Roles of adaptor proteins in regulation of bacterial proteolysis

Aurelia Battesti, Susan Gottesman

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Current opinion in Microbiology Roles of adaptor proteins in regulation of bacterial proteolysis

Aurelia Battesti and **Susan Gottesman***
Bldg. 37, Rm. 5132, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD. 20892

Abstract

Elimination of non-functional or unwanted proteins is critical for cell growth and regulation. In bacteria, ATP-dependent proteases target cytoplasmic proteins for degradation, contributing to both protein quality control and regulation of specific proteins, thus playing roles parallel to that of the proteasome in eukaryotic cells. Adaptor proteins provide a way to modulate the substrate specificity of the proteases and allow regulated proteolysis. Advances over the past few years have provided new insight into how adaptor proteins interact with both substrates and proteases and how adaptor functions are regulated. An important advance has come with the recognition of the critical roles of anti-adaptor proteins in regulating adaptor availability.

I. Introduction

In all cells, proteolysis plays two general roles – quality control, ridding the cell of damaged and aggregated proteins, and regulation of protein abundance. Both require the proteolytic machinery to recognize and degrade the right proteins at the right time. While eukaryotic cells use the ubiquitin-tagging machinery to recognize proteins and mark them for degradation by the proteasome, most prokaryotic species carry out selective degradation without ubiquitin or a comparable system for marking potential substrates. Instead, bacteria have developed adaptor proteins that provide the ability to bring selected substrates to the protease.

We will focus here on the adaptors for the ATP-dependent AAA+ family of proteases that carry out most cytoplasmic degradation in bacteria. Substrate recognition by these proteases is via interactions with the ATPase domains or subunits; once engaged, substrates are unfolded and translocated through the ATPase to the catalytic core, where cleavage takes place (reviewed in [1]). The adaptors improve the recognition of specific classes of substrates by directly interacting with both the substrate and the protease ATPase domains.

Some adaptors mediate regulated proteolysis, allowing degradation of important substrates under some but not all conditions. These adaptors are likely to be essential for proteolysis, and control of their availability and/or activity will be subject to change in response to appropriate environmental and/or developmental cues. Other adaptors change the protease's preference for large classes of proteins, and might be better classified as components of the proteolytic complexes. Such adaptors are often employed in dealing with damaged or

Corresponding author: Gottesms@helix.nih.gov; 301-496-3524. Fax: 301-496-2212.

incomplete proteins, although even these may be utilized for regulated degradation of some substrates.

In recent years, significant progress has been made in understanding how the activity of adaptors can be regulated and in understanding how adaptors improve access to substrates. Here, we emphasize some of the common themes that are emerging.

II. Adaptor proteins for proteolysis: general principles

ATP-dependent protein degradation has been extensively studied in the model organisms *Escherichia coli, Bacillus subtilis*, and *Caulobacter crescentus*. The prokaryotic ATP-dependent proteases must recognize a broad range of substrates and yet not degrade cytoplasmic proteins promiscuously. The importance of this selectivity is demonstrated by the bacteriocidal activity of a novel antibiotic, ADEP, that targets the Clp ATP-dependent proteases. The antibiotic bypasses gating by the Clp ATPases and renders ClpP, the proteolytic core of these proteases, able to degrade many cytoplasmic proteins [2,3].

Substrate recognition by these ATP-dependent proteases generally requires the presence of a degradation tag or “degron”, a peptide sequence that is recognized by the protease, frequently at the N or C-terminal end of the substrate [1]. The substrate must be unfolded and translocated by the central pore of the ATPase before it is exposed to and cleaved by the protease active sites. Partially unfolded proteins can also be substrates for some of these proteases, possibly because hydrophobic regions not generally accessible in folded proteins have become exposed and can be captured in the central pore of the ATPases.

An adaptor protein modulates the affinity of the protease for a given substrate or class of substrates, providing specificity to the interaction of substrate and protease. The adaptor must present the substrate to the protease in such a way that the substrate is engaged by the ATPase pore. Some of the adaptors are themselves degraded, but many are not. Those that are not degraded must interact with the substrate and the protease in a way that prevents them from being recognized as a substrate. These requirements are all consistent with binding of the adaptor to the N-terminal domain of the ATPase, implicated in substrate recognition but not required for unfolding/translocation. These N-domains are frequently at a distance from the unfolding pore of the ATPase and their flexibility may be important for delivering the substrate to the ATPase pore [4,5]. Furthermore, the adaptor must bind the substrate for efficient delivery, but also release it to allow substrate translocation and degradation.

