

The RssB response regulator directly targets σ^S for degradation by ClpXP

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The σ^S subunit of *Escherichia coli* RNA polymerase regulates the expression of stationary phase and stress response genes. Control over σ^S activity is exercised in part by regulated degradation of σ^S . In vivo, degradation requires the ClpXP protease together with RssB, a protein homologous to response regulator proteins. Using purified components, we reconstructed the degradation of σ^S in vitro and demonstrate a direct role for RssB in delivering σ^S to ClpXP. RssB greatly stimulates σ^S degradation by ClpXP. Acetyl phosphate, which phosphorylates RssB, is required. RssB participates in multiple rounds of σ^S degradation, demonstrating its catalytic role. RssB promotes σ^S degradation specifically; it does not affect degradation of other ClpXP substrates or other proteins not normally degraded by ClpXP. σ^S and RssB form a stable complex in the presence of acetyl phosphate, and together they form a ternary complex with ClpX that is stabilized by ATP[γ -S]. Alone, neither σ^S nor RssB binds ClpX with high affinity. When ClpP is present, a larger σ^S -RssB-ClpXP complex forms. The complex degrades σ^S and releases RssB from ClpXP in an ATP-dependent reaction. Our results illuminate an important mechanism for regulated protein turnover in which a unique targeting protein, whose own activity is regulated through specific signaling pathways, catalyzes the delivery of a specific substrate to a specific protease.

[Key Words: ATP-dependent proteolysis; molecular chaperones; ClpA; RpoS; sigma S; acetyl phosphate]

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All organisms use rapid degradation of specific proteins as one way to tightly control important regulatory factors and developmental switches. Regulation of biological functions by proteolysis requires accurate substrate recognition at a precise time. In eukaryotes, fidelity of protein degradation depends on ubiquitin-tagging, a process that occurs in multicomponent complexes involving regulatory factors and various ubiquitin pathway enzymes. The ubiquitinated proteins are then targeted to the proteasome for degradation (for review, see Voges et al. 1999). In *Escherichia coli*, an example of substrate tagging as a means for regulating proteolysis is the degradation of incomplete proteins made from truncated mRNAs. In this case, short peptides coded by the SsrA RNA are added cotranslationally to polypeptides that have become stalled on ribosomes, and the tagged proteins are then degraded (Keiler et al. 1996; Gottesman et al. 1998; Roche and Sauer 1999). In general, *E. coli* proteins regulated by degradation interact directly with the proteases themselves, with each of the five known *E. coli* ATP-dependent proteases degrading a different but somewhat overlapping set of substrates (Gottesman

1996; Wickner et al. 1999). For many of the highly unstable *E. coli* proteins, degradation is rapid under all conditions, and synthesis is tightly controlled.

However, the activity of some proteins in *E. coli* is controlled by regulated degradation. For example, σ^{32} (RpoH), the heat shock sigma factor, is rapidly degraded under normal growth conditions by the AAA protease, FtsH, in a reaction modulated by the DnaJ/DnaK/GrpE chaperone system. During heat shock, σ^{32} is transiently stabilized, and this stabilization results in the rapid increase in the synthesis of the heat shock proteins (Yura and Nakahigashi 1999).

The stationary phase sigma factor, σ^S (RpoS), is another example of a protein whose activity is controlled by regulated proteolysis. σ^S promotes expression of more than 50 genes involved in responses to many stresses, including starvation, osmotic stress, acid shock, cold shock, heat shock, and oxidative damage, as well as the transition to stationary phase (Loewen and Hengge-Aronis 1994; Hengge-Aronis 2000). Although σ^S is present at very low levels during exponential cell growth, owing largely to its rapid degradation (half-life of ~2 min), its stability increases ~10-fold following transition to stationary phase or other stress treatments. Regulated degradation plays a major role in determining the amount of σ^S in the cell, but σ^S accumulation is also regulated at the transcriptional and translational levels (Lange and

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Hengge-Aronis 1994). The protease responsible for σ^S turnover in exponentially growing cells is ClpXP (Schweder et al. 1996), an ATP-dependent protease consisting of a regulatory component, ClpX, and a proteolytic component, ClpP (Gottesman et al. 1993; Wojtkowiak et al. 1993).

Degradation of σ^S requires an additional protein, RssB (Regulator of Sigma S; also referred to as SprE in *E. coli*, MviA in *Salmonella*, and ExpM in *Erwinia*) that is homologous to response regulator proteins (Bearson et al. 1996; Muffler et al. 1996b; Pratt and Silhavy 1996; Andersson et al. 1999). Genetic evidence shows that RssB is required for σ^S degradation but not for another ClpXP substrate, λ O, which indicates that RssB specifically targets σ^S for degradation (Zhou and Gottesman 1998). One of the hallmarks of the response regulator component of two-component signal transduction systems in prokaryotes is the presence of a conserved aspartate that is phosphorylated by the cognate sensor component. The N-terminal domain of RssB contains this conserved aspartate, although a cognate sensor protein has not been identified. RssB, like many other response regulators, is phosphorylated by acetyl phosphate in vitro (Bouche et al. 1998). In addition, phosphorylated RssB forms a stable complex with σ^S in vitro (Becker et al. 1999).

In this report we investigate RssB-regulated degradation of σ^S by reconstituting the pathway of σ^S degradation in vitro with purified RssB and ClpXP. We discovered that RssB acts directly and catalytically in stimulating degradation of σ^S by ClpXP in a reaction requiring acetyl phosphate and ATP. We have isolated and characterized subassemblies of the degradation machinery including σ^S -RssB, σ^S -RssB-ClpX, and σ^S -RssB-ClpXP complexes, and suggest a probable pathway for the degradation of σ^S .

Results

RssB promotes σ^S degradation by ClpXP in vitro

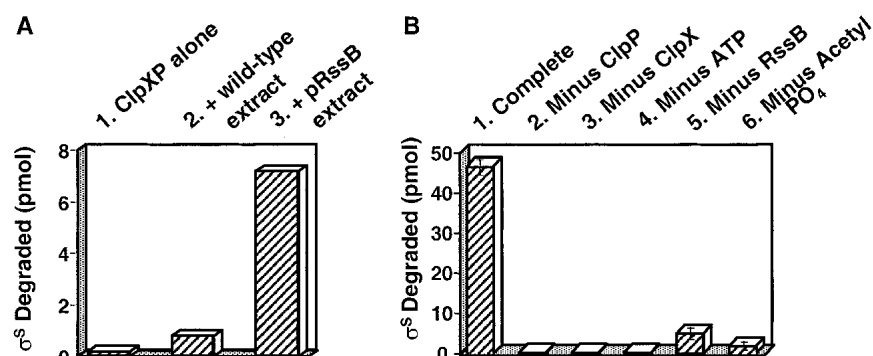
Although genetic evidence pointed to a central role for RssB in σ^S degradation, it was not clear how RssB modu-

lated σ^S degradation and whether or not it acted directly. To study the mechanism of RssB regulation of σ^S degradation by ClpXP, we first developed an in vitro assay to measure σ^S degradation. Purified ClpXP alone degraded σ^S poorly, as measured by acid solubilization of radioactively labeled σ^S (Fig. 1A). Degradation was greatly stimulated by the addition of a crude extract prepared from cells overproducing RssB from a multicopy pACYC plasmid (Fig. 1A). A similar amount of crude extract of cells carrying the vector alone did not stimulate degradation significantly, very likely because the chromosomal copy of *rssB* provided only a small amount of RssB. These observations indicate that RssB is involved in σ^S degradation in vitro.

We purified the protein that promoted degradation of σ^S by ClpXP from an extract of cells expressing RssB from a multicopy plasmid and later from cells producing RssB from a T7 expression system. The identification of RssB as the stimulatory factor was confirmed by N-terminal amino acid sequencing, and the final preparations were >90% pure as judged by SDS-PAGE. Purified RssB stimulated σ^S degradation by ClpXP more than 10-fold (Fig. 1B). ClpX, ClpP, and ATP were essential for σ^S degradation (Fig. 1B). Thus, as seen in vivo (Muffler et al. 1996a; Pratt and Silhavy 1996), σ^S degradation by ClpXP in vitro is dependent upon RssB. Importantly, these results show that the role of RssB in σ^S degradation is direct.

