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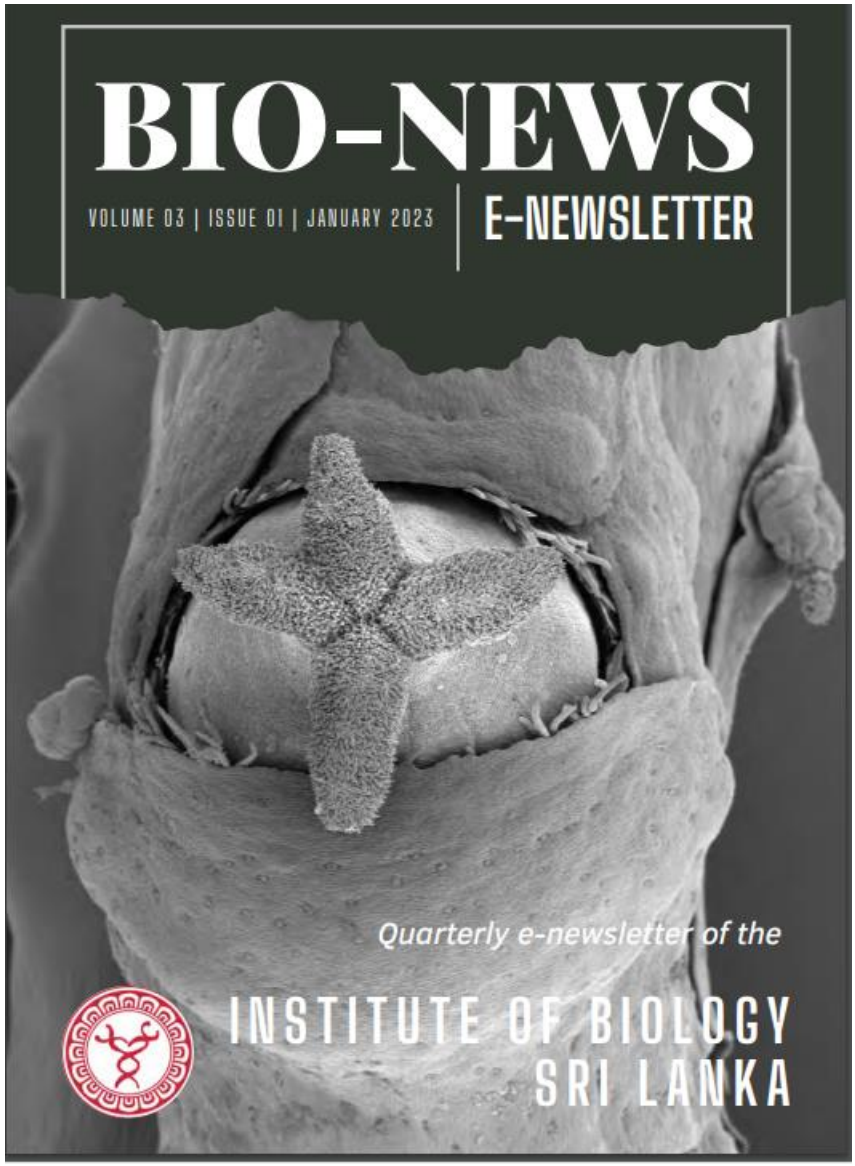

Molecular Chaperone ClpB Becomes a Novel Antimicrobial Target

Introduction

Proteins are the most versatile and complicated biological macromolecules. Protein aggregation is defined as a non-physiological association of misfolded/partially folded polypeptides. Proteostasis prevents or minimizes protein aggregation and keeps proteins soluble and active. In live cells, molecular chaperones and their regulators facilitate protein quality control and proteostasis. A molecular chaperone is a protein that helps other proteins reach their physiologically active native conformation without being present in the client protein's final functional structure. These proteins help *de novo* protein folding and refolding of misfolded/partially folded proteins under cellular stresses and thereby inhibit protein aggregation.

Evolution and biological functions of molecular chaperones

It is interesting to note that certain types of cells have developed a particular type of molecular chaperone with the unique capacity to disassemble protein aggregates and transform them into unstructured polypeptides (a reversal of protein aggregation).