For regulated proteolysis, the availability of the adaptor, or its ability to interact with a substrate, must change under different environmental conditions. Modifications to the adaptor can play this role, but additional protein components, anti-adaptors, are critical modulators of adaptor activity in many systems.

III. What are the adaptors and how are they regulated?

Adaptors that support degradation of cleaved or incomplete proteins

In *E. coli*, ClpS and SspB are general adaptor proteins that recognize two classes of degradation signals associated with protein quality control, the degradation of incomplete or otherwise damaged proteins.

ClpS is an adaptor for the ClpAP protease. ClpS targets substrates with specific N-terminal amino acids (F, L, W or Y in *E. coli*) known as “N-end rule substrates”. The *E. coli* N-end rule substrates might accumulate as a result of other proteolytic cleavage or modification of the N-terminus. ClpS is encoded by the gene immediately upstream of *clpA*, and this linkage

is highly conserved; ClpS is also found in chloroplasts [6]. ClpS may also be used as an adaptor for aggregated proteins, and inhibits degradation of other ClpAP substrates, by blocking their binding to ClpA [6].

Structures are available for both ClpS bound to the N-terminal domain of ClpA and ClpS bound to peptides carrying appropriate N-terminal amino acids [7–9]. These structures and analysis of the requirements for ClpS-dependent degradation of N-end rule proteins led Roman-Hernandez et al to propose a hand-off model in which ClpS binds tightly to the substrate and to the N-domain of ClpA, at a distance from the axial pore of ClpA [9]. Further interactions of ClpS near the pore allow a hand off of the substrate from ClpS to the axial pore of the ATPase [9]. Whether this model will be applicable to the other adaptors discussed here remains to be seen, but it is consistent with what is currently known.

Another class of rapidly degraded proteins result from SsrA-dependent tagging. SsrA, or tmRNA, adds a short C-terminal sequence to incomplete proteins when translation pauses or stops prematurely (reviewed in [10]). The SsrA tagging system is conserved across bacterial species; the tag differs in some species, and while ClpXP is usually the primary protease for these tagged proteins, multiple other proteases contribute to degradation. The variations in the tags have allowed comparative studies that highlight both the specificity of recognition of tags and the conserved features [11,12]. In both *E. coli* and *C. crescentus*, ClpXP's ability to degrade SsrA tagged proteins is improved by the adaptor protein SspB. As for ClpS, SspB requires and binds to the N-terminal domain of its protease, ClpX [13]. Unlike ClpS and N-end rule substrates, degradation of SsrA-tagged proteins is not absolutely dependent upon the adaptor protein.

Adaptors for Regulated Proteolysis

Many adaptors play critical roles in allowing regulated proteolysis of specific proteins. Understanding the regulatory pathway for these adaptors requires knowing the answer to a number of questions beyond those for the adaptors discussed above. What is the default state, stability or instability of the target protein? What are the signal transduction pathways and mechanisms that allow the default state to be modified under the appropriate conditions? How does the cell return to equilibrium, or is the degradation part of a developmental pathway that need not be reversible? Does the adaptor act on many or few (one) targets?

The RssB Adaptor and a set of anti-adaptors in *E. coli*—The best-studied example of regulated protein degradation in *E. coli* is degradation of the master regulator of the general stress response, the sigma factor RpoS. RpoS is rapidly degraded during normal growth conditions. In various stress conditions or during entry into stationary phase, RpoS becomes stable. RpoS proteolysis is mediated by the adaptor protein RssB; RssB delivers RpoS to ClpXP for degradation (Fig. 1) [14,15].

RssB is a member of the response regulator family, characterized by a conserved aspartate residue in the N-terminal domain that can be phosphorylated by histidine kinases, suggesting that RssB phosphorylation status could be a molecular switch to regulate RssB activity and thus RpoS stability. However, cells carrying an *rssB* allele mutant in the site of phosphorylation can still respond to stresses to change the rate of RpoS degradation [16]. Therefore, changes in phosphorylation are not the major mode for regulating RssB activity. RssB is not itself degraded by ClpXP [14].

