Short Conceptual Overview

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Recent advances in the characterization of Crl, the unconventional activator of the stress sigma factor $\sigma^S/RpoS$

Abstract: The bacterial RNA polymerase (RNAP) holoenzyme is a multisubunit core enzyme associated with a $\sigma$ factor that is required for promoter-specific transcription initiation. Besides a primary $\sigma$ responsible for most of the gene expression during active growth, bacteria contain alternative $\sigma$ factors that control adaptive responses. A recurring strategy in the control of $\sigma$ factor activity is their sequestration by anti-sigma factors that occlude the RNAP binding determinants, reducing their activity. In contrast, the unconventional transcription factor Crl binds specifically to the alternative $\sigma$ factor $\sigma^S/RpoS$, and favors its association with the core RNAP, thereby increasing its activity. $\sigma^S$ is the master regulator of the general stress response that protects many Gram-negative bacteria from several harmful environmental conditions. It is also required for biofilm formation and virulence of Salmonella enterica serovar Typhimurium. In this report, we discuss current knowledge on the regulation and function of Crl in Salmonella and Escherichia coli, two bacterial species in which Crl has been studied. We review recent advances in the structural characterization of the Crl-$\sigma^S$ interaction that have led to a better understanding of this unusual mechanism of $\sigma$ regulation.

Keywords: Crl; RNA polymerase; RpoS; Salmonella; sigma S.

Introduction

Bacterial cells encountering multiple environments are constantly exposed to suboptimal conditions, such as nutrient starvation and variations in physical and chemical parameters, to which they adapt by regulating gene expression. One major strategy employed by bacteria to modify expression of their genome is the use of alternative sigma ($\sigma$) subunits of the RNA polymerase (RNAP), directing transcription initiation at different classes of promoters (1–4). Sigma factors direct the expression of specific sets of genes by interacting with the catalytically active RNAP core enzyme ($E, \alpha^2\beta\beta'\omega$) and enabling the holoenzyme $E\sigma$ to bind to specific promoters and initiate transcription. Replacement of one $\sigma$ factor in the RNAP holoenzyme by another one changes the transcription pattern. All bacteria have a housekeeping $\sigma$ factor essential for transcription of the majority of cellular genes during growth, and one or more alternative $\sigma$s, which allow transcription of specific sets of genes in response to environmental conditions. Bacterial $\sigma$ factors have been divided into two structurally and functionally distinct families, the $\sigma^{70}$ and $\sigma^{54}$ families, named after the Escherichia coli housekeeping and nitrogen-stress $\sigma$ factors respectively (3–5). The alternative sigma factor $\sigma^S$, closely related to $\sigma^{70}$ and encoded by the $rpoS$ gene, is the master regulator of the general stress response in E. coli and many other Gram-negative bacteria. $\sigma^S$ remodels the transcriptional program and the cell physiology to promote multiple stress resistance and long-term survival. $\sigma^S$ also plays important roles in biofilm formation and virulence of the food-borne pathogen Salmonella enterica serovar Typhimurium (S. Typhimurium) (6–10).

$\sigma^S$ is tightly regulated at the transcriptional, translational and posttranslational levels to restrict its expression and activity under inappropriate conditions (6, 7). This is because $\sigma^S$ has a negative effect on the expression of several housekeeping genes, making $\sigma^S$ expression a disadvantage for bacterial growth (7, 9, 11, 12). $\sigma^S$ expression is...
Crl: an unconventional transcription activator

The crl gene was named as such because, initially, it was thought to encode a protein that forms fibers called curli at the cell surface of E. coli K12 (13), and was found later to be a regulator of the csg genes encoding the curli protein subunits and secretion apparatus (14–16). Transcription of the csg genes was shown to be dependent on σ^S in the stationary phase of growth (17). Later on, the finding that a crl mutation decreases expression of many other σ^S-regulated genes and does not lower σ^S expression, led to the suggestion that Crl stimulates the activity of σ^S (18). In vitro experiments demonstrated a direct role of Crl in activating σ^S-dependent transcription initiation (19). Crl activation was unusual because Crl stimulated σ^S-dependent transcription at different promoters without binding to the promoter DNA (18–21). Instead, to promote σ^S transcriptional activity, Crl physically interacts with σ^S (22) and enhances the formation of E^S (20, 21, 23) (Figure 1). Crl increases the affinity of σ^S for E by increasing the association constant rate of the binding reaction and has no significant effect on the stability of the E^S complex (23).