Dr. Chathuranga B. Ranaweera, M. I. Biol. (Sri Lanka)
Department of Medical Laboratory Sciences
Faculty of Allied Health Sciences
General Sir John Kotelawala Defence University
Sri Lanka

Generally, nascent polypeptides fold to become functionally active (native state) proteins as they emerge from ribosomes. However, some polypeptides may misfold during this process leading to immediate protein aggregation. Under stress, specific proteins might misfold and lose their native structure. Cells have molecular chaperones to help polypeptides/proteins fold into their native state or guide misfolded polypeptides/proteins back into the functionally active native structure.

Cells produce chaperones GroEL/Hsp 60, DnaK/Hsp 70, DnaJ/Hsp 40, and GrpE/nucleotide exchange factor (NEF) for this purpose. Once protein aggregates are formed, they might concentrate in the cytoplasm and form inclusion bodies. Inefficient aggregate clearance can build up enormous clumps of proteins inside cells, leading to protein toxicity or cell death (Figure 1).

In bacteria, molecular chaperones are referred to as Caseinolytic peptidase B (ClpB), whereas yeast and plants are referred to as heat-shock protein (Hsp)104 and Hsp101, respectively. On the other hand, metazoans do not possess ClpB.

Molecular chaperon ClpB is a member of the Hsp100 family, and the proteins that belong to Hsp100 are members of the AAA+ superfamily. AAA+ superfamily proteins are associated with diverse cellular processes. Therefore, to perform their cellular operations, they need the energy provided by the hydrolysis of ATP. ClpB plays a vital role in bacterial protein homeostasis by reactivating aggregated proteins, which is essential for maintaining optimal cellular functions in a cellular environment. The proper balance of protein homeostasis is critical for maintaining optimal cellular processes.

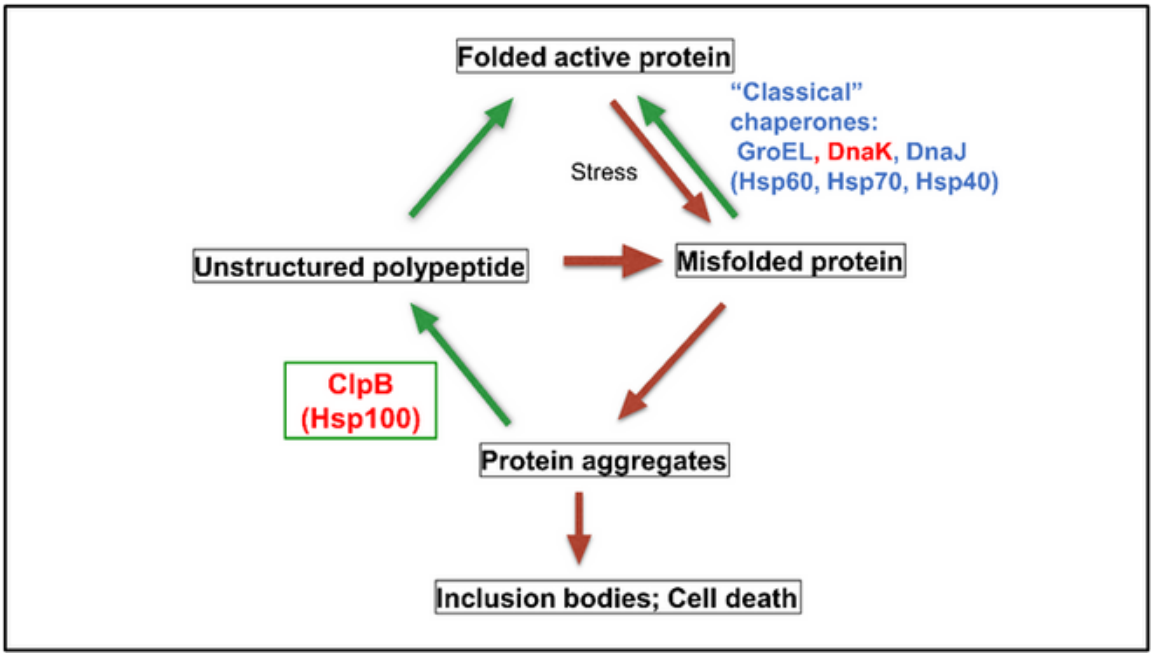

Figure 1 Folding, misfolding, and aggregation of proteins in a cellular milieu
Source: modified from Ranaweera (2021)