The discovery of anti-adaptor proteins now provides an explanation for RpoS stabilization in response to stress. Three anti-adaptor proteins, IraP, IraM and IraD (Inhibitor of RssB activity) are induced under different stress conditions; each interacts with the RssB adaptor

and protects RpoS from degradation [17–20]. Because RssB levels are low, induction of the anti-adaptors rapidly saturates all of the RssB, preventing it from binding to RpoS (Fig. 1).

This partner switching is reminiscent of the anti-sigma and anti-anti-sigma cascades found to regulate transcription in some bacteria [21]. In the absence of proteolysis, RssB does in fact act as an anti-sigma [22,23]; the anti-adaptor should then act as an anti-anti-sigma, releasing RpoS to promote transcription. Although the Ira anti-adaptors all target RssB, they do not share any sequence or predicted structural similarity. Our current work suggests that each anti-adaptor protein interacts differently with RssB to prevent it from degrading RpoS (Battesti et al., unpublished data).

Mixing and matching different promoters to a set of anti-adaptors may have accelerated evolution of different signals for stabilization of RpoS in different species. This is suggested by the significant differences in anti-adaptors and their regulation seen between *E. coli* and *Salmonella* (outlined in Fig. 1). In both species, RpoS is stabilized in response to magnesium starvation via the PhoPQ two-component system, but via different families of anti-adaptors, IraM in *E. coli* and IraP in *Salmonella* [18,24]. Both species use IraP to stabilize RpoS in response to phosphate starvation [17,18].

Intriguingly, some of the anti-adaptors show similarities to other proteins with different functions. IraM, the anti-adaptor regulated by PhoPQ in *E. coli*, is related to another PhoPQ-regulated protein, PmrD. In Salmonella, PmrD acts as a connector between the PhoPQ system and the PmrB/PmrA two component system (reviewed in [25]). PmrD interacts with the PmrA response regulator, stabilizing the phosphorylated state; whether IraM's interaction with the RssB response regulator shares any similarity to this is not yet clear. However, this evolutionary connection, as well as the dependence on the PhoPQ system for induction of both of these proteins, points out the variety of ways in which small proteins may act to modulate signaling cascades.

IraD contributes to RpoS stabilization during the transition to stationary phase and also is needed in response to DNA damage [19,20]. The closest homologs to this 130 aa protein are, surprisingly, found in bacteriophage tail assembly genes and type VI secretion systems. In these systems, the homologs bring together components of the phage tail/secretion pilus. How these proteins have evolved into IraD, or whether IraD has other functions, are intriguing and unanswered questions.

There may be additional anti-adaptors for RpoS, made under other stress conditions [18,26], or other pathways for modulating RpoS degradation, by regulation at other stages of the proteolytic pathway. For instance, low levels of ATP have recently been proposed to interfere with the ability of ClpXP to degrade RpoS without impeding degradation of some other substrates [27].

Adaptors in *B. subtilis:* ClpC and its indispensable set of adaptor proteins— Global responses in Gram-positive bacteria also depend on adaptor proteins for regulated proteolysis. These have been studied primarily in *B. subtilis*, but many of the components are found in the genomes of other gram-positive bacteria.

YjbH is an adaptor protein needed for the degradation of the transcriptional regulator Spx by ClpXP in *B. subtilis* [28]. Spx is degraded under normal growth conditions and is stabilized in response to disulfide stress. A similar system is found in *Staphylococcusaureus* [29]. YjbH function may be regulated by the redox status of the cell, although exactly how is not yet clear [28,29]. Recently, an anti-adaptor protein YirB (YuzO) that interacts with YjbH and stabilizes Spx has been identified [30]. However, the conditions under which this protein is produced and stabilizes Spx are unknown.

The other characterized adaptor proteins in *B. subtilis* interact with the ClpCP ATP-dependent protease and might be considered essential components of the protease (Fig. 2). This protease is unable to assemble into a functional machine without an adaptor; the interaction of ClpC with adaptor proteins favors ClpC oligomerization and stimulates ClpC ATPase activity [31,32]. Three adaptors have been identified for ClpCP.

