-10000, 95/1994; Resato, Roden, The Netherlands), held at 20°C with an external water jacket connected to a cryostat. Finally, at the end of the holding time, decompression was almost instantaneous. After HP treatment, populations were aseptically retrieved from the polyethylene bags, transferred to LB agarose pads placed on a microscopy slide, and subjected to microscopy as described below.

In cases where the same cells were microscopically examined before and after HP shock, cells were first mounted on a microscopy slide as described below and analyzed microscopically while noting their spatial coordinates on the slide. Subsequently, the slide as a whole was subjected to HP shock (300 MPa, 15 min, 20°C) using a 590ml vessel (Warm Isostatic Press, SO. 5-7422-0EPSI, Temse, Belgium) after which the spatial coordinates were used to trace back and microscopically follow-up the same cells on the pressurized slide.
Fluorescence microscopy and image analysis
For imaging, cell suspensions were transferred to agarose pads placed on a microscopy slide, and mounted with a cover glass as described before (40). A Gene Frame (Thermo Scientific) was used to physically fix the cover glass to the microscopy slide. Subsequently, fluorescence microscopy and time-lapse fluorescence microscopy were performed with a temperature controlled (Okolab, Ottaviano, Italy) Ti-Eclipse inverted microscope (Nikon, Champigny-sur-Marne, France) equipped with a 60x objective, a Ti-CT-E motorized condenser, a YFP filter (Ex 500/24, DM 520, Em 542/27), a DAPI filter (Ex 377/50, DM 409, Em 447/60) and a CoolSnap HQ2 FireWire CCD-camera. Images were acquired using NIS-Elements software (Nikon) and resulting pictures were further handled with open source software ImageJ. For further analysis, cell meshes were obtained from the original images using the open source, MATLAB-based software MicrobeTracker (41) and fluorescent spots were manually detected using the SpotFinder tool from MicrobeTracker.

Determination of viability
Cellular viability was determined either through spot-plating experiments or time-lapse microscopy. In spot-plating experiments, the appropriate dilutions of a sample were prepared in 0.85 % KCl and subsequently spot-plated (5 µl) on LB agar. After 24 hours of incubation at 37°C, the plates were counted and the number of survivors in CFU per ml was determined. Please note that the detection limit for these spot-plated samples was 200 CFU/ml. When cellular viability was determined via time-lapse microscopy, the number of cells (out of 100-300 randomly chosen cells per independent experiment) that were able to grow and divide within a 3 h time frame were scored. Please note that the detection limit for viability in the time-lapse...
microscopy samples varies between experiments and depends on the number of randomly chosen cells, from 1/100 cells or 1 % to 1/300 cells or 0.33 %.

**Energy depletion experiments**

To investigate the energy requirements of PA reassembly, different concentrations of CCCP (1-20 µM) were added to nutrient-free agarose pads used for microscopy. In cases where PA reassembly was monitored after CCCP treatment, cells were first treated with CCCP (for 3 h) in a nutrient-free environment, washed, and subsequently placed on LB agarose pads to allow PA reassembly to be monitored under the microscope.

**Data analysis**

The distribution of fluorescent PA-foci was obtained by determining their relative localization along the cell axis using the MicrobeTracker software (41). PA reassembly was determined via time-lapse microscopy, by visually scoring the number of cells that were able to assemble their PAs after HP exposure. Reassembly was considered completed when a cell was able to reassemble its PAs into one or two larger PAs (located in the cell poles) within a time frame of 3 h.

Lag phase measurements were based on the measured area of the meshes generated by the MicrobeTracker program (41). First, an initial area was calculated as the mean of the first three measurements (corresponding to the first 9 minutes after time-lapse recording had begun) for each individual cell. The area of a cell in the subsequent frames was then compared to this initial area, and length of the lag phase was defined as the time corresponding to the frame where a cell’s area had increased over 10 % compared to its initial area, plus the time between the end of
HP exposure and the beginning of recording (typically around 15 min). This 10% increase in initial area was taken as a threshold to prevent random measurement fluctuations from influencing the results and ensure that only lag times of cells that had fully committed to growth were measured. During lag time measurements, the assembly of fluorescent PA-foci was also tracked using the SpotFinder tool (41). This allowed the simultaneous comparison of PA reassembly and growth in individual cells.

