Chapter

Mechanical Properties of Chaperone BiP, the Master Regulator of the Endoplasmic Reticulum

Hilda M. Alfaro-Valdés, Francesca Burgos-Bravo, Nathalie Casanova-Morales, Diego Quiroga-Roger and Christian A.M. Wilson

Abstract

Immunoglobulin heavy-chain-binding protein (BiP protein) is a 75-kDa Hsp70 monomeric ATPase motor that plays broad and crucial roles maintaining proteostasis inside the cell. Its malfunction has been related with the appearance of many and important health problems such as neurodegenerative diseases, cancer, and heart diseases, among others. In particular, it is involved in many endoplasmic reticulum (ER) processes and functions, such as protein synthesis, folding, and assembly, and also it works in the posttranslational mechanism of protein translocation. However, it is unknown what kind of molecular motor BiP works like, since the mechano-chemical mechanism that BiP utilizes to perform its work during posttranslational translocation across the ER is not fully understood. One novel approach to study both structural and catalytic properties of BiP considers that the viscoelastic regime behavior of the enzymes (considering them as a spring) and their mechanical properties are correlated with catalysis and ligand binding. Structurally, BiP is formed by two domains, and to establish a correlation between BiP structure and catalysis and how its conformational and viscoelastic changes are coupled to ligand binding, catalysis, and allosterism (information transmitted between the domains), optical tweezers and nano-rheology techniques have been essential in this regard.

Keywords: immunoglobulin binding protein (BiP), optical tweezers, nano-rheology, posttranslational translocation, molecular motor

1. Introduction

The endoplasmic reticulum (ER) is involved in protein synthesis and the folding, assembly, transport, and secretion of nascent proteins [1]. One of the most important functions of the ER involves the quality control (ERQC) of nascent proteins, which is accomplished by ER chaperones [2, 3]. Chaperones are proteins that assist other proteins in the folding process, facilitating correct folding pathways or providing microenvironments in which folding can occur [4]. One of the most important chaperones is BiP protein (immunoglobulin heavy-chain binding protein).

BiP, a monomeric ATPase, has been referred to as the master regulator of the ER because of the broad and crucial roles that play in ER processes and functions [5], such as protein synthesis, folding, assembly, and translocation across the ER [3, 6]. Although BiP is still in early stages of study at a molecular level, some research groups have published findings of great value. These findings suggest that this protein could be a key player in various fields, such as in detection and treatment of serious diseases (neurodegenerative diseases, cancer, and heart diseases, among others) [7, 8]. Until now, most of the previous studies have been focused on the function of BiP with classical biochemical approaches and have not taken into account the mechanical properties of this protein. The role played by force on macromolecular structure and function is a subject of recent intensive research. Mechanical processes are a key component of many biological events. The coupling of mechanics and chemistry is one of the most important features of enzymes, which is highly specific and regulated [9]. Enzymes need to couple their chemical reactions to mechanical motion. In this way, an enzyme can work like a molecular motor using the hydrolysis or binding of ATP, converting this chemical energy to mechanical work. Allosterism and conformational changes are examples of how a chemical event could be transduced to mechanical events regulated by catalysis and ligand binding events based on changes in the elastic properties of domains [10]. Exploring this coupling may contribute to the understanding of the mechanical properties of enzymes, such as the mechanochemical mechanism of BiP. Understanding viscoelasticity is crucial because biological materials show different phenomena such as stiffening or softening upon ligand binding because proteins behave as springs [11, 12]. Due to recent technological progress, it is possible to measure changes in viscoelasticity in the folded state of proteins and we could correlate these changes with functionality. All these new approaches help to solve biological problems based on a mechanical description of molecular mechanisms to obtain a complete view of how the proteins perform their function with high efficiency.

2. The ATP-regulated Hsp70 chaperone BiP is the master regulator of the endoplasmic reticulum

Approximately, one-third of proteins produced in mammalian cells are folded and assembled in the ER, including secretory, membrane-bound, and some organelle-targeted proteins [13]. In the ER, proteins are translocated into the lumen where they acquire their functional tertiary and quaternary structure [3], and then correctly folded proteins exit the ER and are transported to intracellular organelles and the cell surface. The success of the maturation of a protein in its passage through the secretory pathway is monitored by the ERQC process, which is highly conserved in most eukaryotic organisms [2, 3]. For this, molecular chaperones proteins interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments where folding can occur [4]. However, those proteins that fail to fold properly must be translocated back to the cytoplasm and degraded in the proteasomes through a process known as ER-associated degradation (ERAD) [3]. Two main chaperone systems help to fold the proteins in the ER or target them for ERAD if folding fails: lectins such as calnexin/calreticulin, unique to the ER, and the heat shock protein 70 (Hsp70) system, which has many aspects that are common to all Hsp70s. BiP (also known as glucose-regulated protein 78 kDa, HspA5, or Kar2p in yeast) is the only known conventional Hsp70 chaperone in the ER [14, 15].