MecA is a general adaptor protein for the ClpCP protease, allowing degradation of both unfolded substrates and specific proteins (Fig. 2A). One of the best-studied targets of MecA is ComK, a transcription factor necessary for development of competence. Under normal growth conditions, ComK is rapidly degraded by ClpCP, dependent upon MecA [33,34]. For competence to develop,, degradation of ComK must be blocked. This is accomplished by synthesis of an anti-adaptor, the 46aa ComS protein. ComS is produced in response to quorum-sensing molecules ComX and CSF, when cell density is high or nutrients are limiting [35–39]. Similar regions in ComS and ComK interact with MecA [34], suggesting that ComS directly blocks the ability of MecA to bind to ComK and cause its degradation. This system has obvious parallels to the regulation of RpoS degradation. In both cases, the default situation is that the substrate is degraded, dependent upon an adaptor; regulation of degradation is achieved by regulated synthesis of an anti-adaptor.

The C-terminal region of MecA acts as a degradation tag. It interacts with the N-terminal domain of ClpC, with additional interactions elsewhere on ClpC [31,41]. Intriguingly, there are significant similarities between the interaction of MecA and the ClpC N-terminal domain and the interaction of ClpS with the ClpA N-terminal domain [41]. ATP hydrolysis seems to be essential for the interaction between MecA and ClpC [36]. This interaction activates ClpC ATPase activity, oligomerization and interaction with ClpP [40]. Unlike RssB and its anti-adaptors, which are not degraded, both the adaptor MecA and anti-adaptor ComS are degraded by ClpCP. Cycles of assembly and disassembly of ClpCP are associated with MecA interaction and subsequent degradation [40] (Fig. 2A).

YpbH is a ClpCP adaptor protein with homology to MecA, found only in Bacillus [42]. The C-terminal domain of YpbH has a similar fold to MecA, suggesting parallel interactions with ClpC [41]. The in vivo roles of YpbH are not entirely clear. YpbH does not mediate degradation of ComK or ComS, but does have a role in development of competence and sporulation [42].

A third adaptor for ClpCP, McsB, falls into a different and intriguing category. A primary McsB substrate is CtsR, the global repressor of heat shock proteases. CtsR is degraded during heat stress, dependent on McsB and ClpCP (Fig. 2B) [43]. McsB is an arginine kinase [44]. Its role as an adaptor protein depends on its autophosphorylation but also on the phosphorylation of ClpC on two arginine residues [45]. ClpC phosphorylation is not needed for degradation driven by the MecA adaptor.

During normal growth conditions, McsB interacts with ClpC, which inhibits McsB kinase activity; thus ClpC itself plays the role of an anti-adaptor protein [45] (Fig. 2B). During heat shock, ClpC interacts with unfolded proteins, releasing McsB, which is now free and able to autophosphorylate [32,46]. Once phosphorylated, McsB interacts with the substrate CtsR, leading to degradation of both CtsR and phosphorylated McsB by ClpCP. Thus, the default situation for CtsR is stability; only under the stress condition (heat shock), does CtsR become unstable, leading to induction of ClpC, McsB itself, as well as ClpE and ClpP.

Additional proteins can modulate the basic machinery. McsA, a protein encoded in the same operon as McsB, stimulates adaptor activity by stimulating auto-phosphorylation [32]. YwlE, an arginine phosphatase, dephosphorylates McsB, rendering it inactive [32,46] (Fig. 2B).

McsB can also regulate proteins, including CtsR, under oxidative stress. Under these conditions, McsA is oxidized and no longer associates with McsB, allowing McsB to interact with CtsR and inactivate it without the phosphorylation necessary to target it for degradation [47]. This is an interesting example of an adaptor protein having dual roles in the cell to regulate the same protein. This observation also suggests that McsB may well have a range of other substrates, some targeted to ClpCP degradation and some not. A recent global examination of arginine phosphorylation in *B. subtilis* supports this broader role [48].