Even though it was initially suggested that Crl affects transcription initiation in vitro by other sigmas, such as σ^O and σ^70 (20), it is now established that Crl is exclusively dedicated to σ^S (21, 24–27). In particular, Crl does not bind σ^O and does not modify the affinity of σ^S for the core RNAP enzyme (23).

The in vitro affinity of purified σ^S for RNAP core is the lowest of all six E. coli sigma factors (28, 29), and σ^S levels are lower than that of σ^O, even in the stationary phase of growth when σ^S reaches its highest concentration (30, 31). Therefore, by increasing σ^S affinity for the core RNAP, Crl increases the competitiveness of σ^S and thereby stimulates expression of σ^S-dependent genes (18, 19, 21, 24, 25). However, the magnitude of Crl activation is promoter-specific both in vivo and in in vitro transcription assays (24). These differences in the levels of responsiveness of different promoters to Crl activation may reflect differences in the intrinsic binding constants of these promoters for E^S–RNAP. Promoters that recruit E^S inefficiently would be more affected by an increase in the E^S concentration caused by Crl. Also, feedforward regulatory loops are likely very sensitive to E^S levels and thus to Crl activation (24, 32, 33). However, at particular promoters, Crl might affect downstream steps in the transcription initiation pathway, including E^S-DNA open complex formation (22, 23). It is possible that Crl modifies the positioning of E^S on the promoter region, resulting in an

Figure 1: Main features for Crl expression and function.

The two transcriptional start sites and the ribosome binding site (RBS) of crl are shown. Crl binds to σ^S to favor its association with the core RNAP, thereby increasing its activity. The RssB protein binds σ^S to favor its degradation by the ClpXP protease, unless one of the anti-adaptors (IraP, M or D) is produced and interferes with RssB. Known and potential regulators of Crl expression are indicated. Green arrows and blunt red arrows indicate positive and negative regulation, respectively. Dashed lines indicate unclear regulatory effect. See text for details and references.
altered architecture of the complex, and/or increases the ability of σE to melt DNA. One point that is unclear yet is whether Crl remains associated with Eσ. On one hand, Crl co-purifies with RNAP under certain conditions (34) and binds preformed Eσ in vitro (23). On the other hand, the half-life of the Crl-σE complex is very short in vitro (23), and likely in vivo since fractionation by gel filtration of free and bound Crl from cellular extracts of E. coli (21) and Salmonella (Monteil and Norel unpublished) showed that Crl is found mainly in a free state. Furthermore, there is no data so far demonstrating the participation of Crl in a quaternary complex with Eσ bound to DNA.

The effects of Crl on expression of σE-dependent genes are greatest at low levels of σE, in vitro and in vivo, and increased levels of σE can complement a crl knockout mutation (19–21, 24). Consistently, the physiological impact of Crl on σE-dependent gene transcription is the highest at the entry into stationary phase when σE begins to accumulate (19, 24). In addition, Crl effects have been also revealed in the exponential phase of growth when σE levels are very low (35). Unexpectedly, while a crl knockout mutation lowers σE activity, levels of σE are slightly higher in crl mutants, compared to wild-type strains (18, 19, 21). This finding results from two antagonistic effects of Crl (21). By activating EσE-dependent expression of rssB, Crl increases σE degradation (Figure 1). However, by stimulating σE association with E, Crl indirectly has a stabilizing effect on σE by limiting its interaction with RssB and its subsequent degradation by ClpXP (Figure 1). This dual effect of Crl likely contributes to the tight control of kinetics and levels of EσE formation in the cell.