Because acetyl phosphate is a phosphate donor for the phosphorylation of several response regulators in vitro, including RssB (Bouche et al. 1998), it seemed likely that it might be required for RssB activity and was therefore included in the reaction mixtures. When acetyl phosphate was omitted, σ^S degradation decreased 10-fold (Fig. 1B). Half-maximal activation of degradation occurred with ~7 mM acetyl phosphate. Other potential phosphate donors, including carbamyl phosphate and phosphoenol pyruvate, were ineffective in stimulating σ^S degradation. The observation that RssB is phosphorylated in vitro by acetyl phosphate (Bouche et al. 1998), in combination with our finding that σ^S proteolysis is stimulated by acetyl phosphate, indicates that the phosphorylated form of RssB is required to activate degradation.

Figure 1. σ^S degradation by ClpXP and RssB in vitro. (A) Stimulation of ClpXP-dependent σ^S degradation by extracts of cells overproducing RssB. Degradation of [3 H] σ^S by ClpXP was measured as described in Materials and Methods in the absence of a source of RssB (column 1); in the presence of 20 μ g of an extract of *E. coli* MC4100 *clpP*⁻ (Zhou and Gottesman 1998) carrying the plasmid vector pACYC184 (column 2); or in the presence of 20 μ g of an extract of *E. coli* MC4100 *clpP*⁻ carrying pUM-E, a pACYC184 derivative with a 5.7-kb *EcoRI* fragment that includes *rssB* (Rosl and Kersten 1994) (column 3). (B) Requirements for σ^S degradation with pure proteins. Degradation of [3 H] σ^S was measured as described in Materials and Methods with purified components using 120 pmoles of [3 H] σ^S . ClpP, ClpX, ATP, RssB, and acetyl phosphate were omitted where indicated.



We next addressed the question of whether RssB acts stoichiometrically or catalytically in the degradation reaction. Degradation of σ^S was linear with time in reaction mixtures containing a 60-fold molar excess of σ^S relative to RssB, ClpX, and ClpP (Fig. 2A). After 40 min, 25 pmoles of σ^S had been degraded per pmole of RssB added (Fig. 2A). Thus, RssB functions catalytically in σ^S degradation. By measuring the rate of degradation of σ^S by ClpXP in the presence and absence of RssB at various concentrations of σ^S , we found that RssB increased the apparent affinity for σ^S by >10-fold and increased the rate of the reaction by at least 6-fold (Fig. 2B). Therefore, RssB facilitates the interaction of σ^S with ClpXP.

RssB is specific for σ^S and for ClpXP

In vivo, *rssB* null mutants do not degrade σ^S , but continue to degrade another ClpXP substrate, λ O protein (Gottesman et al. 1993; Wojtkowiak et al. 1993), which indicates that RssB acts on the specific substrate rather than on the protease (Zhou and Gottesman 1998). To test the substrate specificity of RssB in vitro, we examined the effect of RssB on λ O degradation by ClpXP. RssB

Table 1. Substrate and protease specificity of RssB

	Degradation (Percent of protein added)			
	σ^S	σ^{70}	α -casein	RepA
ClpXP	3 \pm 1	<10	<3	<1
ClpXP + RssB	60 \pm 2	<10	<3	<1
ClpAP	12 \pm 1	ND	32	24
ClpAP + RssB	12 \pm 1	ND	31	21

Degradation reactions were carried out as described in Materials and Methods with [3 H] σ^S , σ^{70} , [3 H] α -casein, or [3 H]RepA used as substrates where indicated. σ^{70} degradation was determined by analyzing the reaction products by SDS-PAGE. ND, indicates not done.

stimulated σ^S degradation at all concentrations of ClpX; in contrast, RssB had no effect on λ O degradation by ClpXP (Fig. 2C,D). Thus the in vitro results mimic the in vivo substrate specificity.

We asked whether RssB increased the promiscuity of ClpXP, allowing it to degrade proteins that are not normally recognized by ClpX. σ^{70} , the vegetative sigma subunit of RNA polymerase, was tested as a potential substrate because the C-terminal half of σ^{70} is homologous to σ^S . ClpXP did not degrade σ^{70} in the presence or absence of RssB, using conditions in which σ^S degradation required ClpXP and RssB (Table 1). This result is consistent with the in vivo observation that σ^{70} is stable (Grossman et al. 1983). Similarly, RssB did not enable ClpXP to degrade α -casein or RepA, which are poor substrates for ClpXP but good substrates for another Clp protease, ClpAP (Table 1; Wojtkowiak et al. 1993; Wickner et al. 1994). We also tested whether RssB could stimulate degradation by ClpAP. There was no effect of RssB on the small but significant amount of σ^S degradation by ClpAP (Table 1). There was also no effect of RssB on the rate of degradation of α -casein or RepA by ClpAP (Table 1). These results indicate that RssB acts specifically with ClpX to promote efficient degradation of σ^S . Thus, in vitro as well as in vivo, RssB shows a high degree of specificity both for the substrate σ^S and for the protease to which σ^S is targeted, ClpXP.

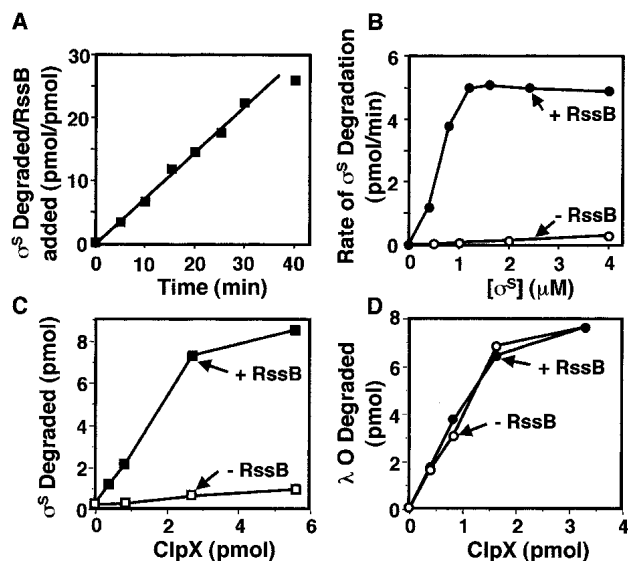


Figure 2. RssB functions catalytically and specifically. (A) RssB acts catalytically. [3 H] σ^S degradation was measured as a function of time as described in Materials and Methods in reaction mixtures with 2 pmoles of RssB, 120 pmoles of [3 H] σ^S , 2 pmoles of ClpX, and 2 pmoles of ClpP. (B) RssB increases the apparent affinity for σ^S and increases the rate of the reaction. [3 H] σ^S degradation was measured in reaction mixtures as described in Materials and Methods with 0.1 μ M ClpX, 0.1 μ M ClpP, and the indicated concentrations of σ^S , either with 0.4 μ M RssB (filled circles) or without RssB (open circles). Reaction times were 2, 4, 6, and 10 min when RssB was included, and 10, 15, and 25 min in the absence of RssB. (C, D) RssB stimulates degradation of σ^S but not λ O. Degradation of [3 H] σ^S (C) and [3 H] λ O (D) was measured in reaction mixtures as described in Materials and Methods with varying amounts of ClpX and 19 pmoles of [3 H] λ O substituted for [3 H] σ^S in panel D, both with RssB (filled symbols) and without RssB (open symbols).

Interaction of RssB and σ^S

To study the mechanism of action of RssB, we designed experiments to dissect the degradation pathway into partial reactions and to determine the role of acetyl phosphate and ATP at the various steps. Evidence for an interaction of σ^S and RssB was obtained by showing that σ^S protects RssB from inactivation by *N*-ethylmaleimide (NEM), a reagent that reacts with accessible sulfhydryl groups. NEM treatment of RssB inhibited the ability of RssB to promote σ^S degradation by ClpXP in vitro by >10-fold (data not shown). When σ^S and RssB were incubated together, σ^S protected RssB from NEM inactivation, and protection required acetyl phosphate and Mg^{2+} . These results suggested that phosphorylated RssB inter-

acts with σ^S , and this intermediate is functional in the degradation pathway.