**Results and discussion**

**HP mediated reversal of protein aggregation in *E. coli***

In order to examine the impact of HP exposure on intracellular PAs, we exposed *E. coli* MG1655 *ibpA-yfp* (in which the IbpA-Yfp fusion protein labels the existing PAs as fluorescent foci; (11)) to 300 MPa for 15 min, an HP intensity shown *in vitro* to be able to dissociate oligomeric proteins into subunits (29). Interestingly, while PAs were typically singular and positioned in one of the cell poles before HP shock (Fig. 1A and B) (as described previously; (11)), a number of smaller PA-foci that were randomly distributed across the cytoplasm appeared after HP shock (Fig. 1C and D). Further analysis confirmed that these smaller PAs arose from the disassembly of the larger and polarly located pre-existing PA rather than from the *de novo* emergence of new PAs, since (i) cells devoid of PAs before HP exposure also remained devoid of PAs after HP (Fig. 1A and B), and (ii) the average cellular fluorescence (and thus IbpA-Yfp concentration) within each cell was not increased after HP shock (Fig. S1A). Interestingly, the extent to which the polar PA became dispersed was very heterogeneous across the population, with the final
number of smaller PAs generated in pressurized cells ranging from 1 to 12 (with an average of 5.45 per cell) (Fig. 1 and S1B). Since the absence of HP gradients during pressurization can be reasonably assumed, this observation might be indicative for the heterogeneity in biophysical properties among polar PAs.

Surprisingly, although their smaller size should in principle have increased their diffusibility, the mobility of HP-dispersed cellular PAs seemed to be abolished (Fig. S1C). Since HP exposure killed the vast majority of MG1655 cells (Fig. S1D), this observation suggested that viability is required to sustain intracellular PA mobility. The ibpA-yfp PA reporter locus was therefore transduced into LMM1010 (a HP resistant derivative of MG1655; (33)), after which the resulting strain was similarly exposed to HP. While PAs in pressurized LMM1010 ibpA-yfp cells were also dispersed (Fig. S2A-C), most of them (ca. 75 %; Fig. S1D) survived HP exposure and were able to (re)assemble their dispersed PAs into their cell pole (Fig. 2), thus restoring the situation before HP shock.

**Cellular requirements for PA assembly and localization**

Subsequently, this model system allowed a closer examination of the structural and physiological requirements underlying intracellular PA dynamics during (re)assembly. To examine the possible role of a number of chaperones and proteases, the effect of their deletion on PA organization before and PA reassembly after HP exposure was scored (Table 1). In unexposed cells, none of these deletions appeared to effect PA organization, leaving cellular PAs typically singular and positioned in one of the cell poles. In HP exposed cells, the dnaK or dnaJ deletions rendered the cells highly sensitive towards the HP treatment and decreased cellular viability dramatically.
with concomitant abolition of PA mobility. In contrast to the dnaK mutant where this reduced viability made it impossible to study the role of this chaperone in PA reassembly, the few surviving cells of the dnaJ mutant were also able to reassemble their PA’s. Taken together, these observations indicate that, in contrast to what was previously reported (25), the chaperone DnaJ is not absolutely required for PA reassembly. The same holds for all the other tested chaperones and proteases: while the survival of their respective deletion strains was not significantly altered, PA reassembly occurred normally in these strains, indicating that these proteins exhibited no significant role in proper PA reassembly (Table 1).