BiP binds transiently to newly unfolded synthesized proteins translocated post-translationally into the ER (**Figure 1**). Binding of BiP to the incoming polypeptide

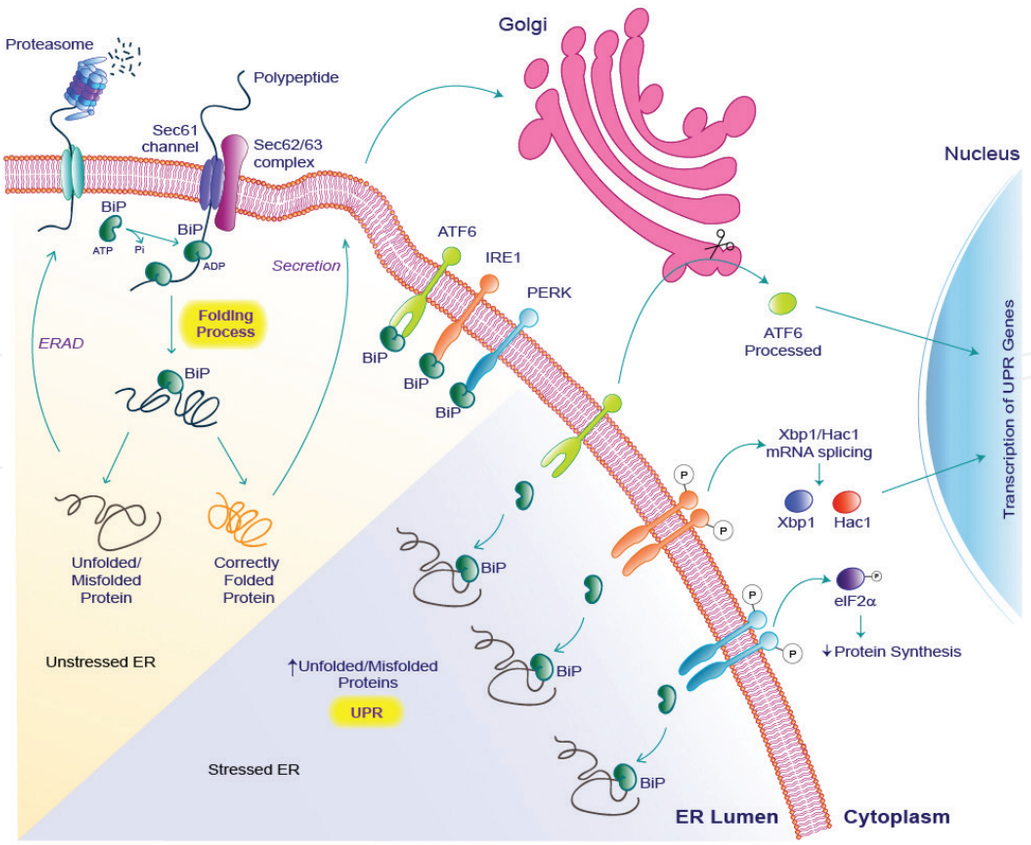

Figure 1.
Hsp70 chaperone BiP is a master ER regulator. Under nonstressed conditions (unstressed ER), BiP binds to hydrophobic regions of unfolded polypeptides fully synthesized to favor their posttranslational translocation into the ER lumen. The high substrate binding affinity of BiP to hydrophobic patches is achieved in the ADP-bound state upon the hydrolysis of ATP to ADP. After the translocation, BiP facilitates correct folding of nascent unfolded proteins or incompletely folded proteins nonglycosylated for their subsequently secretion. The proteins that fail to fold properly are targeted for proteasomal degradation in the cytoplasm through the ER-associated degradation (ERAD) pathway. BiP also interacts with the luminal domains of three ER stress sensors: IRE1, PERK, and ATF6 to maintain them in the ER. However, upon accumulation of unfolded/misfolded proteins in the ER lumen (stressed ER), BiP is released from these molecules to interact with unfolded proteins and favor their correct folding. BiP dissociation from these sensors allows their activation that involves: IRE1 dimerization, autophosphorylation, and splicing of Xbp1 and Hac1 mRNA; PERK dimerization, autophosphorylation, and phosphorylation of eIF2α, which lead to the attenuation of protein translation; and ATF6 transportation to the Golgi where it is processed by proteases. The ATF6 cytoplasmic domain obtained after its processing together with Xbp1 and Hac1 is translocated to the nucleus to activate the transcription of UPR-responsive genes.