Whereas σE is essential for cell viability under nonoptimal growth conditions, it also has negative effects on expression of several housekeeping genes and bacterial growth (7, 10–12), explaining why rpoS mutants, which show growth advantages, are selected in populations of E. coli and Salmonella in the absence of environmental stress (7, 12, 36). For bacterial populations living in changing environments, diversification into individuals with variable levels of σE activity is likely a bet-hedging strategy, in which Crl plays a role. By favoring EσE formation, Crl contributes to the negative effects of σE on gene expression and bacterial growth (24, 37, 38). Thus, it is not unexpected that Δcrl mutants of Salmonella and E. coli have a competitive advantage over wild type strains during stationary phase (24, 38). crl mutants have also been detected among E. coli and Salmonella isolates and Crl was shown to rescue rpoS mutants with reduced σE activity (39–41). These findings suggest that Crl contributes to the fitness advantages of mutants with reduced σE activity in particular environments.

**Regulation of Crl expression**

In S. Typhimurium and E. coli grown in rich medium, maximal levels of Crl are found at the entry into stationary phase (19, 21, 24). Indole has been proposed to act as an extracellular signal for Crl expression in E. coli during the transition between the exponential and stationary phases (25) (Figure 1). However, this cannot be the case in S. Typhimurium, which, unlike E. coli K12, does not produce indole. In late stationary phase, when σE levels are high, σE exerts a negative effect on Crl production (18, 24) (Figure 1). The mechanism underlying this negative correlation is not yet understood but is consistent with the finding that Crl is required at low levels of σE. Crl levels are not limiting for σE activity in Salmonella grown in rich medium (33). Indeed, Crl is present in a 2- to 3-fold excess over σE in late stationary phase and the excess of Crl over σE is even greater at the entry into stationary phase (33).

The crl gene has two overlapping promoters, a σ54-dependent promoter (15) and a downstream σ54-promoter that is up-regulated under nitrogen limitation (42). However, Crl production is silenced under nitrogen limited conditions because the σ54-promoter produces a crl transcript which lacks a ribosome binding site, and the Eσ54 holoenzyme occludes the σ54-dependent crl promoter, thereby preventing the production of the translatable crl mRNA (42) (Figure 1). Under nitrogen-limiting conditions, σ54 production slows growth, and by reducing Crl synthesis this simple regulatory mechanism restrains the activity of σ5 and allows faster growth (42).

Little is known about other possible mechanisms of Crl regulation (Figure 1). Crl has been proposed to be a thermosensor favoring σE activity at 30°C because crl expression in E. coli K12 is increased at low temperature (22). However, in other studies crl expression in E. coli (15, 21, 26) and S. Typhimurium (33) was only mildly affected by temperature. The ferric uptake regulator Fur might both repress crl transcription and interact with Crl in E. coli K12 (43) but not in S. Typhimurium (39). Crl contains a potential ClpX recognition signal and has been captured in a trap for ClpXP substrates, suggesting a role for ClpXP in Crl proteolysis (44). However, a His-tagged Crl protein was not a substrate of the ClpXP proteolytic machinery in in vitro degradation assays (21) and Crl degradation by ClpXP remains to be monitored in vivo.
Distribution and structural features of Crl

RpoS homologues are found in many Gram-negative bacteria of the γ, δ, and β subdivisions (7). In contrast, analysis of the protein sequence databases revealed the narrow distribution of Crl homologues in bacteria (39). Thus, crl is not as widely distributed as rpoS, and it is also less conserved at the sequence level. An alignment of 60 Crl sequences showed that only 17 residues are conserved among all Crl proteins (39). The low level of sequence conservation of Crl in bacterial species raised several questions: do all Crl family members have the same structure? Do they have the same σ^5-activator function? Do they bind to the same region of σ^5?

