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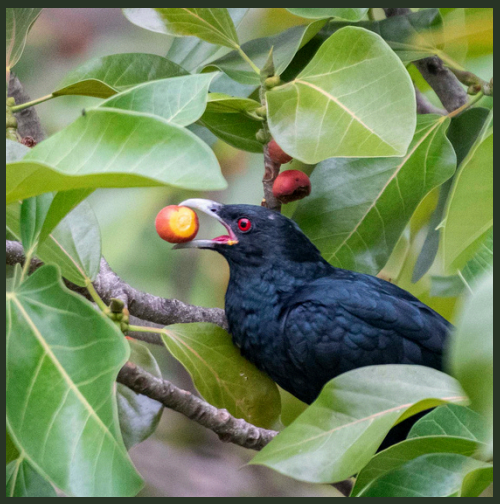

COVER STORY

The Asian koel (*Eudynamys scolopaceus*), commonly known as කොවුලා/කොහා (Kowla/Koha) in Sinhalese, is considered a symbol of the Sinhalese and Hindu New Year in Sri Lanka. The call is often heard during the breeding season around March-June, which aligns with the Sinhala/Tamil New Year, therefore it is called the "New Year Bird". The size of the bird is about 39-46 cm and the male bird has a metallic black tinged blue, long tail, and red eyes. Females are black to brown with white spots and barred underparts.

Common nesting sites are found in home gardens, cultivated and other wooded areas, avoiding dense forests from lowland to hilly areas. It eats almost entirely fruit, including berries of some ornamental garden plants. Because this bird is a nest parasite, it lays its eggs in the nests of other birds such as crows (Jungle crow and House crow) and sometimes in Bablers. Newborn chicks grow up with foster parents.

Photograph by Dr. Praneeth Ratnayake

CONTENTS

Chaperones to the Rescue: Tackling Neurodegenerative Diseases and Potential Therapeutic Applications

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Protein misfolding has been identified as a contributing factor in several neurodegenerative conditions, such as Alzheimer's disease (AD), Huntington's disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Parkinson's disease (PD). One common characteristic observed among individuals affected by these illnesses is the aggregation of deposits consisting of misfolded proteins. The occurrence of abnormal protein folding can lead to toxicity by either impairing or enhancing protein function, or both. One promising treatment strategy for these diseases is to use protein-remodeling factors to correct misfolded proteins and restore protein structure and function back to its native state. Hence, the interaction between chaperones and protein folding/degradation pathways plays a vital role in developing novel therapeutic drugs for these neurodegenerative diseases.

Chaperone-mediated protein homeostasis

Molecular chaperones are indispensable proteins that interact with unfolded and partially folded polypeptide chains, thereby impeding their aggregation and precipitation. Several molecular chaperones were initially characterized as heat shock proteins (Hsp) due to their upregulation in response to higher temperatures.

The folding and re-folding of substrate proteins, also known as clients, are essential for maintaining their functional state and structure. This process is facilitated by a network of molecules called chaperones and co-chaperones, which are highly conserved across various organisms. Molecular chaperones function by interacting with other proteins, thereby stabilizing them and assisting in achieving their native conformation. With a widespread presence in cells, they play a crucial role in facilitating the folding of recently produced proteins, as well as the re-establishment of partially folded proteins into their respective three-dimensional configurations.

Chaperones play a crucial role in maintaining intracellular protein homeostasis by engaging protein degradation pathways responsible for regulating the continuous turnover of proteins and eliminating misfolded proteins. Molecular chaperones in bacteria are called Caseinolytic peptidase B (ClpB), while in yeast and plants, they are called heat-shock protein (Hsp)104 and Hsp101, respectively. Conversely, metazoans lack ClpB. In recent times, ClpB and Hsp104 have become the main focal points of protein research and the development of novel therapeutic drugs.

Caseinolytic peptidase B (ClpB)/heat-shock protein (Hsp)104 regulation

In an ATP-dependent manner, Hsp100 disaggregases (known as Hsp104 in *Saccharomyces cerevisiae* and ClpB in *Escherichia coli*) drive aggregated substrates as single polypeptides through their central pores, forming hexameric rings.

