Control of Protein Function through Regulated Protein Degradation: Biotechnological and Biomedical Applications

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Abstract
Targeted protein degradation is crucial for the correct function and maintenance of a cell. In bacteria, this process is largely performed by a handful of ATP-dependent machines, which generally consist of two components – an unfoldase and a peptidase. In some cases, however, substrate recognition by the protease may be regulated by specialized delivery factors (known as adaptor proteins). Our detailed understanding of how these machines are regulated to prevent uncontrolled degradation within a cell has permitted the identification of novel antimicrobials that dysregulate these machines, as well as the development of tunable degradation systems that have applications in biotechnology. Here, we focus on the physiological role of the ClpP peptidase in bacteria, its role as a novel antibiotic target and the use of protein degradation as a biotechnological approach to artificially control the expression levels of a protein of interest.

Introduction
Proteins are involved in a vast array of biological processes in the cell. Some are constantly required by the cell as they perform essential cellular tasks (i.e. housekeeping proteins) – these proteins are often long-lived and highly abundant. Other proteins are only transiently required by the cell to manage, for example, specific environmental or cellular stresses – these proteins are usually short-lived and often found at low levels within the cell [Ishihama et al., 2008]. Regardless of their abundance or stability, most proteins need to maintain their structure in order to perform their desired biological function. Therefore, for cells to function properly, they must not only maintain the structure of their proteins, but also the correct cellular concentration of these proteins, in a time-resolved manner. To do so, cells contain a sophisticated protein surveillance network, commonly known as the protein quality control network, which is composed of two groups of proteins – molecular chaperones and proteases. Chaperones are primarily responsible for the maintenance of proteins in their folded and functional state [Hartl et al., 2011], while proteases are responsible for the removal of any misfolded or aggregated proteins that may accumulate [Gur et al., 2013; Sauer and Baker, 2011]. Proteases also mediate the removal of unwanted regulatory proteins such as transcription factors, which are only transiently required by the cell to activate and/or shutdown specific stress response pathways [Micevski and Dougan, 2013]. In bacteria, the removal of these misfolded and/or unwanted cytosolic proteins falls to one of several energy-
dependent machines. These machines are generally composed of two components – a peptidase component and an unfoldase belonging to the AAA+ (ATPase associated with diverse cellular activities) superfamily [Neuwald et al., 1999]. As such, these machines are commonly referred to as AAA+ proteases [Sauer and Baker, 2011]. In Gram-negative bacteria, such as Escherichia coli, there are five different AAA+ proteases [ClpAP, ClpXP, HslUV, Lon (sometimes referred to as LonA) and FtsH], while Gram-positive bacteria, such as Bacillus subtilis, contain up to seven related proteases [Moliere and Turgay, 2009, 2013]. In this review, we will focus on the ClpP-containing proteases, which are amongst the most highly conserved and best-characterized proteolytic machines in bacteria. We will summarize our current understanding of regulated protein degradation by ClpP and discuss how recently identified novel antibiotics hijack this peptidase to trigger cellular suicide. The review will also cover the use of proteolysis as a biotechnological tool to control protein expression in a heterologous host.

‘ClpP-Containing’ Proteolytic Machines

ClpP is a serine protease, which exhibits chymotrypsin-like activity [Arribas and Castano, 1993; Thompson et al., 1994]. It is highly conserved, not only in bacteria, but also in many eukaryotic species. In eukaryotes, ClpP is targeted either to the mitochondrion (in mammals, worms, plants and some fungi), the chloroplast (in plants), or in the case of Plasmodium falciparum ClpP (PfClpP) to the apicoplast (a plastid-like organelle found in members of the phylum Apicomplexa). Currently, there are over 30 ClpP structures from a variety of organisms deposited in the Protein Data Bank. Despite the relatively low sequence identity among these proteins, ClpP subunits share a high degree of structural similarity (online suppl. table S1; see www.karger.com/doi/10.1159/000352043 for all online suppl. material). In its simplest form, ClpP is a barrel-shaped oligomer composed of 14 identical subunits in which the subunits are arranged into two heptameric rings, stacked back-to-back (fig. 1a). Each monomer of ClpP resembles a hatchet and consists of three subdomains: a handle, a globular head and an N-terminal axial loop (fig. 1b). The heptameric ring is formed by the interaction of seven subunits through the head subdomain, and the tetradecamer is formed by the interaction of two heptameric rings through the handle subdomain (fig. 1a). This arrangement ensures that the catalytic triad (Ser-His-Asp) of the peptidase (fig. 1b) located within the proteolytic chamber are sequestered away from the exposed solvent. Importantly, entry into the proteolytic chamber of ClpP is limited to a narrow axial pore located at both ends of the cylinder (fig. 2a, c), which acts as a ClpP-‘safety switch’, preventing the uncontrolled degradation of correctly folded cellular proteins. Although this arrangement prevents the entry of folded proteins into the catalytic chamber, small peptides and even unfolded proteins can enter and hence be degraded in an ATPase-independent fashion. Nevertheless, unfolded proteins, in the absence of the ATPase, are degraded very slowly [Jennings et al., 2008b].