Interaction between RssB and σ^S was further assessed using gel filtration. σ^S , a 38-kD protein, alone eluted earlier than expected, at the position predicted for a globular protein of 48 kD. RssB, a 37-kD protein, alone eluted later than expected, at the position predicted for a globular protein of 25 kD (the positions where σ^S and RssB eluted when chromatographed separately are indicated by arrows at the top of Fig. 3). The possibility that RssB interacts with the column matrix has not been ruled out.

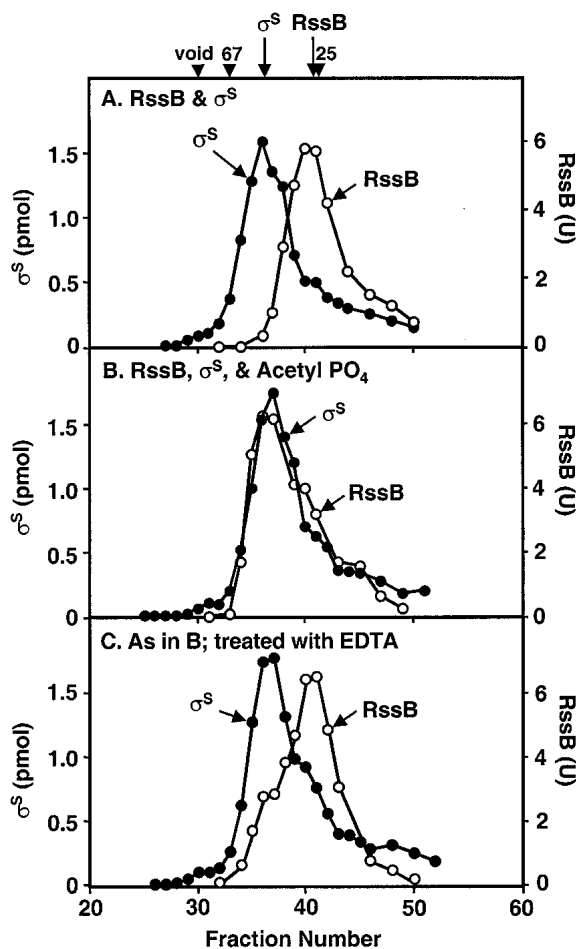


Figure 3. Interaction of σ^S and RssB. (A) RssB (150 pmoles) and [3H] σ^S (320 pmoles) were incubated in the absence of acetyl phosphate and analyzed by gel filtration in the absence of acetyl phosphate as described in Materials and Methods. (B) [3H] σ^S and RssB were incubated together with acetyl phosphate and analyzed by gel filtration in the presence of acetyl phosphate. (C) [3H] σ^S and RssB were incubated with acetyl phosphate for 10 min, treated with 20 mM EDTA for 1 h at 0°C, and then analyzed by gel filtration in the absence of acetyl phosphate and Mg^{2+} . The positions where σ^S and RssB eluted when chromatographed separately, with or without acetyl phosphate, are indicated with arrows. Filled circles indicate the elution of σ^S , and open circles indicate RssB in A, B, and C. Molecular weight standards, Blue dextran 2000 (2000 kD), bovine serum albumin (67 kD), and chymotrypsinogen A (25 kD), eluted as indicated by arrows labeled void, 67, and 25, respectively.

Acetyl phosphate did not affect the elution of either protein. When σ^S and RssB were incubated together in the absence of acetyl phosphate and analyzed by gel filtration, they eluted separately and at the same positions obtained when each was chromatographed separately (Fig. 3A). Following incubation of σ^S and RssB with acetyl phosphate and Mg^{2+} , the two proteins coeluted at a position indistinguishable from the position of σ^S alone (Fig. 3B). Both acetyl phosphate and Mg^{2+} were required for the altered elution pattern. Because the elution profiles of σ^S and RssB alone were not as expected, we confirmed the interaction of σ^S and RssB by affinity chromatography using either His₆-RssB or His₆- σ^S (data not shown). The interaction was also detected by Far-Western blotting of His₆-RssB associated with membrane-bound σ^S using RssB antibody (data not shown). Physical interaction between σ^S and tagged RssB was also shown by Hengge-Aronis and coworkers by affinity chromatography (Becker et al. 1999) and by Foster and coworkers using a two-hybrid system (Moreno et al. 2000). In light of independent demonstrations of a σ^S -RssB complex, the gel filtration experiments further indicate that the σ^S -RssB interaction results in significant conformational changes in σ^S .

To test whether or not the σ^S -RssB complex could be dissociated, we formed σ^S -RssB complexes in the presence of acetyl phosphate and Mg^{2+} and then added EDTA to chelate Mg^{2+} , a metal ion essential for the interaction of σ^S and RssB. After 60 min, the mixture was analyzed by gel filtration. σ^S eluted free from RssB (Fig. 3C). Taken together, these observations are consistent with a reversible acetyl phosphate-dependent physical interaction between RssB and σ^S .

σ^S , RssB, and ClpX form a ternary complex

Because ClpX is responsible for unfolding and delivering substrates to ClpP, we expected that σ^S alone or the σ^S -RssB complex interacts with ClpX. Using ultrafiltration membranes with a molecular weight cutoff of 100,000, it was observed that σ^S was retained on the membranes with ClpX when RssB was present (Fig. 4). σ^S was not retained when ClpX was omitted.

To distinguish whether RssB transferred σ^S to ClpX or whether σ^S and RssB formed a ternary complex with ClpX, mixtures of σ^S , ClpX, and RssB were incubated in the presence of acetyl phosphate, adenosine-5'-O-(3-thiotriphosphate) (ATP[γ -S]), and Mg^{2+} and analyzed by gel filtration. The three proteins eluted together in the excluded volume, showing that RssB assembles into a ternary complex with σ^S and ClpX (Fig. 5A). In control experiments, ClpX, σ^S , and RssB alone eluted separately (at the positions indicated by arrows at the top of Fig. 5). Complex formation required ATP[γ -S] and Mg^{2+} . When acetyl phosphate was omitted, ~20% as much σ^S and RssB eluted with ClpX as when acetyl phosphate was present. A very small amount of RssB associated with ClpX in the absence of σ^S , and a small amount of σ^S associated with ClpX in the absence of RssB, indicating weak interactions between the individual proteins and

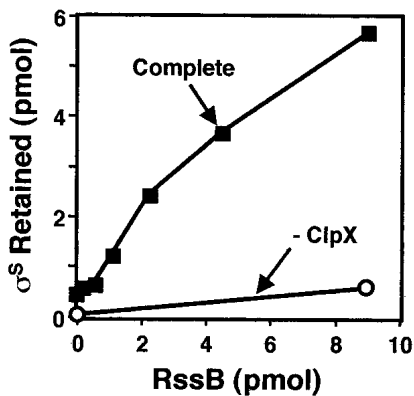


Figure 4. Binding of σ^S to ClpXP promoted by RssB. Reaction mixtures containing 9 pmoles of [^3H] σ^S , 60 pmoles of ClpXP, and the indicated amounts of RssB were incubated with ATP[γ -S] and acetyl phosphate as described in Materials and Methods. The mixtures were then centrifuged through microcon 100 ultrafiltration devices, and radioactivity in the retentates was measured as described. Complete (filled squares); minus ClpX (open circles).

ClpX (Fig. 5B,C). RssB and σ^S were released from ClpX following the exchange of ATP[γ -S] with ATP by the addition of a 10-fold molar excess of ATP to ATP[γ -S] (data not shown). These data indicate that RssB is an integral part of the degradative complex and has a role beyond simply promoting a conformation of σ^S that is recognized by ClpX.