The first X-ray crystal structure of Crl, from Proteus mirabilis, was released in the protein data bank (PDB) by the Midwest Center for Structural Genomics consortium [PDB code 3RP], later reported in (45)]. Cavaliere et al. also solved the crystal structure of Crl from Proteus mirabilis [PDB code 4Q11, (46)] and the solution nuclear magnetic resonance (NMR) structure of Crl from S. Typhimurium (47, 48) (Figure 2). These structural studies, and complementary biophysical and functional analyses, demonstrated that Crl proteins from different bacterial species display similar structural features and σ^5-enhancer activity (45, 46). Moreover, they bind to the same region of σ^5, suggesting a common functionality in Crl family members (45, 46). Although Crl forms dimers in the X-ray crystal structures (45, 46), biophysical analyses and the NMR studies have demonstrated the monomeric state of Crl in solution (46–48). Crl has a globular fold with a single α/β-domain in which an exposed cavity, formed by antiparallel β-sheets, is enclosed by flexible loops (Figure 2). Both the cavity and the flexible loops have a fundamental role in the recognition and binding to σ^5 (45–48). Conserved residues important for Crl activity have been identified in the cavity and loop 2 (39, 45, 46, 48).

σ^5-Crl binding interface

There is no tridimensional structure available for σ^5 and for other isolated full-length σ factors. However, crystal structures were solved for housekeeping σ factors in the RNAP holoenzyme, and for other σ factors in complex with anti-sigmas (2, 4). For the couple σ^5-Crl, crystallization trials have failed [Ref. (48) and unpublished works], probably in part because of the instability of the Crl-σ^5 complex. Indeed, the interaction between σ^5 and Crl is not strong. The K_d value of 0.8 μM, measured by isothermal titration calorimetry (ITC) for the Salmonella proteins (46), is very high compared to K_d values obtained for interaction between sigma factors and the core RNAP that are in the nanomolar range (28). Moreover, surface plasmon resonance (SPR) experiments have shown that the half-life of this complex is of about 3 s (23). The σ^5-Crl binding reaction is characterized by negative values of enthalpy changes (Δ_H), suggesting that mainly electrostatic interactions drive the formation of this complex (46).

σ^5 belongs to the σ^70-family of σ factors whose members contain at least two structural domains connected by flexible linkers: domain 2 and domain 4 (4, 5). The Crl binding region on σ^5 was initially spotted within domain 2 by using the bacterial two-hybrid system (41). Domain 2 is the most highly conserved domain of σ factors, and is composed of five regions (1.2, 2.1, 2.2, 2.3 and 2.4) with specific roles in RNAP and promoter DNA binding (2, 4, 5). Biochemical, biophysical and mutational analyses have identified two noncontiguous regions in σ^5 domain 2 required for Crl binding, one in region 1.2 and one in region 2.3 (27, 41, 48). The Crl binding motif in σ^5 region 2.3 is not conserved in σ^70, and the other Crl binding site is at a position in σ^5 region 1.2 where a large non-conserved region (NCR) interrupts the sequence of σ^70, explaining why Crl does not recognize σ^70 (23, 27). Indeed, a σ^70 chimeric protein lacking NCR but containing the Crl-binding motifs of σ^5 interacts with Crl (27).

Figure 2: Tridimensional structures of Crl.

The structure of Crl is characterized by a single α/β-domain in which an exposed cavity, formed by antiparallel β-sheets, is enclosed by three flexible loops (loops 1, 2 and 3). In panel (A) the X-ray crystal structure of Crl from Proteus mirabilis [4Q11 (46)] is shown and in panel (B) are shown 10 conformers of Salmonella Crl obtained by NMR [2MZ8 (47, 48)]. The σ^5 binding regions are highlighted in red.
Cavaliere et al. (48) recently added a new piece of information to the identification of the Crl-σ5 interface. They took advantage of the sequence evolution of conserved domain 2 of σ5 in bacterial species that do not contain a crl gene, such as *Pseudomonas aeruginosa*, to identify and assign a critical σ5 arginine residue to the σ5-Crl interface. Whereas this arginine is conserved in σ5 proteins from crl-proficient species (R82, Figure 3), a leucine is present at the corresponding position in the *P. aeruginosa* σ5 protein. Remarkably, *P. aeruginosa* σ5 does not bind Crl unless the leucine is substituted by an arginine (48). The key arginine residue is located within the first Crl binding site, in region 1.2 of σ5, which consists of an α-helix in the structural model of σ5 (27, 41, 48) (Figure 3). The loop just on top of this α-helix constitutes the second Crl binding site in region 2.3, which is formed by conserved residues D135, P136 and E137 (DPE motif) (27, 48).