For the degradation of native and/or folded proteins, ClpP requires the assistance of an unfoldase, which invariably belongs to the AAA+ superfamily. Interestingly, with respect to unfoldase docking, ClpP is a promiscuous peptidase. To date, a total of six different AAA+ proteins (ClpA, ClpC, ClpD, ClpE, ClpL and ClpX) are known to interact with ClpP. Although many of these proteins are limited in their distribution (i.e. ClpE and ClpL are specific to Gram-positive bacteria [Frees et al., 2007] and ClpD is exclusively found in some plants [Adam et al., 2001]), others (e.g. ClpA and ClpC) are widely distributed across a broad range of species. However, only ClpX is universally found in all ClpP-containing species, from bacteria to humans [Truscott et al., 2010]. Not surprisingly, the ClpXP proteases play a number of crucial roles in a wide variety of organisms, from the regulation of virulence in several pathogenic bacteria [Frees et al., 2013] to the control of various different stress response pathways, not only in bacteria, but also in eukaryotes [Barchinger and Ades, 2013; Kirstein-Miles and Morimoto, 2010; Kwasniak et al., 2012; Micevski and Dougan, 2013; Truscott et al., 2011]. Although not as widely conserved, the ClpAP and ClpCP proteolytic machines also control a number of key proteolytic pathways in bacteria [Kirstein et al., 2009b]. ClpCP also appears to play an important role in proteostasis within the plastid of plants [Clarke, 2012; Oliena et al., 2011].

Independent of the unfoldase component involved, protease complexes may be either single- or double-headed complexes. Single-headed (1:1) complexes contain an unfoldase component at one end of ClpP, while double-headed (2:1) complexes contain an unfoldase component at both ends of ClpP. Double-headed complexes may be either symmetric (i.e. only contain a single type of unfoldase) or asymmetric (i.e. contain different unfoldase components at either end). Despite the fact that asymmetric double-headed complexes have been observed in vitro, it remains unclear if these complexes are physiologically rel-
relevant [Grimaud et al., 1998]. Nevertheless, symmetric double-headed complexes (of ClpAP) are proposed to be more efficient at substrate processing than single-headed complexes [Maglica et al., 2009]. Regardless of whether the complexes are single or double headed, all ‘ClpP-ATPase’ complexes exhibit a unique symmetry mismatch between the unfoldase (a hexamer) and the peptidase (a heptamer) components. This symmetry mismatch poses some interesting questions: how do these two rings interact to form a functional complex, and how many subunits are required for a functional interaction? Current evidence suggests that interaction of the ATPase, with the peptidase component, is mediated by two sets of contacts; one at the periphery of the interface and the other near the central pore which is supported by nucleotide binding [Hersch et al., 2005]. The peripheral contact occurs between a flexible

![Fig. 1. Ribbon representation of the structure of E. coli ClpP.](image_url)

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Protein Microcompartmental Machines for Protein Folding

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loop on the unfoldase, which contains a conserved tripeptide motif ([L/I/V]-G-[F/L]), and a hydrophobic pocket on the surface of ClpP. This motif is common to all ClpP-binding unfoldases and is essential for interaction with ClpP [Kim et al., 2001; Singh et al., 2001]. The second contact is highly dynamic and occurs between two loops; the axial pore-2 loop of the unfoldase and the N-terminal loop of ClpP [Bewley et al., 2006; Gribun et al., 2005; Martin et al., 2007]. Although these complexes are not symmetric, both loops appear to be flexible enough to contact each subunit of the unfoldase. Indeed, loss of a single [L/I/V]-G-[F/L]-loop within the hexamer of ClpX is sufficient to reduce ClpP binding and activity, while loss of more than one contact per hexamer completely abolishes ClpP binding [Martin et al., 2007].