It is believed that Hsp70 first detects the substate and binds to the surface of aggregates, facilitating the transfer of the aggregated substrate to Hsp100. Subsequently, Hsp100 disaggregases break down the aggregates by pulling peptide loops through their central channel in an ATP dependent manner.

The reactivation and breakdown of protein aggregation controlled by ClpB are closely linked to two additional molecular chaperones, namely DnaK/Hsp70 and DnaJ, as well as a nucleotide exchange factor known as GrpE that operates in a cyclic manner. Nevertheless, the precise mechanism by which ClpB facilitates the reactivation and disintegration of protein aggregates remains unknown. The hexameric form of bacterial ClpB, which possesses biological activity, has a molecular weight of 575 kDa. When nucleotides such as ATP or ADP are present, six monomers of ClpB, each weighing 95 kDa, assemble to form a functional hexameric ClpB complex (Figure 1).

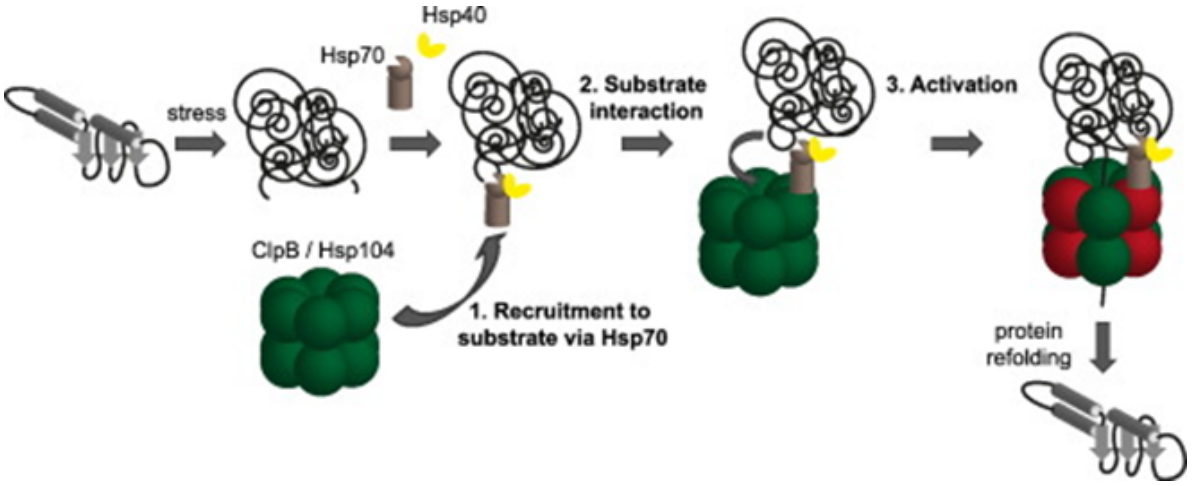


Figure 1 Substrate interaction of hexameric ClpB and Hsp104. Co-chaperone Hsp40 and Hsp70 are important in substrate recognition and interaction. Folded proteins become misfolded proteins due to cellular stress. These misfolded proteins are recognized by Hsp70 (brown) and Hsp40 (yellow) and guided towards ClpB/Hsp104 (green). Misfolded proteins are exraected trhough its central channel of ClpB/Hsp104 by ATP hydrolysis (red). These exreacted linear polypeptides are folded/recycled back to active proteins again.
Source: Kummer et al (2016)

Each ClpB monomer (also Hsp104) has a structure made up of many unique domains, including an N-terminal domain (NTD), two AAA+ nucleotide-binding domains (NBD1 and NBD2) that bind to ATP and hydrolyze ATP, a middle domain (MD) inserted into NBD1 that has a coiled-coil structure, and a short region at the C-terminal. When these monomers undergo self-association, they create a constricted channel located in the middle of the hexamer. ClpB breaks the aggregated proteins and releases them as single polypeptides by using ATP as the energy source (Figure 2).

The structure of each Hsp104 monomer (each weighing 104 kDa) shares a similar domain arrangement with ClpB. The Hsp104 hexamer has a central channel that spans its entire length. Polypeptides extracted from aggregates are propelled through the central channel of both ClpB and Hsp104 using ATP. *In vitro* experiments have demonstrated that Hsp104 chaperones can reconstruct amyloid conformers and denatured proteins. Sequence variations in the middle domain (M-domain) of ClpB and Hsp104 are responsible for chaperone activation. The M-domain also regulates the ATPase activities of ClpB and Hsp104 and is responsible for the interaction with DnaK/Hsp70 chaperones (Figure 2).