contributes to efficiency and unidirectionality of transport due to its role as a molecular motor in the posttranslational translocation (will be discussed below). As a molecular chaperone, binding of BiP to hydrophobic patches exposed on nascent unfolded proteins that enter into the ER lumen or incompletely folded nonglycosylated proteins prevents nascent polypeptide chains from folding incorrectly and their interaction with nascent immature secretable proteins synthesized from membrane-bound polysomes. This prevents immature protein denaturation or degradation and ensures proper folding and its secretion (**Figure 1**).

Any condition perturbing the correct functioning of the ER, leading to an increase in protein synthesis or to the generation and accumulation of misfolded proteins inside the ER, is known as ER stress [16]. Moreover, misfolded proteins can also aggregate into insoluble higher order structure that has been associated with numerous neurodegenerative human diseases [17]. Adaptation to protein-folding stress is mediated by the activation of the unfolded protein response (UPR), which has evolved to detect the accumulation of misfolded proteins and activate a cellular response to maintain homeostasis and a normal flux of proteins in the ER, by increasing its folding capacity [18]. In this context, BiP serves as a

master UPR regulator and plays essential roles in activating three distinct ER stress sensors: IRE1, PERK, and ATF6 (**Figure 1**). Under nonstressed conditions, BiP binds to IRE1, PERK, and ATF6 by their luminal domains to maintain them in the ER. The accumulation of unfolded/misfolded proteins induces dissociation of BiP from IRE1 and PERK to permit their dimerization, trans-autophosphorylation, and activation [19]. Activated IRE1 initiates mRNA splicing of two transcriptional factors (Xbp1 and Hac1) to generate potent transcriptional activation of UPR target genes. PERK activation involves phosphorylation of the translational elongation factor eLF2 to attenuate protein synthesis. The release of ATF6 favors its transport to Golgi where is cleaved to generate the cytosolic domain of ATF6 that translocate to the nucleus to activate transcription of UPR-responsive genes [20]. Therefore, the activation of these sensors results in the attenuation of translation to reduce the workload of the ER, the transcriptional upregulation of genes encoding ER chaperones to increase the folding capacity of the ER, and the overexpression of the ERAD component to favor the degradation of these unfolded proteins by the proteasome [21, 22]. Thus, BiP participates not only in assisting protein folding, assembly and translocation but also in protein degradation and in the stress adaptability of the ER [1]. One big difference between BiP and lectins is that BiP detects only the unfolded regions of the nascent polypeptide chains, whereas lectins can detect both N-linked glycans of the peptides and unfolded regions [23]. However, it is not yet completely clear how BiP binds to its unfolded substrate because usually peptides are used as substrates instead of complete unfolded proteins. Recently, we developed a new method to study this process by specifically unfolding a complete protein substrate for BiP and measuring in optical tweezers the time that BiP remains bound to its substrate [24]. Previously, a work with DnaK (a close homolog of BiP) shows that it binds and stabilizes also partially folded protein structures [25]. BiP has a crucial role during posttranslational translocation, acting as a molecular motor. Molecular chaperones in the cytoplasm and ER lumen are involved in polypeptide translocation across the ER. Proteins enter the ER by a channel protein complex known as the translocon, discovered in yeast in Randy Schekman's laboratory [26]. In eukaryotic cells, the translocation of proteins is carried out by the Sec61 complex [6, 27]. Sec61 complex consists of three subunits, α, β, and γ, in which the pore to transport the polypeptide chain is created by the α-subunit of Sec61 protein. This complex functions as a passive channel that requires accessory proteins to provide a driving force to facilitate the vectorial translocation of the polypeptide chain through the membrane. Those accessory proteins are molecular motors [28]. Motor enzymes use the energy of nucleotide binding/hydrolysis or product release to generate mechanical work. The two mechanisms of translocation across the ER are co-translational translocation and posttranslational translocation [29]. In the co-translational mechanism, which has been well studied in mammalian systems, the signal sequence at the N-terminus of the nascent polypeptide interacts with the signal recognition protein (SRP) in the cytoplasm, keeping the ribosome attached to the Sec61 complex [6]. In this mechanism, the ribosome acts as an "auxiliary protein," since the driving force for translocation is given by GTP hydrolysis during the elongation of the polypeptide chain [30]. However, the driving force delivered by the ribosome is missing for posttranslationally translocated proteins. In this case, the driving force for polypeptide chain translocation comes from BiP protein [30]. Thus, in post-translational translocation, after the polypeptides are fully synthesized, cytoplasmic molecular chaperones keep them unfolded to be transported through the Sec61 complex. In this mechanism, the channel partners with another membrane-protein complex, the Sec62/Sec63 complex, and with the lumenal chaperone BiP. However, in spite of the crucial roles of BiP during translocation, it is not fully understood