Irrespective of the machine involved, all ClpP-containing proteases use three basic steps to degrade folded proteins. In the first step, the substrate is recognized by the unfoldase, which in some cases may be facilitated by an adaptor protein (see below). In general these machines recognize short sequence-specific motifs (termed degrons), which are usually located at the N- or C-terminus of the substrate. Following recognition by the unfoldase, the substrate is then unfolded in an ATP-dependent fashion. The unfolded substrate is then translocated into the associated peptidase, where it is hydrolyzed into small peptide fragments (~3–8 amino acids long) [Choi and Licht, 2005]. During this process ClpP is proposed to undergo dynamic changes in its structure [Kimber et al., 2010; Zhang et al., 2011]. Engagement with the unfoldase

Fig. 2. Surface representation of ClpP tetradecamers. E. coli ClpP (1YG6; a); E. coli ClpP bound to ADEP1 (3MT6; b); B. subtilis ClpP (3KTG; c) and B. subtilis ClpP bound to ADEP2 (3KTK; d). ADEP1 (green) and ADEP2 (orange) molecules are shown in space fill representation. The N-terminal residues of ClpP (residues 1–18) are colored in blue. This figure was generated using PyMOL.
is proposed to occur with the ‘extended’ ClpP conformation, which permits substrate entry into the catalytic chamber [Kimber et al., 2010; Zhang et al., 2011]. Based on limited structural evidence, transition to a compressed ClpP conformation through a kink in the handle subdomain closes the unfoldase-binding pockets triggering transient release of the unfoldase, which has been proposed to facilitate peptide release [Zhang et al., 2011]. Peptide release is thought to occur either through the axial pore or holes in the sidewall of the peptidase [Gribun et al., 2005; Sprangers et al., 2005].

Variations to the ‘Basic’ ClpP Machine

Although much of our basic understanding of ClpP structure and function is based on E. coli ClpP (the first homologue to be identified and characterized), the processing, assembly and the composition of ClpP proteases can vary significantly from species to species. For example, E. coli contains a single ClpP homologue, which is synthesized as a zymogen containing an N-terminal propeptide [Maurizi et al., 1990] and autocatalytically cleaved upon oligomerization, resulting in the formation of a proteolytically active tetradecamer. Although this is true for many species, several organisms contain more than one ClpP homologue, for example Cyanobacteria contain three clpP genes, and plants such as Arabidopsis thaliana contain up to six clpP genes. Moreover, some organisms contain ClpP homologues that lack a propeptide region. For instance, Mycobacterium spp. contain two clpP genes, both of which are essential but only one encodes a propeptide region [Raju et al., 2012]. Therefore, given that ClpP homologues lacking a propeptide appear to exhibit normal proteolytic activity, it currently remains unclear why the propeptide has been retained in some ClpP homologues. It also remains unclear why some species contain multiple ClpP homologues. One possibility, however, is that specific ‘ClpP-ATPase’ combinations may be formed in organisms that also contain multiple unfoldase homologues. Alternatively, different ClpP homologues may come together to form heterooligomeric ClpP complexes, which exhibit altered proteolytic activity, as has been proposed in plants [Peltier et al., 2004]. The complexity of these machines may be further enhanced in specific organisms (e.g. A. thaliana, P. falciparum and Cyanobacteria) that contain catalytically inactive ClpP homologues, known as ClpR. In P. falciparum and Cyanobacteria only a single ClpR homologue has been identified, while A. thaliana contains five different ClpR homologues. Interestingly, although ClpP/ClpR heterooligomers are proposed to form in Cyanobacteria and plants [Olinares et al., 2011; Peltier et al., 2004; Stanne et al., 2007], biochemical and structural evidence suggests that stable ClpP/ClpR heterooligomers do not form in P. falciparum [El Bakkouri et al., 2013]. Consistent with the inability to form heterooligomeric complexes, the structure of the P. falciparum ClpR homologomer [El Bakkouri et al., 2013] is considerably larger and flatter than most ClpP structures (including P/ClpP). Despite this the overall fold of ClpR, with the exception of a unique insertion termed the R-motif, is similar to ClpP (online suppl. table S1). Currently, however, the precise role of the R-motif (or indeed ClpR) remains unclear in many of the organisms in which they are found.