Adaptors and Alternative Mechanisms for Regulated Proteolysis in *C. crescentus*—Regulated proteolysis is a common mechanism for regulation of the cell cycle in *C. crescentus* (reviewed in [49]). However, thus far only one adaptor protein for this process, CpdR, has been demonstrated. CpdR, a single domain response regulator, is necessary for the degradation of PdeA, a phosphodiesterase involved in cell cycle regulation [50,51]. The degradation of PdeA depends on the phosphorylation status of CpdR, which is also cell-cycle timed [52]; unphosphorylated CpdR leads to PdeA degradation by ClpXP [50]. The dephosphorylation of CpdR occurs during the swarmer to stalk transition.

In other cases, mechanisms that ensure localization of the protease and the substrate to the same place at the same time may play the role that adaptors play, allowing the cell to modulate the conditions under which substrate degradation takes place. For instance, CtrA, a transcription factor that plays a major role in the developmental cell cycle of *C. crescentus*, is degraded by ClpXP at the transition from G1 to S, and this degradation is essential for the proper cell cycle (reviewed in [49,53]). For proteolysis to occur, CtrA and ClpXP have to be properly localized to the pole. ClpXP localization depends on the response regulator CpdR and CtrA localization depends on PopA, another response regulator-like protein. Thus, these proteins act as in vivo adaptor-like proteins, but were not found to affect in vitro degradation; their role in regulating CtrA is apparently only for localization [11]. Another accessory protein, RcdA, is strictly required for CtrA degradation in vivo, but not in vitro, again suggesting that this protein does not strictly fit the definition of an adaptor protein [54]. Studies in Caulobacter highlight some of the alternative ways in which protein stability can be regulated without adaptors.

IV. Conclusions and Implications

The bacterial ATP-dependent proteases play broad roles in both quality control and regulated proteolysis. Because of the range of substrates that each protease degrades, changing the levels of the protease is not an efficient way to change the fate of a specific substrate. The growing list of adaptor proteins and anti-adaptors provides a solution to the problem of how to regulate specific protein turnover in response to multiple signals, using a limited number of ATP-dependent proteases. Environmental and developmental signals feed into specific degradation pathways by regulating synthesis of anti-adaptors and modification of both adaptors and anti-adaptors.

The mechanistic details of how the adaptors work are far from fully understood, but are starting to emerge. In all studied cases, the adaptors interact with flexible N-terminal domains of the ATPase components of the proteases. Secondary binding sites nearer the ATPase pore may also be involved in hand-off of the substrate to the ATPase. While adaptors vary greatly, it is striking that a number of them contain conserved response regulator domains. Those that are not subject to degradation may be protected in part by C-terminal sequences that are not favored by the pore binding sites of the ATPase, or may be too stable to be unfolded. As more structures of adaptor complexes become available, it should become clearer how much different adaptor pathways have in common.

Anti-adaptors also differ significantly. Their mode of action appears to be direct protein-protein binding, leading to sequestration of the adaptor from its substrate. Regulation of levels of both adaptor and anti-adaptor must be precise to allow anti-adaptor function and return to equilibrium after the anti-adaptor is no longer needed.

Proteolysis of specific substrates is modulated without adaptors as well. Substrate modifications can change susceptibility to proteolysis, and substrates can be both protected from proteolysis or made more susceptible by interactions with protein partners, or DNA sites. For instance, UmuD′ protein, a subunit of DNA polymerase V, becomes a substrate for ClpXP by interacting with UmuD, the precursor for UmuD′ [55,56]. In these cases, regulation may reflect changes in the availability of those partners. The need for proper localization of protease and substrate in Caulobacter highlights another pathway. Just as synthesis is regulated at multiple levels, it is not surprising that proteolysis is as well.

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Highlights

- Regulated degradation by ATP-dependent proteases frequently depends upon delivery of the substrate by adaptors.
- Regulation of activity of adaptors by anti-adaptors allows environmental modulation of protein turnover.
- Structural studies are uncovering how adaptors bind and hand over the substrate to the protease.