Because none of the tested chaperones or proteases were clearly found to be required for PA assembly, we set forward to identify other required cellular factors. One of such factors, although currently still controversial, is the possible dependence of PA assembly on intracellular energy. To investigate this, we exposed LMM1010 ibpA-yfp cells after HP treatment to CCCP (carbonyl cyanide m-chlorophenyl hydrazine), an oxidative phosphorylation uncoupling agent that was previously shown to reduce cellular energy (2). Moreover, by exposing these cells to CCCP in an environment without nutrients, we also attenuate other cellular sources of energy such as substrate-level phosphorylation in glycolysis. Compared to pressurized cells without CCCP, the resulting cells displayed a strongly reduced mobility of their dispersed PAs and a concomitantly reduced capacity to reassemble them, with this effect being proportional to the CCCP concentration used (Fig. 3A). Importantly, none of the treatments with CCCP affected cellular viability (Fig. S3), indicating that the observed decrease in PA mobility and assembly was not due to a decrease in cellular survival but to the presence and action of the compound itself. Moreover, when HP exposed cells were treated with CCCP (for 3 h), washed, and subsequently placed on LB agarose pads, PA reassembly, which was halted until then due to the action of
CCCP, again proceeded normally (Fig. 3B and C). These findings indicate that the energy-depletion effect of the CCCP uncoupler is reversible and energy repletion occurs when fresh nutrients are supplied. It is therefore important to continuously expose the cells to CCCP or other ATP-depleting compounds in a nutrient-free environment (an approach that contrasts those previously used in studies where the energy-dependence of PA assembly in E. coli was investigated (2, 25)), in order to be able to truly investigate the energy dependency of this process. Although these results demonstrate PA reassembly to be an energy-dependent process, the synthesis of novel proteins did not account for this dependence, since a translation inhibitor such as chloramphenicol did not prevent PA reassembly in HP exposed cells (Fig. 3A).

Interestingly, while an active PA assembly process might at first sight contradict previous studies that have shown and modeled PA movement and assembly to be strictly diffusion-based (2, 28), a unifying explanation might stem from the most recently observed glass-like properties of the bacterial cytoplasm (42). More specifically, by tracking the intracellular movement of protein filaments, plasmids, storage granules, and foreign particles of different sizes, Parry et al. (42) found that the bacterial cytoplasm displays glass-like properties which restrict the mobility of cytoplasmic components in a size-dependent fashion. Strikingly, they also found that cellular metabolism fluidizes the cytoplasm, allowing larger components, normally increasingly constrained with increasing size, to escape their local environment and explore larger regions of the cytoplasm. As such, PAs might be seen as macromolecules of which the diffusive movement not only requires cellular energy (in order for metabolic activity to be able to fluidize the cytoplasm) but where the amount of energy needed is also dependent on macromolecular crowding and PA size. This in turn offers an explanation for studies in which protein aggregation...
was reported to be energy-independent and their movement strictly diffusive, since these studies mainly focused on smaller PAs (2, 28).

The impact of the nucleoid on assembling and localizing cellular PAs to one of the poles has been debated as well (2, 25, 28), and to examine this in our model system, a Δ-recA derivative of LMM1010 ibpA-yfp was constructed because of its natural propensity to frequently produce anucleate cells (43). When the latter cells of such an HP exposed population were examined, their behavior was similar to wild type nucleated cells in terms of reassembling dispersed PAs into a singular PA (Fig. 4A). In strong contrast to the situation in nucleated cells, however, this PA was not confined to the cell pole anymore, but instead roamed freely throughout the entire cytoplasm (Fig. 4B). In addition, when looking at the relative localization of PAs in nucleate and anucleate cells after PA reassembly, we found that, while PAs in nucleated cells were again preferentially located in the cell pole, PAs in anucleate cells displayed a more random localization (Fig. 4C and D). In support of these findings, PAs in anucleate control cells, both in LMM1010 and MG1655 ibpA-yfp Δ-recA cells, also appeared to roam freely throughout the entire cell without being restricted to a specific (polar) location (Fig. S4A and B). These observations clearly prove that the presence of the nucleoid enforces a polar localization on assembled PAs, although it does not per se interfere with the assembly process itself.