Regarding Crl, mutational analyses and structural data have demonstrated that two conserved and surface exposed residues, D36 located in the cavity and R51 located in loop 2, are directly involved in the Crl-σ5 complex formation (39, 45, 46, 48) (Figure 3). Interestingly, NMR experiments using labeled 15N/13C Crl and unlabeled σ5 proteins from *Salmonella* revealed that chemical shift perturbations extend beyond the region directly involved in σ5 binding (48). These perturbations might be due to rearrangements in the flexible loops to allow breathing of the cavity and to accommodate σ5. Using docking programs, Cavaliere et al. (48) proposed structural models for the σ5-Crl complex, compatible with all the structural data and mutational analyses. In these models, salt bridges can be established between the two pairs of residues Crl-D36/σ5-R82 and Crl-R51/σ5-E137, leading to a binding interface with the α-helix of σ5 docking into the Crl cavity and the DPE motif in σ5 interacting with the Crl loop 2 (Figure 3). This interface, based on electrostatic interactions, endorses the finding of the electrostatic driven mode of σ5-Crl complex formation (46).

Characterization of a Crl protein in which the key residue D36 is substituted by an alanine might shed light on the transient nature of the Crl-σ5 complex (48). In the native structure of Crl, residue D36 interacts intramolecularly with residue R24, which is not required for σ5 binding (46, 48) (Figure 3). Upon σ5 binding, this interaction is likely disrupted to allow interaction between D36 in Crl and R82 in σ5 (Figure 3). The NMR spectra of the variant Crl D36A showed how the disruption of the intramolecular contact D36-R24 is sensed by the whole Crl structure, in particular by loop 1, suggesting a scenario in which disruption of the D36-R24 interaction due to σ5 binding destabilizes the Crl structure, leading to a rapid dissociation of the Crl-σ5 complex.

**Conclusion**

Despite the greater knowledge accumulated recently on the structural determinants of the Crl-σ5 interaction, the mechanism by which Crl increases σ5 affinity for the core RNAP is still not fully understood. Crl binds to domain 2 (σ5) of σ5, the most highly conserved domain of σ factors. The σ-core RNAP interface involves several regions of σ, but the contact area of the σ5-core interface is the largest among the σ domains (2, 4, 5). σ5 interacts with the β′ subunit in core RNAP mainly through region 2.2 and to a lesser extent through region 2.1 (2, 4, 5). Thus, the Crl binding sites on σ5 are in close proximity with the RNAP binding regions. In some housekeeping σ, such as σ30, the NCR inserted between regions 1.2 and 2.1 is also implicated in binding to the β′ subunit of RNAP (2, 4). In the structure of domain 2 of σ30 [PDB 1SIG, (49)], the C-terminus of region 1.2 is close to the N-terminus of conserved region 2.1. Crl binding to this position in σ30 might facilitate interactions between the β′ subunit and σ5, as suggested (27). The short half-life of the Crl-σ5 complex suggests a scenario where Crl acts as a ‘bind and deliver’ chaperone of σ5, increasing the rate of σ5 association with the core RNAP and preventing σ5 to remain free in the cell. Crl might induce conformational changes in σ5 unmasking key β′ binding determinants and/or repositioning σ5 in the holoenzyme. Crl has no major effect on the stability