σ^S , RssB, ClpX, and ClpP form a quaternary complex

Knowing that ClpX and ClpA form stable complexes with their specific substrates and with ClpP, we anticipated that larger proteolytic complexes containing σ^S and ClpXP might also be stable. To show this, σ^S , RssB, ClpX, and ClpP were incubated with a nonhydrolyzable ATP analog, adenylyl-imidodiphosphate (AMP-PNP), acetyl phosphate, and Mg^{2+} and applied to a gel filtration column. σ^S and RssB eluted together with ClpXP in the excluded volume of the column, demonstrating a stable quaternary complex (Fig. 6A).

In the absence of ClpP, ClpX did not associate with σ^S and RssB in the presence of AMP-PNP, in contrast to the ternary complex formed in the presence of ATP[γ -S] (cf. Figs. 6B and 5A). When we attempted to form σ^S -RssB-ClpXP complexes in the presence of ATP[γ -S], we found that σ^S was degraded (data not shown). Kinetic experiments showed that the rate of σ^S degradation was 8-fold slower with ATP[γ -S] than with ATP (data not shown). ClpX hydrolyzes ATP[γ -S] slowly, and ClpXP degrades another substrate, λ O, in the presence of ATP[γ -S], indicating that hydrolysis of ATP[γ -S] serves as the energy source for proteolysis (J. Rozycki and M.R. Maurizi, unpubl.). These results indicate that ClpP, when bound to ClpX, promotes conformational changes in the nucleotide-binding cleft of ClpX that allow AMP-PNP and ATP[γ -S] to bind.

RssB is released from ClpXP as σ^S is degraded

We wanted to know whether RssB was released from ClpXP as σ^S was degraded or whether it remained associated with ClpXP for multiple cycles of σ^S degradation. Therefore, σ^S , RssB, and ClpXP were incubated in the presence of AMP-PNP, acetyl phosphate, and Mg^{2+} , and quaternary complexes were isolated by gel filtration as described above. The isolated complexes were incubated with a 10-fold molar excess of ATP over AMP-PNP to allow degradation, and then the reaction mixture was reapplied to the gel filtration column (Fig. 6C). As expected, σ^S was degraded; the radioactively labeled σ^S peptides eluted in the fully included volume of the column

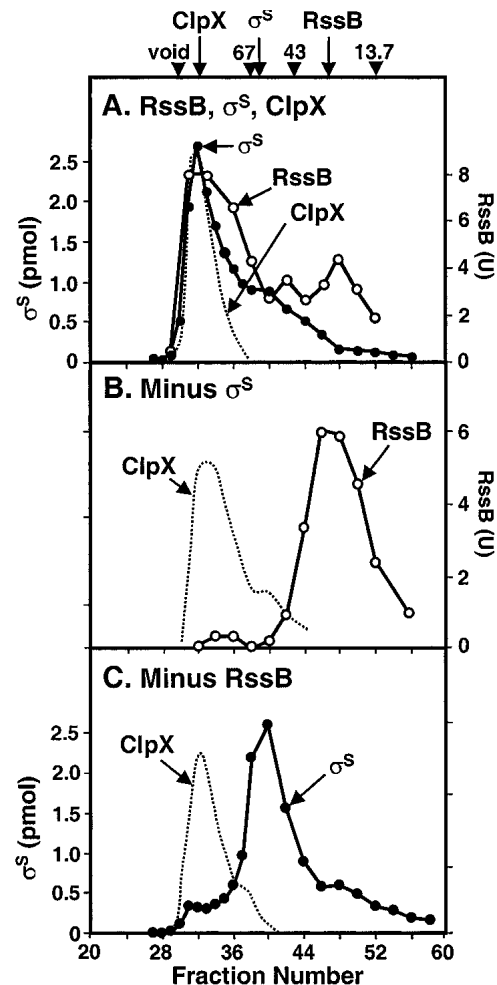


Figure 5. Formation of ternary σ^S -RssB-ClpX complexes. (A) [^3H] σ^S (215 pmoles), 150 pmoles of RssB, and 250 pmoles of ClpX were incubated in the presence of acetyl phosphate and ATP[γ -S] and analyzed by Sephacryl S-200 gel filtration as described in Materials and Methods. (B) σ^S was omitted. (C) RssB was omitted. The positions where ClpX, [^3H] σ^S , and RssB eluted when chromatographed separately are indicated with arrows. σ^S is indicated by filled circles, RssB by open circles, and ClpX by dotted line. The positions where Blue dextran 2000 (2000 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), and ribonuclease A (13.7 kD) eluted are indicated by arrows labeled void, 67, 43, and 13.7, respectively.

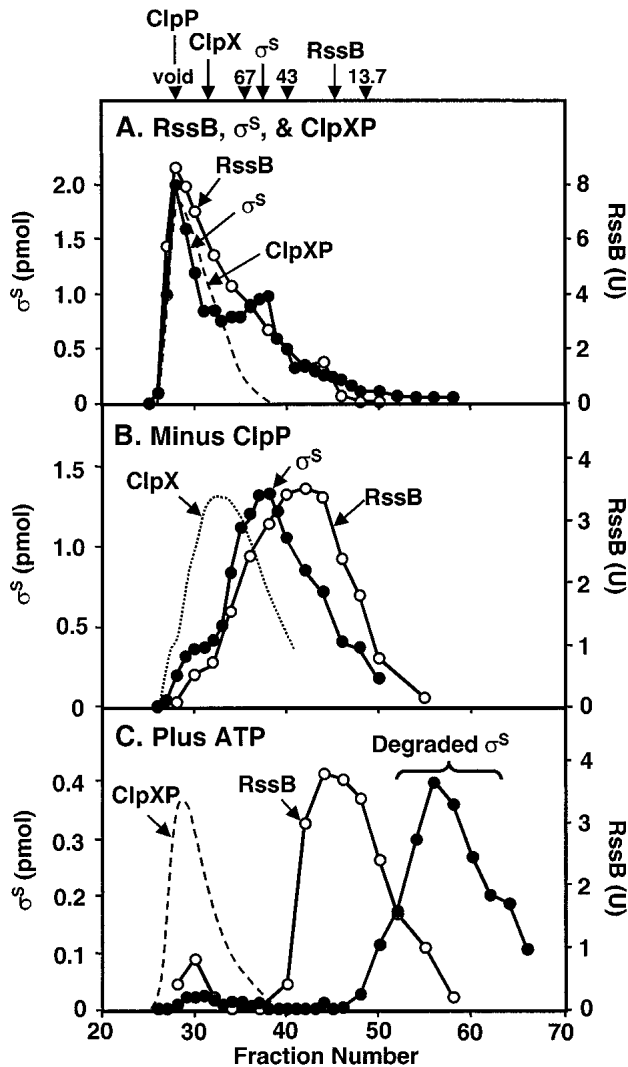


Figure 6. Formation of quaternary σ^S -RssB-ClpXP complexes and release of RssB with σ^S degradation. (A) [^3H] σ^S (215 pmol), 150 pmol of RssB, 250 pmol of ClpX, and 250 pmol of ClpP were incubated in reaction mixtures containing acetyl phosphate and AMP-PNP in Buffer B and then analyzed by Sephacyl S-200 gel filtration as described in Materials and Methods. (B) Reactions were as in A, but ClpP was omitted. (C) [^3H] σ^S , RssB, ClpX, and ClpP were incubated as in A, and complexes were isolated. Fractions containing σ^S -RssB-ClpXP complexes were pooled (fractions 29–31) and incubated in 200 μL with 10 mM ATP for 10 min at 24°C. Following incubation, the sample was chromatographed on the gel filtration column. In panel C, TCA-soluble radioactivity was measured in a portion of each fraction, and the radioactivity eluting between fractions 50 and 68 was soluble. The positions where ClpX, ClpP, σ^S , and RssB eluted when chromatographed separately are indicated with arrows. The positions where Blue dextran 2000 (2000 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), and ribonuclease A (13.7 kD) eluted are indicated by arrows labeled void, 67, 43, and 13.7, respectively. In all three panels, the proteins were measured as described in Materials and Methods. σ^S is indicated by filled circles, RssB by open circles, ClpX by dotted line, and ClpXP by dashed line.

and were acid-soluble. We found that RssB was released from ClpXP. It eluted as expected for free RssB and was able to stimulate σ^S degradation by ClpXP in the RssB assay (Fig. 6C). Thus, RssB is not degraded by ClpXP concomitantly with σ^S degradation, nor is it inactivated upon release. When [^3H] σ^S was added to aliquots of pooled fractions containing ClpXP (fractions 30–31), <1 pmole of σ^S was degraded in the absence of added RssB, and 6 pmol of σ^S was degraded in the presence of RssB. Thus, prior interaction of ClpXP with RssB does not activate ClpXP to degrade additional σ^S .