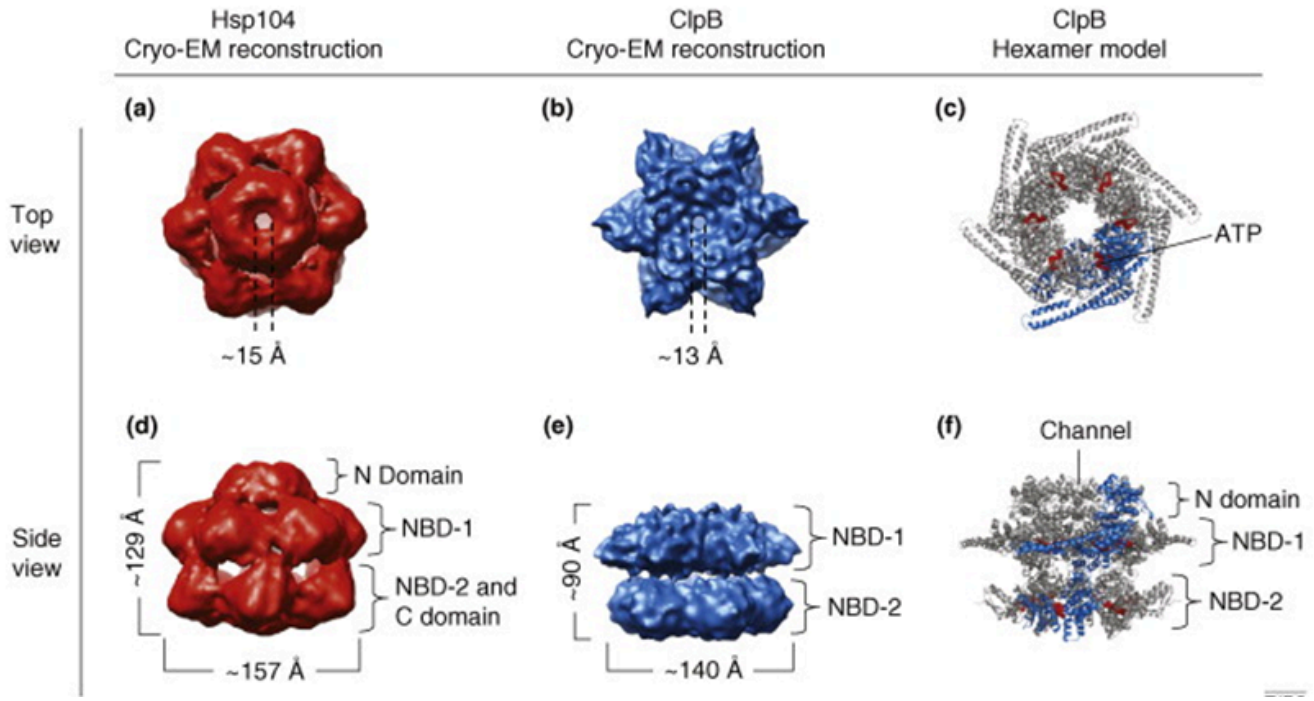


Figure 2 Three-dimensional cryo-EM reconstruction of hexameric ClpB and Hsp104 models. The length, width and the size of the central channel is shown in Angstroms (Å). NBD: Nucleotide Binding Domain. (a) and (d) are Hsp104 protein viewed from top and side. (b) and (e) are ClpB protein, viewed from top and side. Hsp104 and ClpB has a central channel of 15 Å and 13 Å, respectively. Hsp104 has following dimensions: length 129 Å, width 157 Å. ClpB has following dimensions: length 90 Å, width 140 Å. ClpB hexamer bound to ATP is shown in (c) and (f). A single protomer is shown in blue and the position of ATP in red as a CPK model in (c) and (f).
Source: Doyle et al (2009)