**Figure 3:** The σ5-Crl binding interface model. In the σ5-Crl complex model obtained using docking programs, two possible electrostatic interactions involve conserved σ5 and Crl residues of paramount importance for complex formation: Crl R51-σ5 E137 and Crl D36-σ5 R82 (48). In the tridimensional structure of unbound Crl, residue R51 is free while D36 can establish an electrostatic interaction with the Crl residue R24 (46, 48). σ5 is depicted in orange and Crl in pale cyan.
of the Es\(\sigma\) holoenzyme, but a weak interaction has been detected between Crl and E (23). Moreover, Crl can bind Es\(\sigma\) and copurify with RNAP in some conditions (23, 34). In addition, Crl may aid Es\(\sigma\) assembly indirectly, by breaking \(\sigma\) intra-molecular or inter-molecular interactions, since Crl favors the solubility of \(\sigma\) and \(\sigma\)'s, proteins, which have a tendency to form dimers and aggregate at high concentrations (46). Altogether these findings suggest that our current knowledge on the Crl-Es\(\sigma\) interaction is the tip of the iceberg.

The NCR of housekeeping sigmas and the equivalent position in other \(\sigma\) factors (where Crl binds to \(\sigma\)) might constitute a target for transcription regulation (50). At least two transcriptional activators, GrgA and RbpA, interact with the NCR of \(\sigma\) factors (48–50). RbpA binding to this position in the housekeeping factor \(\sigma\) might facilitate interaction between RbpA and the -10 promoter element, and favor open complex formation (51, 52). Assuming that Crl stays associated with Es\(\sigma\) in the transcription initiation complex, and/or modifies the positioning of \(\sigma\) in the holoenzyme and in its complex with DNA, Crl could also affect the formation of open complexes at specific promoters, as suggested (22, 23). This activity of Crl might be facilitated by the close proximity on \(\sigma\)'s of the Crl binding sites and regions 1.2 and 2.3, which interact with the promoter discriminator and -10 elements and are involved in open complex formation in the context of housekeeping RNAP (2, 4, 5). For deeper understanding of the Crl mechanism, it would be important to determine whether and how Crl modifies the interaction between Es\(\sigma\) and the promoter DNA. In vivo, Crl appears to stimulate the expression of \(\sigma\)-dependent genes independently of any specific promoter motif (21). In vitro transcription profiling (53), using Es\(\sigma\)/Crl and the whole bacterial genome as DNA template, might reveal structural features of Crl-dependent promoters. Studies of transcriptional activators that show no sequence similarity to Crl, but bind to an equivalent region on \(\sigma\) factors and perform analogous tasks should provide important novel insights. It would be interesting to determine how widespread these unconventional transcription activators are, and to which extent their mechanisms of action share common features.

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List of abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>RNAP</td>
<td>RNA polymerase</td>
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<tr>
<td>(\sigma)</td>
<td>sigma factor</td>
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<td>E</td>
<td>core RNA polymerase</td>
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<td>SPR</td>
<td>surface plasmon resonance</td>
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<td>PDB</td>
<td>protein data bank</td>
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<td>ITC</td>
<td>isothermal titration calorimetry</td>
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<td>(\Delta H)</td>
<td>binding enthalpy change</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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References