These results, together with the results showing that RssB acts catalytically (Fig. 2A), indicate that RssB shuttles σ^S to ClpXP; the RssB- σ^S complex has high affinity for ClpX, whereas RssB and σ^S each alone has low affinity for ClpX.

σ^S is protected from degradation when associated with core RNA polymerase

In vivo, σ^S exists either free or in a complex with core RNA polymerase, forming an active holoenzyme. To determine if the interaction of σ^S with core affects RssB-promoted degradation of σ^S , we mixed core with an equimolar amount of σ^S , and, after a short incubation, measured degradation of σ^S by ClpXP in the presence of RssB. We found that core RNA polymerase inhibited σ^S degradation ~90%, demonstrating that σ^S is not a substrate for proteolysis when associated with core (Fig. 7A). Because σ^S and σ^{70} are known to compete for binding core, we tested whether the addition of σ^{70} would allow σ^S degradation in the presence of core. Addition of σ^{70} in an equal or greater molar ratio to core resulted in degradation of the σ^S . This result indicates that RssB recognition sites in σ^S or ClpX recognition sites in the σ^S -RssB complex are inaccessible when σ^S is associated with core RNA polymerase.

To determine if the complex of σ^S -RssB can be bound by core RNA polymerase, σ^S and RssB were first incubated with acetyl phosphate to generate σ^S -RssB complexes and then core RNA polymerase was added. Following incubation, the mixture was analyzed by gel filtration column chromatography (Fig. 7B), and we found that >90% of the σ^S eluted with core RNA polymerase, whereas >90% of the RssB eluted as expected for monomeric RssB. Therefore, σ^S interacts with either RssB or with core RNA polymerase, but not with both together. These experiments also show that with equimolar concentrations of core and RssB, core displaces RssB from σ^S -RssB complexes, indicating that σ^S has a higher affinity for core than for RssB.

Discussion

In *E. coli*, regulating σ^S degradation quickly and reversibly in response to environmental signals is a key aspect of transitions between growth states, such as from exponential to stationary phase or between normal and stress response states. Mutations that block σ^S degradation

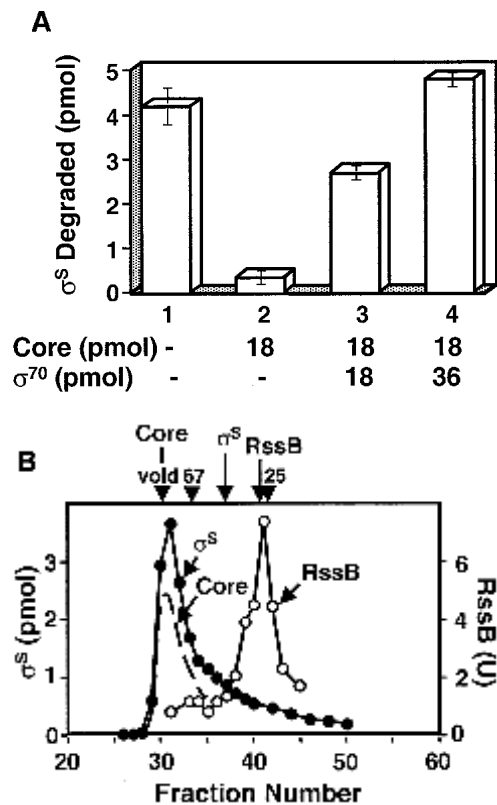


Figure 7. Competition between RssB and core RNA polymerase for σ^S binding. (A) Protection of σ^S from degradation by core RNA polymerase. [^3H] σ^S (18 pmoles) alone (columns 1 and 2) or with 18 pmoles of σ^{70} (column 3) or 36 pmoles (column 4) of σ^{70} was added to 20- μL reaction mixtures containing Buffer B. Then, 18 pmoles of core RNA polymerase was added to reaction mixtures shown in columns 2, 3, and 4. After 20 min at 30 $^\circ\text{C}$, RssB, ClpX, ClpP, acetyl phosphate, and ATP were added to all samples in a final volume of 25 μL as described for degradation assays in Materials and Methods. Following incubation for 30 min at 24 $^\circ\text{C}$, TCA-soluble radioactivity was measured. (B) Core binding by σ^S excludes RssB binding. [^3H] σ^S (160 pmoles) and RssB (160 pmoles) were incubated for 20 min with acetyl phosphate as described in Materials and Methods for the formation of σ^S -RssB complexes. Then 160 pmoles of core RNA polymerase was added and after 10 min, the mixture was analyzed by Sephacryl S-100 gel filtration as described for the isolation of σ^S -RssB complexes. The positions where core, σ^S , and RssB eluted when chromatographed separately are indicated by arrows. Molecular weight standards, Blue dextran 2000 (2000 kD), bovine serum albumin (67 kD), and chymotrypsinogen A (25 kD), eluted as indicated by arrows labeled void, 67, and 25, respectively. The elution of σ^S (filled circles), RssB (open circles), and core (dashed line) are indicated.

lead to inappropriate expression of σ^S -dependent genes during exponential growth (Muffler et al. 1996a) and to decreased virulence in *Salmonella* and *Erwinia* (Swords et al. 1997; Andersson et al. 1999).

We have shown that in vitro, as in vivo, σ^S is degraded by the ClpXP protease. Degradation is greatly stimulated by RssB, and the stimulation by RssB depends on acetyl phosphate. RssB promotes σ^S degradation while having no effect on ClpXP activity with other substrates. More-

over, RssB itself is not consumed during the reaction. Thus, RssB is a specificity factor that delivers σ^S to ClpXP for degradation.

Our model for the pathway of degradation of σ^S is shown in Figure 8. σ^S is able to interact with both core RNA polymerase and RssB, but not with both simultaneously (Fig. 7). When bound by core, σ^S is protected from degradation. In the degradation pathway, σ^S initially binds the phosphorylated form of RssB with acetyl phosphate serving as the phosphate donor (Fig. 3; Becker et al. 1999). In vivo, acetyl phosphate contributes to phosphorylation of RssB (Bouche et al. 1998), but other phosphate donors must exist that have not been identified yet. The σ^S -RssB complex is bound by ClpX in the presence of ATP[γ -S] with high affinity, although neither RssB nor σ^S alone binds ClpX with high affinity (Fig. 5). These observations indicate that motifs on both RssB and σ^S contribute to the tight binding seen in the ternary complex and/or that complex formation exposes a motif on σ^S that ClpX recognizes and binds with high affinity. Because RssB has been found associated with ClpX by other methods (Moreno et al. 2000; R. Hengge-Aronis, unpubl.), RssB may have a direct role in interacting with ClpX in the ternary complex, possibly orienting σ^S to favor its binding to ClpX. When ClpP is present, a quaternary complex of σ^S -RssB-ClpXP is formed in the presence of a nonhydrolyzable ATP analog, AMP-PNP (Fig. 6). This proteolytic complex catalyzes σ^S degradation upon the exchange of AMP-PNP with ATP. ClpX most likely catalyzes ATP-dependent unfolding of σ^S , as has been shown for other ClpXP substrates (Kim et al. 2000; Singh et al. 2000). The unfolded σ^S is then translocated through the narrow opening of ClpP into the interior chamber, where the protease active sites reside (Wang et al. 1997). Concurrent with or following σ^S degradation, RssB is released and is able to carry out multiple cycles of σ^S binding and delivery to ClpXP (Fig. 6).