In addition to the different composition of certain ClpP complexes, some ClpP homologues also require assistance to reach their final active oligomer. In contrast to E. coli ClpP, which assembles into a stable tetradecamer without assistance, B. subtilis ClpP (which lacks a propeptide) requires its cognate unfoldase (ClpG, together with the adaptor protein MecA) to form not only the stable tetradecamer, but also the heptamer [Kirstein et al., 2006]. In contrast, human ClpP only requires assistance from its cognate unfoldase (i.e. ClpX) to form the final active tetradecamer, as the heptameric intermediate can be formed without assistance of human ClpX [Kang et al., 2005].

ClpP as a Novel Antibiotic Target

The architecture of ClpP appears to have been carefully designed to regulate substrate access into its proteolytic chamber and thereby prevent widespread and indiscriminate degradation of cellular proteins. The axial pore of ClpP is proposed to block indiscriminate substrate entry into the catalytic chamber. Specifically, it is believed that substrate entry into the catalytic chamber of ClpP is gated by the N-terminal peptides that create the axial pore. Unfortunately, due to the symmetry mismatch between the unfoldase and peptidase components, structural evidence for such a model has remained elusive. Nevertheless, consistent with this gating mechanism, the ClpP-mediated degradation of an unfolded substrate is accelerated by the addition of a cognate unfoldase component, while deletion of the N-terminal loops from ClpP is able to accelerate the degradation of short peptides [Jennings et al., 2008a]. Collectively these data suggest that gated entry of a substrate into ClpP is activated by the unfoldase, as has been observed for the proteasome.
[Kohler et al., 2001]. Consistent with this idea, recent cryo-EM reconstructions of ClpAP complexes have shown that ClpA-binding to ClpP triggers a change in the N-terminal loops of ClpP, from a ‘down’ conformation where they block entry to the catalytic chamber, to an ‘up’ conformation which permits access to the chamber [Effantin et al., 2010].

Recently a series of small molecules [known as acyldepsipeptides (ADEPs) and activators of compartmentalized proteolysis (ACPs)] were shown to hijack ClpP in the absence of the unfoldase component by overriding this gated safety switch [Brotz-Oesterhelt et al., 2005; Dougan, 2011; Kirstein et al., 2009a; Lee et al., 2010; Leung et al., 2011; Li et al., 2010]. The small molecule-mediated activation of ClpP results in the unregulated degradation of nascent polypeptides and unfolded proteins in the cell [Brotz-Oesterhelt et al., 2005; Kirstein et al., 2009a]. Indeed, ADEP was recently shown to trigger the ClpP-mediated degradation of FtsZ (a key protein required for septum formation) and hence inhibit cell division of Gram-positive bacteria [Sass et al., 2011]. These novel molecules appear to exhibit both bactericidal and bacteriostatic properties against several pathogenic and drug-resistant bacteria [Leung et al., 2011; Sass et al., 2011].

Through a series of biochemical and structural studies, these chemical activators of ClpP have been shown to dock into a hydrophobic pocket located on the surface of ClpP (fig. 2b, d). Firstly, and most importantly, binding to this hydrophobic pocket appears to result in opening of the pore (from ~10 Å in the absence of ADEP to ~21–27 Å in the presence of different forms of ADEP). This ‘gated opening’ of the ClpP pore is proposed to be sufficient to allow entry of unfolded proteins into the proteolytic chamber of ClpP (where the catalytic residues are located) and possibly the primary reason for degradation of unfolded substrates. Interestingly, in the case of B. subtilis ClpP, ADEP not only triggers opening of the pore, but also triggers oligomerization of ClpP from free ‘inactive’ monomers to ‘active’ tetradecamers [Kirstein et al., 2009a], a step that is normally controlled by the cognate unfoldase, ClpC [Kirstein et al., 2006]. Similarly, ADEP activation of human ClpP for unregulated degradation [Lowth et al., 2012] is also likely to result from assembly of the ClpP tetradecamer, a process that normally requires the assistance of human ClpX [Kang et al., 2005].