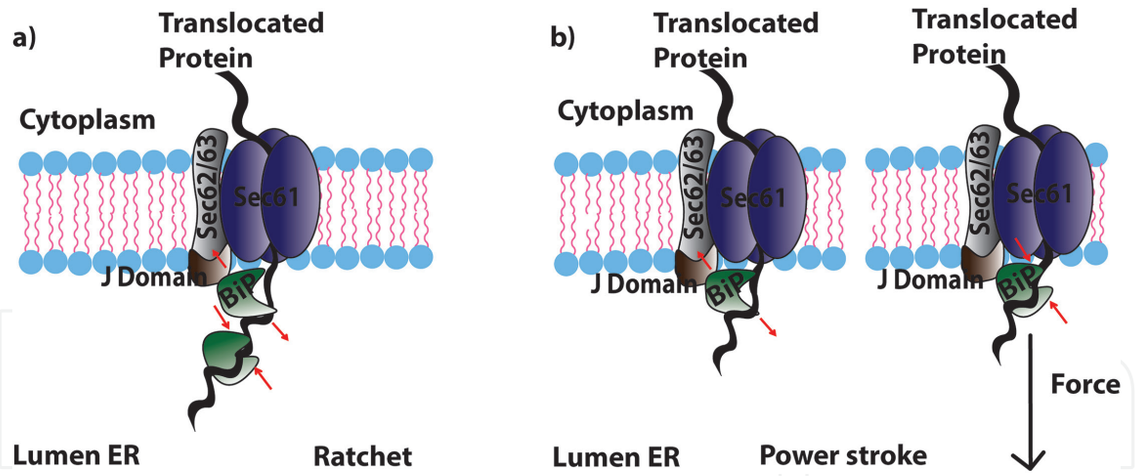

Figure 2.
Schematic representation of the two mechanisms of BiP in translocation. The figure shows how BiP could be involved in the transport process of the protein into the ER. (A) The ratchet theory is shown in which several BiP molecules would be interacting with the incoming chain, and in this way, the chain will not be returned to the cytoplasm. (B) The theory of power stroke is shown, where BiP binds to the polypeptide chain exerting a force greater than that of the thermal bath. Open domains of BiP are represented by red arrows outward and domains closed by red arrows inward.

if the action of BiP is through an active mechanism of pulling (as a power stroke), mediated by the binding/hydrolysis of ATP, or as a ratchet mechanism (**Figure 2**). In the latter, the polypeptide chain enters the channel passively by Brownian motion, and the BiP protein prevents it from returning to the cytoplasm. The hypothesis of the ratchet mechanism has been supported by employing antibodies against the polypeptide chains passing through the ER lumen [31]. Evidence for the translocation mechanism has been obtained using coarse-grained model simulations [32]. This study suggests that Hsp70 chaperones use an "entropic pulling mechanism," applying a force of about 15pN, and proposes that the Hsp70's would use a combination of ratchet and power stroke mechanisms [33]. Translocation in all eukaryotes is likely to be similar to yeast because of the high identity of amino acids between their channels. The channel interacts with the Sec62/Sec63 complex, with BiP acting as a molecular motor to bias the passive movement of a polypeptide in the Sec61 channel. In bacterial posttranslational translocation, the channel interacts with the cytoplasmic ATPase SecA. SecA moves polypeptides through the SecY channel to the periplasm by a "push and slide" mechanism [34]. Archaea probably use both cotranslational and posttranslational translocation, but it is unknown how posttranslational translocation occurs because these organisms lack SecA, Sec62/Sec63 complex, and BiP [6, 30]. In double membrane system, as in chloroplast, it is mediated by translocon at the outer envelope membrane of chloroplasts (TOC) and translocon at the inner envelope membrane of chloroplasts (TIC), which facility the import of translated proteins with assistant of a TIC associated ATP-driven import motor [35]. However, in mitochondria, the import of preproteins is carried out by translocases called as TOM complex (translocon outer mitochondria membrane) and TIM23 complex (translocon at the inner mitochondrial membrane), where proteins with a hydrophobic sorting signal can be released into the inner membrane and hydrophilic proteins are imported into the matrix by one presequence translocase-associated motor (PAM) in which the force is driven by chaperone mtHsp70 [36]. Therefore, the mHsp70 pulls the presequence by power stroke or Brownian ratchet mechanism to finally translocate the presequence at the mitochondria matrix. This suggests that the mechanism of Hsp70 in the import of preprotein in mitochondria and ER could have similar basic mechanism.