In vitro, acetyl phosphate greatly stimulates σ^S degradation by ClpXP and RssB and is required for the interaction of RssB and σ^S (Figs. 1, 3; Becker et al. 1999). These observations suggest that signals that affect the phosphorylation state of RssB modulate the activity of RssB. Phosphorylation very likely stabilizes a conformation of RssB that has high affinity for σ^S . However, phosphorylation may not be essential. We observed that in the absence of acetyl phosphate some σ^S and RssB associated with ClpX, although the amount was fivefold less than in the presence of acetyl phosphate. Moreover, when overexpressed, a mutant of RssB that cannot be phosphorylated can promote σ^S degradation in vivo (Bouche et al. 1998; unpubl.). Nonetheless, the higher affinity of σ^S for the phosphorylated form of RssB and the acetyl phosphate requirement for degradation support the critical role for RssB phosphorylation in promoting σ^S degradation under normal in vivo conditions.

The observation that core RNA polymerase protects σ^S from degradation suggests a second possible level of regulation of σ^S degradation (Fig. 7). Under the unusual conditions of high RssB expression and absence of ClpXP protease, RssB interferes with σ^S activity in vivo (Zhou

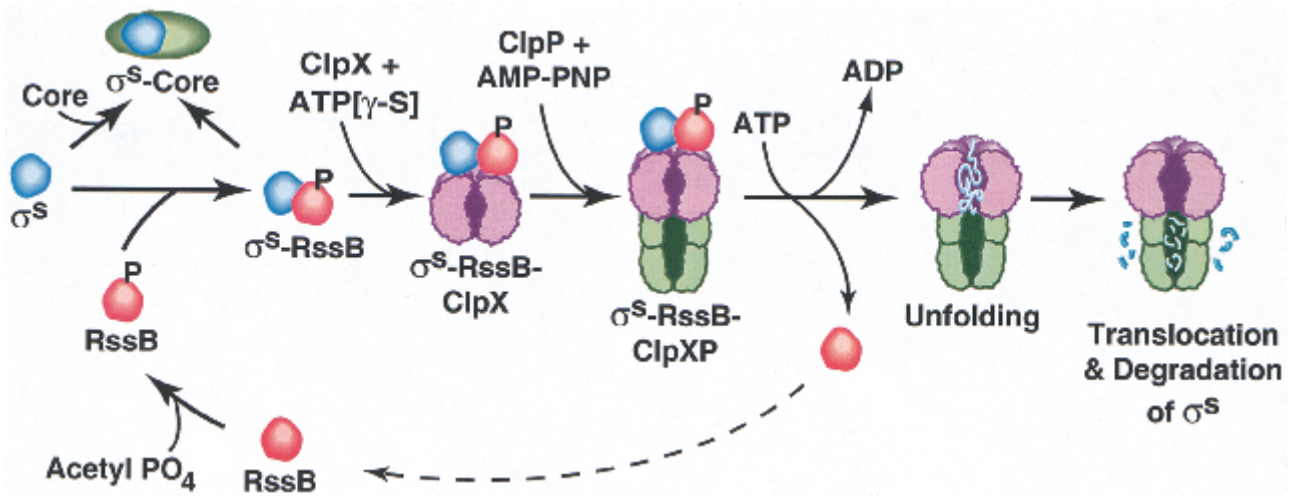


Figure 8. Model for the mechanism of action of RssB in regulating σ^S degradation by ClpXP. See text for discussion.

and Gottesman 1998; Becker et al. 2000). These observations are consistent with a role for competition between RssB and core RNA polymerase for σ^S under some conditions and provide support for the direct interaction between σ^S and RssB that has been detected in vitro. If the ability of σ^{70} or other sigma factors to interact with core were diminished under some conditions, core would be more available to bind σ^S , protecting it from rapid degradation. Core RNA polymerase appears to be limiting during stationary phase growth, because an *rpoS* mutant allows better in vivo use of σ^{70} (Farewell et al. 1998). Thus, sigma factor competition for core may play a significant regulatory role in vivo. The identification of a stationary phase-specific factor, Rsd, that interacts with σ^{70} and interferes with the activity of σ^{70} (Jishage and Ishihama 1998) is consistent with the idea that sigma-core interactions may be regulated and may in turn regulate the susceptibility of σ^S to degradation. Degradation of σ^{32} also appears influenced by its interactions with core polymerase (Błaszczak et al. 1999).

RssB is an example of a *trans*-targeting protein, specifically enabling the degradation of σ^S by ClpXP. Use of such specific and regulatable targeting proteins may be essential for the regulated degradation of physiologically important substrates. The concept of *trans*-recognition was proposed by Varshavsky and coworkers (Johnson et al. 1990) for targeting of certain proteins for degradation in *Saccharomyces cerevisiae*. They showed that the presence of an amino acid that acts as a degradation signal at the amino terminus of one subunit of an oligomeric protein was sufficient to target the degradation of another subunit, which lacked its own degradation signal but carried a proximal site for ubiquitin conjugation. Recognition of the unusual N terminus was necessary to attract the ubiquitination machinery, but ubiquitin was conjugated to the subunit carrying the ubiquitination site, and only that subunit was degraded when the complex was presented to the 26S proteasome.

In addition to the *trans*-targeting of σ^S by RssB to ClpXP reported here, two other examples have been re-

cently described for the *trans*-targeting of substrates to ClpXP in *E. coli*. UmuD', a processed and active form of the UmuD error-prone mutagenesis protein of *E. coli*, is degraded by ClpXP only when in a heteromeric complex with UmuD (Gonzalez et al. 2000). The unprocessed N terminus of UmuD is an essential element for recognition of UmuD' by ClpXP, but only UmuD' is degraded. Degradation of UmuD' acts to down-regulate mutagenesis as the cell recovers from DNA damage. Another example of *trans*-targeting is the stimulation of ClpXP degradation of SsrA-tagged proteins by SspB. It has recently been found that a ribosome-associated protein, SspB, binds specifically to SsrA-tagged proteins and enhances the recognition of these substrates by ClpXP without itself being degraded (Levchenko et al. 2000). SspB stimulates degradation of SsrA-tagged proteins both in vivo and in vitro.

It is intriguing that both RssB and SspB act by increasing the affinity of a specific substrate for ClpX. If, alternatively, the *trans*-targeting proteins modified the protease itself, degradation of many substrates would be affected. In eukaryotic cells, the diversity of ubiquitin ligase complexes provides a similar means for regulating access of specific substrates under given conditions without perturbing other degradative pathways. Possibly the small number of examples of regulated proteolysis in prokaryotes reflects the absence of a generalized regulatory network such as ubiquitin tagging, and therefore the evolution of novel solutions for each situation.

Materials and methods

Materials

ATP, AMP-PNP, and ATP[γ -S] were obtained from Boehringer Mannheim. Sephacryl S-100 and S-200 High Resolution gel filtration media were obtained from Pharmacia.

Proteins

ClpX (Grimaud et al. 1998), ClpP (Maurizi et al. 1994), ClpA (Maurizi et al. 1994), P1 RepA (Wickner 1990), and λ O (Wickner

and Zahn 1986) were purified as described. σ^{70} was a gift from Jeffery Roberts (Cornell University). The core RNA polymerase used in the experiments described in Figure 7A was a gift from Ding Jin (NIH); the core used in Figure 7B was purchased from Epicentre Technologies. σ^S was isolated from cells carrying pLHN14 and expressing σ^S (Nguyen et al. 1993) using a described procedure (Tanaka et al. 1993) with some modifications. σ^S , λ O, RepA, and α -casein were labeled in vitro as previously described (Hoskins et al. 1998). The final specific activities of [3 H] σ^S , [3 H] λ O, [3 H]RepA, and [3 H] α -casein were 1150, 450, 22,000, and 5500 cpm/pmole, respectively.

Throughout, proteins are expressed as moles of RssB monomers, ClpX hexamers, ClpP tetradecamers, ClpA hexamers, σ^S monomers, σ^{70} monomers, λ O dimers, RepA dimers, and α -casein monomers.