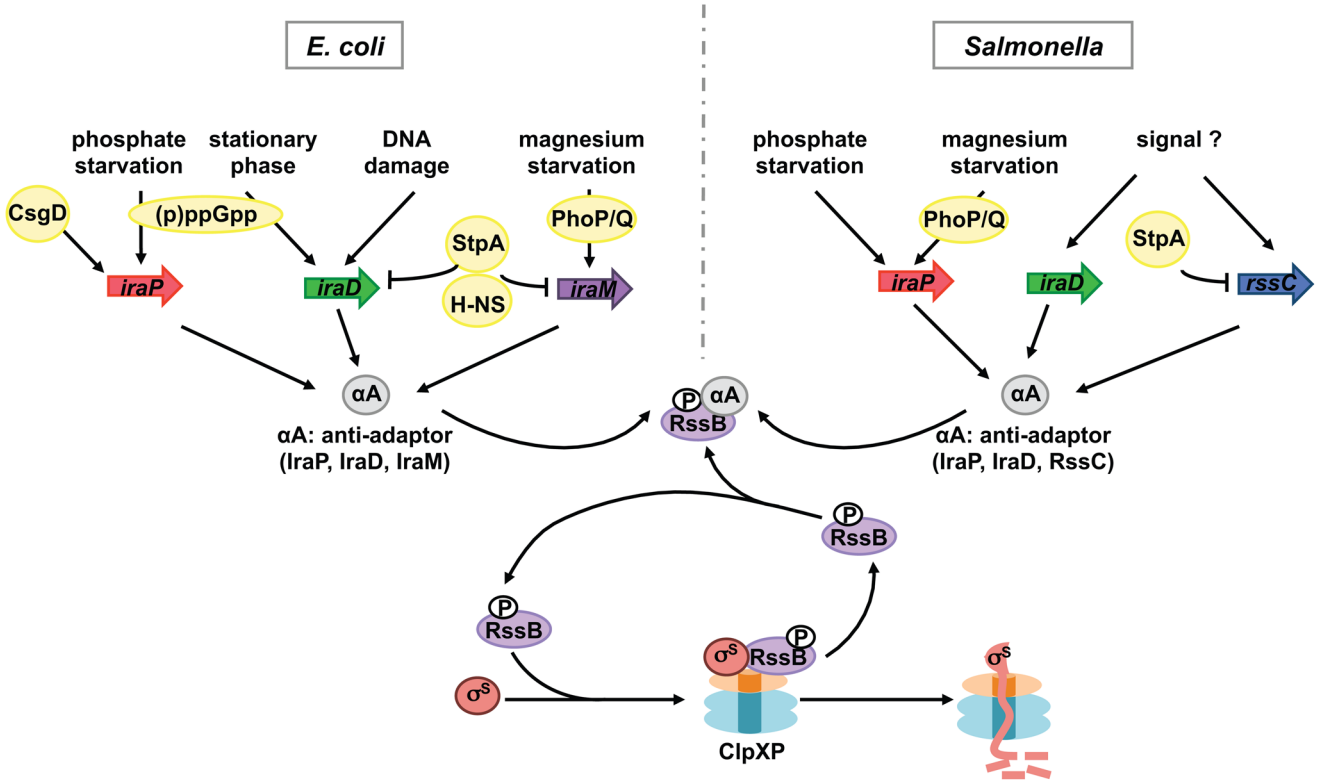

Figure 1. Regulation of RpoS proteolysis in *E. coli* and *Salmonella*

RpoS (σ^S) is degraded by the ClpXP protease via its interaction with the adaptor protein RssB (purple oval). The known anti-adaptor proteins (αA; grey circle) that contribute to RpoS stabilization under different stress conditions in *E. coli* and *Salmonella* share some but not all inducing signals, and the same inducing signal (magnesium starvation via PhoQP) is used for different proteins in different species. Multiple regulatory factors (yellow ovals) control the expression of the anti-adaptors.