2.1 BiP structure and catalysis

The effective application of work depends on the elastic properties of a motor based on the softening and stiffening of some domains [37], and it is important to understand how the information is transmitted through domains by BiP. BiP is formed by two domains: a nucleotide-binding domain (NBD), with ATPase activity, connected by a flexible hydrophobic linker to the substrate-binding domain (SBD) (**Figure 3**). The SBD can be further divided into a compact β-sandwich domain harboring, a cleft for substrate binding, and an α-helical domain at its C-terminal end, the so-called "lid" [38]. Many conformational changes, such as the open and close movement of the lid and the variation in the distance between the SBD and NBD, have been associated with the ATPase cycle of BiP in the ER. Once BiP binds K^+ and ATP, its NBD and SBD come into close proximity to each other and the lid of the SBD opens, which results in a form that binds substrates with low affinity [3]. Also, a number of BiP cofactors have been discovered that assist in controlling the substrate-binding cycle and its localization within the ER. Nucleotide exchange factors (NEFs) help in the transition from the ADP to the ATP bound state for BiP, catalyzing the release of substrate. Hsp70 hydrolysis of ATP to ADP is accelerated by Hsp40 family members or so-called J domain proteins. The J-domain binds to Hsp70 and stimulates its ATPase activity [39]. In addition to controlling the localization and activity of Hsp70's, J-domain proteins may also bind the substrate themselves and help with the initial delivery of the substrate to Hsp70 chaperone. In the mammalian ER, there are seven J-domain proteins (ERdj1–7) that assist with the diverse functions of BiP [40]. After the Mg^{2+}-dependent hydrolysis of ATP, BiP enters a state with low on and off rates for substrates [3]. For elongated/peptide substrates, the lid closes over the bound substrate; for globular substrates, there are direct interactions between the lid and the substrate, but the lid may not close completely [3]. The SBD and NBD become farther apart upon substrate binding and ATP hydrolysis, which is less pronounced for globular substrates. ADP must be exchanged for ATP in order to release the substrate and make BiP available for another round of client binding. Ca^{2+} increases the affinity for ADP, whereas NEFs Grp170 and Sil1 facilitate the nucleotide exchange reaction [3]. Conformational changes in murine BiP during ATPase cycle have been determined by Förster Resonance Energy Transfer (FRET) at the single molecule level, showing that NBD and SBD come into close

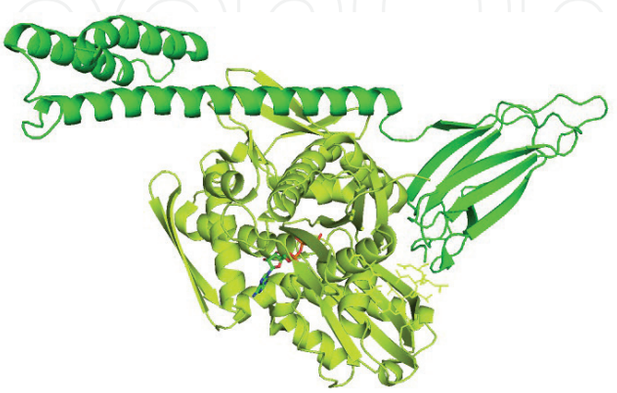

Figure 3.
Structure of ATP-bound BiP in the open conformation. BiP has two domains, NBD (light green) and SBD (dark green). The latter has a subdomain that acts as an α helix lid that covers the binding pocket for polypeptides formed by β sheets. In the ATP-bound BiP conformation, the lid is open. This structure corresponds to protein data bank number: 5E84 and was drawn as a ribbon diagram, using PyMOL molecular visualization system.

contact with a mean distance 58–75 Å [41]. Additionally, by using NMR residual dipolar coupling, spin labeling, and dynamics methods, it has been determined in DnaK that the NBD and SBD are loosely linked and can move in cones of 35° with respect to each other [42]. Moreover, the distance between the base and the lid of the SBD domain in Hsp70 has been calculated to be 77 Å by means of FRET [43]. Also, there is a crystal structure of human BiP bound to ATP that shows similar distances [44]. The conformational changes and movements of BiP are not independent for each domain because an important communication and coupling exists between them.