Proposed mechanism of the ClpB mediated protein disaggregation

ClpB-mediated protein aggregation reactivation and breakdown are associated with two other molecular chaperones, DnaK and DnaJ, and a NEF/GrpE that works in cycles. However, the exact ClpB-mediated protein aggregate reactivation and breakdown mechanism is still a mystery. Biologically active bacterial ClpB is a 575 kDa hexamer. In the presence of nucleotides (ATP or ADP), six ClpB monomers (each 95 kDa) combine into an active hexametric ClpB unit. The self-associating monomers form a narrow channel at the center of the hexamer. ClpB hydrolyses ATP to release polypeptides from aggregates. ClpB disassociates protein aggregates by threading either polypeptide ends (N or C terminus) or exposed loops of polypeptides through its central channel via ATP hydrolysis. DnaK is needed for ClpB to recognize protein aggregates and target them within a cell. ClpB can pull the peptide by both arms of the loop or switch to a single-arm translocation if resistance is encountered when extracting a polypeptide loop.

As the extracted polypeptide exits ClpB's central channel, the substrate can refold on its own or be transferred to other co-chaperones (Dnak, DnaJ-GrpE or GroEL-GroES). If peptide extraction from aggregates is unfeasible, a ClpB hexamer dissociates into its monomers and reassembles to engage a new loop or terminal. Multiple ClpB machines can work simultaneously on the same aggregate making aggregate breakdown fast and efficient (Figure 2).

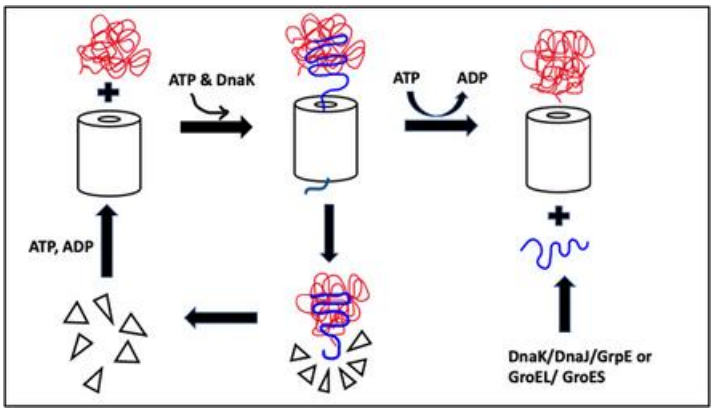

Figure 2 Proposed ClpB disaggregation mechanism. Six monomers are shown as triangles. Hexameric ClpB is shown as a cylinder with a central channel. Aggregated polypeptides are shown in red, and the extracted polypeptide is shown in blue.
Source: modified from Zolkiewski et al. (2012)

Therapeutic potential of ClpB as a novel antimicrobial target

To be considered as antimicrobial targets, targets must be essential for microbe life, have no analogous targets in humans, and be druggable *in vitro* and *in vivo* with small compounds. Few research groups have shown that the host-pathogen stress response mechanism can be a unique antibacterial target for medication development. Hsp100 chaperones are required for invasiveness and/or in-host survival of multiple significant bacterial and protozoan pathogens, including the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).

Moreover, bacterial heat shock response has emerged as a distinct target for antibiotic development. Pathogens endure heat shock and oxidative stress during infection, and their survival depends on molecular responses. The pathogen stress response is key to generating new antimicrobials.

No successful suppression of pathogen stress-response machinery has been developed due to the highly conserved nature of the Hsp sequence across many domains of life.

Loss of ClpB activity is deleterious to the survival of many pathogens, and no mammalian ClpB orthologs are known. Inhibiting Hsp100 may lower the infectivity and survival of therapeutically important pathogens without harming the harboring host. So far, no Hsp100-selective high-affinity inhibitors exist. Therefore, AAA+ ATPase inhibitors unrelated to Hsp100 could be exploited to generate Hsp100-selective ligands.

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