Dispersed PAs suspend growth resumption

Upon further analyzing cellular PA reassembly in a HP exposed LMM1010 ibpA-yfp population more quantitatively (Fig. 5), we noticed that cells never seemed to commit to growth (defined
here as a microscopically detectable increase in cell surface) as long as intracellular PAs were still dispersed. In fact, regardless of the extent of HP-induced PA dispersion (which ranged from 1 to 12 PAs per cell; Fig. 5A and S2D), cells did not resume growth until the reassembly process yielded one (in 61.5 % of cases; 104/169), two (in 36.7 % of cases; 62/169) or –exceptionally– three (in 1.8 % of cases; 3/169) PAs (Fig. 5B), of which the location seem to match the three stable PA-repositories in the cell (i.e. both poles and midcell) reported earlier (2, 11). In addition, a good correlation was also found between individual cellular lag times (defined as the time needed for a cell to resume growth after HP exposure) and the corresponding times needed to reassemble their dispersed PAs (Fig. 5B). Although it remains to be established whether there is a causal relationship between these two phenomena, it could be hypothesized that dispersed PAs functionally interfere with essential cellular processes responsible for growth (e.g. because the cytotoxic PA surface area exposed in the cytoplasmic milieu increases significantly upon dispersion) or even that such processes might be deliberately halted until the proper assembly of PAs allows their asymmetric segregation (and subsequent riddance) among siblings. Either way, such behavior would prevent cytotoxic PAs to be randomly partitioned among siblings, and would ensure a rapid spatial clearance of molecular damage throughout the emerging population, which has previously been forwarded to be most effective in maintaining high growth rates (44-46). Nevertheless, repair or other resuscitation processes different from PA assembly are likely to contribute to cellular lag times after HP stress as well.

In a related context, it has most recently been shown in Caulobacter crescentus that proteotoxic stress stimulates the production and activity of the Lon protease, in turn leading to the degradation of the DnaA replication initiator and a subsequent arrest in chromosome replication (47). Although it remains to be established whether the PA-based growth arrest suggested in this
study and the Lon/DnaA effect revealed by Jonas et al. (47) are mechanistically similar, both could support a more universal cellular strategy in which cellular proliferation is halted until the proper spatial alleviation or structural repair of protein damage.

**Conclusion**

In summary, we have elaborated a unique model system to study the assembly of PAs and its consequences on cellular physiology, based on the thermodynamically enforced *in vivo* dispersion of pre-existing PAs inside the cell by high hydrostatic pressure. Using this model system, we were able to clearly establish that the assembly of PAs is dependent on intracellular energy and metabolic activity, with the resulting (larger) PA being confined to one of the cell poles by nucleoid occlusion. Moreover, by closely monitoring the PA assembly process and its impact on the timing of resuscitation, we provide possible evidence for a cellular strategy that postpones growth and proliferation until proper PA assembly is achieved.

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**References**


**Figure Legends**

**Figure 1.** *In vivo* HP exposure leads to PA disassembly in *E. coli* MG1655 *ibpA*-yfp.

(A and C) Microscopic images show the same MG1655 *ibpA*-yfp cells (A) before and (C) after HP exposure (300 MPa, 20°C, 15 min). Phase contrast images are superimposed with YFP epifluorescence images (reporting PAs), and the scale bar corresponds to 1 µm. The black arrow indicates a cell devoid of PAs. (B and D) Binned histograms show PA distribution along the relative axial position of the cells as detected in (B) untreated and (D) HP exposed (300 MPa, 20°C, 15 min) MG1655 *ibpA*-yfp cells. Average number of PA-foci per cell was 1.00 for control cells (n = 427) and 5.45 for HP exposed cells (n = 640).

**Figure 2.** HP-induced disassembly and reassembly of PAs in *E. coli* LMM1010 *ibpA*-yfp.

Representative images of a time-lapse fluorescence microscopy image sequence of the same cell before and after HP exposure (300 MPa, 20°C, 15 min), showing the reassembly of dispersed PAs into one larger polar PA and subsequent outgrowth of the cell. YFP epifluorescence images
Figure 3. PA reassembly after HP exposure is an energy-dependent process, not dependent on the synthesis of novel proteins.