2.2 BiP allosteric mechanism

Most HSP70 do their work coupling the Mg^{2+}-dependent hydrolisis of ATP to large conformational changes, involving movements of its structural domains (SBD and NBD) and the interdomain linker. So, HSP70 protein function rely on a dynamic ATP dependent cycle in which several conformations are visited, with different substrate binding affinities in them [45–47]. For example in DnaK, ATP binding favors a compact, domain-docked, and linker-bound conformation, which has low ATPase activity [3, 45]. Substrate binding to this state stabilizes a transient domain-undocked conformation, with a linker-bound state, that has high ATPase activity and fast substrate binding and release kinetics but low substrate affinity. Then, when ATP is hydrolyzed to ADP, it is favored a domain-undocked conformation, linker-unbound state, which has high substrate affinity but very slow substrate binding and release kinetics [45–48]. Recently, X-ray structures of ATP-bound DnaK [49] and human BiP [44] have shown that both proteins have big structural similarity, but their functional activity (and between different Hsp70s) varies significantly between them [3]. On this ground, considering the fact that in spite of the structural similarity between different Hsp70, they have different functional activity; it was suggested that an important feature that should modulate Hsp70 function was its allosteric communication between both structural domains, mediated by the interdomain linker [44, 50]. Basically, the allosteric mechanism transmits information on the nucleotide state from NBD to SBD and on the substrate occupancy state from SBD back to NBD [51]. At the beginning, three different ideas explaining how interdomain communication occurs have been suggested. In the *E. coli* Hsp70 DnaK, bound nucleotide is sensed by residues in NBD that are closer to the bound ATP, in particular, a proline residue (Pro143) and a surface-exposed arginine (Arg151), and the communication with the SBD domain is thought to be via this proline, which can likely undergo cis/trans isomerization [52]. Replacement of the arginine completely disrupted the mutual allosteric communication between ATPase domain and substrate binding domain. Moreover, arginine had been shown to be an important residue in allosteric communication in other proteins [53]. Replacement of the proline destabilized the allosteric communication, increasing the rate of spontaneous transition between ATP-like and ADP-like states. Interestingly, all residues of the proposed DnaK sensor-relay system are conserved in BiP [3, 54]. In addition to this putative proline-focused sensor-relay system, threonine in position 37 (Thr37) in NBD plays a particularly important role as a nucleotide sensor in a hamster BiP [55], likely due to a direct interaction of its hydroxyl group with the γ-phosphate oxygen of bound ATP. Once this position was mutated, no more conformational change occurred, while nucleotide binding and hydrolysis were not affected [3]. The third known communication path between NBD and SBD occurs through the conserved hydrophobic linker, which connects both domains.

Upon ATP binding, the linker binds to a cleft in NBD, which is important in transmitting the nucleotide state of NBD to SBD and increases ATP hydrolysis of the NBD once bound to the cleft. Basically, it has been suggested that allostery results from an energetic tug-of-war between domain conformations and formation of two orthogonal interfaces: between the NBD and SBD and between the helical lid and the β subdomain of the SBD [46]. More recently, "soft" amino acid substitutions have been performed in BiP, trying to affect the allosteric communication between SBD and NBD, uncoupling the substrate-binding site with the NBD-SBD interdomain interface. In particular, Val461 was mutated to Phe; Ile526 to Val; Ile437 to Val; and Ile538 to Val. It has been reported that in the presence of ATP, all these "soft" mutations affected the equilibrium between the domain-docked and domain-undocked conformations, suggesting that this residue enables allosteric control of BiP conformational ensemble [45].

Moreover, allosterism in BiP has been studied at the single molecule level with optical tweezer manipulation [24]. The results showed that BiP binds reversibly to the unfolded state of MJ0366 (substrate protein), preventing its refolding, and that this effect depends on both the type and concentration of nucleotides. Additionally, more clues about BiP allosteric mechanism have arisen from BiP ensemble measurements performed with nano-rheological experimental setup, which will be explain later.