Indeed, this activation also appears to be a competitive inhibitor of unfoldase binding to ClpP, preventing the regulated degradation of substrates that would normally be delivered to ClpP by the unfoldase component [Kirstein et al., 2006]. As such, the ADEP-bound conformation of ClpP has been proposed to mimic the unfoldase-bound conformation of ClpP. However, it currently remains unclear if ADEP-binding triggers the same effect on ClpP as ClpA-docking, which based on a series of cryo-EM structures [Effantin et al., 2010] appears to have little effect on the size of the ClpP pore (diameter ~12 Å). One explanation for the observed difference in pore opening could be that the size of the pore may vary with translocation of different substrates [Alexopoulos et al., 2012]. Therefore, it is yet to be seen whether the ordered (fig. 2b) or the disordered (fig. 2d) arrangement of the N-terminal loops of ClpP resemble the unfoldase-bound complex or simply different states in the reaction cycle. Hence, although ADEP is proving to be a useful tool to understand activation of ClpP, the mechanism of unfoldase activation currently remains poorly understood.

Regulated Proteolysis as a Means to Regulate Protein Levels

The manipulation of protein concentration within a cell is a useful tool to examine the physiological function of a protein. Although overexpression of a protein of interest (POI) can provide some insight into protein function, often it is necessary to deplete or delete the POI in order to obtain some phenotypic information. Commonly, protein levels within the cell are altered through the deletion of the gene or via targeted degradation of the mRNA. These approaches are particularly poorly suited to the study of essential or long-lived proteins. In contrast, protein degradation is a rapid and efficient way to regulate protein levels within a cell. Indeed, to date several different proteolytic pathways (in both pro- and eukaryotic cells) have been exploited to control protein levels in homologous as well as heterologous hosts [Banaszynski and Wandless, 2006]. However, for the purpose of this review, we shall focus only on the use of two well-characterized recognition tags to control protein levels: (1) an N-degron and (2) the SsrA-tag.

A critical feature of regulated protein turnover, and hence the application of these systems to artificially control protein levels, is the specific recognition of the POI. In bacteria, this step is generally mediated by the unfoldase component; however, in some cases it can be modulated by a specific adaptor protein. In eukaryotes, substrate recognition is mediated by a specific ubiquitin (Ub) E3 ligase. Following recognition, the substrate is ubiquitylated at an internal Lys residue, via a multistep pathway. The modified protein is then recognized by the protea-
To date, arguably the best-characterized Ub-dependent degradation pathway is the N-end rule degradation pathway [Dougan et al., 2012; Mogk et al., 2007; Varshavsky, 2011]. This pathway is not only highly conserved but also extremely specific as initial substrate recognition is solely dependent on the identity of the N-terminal amino acid of the protein. In eukaryotes, only proteins bearing a basic (R, K and H) or bulky hydrophobic (L, F, Y, W and I) residue at their N-terminus are recognized and hence degraded, while in bacteria recognition (and degradation) is limited to proteins bearing an N-terminal bulky hydrophobic residue (L, F, Y or W). In bacteria the N-terminal residue is specifically recognized by the adaptor protein ClpS [Erbse et al., 2006; Schuenemann et al., 2009; Wang et al., 2008], while in eukaryotes recognition is performed by a family of Ub E3 ligases [Tasaki et al., 2005]. For a recent review of the bacterial N-end rule pathway see Dougan et al. [2010] and Varshavsky [2011].

Although the N-end rule pathway has currently not been developed for use in bacteria, several systems have been developed for use in eukaryotes [Dohmen and Varshavsky, 2005; Kearsey and Gregan, 2009; Taxis et al., 2009]. The first system to artificially control protein levels...
using the N-end rule pathway was developed by Var-
shavsky and colleagues. In this case, the POI was fused to
Ub-dihydrofolate reductase (DHFR), in which a temper-
ature-sensitive (ts) mutant of DHFR contained an Arg
residue C-terminal of the Ub protein (R-DHFR\textsuperscript{ts}). Upon
induction of a deubiquitylating enzyme, the N-terminal
Ub is cleaved, exposing a destabilizing Arg residue at the
N-terminus of DHFR\textsuperscript{ts}, which under permissive condi-
tions (i.e. at 23°C) is stable. However, upon shifting the
cells to nonpermissive conditions (i.e. at 37°C), the R-
DHFR\textsuperscript{ts} fusion protein is rapidly degraded by the protea-
some (fig. 3a). This conditional degradation system offers
excellent control, and although in principle it could be
used in many eukaryotic species, the nature of its induc-
tion (i.e. temperature) has limited its use largely to yeast
[Dohmen and Varshavsky, 2005; Kearsey and Gregan,
2009]. More recently, an alternative method to regulate
protein levels using an N-degron has been developed
[Taxis et al., 2009]. In this method, the N-degron is pro-
tected by a N-terminal fusion protein/reporter, which
contains a tobacco etch virus (TEV) protease cleavage site
(fig. 3b). In the absence of TEV protease, the N-degron is
protected and hence the POI is stable. Upon induction of
TEV protease, the dormant N-degron is exposed, result-
ing in its degradation by the proteasome. As such, the
method has been termed TIPI (TEV protease-induced
protein inactivation). An important feature of this system
is the flexibility of its induction, which is controlled by
TEV protease expression. Indeed, through the use of spe-
cific promoters to drive TEV protease expression, the lev-
els of an individual POI could be controlled in a particu-
lar cell type, tissue or developmental stage.

