Dispose to the pole—protein aggregation control in bacteria

Szymon Ziętkiewicz and Krzysztof Liberek*

Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology, University of Gdańsk, Gdańsk, Poland

*Correspondence to: liberek@biotech.ug.gda.pl

The EMBO Journal (2010) 29, 869–870. doi:10.1038/emboj.2010.12

The disaggregation and refolding of polypeptides aggregated in bacterial cells in response to environmental stress is crucial for the development of thermotolerance. Although the mechanistic aspects of the chaperone-dependent disaggregation reaction are known, the spatio-temporal organization of this process is less understood. In this issue, Winkler *et al* (2010) present the results of technically advanced studies on the organization of this process and its biological consequences in bacterial cells.

Most cellular processes require the structure of a protein to be dynamic and flexible. As a consequence, proteins evolved to possess a relatively small margin of stability (Tartaglia *et al*, 2007). Minor changes in intracellular physicochemical conditions, for instance an increase in temperature over the physiological limit, destabilize proteins. Hydrophobic amino acid residues, buried inside proteins in their native conformation, become exposed under destabilizing conditions and thus promote intracellular protein aggregation. Such aggregation is a serious problem for all organisms, especially those like bacteria, which cannot regulate their internal temperature and are constantly challenged by environmental changes.

With protein aggregation occurring in response to heat exposure, the cell uses chaperone proteins to control and counteract this process. For example, the Hsp70- and Hsp104-dependent bi-chaperone system is capable of disaggregating aggregated polypeptides and subsequently refolding the liberated polypeptides into native proteins (Skowrya *et al*, 1990; Glover and Lindquist, 1998). The alternative scenario involves the proteolysis of the aggregated polypeptides. The

importance of the disaggregation pathway for the cell was shown by studies in which bacterial Hsp100 was modified so that it acted in the degradation pathway instead of disaggregation. As a result, a lack of thermotolerance was observed, even though intracellular aggregates were efficiently eliminated by proteolysis (Weibezahn *et al*, 2004).

Mechanistic studies of bi-chaperone-dependent disaggregation of aggregated polypeptides are relatively advanced (for a review, see Doyle and Wickner, 2009), but much less is known about the spatio-temporal organization of this process in a living bacterial cell. The featured study of the Bukau group makes an important contribution to our understanding of this process. Previously, it was established that following heat, stress-denatured proteins form aggregates at the cell poles of bacteria (Ignatova and Gierasch, 2004; Lindner *et al*, 2008; Rokney *et al*, 2009). Winkler *et al*, by analysing the fate of GFP-tagged, aggregation-prone proteins in real time, confirm polar aggregate localization and in addition show that disaggregating chaperones, as opposed to chaperones involved in protein degradation or folding, dynamically associate with these aggregates (Figure 1). It is suggested that the movement of misfolded proteins to the cell poles is based on their diffusion to regions free of the bacterial chromosome, and this movement seems to be energy independent. However, some controversy remains, as the work of Rokney *et al* (2009) suggests that the transport of misfolded aggregated proteins to the poles requires energy input.

The movement of misfolded proteins to the cell poles can be easily re-targeted to other locations by creation of artificial

Figure 1 Model of protein aggregation control in bacteria. Under heat shock conditions, the destabilized proteins form amorphous aggregates in nucleoid free space. They are assisted by disaggregating chaperones, which solubilize and reactivate the denatured proteins. The fact that the aggregates are placed at opposite cell poles leads to their asymmetric inheritance during subsequent cell divisions. This gives rise to generations of rejuvenated cells that are less—or not at all—burdened with aggregates.

aggregation seeds consisting of an aggregation-prone protein either anchored to the inner membrane or bound to DNA. These results also argue against active guidance of misfolded protein to the poles. As the membrane- or nucleoid-localized aggregates are not found in the absence of aggregation seeds, it suggests that proteins evolved such that initial protein aggregation events took place in the cytoplasm. As the misfolded proteins are deposited in the nucleoid free space, aggregates at the cell poles are formed. Consequently, this leads to the asymmetrical inheritance of denatured proteins, which are a burden to the cell. This was indeed observed, initially by Lindner *et al* (2008) for spontaneous protein aggregation under non-stressed conditions. The Bukau and Mogk group demonstrate this point further by performing single-cell real-time microscopic measurements of bacterial growth following heat shock. Their results show that the

daughter cells that are less burdened with heat-induced protein aggregates divide faster, as opposed to cells that inherit the majority of the mother cell's aggregates.

The featured report of Winkler *et al* provides a detailed analysis of the spatio-temporal organization of heat-induced protein aggregation in bacterial cells, allowing for a better understanding of heat shock recovery, elimination of aggregates and rejuvenation of bacterial cells.

Acknowledgements

We thank Foundation for Polish Science for support under TEAM programme.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Doyle SM, Wickner S (2009) Hsp104 and ClpB: protein disaggregating machines. *Trends Biochem Sci* **34**: 40–48
- Glover JR, Lindquist S (1998) Hsp104, Hsp70, & Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* **94**: 73–82
- Ignatova Z, Gierasch LM (2004) Monitoring protein stability and aggregation *in vivo* by real-time fluorescent labeling. *Proc Natl Acad Sci USA* **101**: 523–528
- Lindner AB, Madden R, Demarez A, Stewart EJ, Taddei F (2008) Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc Natl Acad Sci USA* **105**: 3076–3081
- Rokney A, Sagan M, Kassel M, Smith Y, Rosenshine I, Oppenheim AB (2009) *E. coli* transports aggregated proteins to the poles by a specific energy-dependent mechanism. *J Mol Biol* **392**: 589–601
- Skowyra D, Georgopoulos C, Zylicz M (1990) The *E. coli dnaK* gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. *Cell* **62**: 939–944
- Tartaglia GG, Pechmann S, Dobson CM, Vendruscolo M (2007) Life on the edge: a link between gene expression levels and aggregation rates of human proteins. *Trends Biochem Sci* **32**: 204–206
- Weibezahn J, Tessarz P, Schlieker C, Zahn R, Maglica Z, Lee S, Zentgraf H, Weber-Ban EU, Dougan DA, Tsai FTF, Mogk A, Bukau B (2004) Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. *Cell* **119**: 653–665
- Winkler J, Seybert A, König L, Pruggnaller S, Haselmann U, Sourjik V, Weiss M, Frangakis AS, Mogk A, Bukau B (2010) Quantitative and spatio-temporal features of protein aggregation in *E. coli* and consequences on protein quality control and cellular aging. *EMBO J* **29**: 910–923