Finally, it has been studied how the posttranslational modification of BiP by AMPylation onto Thr518 [56] could affect BiP conformational cycle, modulating in this way the allosteric mechanism of BiP. The results showed that effectively, this modification shifted BiP conformational equilibrium toward the domain-docked conformation in the presence of ATP, stabilizing the domain docking in the absence of ATP and demonstrating posttranslational fine tuning of BiP conformational equilibrium [45].

As a general overview, BiP allosteric mechanism has a high level of complexity, as it has different layers of control. From a structural point of view, there are residues that exert the communication between the SBD and NBD domains, and other residues that are involved in stabilizing conformational ensembles of BiP that affect allosteric communication. Moreover, changes in the mechanical properties of BiP are also involved in the allosteric mechanism regulation, as it has been demonstrated with the nano-rheological studies. Finally, posttranslational modifications also play a role in this chaperone function, as their importance in shuffling conformational ensembles, involved in this ATP and Mg^{2+} dependent cycle, has been demonstrated.

2.3 Mechanical aspects

Considering that translocation through the ER is a crucial process to maintain homeostasis inside the cell, it is essential to have a mechanistic understanding of the role that BiP has in translocation to maintain proteostasis. Therefore, classical biochemical assays, or ensemble studies, have been conducted to study each of these processes without taking into account the measurement of forces and changes in elasticity. Single molecule level studies, called *in singulo* studies, have become very relevant during recent years. These studies have become the gold standard to study biomolecular mechanisms because of their advantages when it comes to obtaining specific information about biological phenomena, and it also permits the application of force in molecules [57]. *In singulo* studies are very direct approaches, following the behavior of an individual molecule in time, thus making it possible to obtain not just the average behavior of many molecules, but rather the

whole distribution and individual behaviors of a population that may not be homogeneous. It is possible to study a single biomolecule by visualizing it or manipulating it, with the most common approaches being single molecule fluorescence and single molecule force spectroscopy [9]. With force spectroscopy, it is possible to mechanically manipulate and apply forces to molecules in a highly specific manner [58]. This technique lets us measure the mechanical stability of particular domains, instead of the whole protein, thus allowing us to determine the energetic coupling between one domain and the other. Techniques such as atomic force microscopy (AFM), magnetic tweezers, and optical tweezers allow the direct application of mechanical forces to biological macromolecules and let us study the conformational changes [9, 59]. One example of single molecule studies with BiP has been the analysis of the conformational cycle of BiP achieved by single molecule and ensemble FRET measurements. In this study, the authors determined that nucleotide binding resulted in concerted domain movements of BiP. Conformational transitions of the lid domain allowed BiP to discriminate between peptide and protein substrates [41]. Also, we recently developed a method to measure how BiP binds to its substrate using optical tweezers [24]. Without single molecule approaches, it is very difficult to learn about how BiP binds to its substrate, since the substrate of BiP is an unfolded peptide, and if we unfold the substrate, we may also unfold BiP. However, by optical tweezers manipulation, we can specifically unfold the substrate without affecting BiP. Another study, by directly pulling DnaK using optical tweezers, the authors were able to study the mechanics and the order of events of unfolding of each domain of this Hsp70 [60, 61]. This study shows that DnaK has more than two mechanical intermediates in each domain. All the single molecule techniques that exert force on the protein are not able to measure small changes in distance at subnanometer resolution at low forces (below 1–5 pN), and so it is difficult to correlate the elastic properties of the folded protein with ligand binding. A new technique called nano-rheology developed in Giovanni Zocchi's laboratory at the University of California at Los Angeles (UCLA) allows measurement of elasticity in folded proteins [62]. Nano-rheology is a traditional rheology experiment, in which an oscillatory force is directly applied to the protein and where average deformation is measured [63]. This technique exploits sub-Angstrom resolution to study the mechanical properties of the folded state of proteins by applying low force to the proteins in bulk [12]. The universal mechanical property of the folded state is the viscoelastic behavior, meaning, when a protein is subjected to a force, it can behave as an elastic or viscous material, getting stiffer or softer (flexible). Then, stiff and soft here refer to both elastic and viscous mechanical responses; the two are coupled because the structure is viscoelastic [63]. This behavior is relevant for the large conformational changes of protein which often accompany substrate binding in proteins [12, 64]. Using this technique (**Figure 4**), we studied the mechanical properties of BiP, considering the viscoelastic behavior upon ligand binding. We observed that the folded state of the protein behaves like a viscoelastic material, getting softer when it binds nucleotides but stiffer when it binds peptide substrate. The explanation for this mechanical behavior is related to the ATPase cycle of BiP. As shown **Figure 4B**, when the protein is in the presence of ATP, the protein is softer state because the lid is more flexible and the NBD and SBD domains are closer [50, 65]. Also, the protein is in softer state in the presence of ADP, but the structural reason is different. The hydrophobic linker is more flexible, and the domains seem to be in a dynamic distance distribution [3, 6]. Finally, the protein is more rigid or stiffer in the presence of the HTFPAVL peptide substrate. The structural reason is because the lid is close [6]. Additionally, it was observed in presence of peptide the dissociation constant (KD) for ADP decreased