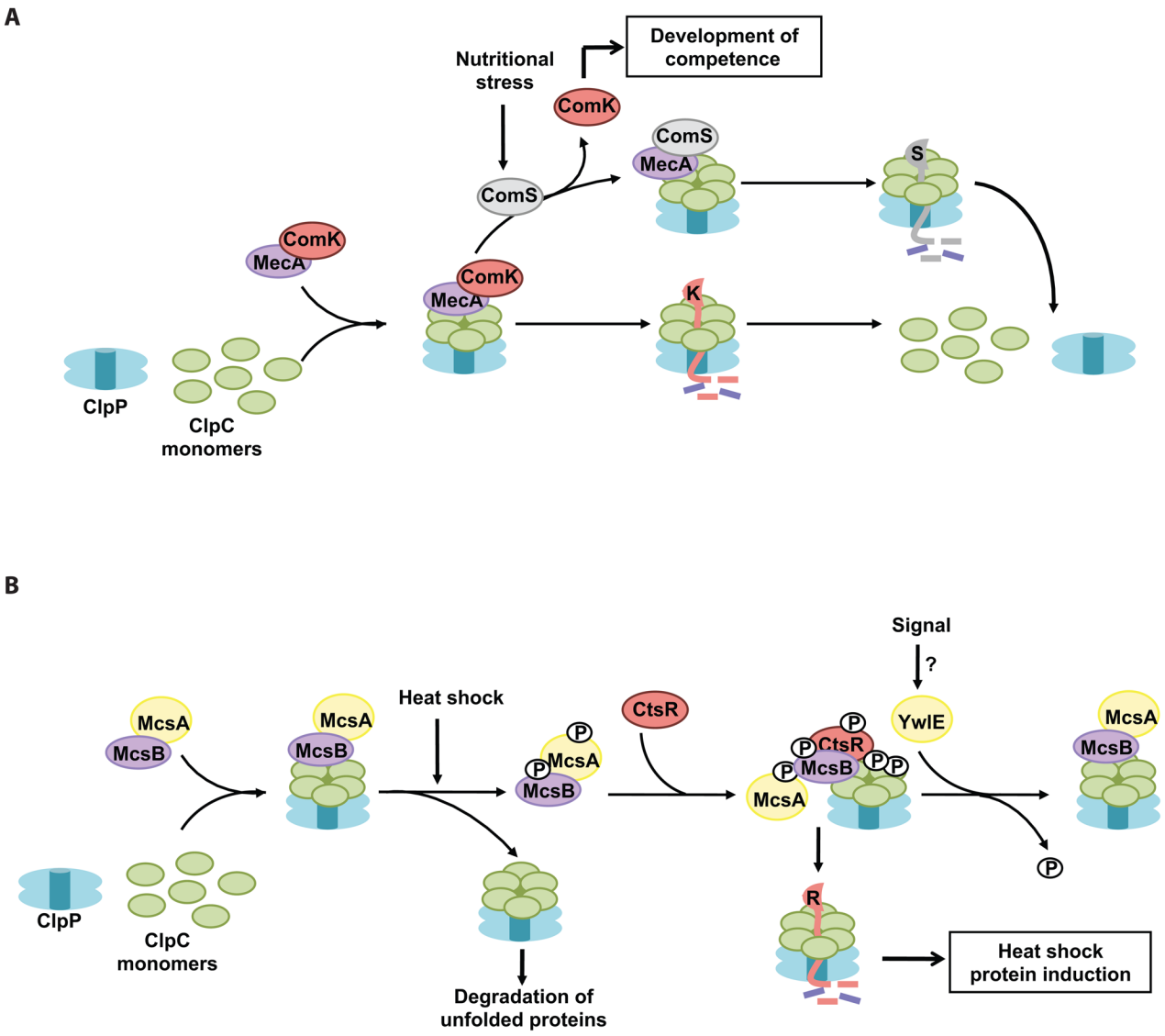

Figure 2. ClpC interaction with various adaptor proteins modulates substrate specificity in *B. subtilis*

ClpC oligomerization and interaction with ClpP depends on its interaction with adaptor proteins. A. MecA allows the degradation of ComK but is titrated, in the case of nutritional stress, by the anti-adaptor protein ComS, allowing development of competence by sparing ComK. ComS and ComK are themselves degraded by ClpCP. MecA degradation leads to ClpCP disassembly.

B. McsB activity depends on its phosphorylation state but also requires ClpC phosphorylation. Under normal growth conditions, McsB kinase activity is inhibited by its interaction with ClpC. During heat shock, ClpC interacts preferentially with unfolded proteins, allowing McsB phosphorylation which is also favored by the protein McsA. This activation leads to the degradation of the repressor, CtsR. In thus far undefined conditions, the YwlE phosphatase inactivates McsB.