Purification of RssB

For the preparation of RssB, the *rssB* gene was amplified by the polymerase chain reaction and inserted in a T7 expression vector, plasmid pVex11 (Studier et al. 1990). The sequence of *rssB* was verified by DNA sequencing. Cultures of *E. coli* BL21 *lysS*, *rssB*⁻ (YN305) carrying the plasmid pT7-RssB were grown to an O.D.₆₀₀ of 0.5, induced by the addition of 1 mM IPTG, incubated for 2 h, collected, and frozen. Thawed cells were adjusted to 20 mM Tris-HCl, 0.2 M NaCl, 1 mM dithiothreitol (DTT), and 0.1 mM EDTA, and passed through a French pressure cell (Aminco) at 20,000 psi at 0°C. RssB activity was followed by measuring the stimulation of σ^S degradation by ClpXP in the assay described below. The lysate was centrifuged at 30,000g for 60 min at 4°C, and the pellet was resuspended in 8 M urea to give 12 mg/mL protein. After centrifuging as above, the supernatant was dialyzed against Buffer A (20 mM Tris-HCl, 10% [v/v] glycerol, 1 mM DTT, and 0.1 mM EDTA) for 2 h and diluted to a final NaCl concentration of 0.05 M. The sample was applied to a Q-sepharose column that was equilibrated with Buffer A. The column was washed with Buffer A, and protein was eluted with a linear gradient from 0 to 0.5 M NaCl in Buffer A. RssB eluted with 0.2–0.3 M NaCl. The protein was further purified by Sephacryl S-100 high resolution gel filtration in Buffer A containing 0.2 M NaCl, where it eluted as expected for a monomer. The RssB fractions were stored at -70°C. The final preparation was >90% pure as determined by comassie staining following SDS-PAGE.

σ^S in vitro degradation assay

Reaction mixtures were assembled in 20 μ L of Buffer B (20 mM Tris-HCl at pH 7.5, 10 mM MgCl₂, 140 mM KCl, 1 mM DTT, 0.1 mM EDTA, and 5% glycerol [v/v]) containing 5 mM ATP, 25 mM acetyl phosphate, 2 pmole ClpX, 2 pmole ClpP, 19 pmole [3 H] σ^S , and 1 pmole RssB, unless otherwise indicated. The mixtures were incubated at 24°C for 20 min unless otherwise indicated. TCA (trichloro acetic acid) was added to 20% (w/v), and [3 H] σ^S degradation was quantitated by measuring acid soluble radioactivity. In the absence of ClpX, ClpP, and RssB, <1% of the [3 H] σ^S was TCA-soluble. One unit (U) of RssB stimulates the degradation of 1 pmole of σ^S in 20 min with the standard assay conditions. When crude fractions were used as the source of RssB, 25 mM phosphocreatine and 1 μ g of creatine kinase were added to reaction mixtures. Degradation by ClpAP was measured similarly in 20- μ L reaction mixtures containing 1 mM ATP, 1 pmole ClpA, 1 pmole ClpP, 1 pmole of RssB (where indicated), and 1 pmole of [3 H] α -casein, 1 pmole of [3 H]RepA, or 18 pmole of [3 H] σ^S in Buffer B.

Isolation of RssB- σ^S , RssB- σ^S -ClpX and RssB- σ^S -ClpXP complexes by gel filtration chromatography

To isolate σ^S -RssB complexes, 100- μ L mixtures containing 320 pmole of [3 H] σ^S , 150 pmole of RssB, and 25 mM acetyl phosphate in Buffer B were incubated for 15 min at 23°C. The mixtures were applied onto a Sephacryl S-100 HR column (0.7 cm \times 7 cm) equilibrated with Buffer B containing 25 mM acetyl phosphate and 50 μ g/mL of bovine serum albumin. Fractions (120 μ L) were collected, and σ^S was determined by measuring radioactivity by scintillation counting. RssB was determined using the σ^S degradation assay described above.

For the isolation of σ^S -RssB-ClpX complexes, 215 pmole of [3 H] σ^S , 250 pmole of ClpX, 150 pmole of RssB, 5 mM ATP[γ -S], and 25 mM acetyl phosphate in Buffer B were incubated in 100- μ L reaction mixtures for 15 min at 23 °C. The reaction mixtures were then applied onto a Sephacryl S-200 HR column (0.7 cm \times 7 cm) equilibrated with Buffer B containing 1 mM ATP[γ -S] and 25 mM acetyl phosphate. Fractions (120 μ L) were collected. σ^S and RssB were determined as above. ClpX was measured by densitometry of SDS-PAGE of the fractions. To isolate σ^S -RssB-ClpXP complexes, 215 pmole of [3 H] σ^S , 250 pmole of ClpX, 150 pmole of RssB, 250 pmole of ClpP, 5 mM AMP-PNP, and 25 mM acetyl phosphate in Buffer B were incubated in 100- μ L reaction mixtures for 15 min at 23°C. The reaction mixtures were then applied onto a Sephacryl S-200 HR column as described above for S-100 column chromatography and equilibrated with Buffer B containing 1 mM AMP-PNP and 25 mM acetyl phosphate, and 120- μ L fractions were collected. σ^S , RssB, and ClpX were determined as above, and ClpP was measured by densitometry after SDS-PAGE of the fractions.

Ultrafiltration of σ^S , RssB, and ClpX

In 100- μ L reaction mixtures, 9 pmole of [3 H] σ^S was incubated with 60 pmole of ClpX, various amounts of RssB, 1 mM ATP[γ -S], 20 mM acetyl phosphate, 0.01% (v/v) Triton X-100, and 25 μ g/mL bovine serum albumin in Buffer B for 30 min at 24°C. NaCl (1 M) was added, and the mixtures were incubated for 10 min at 24°C. Reaction mixtures were centrifuged through Microcon 100 ultrafiltration devices (Amicon) for 10 min at 3200g. Retentates were recovered with 100 μ L of 10% (w/v) SDS, and the radioactivity was measured.

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References

- Andersson, R.A., Palva, E.T., and Pirhonen, M. 1999. The response regulator expM is essential for the virulence of *Erwinia carotovora subsp. carotovora* and acts negatively on the σ factor RpoS (σ^S). *Mol. Plant Microbe Interact.* **12**: 575–584.
- Bearson, S.M., Benjamin, Jr., W.H., Swords, W.E., and Foster, J.W. 1996. Acid shock induction of RpoS is mediated by the mouse virulence gene *mviA* of *Salmonella typhimurium*. *J.*

Zhou et al.