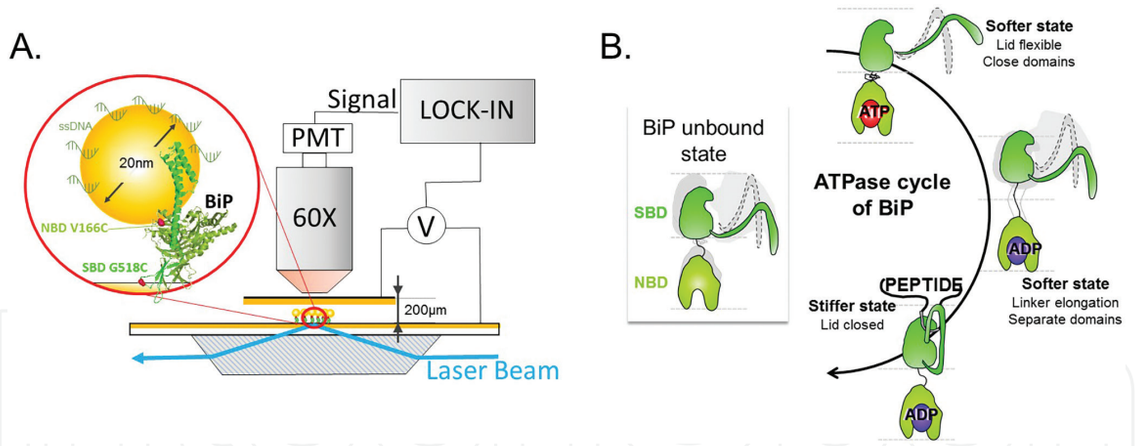

Figure 4.
Mechanical aspects of BiP. (A) BiP nano-rheology setup shows the flow chamber with BiP attached to both gold surfaces, the parallel plates capacitor geometry used for mechanical excitation, and the evanescent wave scattering optics used for read out. BiP was directly tethered between a gold film surface evaporated on a glass slide and 20 nm diameter GNPs, constituting the lower part of a thick flow chamber. BiP attachment proceeds via two exposed cys residues at positions 166 and 518, located in NBD and SBD, respectively. GNPs are covered with ssDNAs on the surface to negatively charge them. (B) Model for mechanical response of BiP in the presence of different ligands in ATPase cycle. The illustration shows BiP unbound state. In ATPase cycle of BiP, the structure is softer in two cases: first, in the presence of ATP, the lid is more flexible and the domains are closer leading to an important rigidity decrease. Second, in the presence of ADP, the domains are separated by the linker elongation. SBD seems to be in a dynamic distance distribution with a general trend toward domain separation. Finally, the structure is stiffer in the presence of peptide because the lid of BiP is closed, then generating a compact state.

1000 times, demonstrating that peptide binding dramatically increases the affinity for ADP which evidences the allosteric coupling between SBD and NBD domains [66].

3. Conclusion

Changes in the conformational state and viscoelastic properties of BiP triggered by ATP binding and/or hydrolysis are essential for allosteric communication between its domains (NBD and SBD), as these could supply the mechanical work to move the peptides through the Sec61 channel, with BiP behaving as a molecular motor. It is still not completely known how BiP applied the force in the peptide that is translocating or if it just uses the water bath. Taking into account the important role of BiP in proteostasis and diseases, an in-depth study of the functioning of the mechanics of BiP with new technology has major relevance to future research and development in science, biomedicine, and health, as well as in technological developments in biotechnology and even education, thus opening up new investigative directions of great potential and impact for science worldwide.

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Conflict of interest

None conflict of interest.

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