(A) Treatment of an LMM1010 *ibpA-yfp* population with indicated concentrations of CCCP after HP exposure (300 MPa, 20°C, 15 min) decreases the fraction of cells (n ≥ 115 per independent experiment) that are able to assemble their PAs within 3 hours. Asterisks indicate statistically significant differences from the control (i.e. no addition of CCCP) (Student’s t-test, α = 0.01, p = 0.003 for 20 µM and 5 µM CCCP, and p = 0.005 for 1 µM CCCP). Addition of chloramphenicol (30 µg/ml) to the growth medium of an HP exposed (300 MPa, 20°C, 15 min) LMM1010 *ibpA-yfp* population does not influence the ability of cells (n ≥ 120 per independent experiment) to reassemble their PAs (Student’s t-test, p = 0.28). Reassembly was considered completed when a cell was able to reassemble its PAs into one or two larger PAs (located in the cell poles) within 3 hours after HP exposure. The means of three independent experiments are shown with error bars representing the standard deviation. (B) and (C) Representative images of two time lapse fluorescence image sequences indicating the reversibility of CCCP-induced energy depletion. (B) PA assembly does not occur after HP exposure (300 MPa, 20°C, 15 min) in the presence of CCCP (20 µM). (C) PA assembly proceeds normally after CCCP is washed away and cells are supplied with fresh nutrients. YFP epifluorescence images (reporting PAs) in combination with cell outlines are shown at the indicated times after HP exposure. The scale bar corresponds to 1 µm.
Figure 4. Polar positioning of PAs is imposed by the nucleoid.

(A and B) Representative images of a time-lapse fluorescence microscopy image sequence of (A) a nucleoid-bearing LMM1010 *ibpA-yfp* cell, and (B) an anucleate LMM1010 *ibpA-yfp*Δ*recA* cell after HP exposure (300 MPa, 20°C, 15 min). DAPI (reporting the nucleoid) or YFP (reporting PAs) epifluorescence images in combination with cell outlines are shown at the indicated times after HP exposure. The scale bar corresponds to 1 µm. (C and D) Binned histograms show PA distribution along the relative axial position of the cells as detected in (C) nucleoid-bearing LMM1010 *ibpA-yfp* and (D) anucleate LMM1010 *ibpA-yfp*Δ*recA* cells after PA reassembly after HP exposure (300 MPa, 20°C, 15 min) was completed. Average number of PA-foci per cell was 1.18 for nucleoid-bearing cells (*n* = 224) and 1.00 for anucleate cells (*n* = 84).

Figure 5. Growth resumption is correlated with PA reassembly.

(A) Cumulative lag time distribution of LMM1010 *ibpA-yfp* cells after HP exposure (300 MPa, 20°C, 15 min) was combined with the PA reassembly process in these same cells (*n* = 169; derived from three independent experiments). The time individual cells needed to resume growth after HP exposure was determined and binned to create the cumulative lag time distribution. The mean cumulative lag time distribution of three independent experiments is shown with error bars representing the standard deviation per bin. At the same time, the number of individual PAs (visible as IbpA-YFP foci) inside these cells were monitored. Every line represents the PA assembly process of a single cell. Because of visibility, not all the monitored cells are included. (B) Correlation between the time a cell needs to reassemble its PAs and its corresponding lag time.
time, based on the dataset of panel A. A subdivision was made depending on the number of larger PAs a cell assembles its dispersed PAs into prior to growth resumption. Linear regression line (trend line, black) and Pearson’s correlation coefficient ($r = 0.4945$, p-value $= 2.35 \times 10^{-11}$) are based on all the cells together. Inset: fraction of cells resuming growth after HP treatment when PAs are reassembled into one, two or three larger PAs. The means of three independent experiments are shown with error bars representing the standard deviation.
TABLE 1 Overview of the effect of different chaperone or protease deletions on PA management in control and HP treated populations of LMM1010 \(ibpA-yfp\). Averages (Av.) and standard deviations (Stdev.) of three independent experiments are shown for both PA assembly and survival after HP treatment. Results are indicated as the relative fraction of cells.

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*Values were below the detection limit of 0.01