- Bacteriol.* **178**: 2572–2579.
- Becker, G., Klauck, E., and Hengge-Aronis, R. 1999. Regulation of RpoS proteolysis in *Escherichia coli*: The response regulator RssB is a recognition factor that interacts with the turnover element in RpoS. *Proc. Natl. Acad. Sci. USA* **96**: 6439–6444.
- . 2000. The response regulator RssB, a recognition factor for σ^S proteolysis in *Escherichia coli*, can act like an anti- σ^S factor. *Mol. Microbiol.* **35**: 657–666.
- Blaszczak, A., Georgopoulos, C., and Liberek, K. 1999. On the mechanism of FtsH-dependent degradation of the σ^{32} transcriptional regulator of *Escherichia coli* and the role of the DnaK chaperone machine. *Mol. Microbiol.* **31**: 157–166.
- Bouche, S., Klauck, E., Fischer, D., Lucassen, M., Jung, K., and Hengge-Aronis, R. 1998. Regulation of RssB-dependent proteolysis in *Escherichia coli*: A role for acetyl phosphate in a response regulator-controlled process. *Mol. Microbiol.* **27**: 787–795.
- Farewell, A., Kvint, K., and Nystrom, T. 1998. Negative regulation by RpoS: A case of σ factor competition. *Mol. Microbiol.* **29**: 1039–1051.
- Gonzalez, M., Rasulova, F., Maurizi, M.R., and Woodgate, R. 2000. Subunit-specific degradation of the UmuD/D' heterodimer by the ClpXP protease: The role of *trans* recognition in UmuD' stability. *EMBO J.* **19**: 5251–5258.
- Gottesman, S. 1996. Proteases and their targets in *Escherichia coli*. *Annu. Rev. Genet.* **30**: 465–506.
- Gottesman, S., Clark, W.P., de Crecy-Lagard, V., and Maurizi, M.R. 1993. ClpX, an alternative subunit for the ATP-dependent Clp protease of *Escherichia coli*. Sequence and in vivo activities. *J. Biol. Chem.* **268**: 22618–22626.
- Gottesman, S., Roche, E., Zhou, Y., and Sauer, R.T. 1998. The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Genes & Dev.* **12**: 1338–1347.
- Grimaud, R., Kessel, M., Beuron, F., Steven, A.C., and Maurizi, M.R. 1998. Enzymatic and structural similarities between the *Escherichia coli* ATP-dependent proteases ClpXP and ClpAP. *J. Biol. Chem.* **273**: 12476–12481.
- Grossman, A.D., Burgess, R.R., Walter, W., and Gross, C.A. 1983. Mutants in the *lon* gene of *E. coli* suppress a mutation in the σ subunit of RNA polymerase. *Cell* **32**: 151–159.
- Hengge-Aronis, R. 2000. The general stress response in *Escherichia coli*. In *Bacterial stress responses* (eds. G. Storz and R. Hengge-Aronis), pp. 161–178. ASM Press, Washington, DC.
- Hoskins, J.R., Pak, M., Maurizi, M.R., and Wickner, S. 1998. The role of the ClpA chaperone in proteolysis by ClpAP. *Proc. Natl. Acad. Sci. USA* **95**: 12135–12140.
- Jishage, M. and Ishihama, A. 1998. A stationary phase protein in *Escherichia coli* with binding activity to the major σ subunit of RNA polymerase. *Proc. Natl. Acad. Sci. USA* **95**: 4953–4958.
- Johnson, E.S., Gonda, D.K., and Varshavsky, A. 1990. *Cis-trans* recognition and subunit-specific degradation of short-lived proteins. *Nature* **346**: 287–291.
- Keiler, K.C., Waller, P.R., and Sauer, R.T. 1996. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* **271**: 990–993.
- Kim, Y.I., Burton, R.E., Burton, B.M., Sauer, R.T., and Baker, T.A. 2000. Dynamics of substrate denaturation and translocation by the ClpXP degradation machine. *Mol. Cell* **5**: 639–648.
- Lange, R. and Hengge-Aronis, R. 1994. The cellular concentration of the σ^S subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation, and protein stability. *Genes & Dev.* **8**: 1600–1612.
- Levchenko, I., Seidel, M., Sauer, R.T., and Baker, T.A. 2000. A specificity-enhancing factor for the ClpXP degradation machine. *Science* **289**: 2354–2356.
- Loewen, P.C. and Hengge-Aronis, R. 1994. The role of the sigma factor sigma S (KatF) in bacterial global regulation. *Annu. Rev. Microbiol.* **48**: 53–80.
- Maurizi, M.R., Thompson, M.W., Singh, S.K., and Kim, S.H. 1994. Endopeptidase Clp: ATP-dependent Clp protease from *Escherichia coli*. *Methods Enzymol.* **344**: 314–331.
- Moreno, M., Audia, J.P., Bearson, S.M., Webb, C., and Foster, J.W. 2000. Regulation of σ^S degradation in *Salmonella enterica* var *typhimurium*: In vivo interactions between σ^S , the response regulator MviA (RssB) and ClpX. *Mol. Microbiol. Biotechnol.* **2**: 245–254.
- Muffler, A., Fischer, D., Altuvia, S., Storz, G., and Hengge-Aronis, R. 1996a. The response regulator RssB controls stability of the σ^S subunit of RNA polymerase in *Escherichia coli*. *EMBO J.* **15**: 1333–1339.
- Muffler, A., Traulsen, D.D., Lange, R., and Hengge-Aronis, R. 1996b. Posttranscriptional osmotic regulation of the σ^S subunit of RNA polymerase in *Escherichia coli*. *J. Bacteriol.* **178**: 1607–1613.
- Nguyen, L.H., Jensen, D.B., Thompson, N.E., Gentry, D.R., and Burgess, R.R. 1993. In vitro functional characterization of overproduced *Escherichia coli* *katF/rpoS* gene product. *Biochemistry* **32**: 11112–11117.
- Pratt, L.A. and Silhavy, T.J. 1996. The response regulator SprE controls the stability of RpoS. *Proc. Natl. Acad. Sci. USA* **93**: 2488–2492.
- Roche, E.D. and R.T. Sauer. 1999. SsrA-mediated peptide tagging caused by rare codons and tRNA scarcity. *EMBO J.* **18**: 4579–4589.
- Rosl, M. and Kersten, H. 1994. Organization and functions of genes in the upstream region of *tyrT* of *Escherichia coli*: Phenotypes of mutants with partial deletion of a new gene (*tgS*). *J. Bacteriol.* **176**: 221–231.
- Schweder, T., Lee, K.H., Lomovskaya, O., and Matin, A. 1996. Regulation of *Escherichia coli* starvation σ factor (σ^S) by ClpXP protease. *J. Bacteriol.* **178**: 470–476.
- Singh, S.K., Grimaud, R., Hoskins, J.R., Wickner, S., and Maurizi, M.R. 2000. Unfolding and internalization of proteins by the ATP-dependent proteases ClpXP and ClpAP. *Proc. Natl. Acad. Sci. USA* **97**: 8898–8903.
- Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**: 60–89.
- Swords, W.E., Giddings, A., and Benjamin, Jr., W.H. 1997. Bacterial phenotypes mediated by *mviA* and their relationship to the mouse virulence of *Salmonella typhimurium*. *Microb. Pathog.* **22**: 353–362.
- Tanaka, K., Takayanagi, Y., Fujita, N., Ishihama, A., and Takahashi, H. 1993. Heterogeneity of the principal sigma factor in *Escherichia coli*: The *rpoS* gene product, sigma 38 is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **90**: 3511–3515.
- Voges, D., Zwickl, P., and Baumeister, W. 1999. The 26S proteasome: A molecular machine designed for controlled proteolysis. *Annu. Rev. Biochem.* **68**: 1015–1068.
- Wang, J., Hartling, J.A., and Flanagan, J.M. 1997. The structure of ClpP at 2.3 Å resolution suggests a model for ATP-dependent proteolysis. *Cell* **91**: 447–456.
- Wickner, S.H. 1990. Three *Escherichia coli* heat shock proteins are required for P1 plasmid DNA replication: Formation of an active complex between *E. coli* DnaJ protein and the P1 initiator protein. *Proc. Natl. Acad. Sci. USA* **87**: 2690–2694.

- Wickner, S.H. and Zahn, K. 1986. Characterization of the DNA binding domain of bacteriophage λ O protein. *J. Biol. Chem.* **261**: 7537–7543.
- Wickner, S., Gottesman, S., Skowrya, D., Hoskins, J., McKenney, K., and Maurizi, M.R. 1994. A molecular chaperone, ClpA, functions like DnaK and DnaJ. *Proc. Natl. Acad. Sci. USA* **91**: 12218–12222.
- Wickner, S., Maurizi, M., and Gottesman, S. 1999. Posttranslational quality control: Folding, refolding, and degrading proteins. *Science* **286**: 1888–1893.
- Wojtkowiak, D., Georgopoulos, C., and Zylicz, M. 1993. Isolation and characterization of ClpX, a new ATP-dependent specificity component of the Clp protease of *Escherichia coli*. *J. Biol. Chem.* **268**: 22609–22617.
- Yura, T. and Nakahigashi, K. 1999. Regulation of the heat-shock response. *Curr. Opin. Microbiol.* **2**: 153–158.
- Zhou, Y. and Gottesman, S. 1998. Regulation of proteolysis of the stationary-phase σ factor RpoS. *J. Bacteriol.* **180**: 1154–1158.



The RssB response regulator directly targets ζ^S for degradation by ClpXP

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