Another simple yet well-characterized substrate tag de-
derived from bacteria is the SsrA-tag. In E. coli this tag is
relatively short, it is composed of eleven amino acids
(AANDENYALAA) and in contrast to an N-degron it is
found at the C-terminus of a protein. In bacteria, the SsrA-
tag is normally attached to aborted translation products
(for a detailed review of the SsrA-tagging system see Kar-
zai et al. [2000]). The tag is directly recognized by ClpX
[Flynn et al., 2001], leading to progressive degradation of
the entire protein by ClpP. Importantly, ClpX recognition
of the SsrA-tag can be specifically enhanced by the adap-
tor protein, SspB [Dougan et al., 2003; Levchenko et al.,
2003; Wah et al., 2003]. Using this detailed understanding
of SsrA-tag recognition by ClpXP and SspB [Flynn et al.,
2001], Sauer and colleagues developed a modified tag
(AANDENYSENADAS, termed DAS+4) which exhibited
reduced affinity to ClpX and a greater dependence on
SspB for its ClpXP-mediated degradation [McGinnness et
al., 2006] (fig. 3c). This improved SspB-mediated recogni-
sion system permitted the precise control of POI concen-
tration through the induction of SspB not only in E. coli,
but also in heterologous systems, such as mycobacteria
[Kim et al., 2011], B. subtilis [Griffith and Grossman,
2008] and Saccharomyces cerevisiae [Grilly et al., 2007].

In order to further improve the control of this system, Sauer and colleagues engineered SspB into two separate parts
[Davis et al., 2011]. The design of this split adaptor was based on an intimate knowledge of SspB, which is com-
posed of two functional regions (an N-terminal domain
for substrate recognition and a C-terminal tail for docking
to the N-terminal domain of ClpX) separated by a flexible
linker [Dougan et al., 2003; Levchenko et al., 2003; Wah
et al., 2003]. Importantly, when split into two parts each part
alone was inactive; however, when fused together by the
addition of a small molecule (rapamycin) ‘full’ adaptor ac-
tivity was restored [Davis et al., 2011].

Concluding Remarks

Regulated proteolysis in bacteria is proving not only to
be a fascinating area of basic biological research but also
proving to be a rich source of biomedical and biotechno-
logical applications.

References

Adam Z, Adamska I, Nakabayashi K, Ostersetzer
O, Haussuhl K, Manuell A, Zheng B, Vallon
O, Rodermel SR, Shinozaki K, Clarke AK: Chloro-
plast and mitochondrial proteases in Arabidopsis: a pro-

Alexopoulos JA, Guerne A, Ortega J: ClpP: a
structurally dynamic protease regulated by
210.

Arribas J, Castano JG: A comparative study of the
chymotrypsin-like activity of the rat liver mul-
ticatalytic protease and the ClpP from Esch-

Banaszynski LA, Wandless TJ: Conditional con-
trol of protein function. Chem Biol 2006;13:
11–21.

Barchinger SE, Ades SE: Regulated proteolysis:
control of the Escherichia coli sigma(E)-de-
pendent cell envelope stress response. Subcell

Bewley MC, Graziano V, Griffin K, Flanagan JM:
The asymmetry in the mature amino-termi-
nus of ClpP facilitates a local symmetry match

Brotz-Oesterhelt H, Beyer D, Kroll HP, Ender-
mann R, Ladel C, Schroeder W, Hinzen B,
Raddatz S, Paulsen H, Henninger K, Bandow
JE, Sahi HG, Labischinski H: Dysregulation of
bacterial proteolytic machinery by a new class


Protein Microcompartamental Machines for Protein Folding