Human protein disaggregase system

For a long time, it has been thought that humans might possess protein disaggregase related to the Hsp100 family found in bacteria, fungi, and plants. However, identifying such a protein disaggregase has proven challenging until the revelation that the collaboration between Hsp110, Hsp70, and Hsp40 can effectively disaggregate and reactivate proteins. Hsp110, is an essential component of the mammalian cytosolic disaggregase machinery, exhibits the ability to collaborate with Hsp70 and Hsp40 in the process of disaggregating preformed aggregates and amyloids. Hsp110 engages in collaborative and synergistic interactions with Hsp70 and two distinct types of Hsp40 cochaperones to effectively resolve substantial protein aggregates. Given the extensive array of potential complexes that may form between various Hsp70s and Hsp 40s, it is hypothesized that distinct and specific complexes could be utilized to dissolve various protein aggregates. While it is possible that a particular combination may be utilized in specific neuronal subtypes, it is also possible that a specific combination could target α-synuclein disaggregation, while another combination could target tau disaggregation.

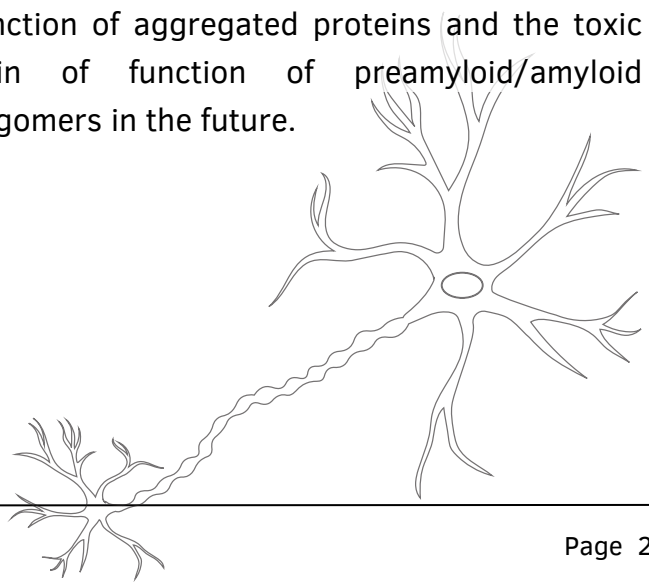
Powerful amyloid-remodeling system

The term "intracellular inclusions" refers to the signature lesion of PD, which consists of Lewy bodies made of alpha synuclein protein (alpha-syn), amyloid versions of the tiny pre-synaptic protein. Inhibition of fibrillization by Hsp104 was potent for both alpha-syn and PD-linked variants.

The hallmarks of AD include neurodegeneration in certain subcortical regions and the cerebral cortex, as well as widespread shrinkage of the brain. Internal neurofibrillary tangles containing amyloid protein tau and external neuritic plaques containing primarily beta amyloid 42 and beta amyloid 40 (Ab42 and Ab40) are the defining pathogenic lesions of AD. It has been reported that Hsp104 inhibits the *de novo* fibrillization of Ab42.

Potential future directions

The amyloid-remodeling activity of Hsp104 is exceptionally potent, and it combines the hydrolysis of ATP with the rapid dismantling of amyloid fibers. It has not been possible to definitively identify a metazoan homologue or analogue of Hsp104. Furthermore, no activity has been discovered in metazoa that combines protein disaggregation with renaturation processes. The unique ability of Hsp104 to deconstruct cross beta sheets and preamyloid oligomers opens a potential new avenue of application in metazoan systems. Reversing amyloid formation and breaking down formed amyloids could serve as an initial solution for addressing amyloid associated disorders. By employing these chaperones in a metazoan system, we may address both the loss of function of aggregated proteins and the toxic gain of function of preamyloid/amyloid oligomers in the future.



Surprisingly, despite being a yeast protein, Hsp104 is extremely well tolerated in metazoan systems and does not exhibit any overt toxicity. However, to apply these systems in a mammalian setup, initial studies on ClpB for its specific substrate recognition mechanisms are vital, as ClpB and Hsp104 share certain structural and functional features.

The concept of modifying the proteostasis network has limitations despite its great potential. For example, while increased expression of protein-remodeling proteins may help improve protein folding and combat neurodegenerative diseases, it may also stimulate cell division, potentially leading to cancer. To address neurodegenerative disease effectively, the development of novel strategies for small-molecule modulators of protein-remodeling systems and the creation of customized protein-remodeling systems will be crucial for rewiring and restoring the proteostasis network.

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