15. Arnqvist A, Olsen A, Pfeifer J, Russell DG, Normark S. The Crl protein activates cryptic genes for curli formation and fibronec- 
16. Taylor JD, Matthews SJ. New insight into the molecular control of 
17. Olsén A, Arnqvist A, Hammar M, Sukupolvi S, Normark S. The 
RpoS sigma factor regulates H-NS-mediated transcriptional 
repression of csgA, the subunit gene of fibronecin-binding curli in 
18. Pratt LA, Silhavy TJ. Crl stimulates RpoS activity during sta-
19. Robbe-Saule V, Ja mou il lè V, Prévost MC, Guadagnini S, 
Talhouarne C, Mathot H, Kolb A, Norel F. Crl activates transcrip-
tion initiation of RpoS-regulated genes involved in the multicel-
20. Gaal T, Mandel MJ, Silhavy TJ, Gourse RL. Crl facilitates RNA 
21. Typas A, Barembuch C, Possling A, Hengge R. Stationary phase 
reorganisation of the *Escherichia coli* transcription machinery by 
Crl protein, a fine-tuner of σ54 activity and levels. EMBO J 2007; 26: 1569–78.
22. Bougdour A, Lelong C, Geiselm ann J. Crl, a low temperature-
induced protein in *Escherichia coli* that binds directly to the 
Kolb A. Binding of the unorthodox transcription activator, Crl, 
24. Robbe-Saule V, Lopes MD, Kolb A, Norel F. Physiological effects 
of Crl in *Salmonella* are modulated by sigmaS level and pro-
25. Faure D, Frederick R, Wloch D, Portier P, Blot M, Adams J. 
Genomic changes arising in long-term stab cultures of *Escheri-
to domain 2 of sigma(S) and confers a competitive advantage on 
a natural rpoS mutant of *Salmonella enterica* serovar Typhi. 
27. Zafar MA, Carabetta VJ, Mandel MJ, Silhavy TJ. Transcriptional 
occlusion caused by overlapping promoters. Proc Natl Acad Sci 
regulation of Crl and Fur in *Escherichia coli*. Mol Cell 
29. Banta AB, Cuff ME, Lin H, Campbell EA, Burgess RR, 
Gourse RL. Key features of sigmaS required for specific recogni-
tion by Crl, a transcription factor promoting assembly of RNA 
30. Maeda H, Fujita N, Ishihama A. Competition among seven 
*Escherichia coli* sigma subunits: relative binding affinities 
to the core RNA polymerase. Nucleic Acids Res 2000; 28: 
3497–503.
31. Piper SE, Mitchell JE, Lee DJ, Busby SJ. A global view of *Escherichia 
analysis reveals differential regulation of the σ54-dependent 
ycGFE(katN) locus by YncC and H-NS in *Salmonella* and *Escheri-
33. Robbe-Saule V, Carreira I, Kolb A, Norel F. Effect of growth 
temperature on Crl-dependent regulation of sigmaS activity in 
*Salmonella enterica* serovar Typhimurium. J Bacteriol 2008; 190: 
4453–9.
34. Lee DJ, Busby SJ, Westblade LF, Chait BT. Affinity isolation and 
I-DIRT mass spectrometric analysis of the *Escherichia coli* 0157:H7 
35. Dong T, Kirchhof MG, Schellhorn HE. RpoS regulation of gene 
expression during exponential growth of *Escherichia coli* K12. 
36. Robbe-Saule V, Algorta G, Rouilhac I, Norel F. Characterization of 
the RpoS status of clinical isolates of *Salmonella enterica*. Appl 
37. Schnetz K. Silencing of the *Escherichia coli* bgl operon by RpoS 
38. Madan R, Moorthy S, Mahadevan S. Enhanced expression of 
the bgl operon of *Escherichia coli* in the stationary phase. FEMS 
amino acid residues of the *Salmonella* sigmaS chaperone 
Crl involved in Crl-sigmaS interactions. J Bacteriol 2010; 
40. Madsen J, Neher SB, Kim YI, Sauer RT, Baker TA. Proteomic 
discovery of cellular substrates of the ClpXP protease reveals five 
41. Banta AB, Chumanov RS, Yuan AH, Lin H, Campbell EA, Burgess RR, 
Gourse RL. Structure of the RNA polymerase assembly factor Crl 
and identification of its interaction surface with sigma S. J 
Raynal B, Monteil V, Bellalou J, Haouz A. Structural and func-
tional features of Crl proteins and identification of conserved 
surface residues required for interaction with the RpoS/sigmaS 
43. Cavaliere P, Norel F, Sizun C. (1)H, (13)C and (15)N resonance assign-
ment reveals differential regulation of the σ54-dependent 
ycGFE(katN) locus by YncC and H-NS in *Salmonella* and *Escheri-
44. Cavaliere P, Sizun C, Levi-Acobas F, Nowakowski M, Monteil V, 
Bontems F, Bellalou J, Mayer C, Norel F. Binding interface 
between the *Salmonella* sigma(S)/RpoS subunit of RNA