4. Results and Discussion

4.1 Differential ability of σ^S and σ^{70} of *Escherichia coli* to utilise promoters containing half or full UP-element sites

Typas A. and Hengge R. (2005), *Mol Microbiol*, **55**(1), 250-60

A.T. and R.H. designed research, A.T. performed research and analysed data, A.T. and R.H. wrote the paper

In a previous study it was shown that the presence of a distal UP-element sub-site in *csiD*p contributed specifically to Eσ^s-dependent promoter transcription. When this half-site was optimised to a full UP-element, Eσ^{70} could utilise the promoter equally well as Eσ^s (in collaboration with a class I acting CRP; (Germer et al., 2001). We aimed to clarify whether this ability of Eσ^s and Eσ^{70} to oppositely benefit from different UP-element configurations had a broader impact in promoter selectivity in *E. coli*. If yes, then what was the molecular mechanism that lied behind it?

The presence of a distal UP-element sub-site confers Eσ^s promoter selectivity, only when the promoter possesses a recognisable –35 hexamer

An alignment of ~80 mapped σ^s-dependent promoters revealed a high conservation of distal UP-element half-sites: 35-40% of them carried sequences that resembled a distal UP-element sub-site (see Table 1 in Figure Appendix for an updated table; all experimentally verified σ^s-dependent promoters are grouped according to their UP-element configuration). On the contrary, promoters with full UP-element sites or proximal half-sites are very rare. Three natural σ^s-dependent promoters, containing putative distal UP-element sub-sites, were selected for further studying. The distal UP-element sub-sites turned out to be functional in all three cases (*bolA*, *osmY* and *csiE*), but only in *osmY* and *csiE* promoters did the presence of such a sub-site favour Eσ^s-derived transcription. On the other hand, Eσ^{70} could utilise those two promoters more efficiently when the distal UP-element sub-site was completed to a full site.

To avoid the complex regulation that natural promoters frequently exhibit, we continued our studies using a series of synthetic promoters, which derived from the p_{tac} (known to lack additional regulation by transcriptional factors) and possessed different degree of Eσ^s-selectivity. UP-element half sites or full sites were implanted in front of all the selected

synthetic promoters. Consistently with the data obtained for the natural promoters, the synthetic promoter carrying canonical –10 and –35 elements (recognisable by both RNAPs, $E\sigma^s$ and $E\sigma^{70}$) became completely $E\sigma^{70}$-dependent when either a proximal UP-element sub-site or a full UP-element site were introduced in front of it, whereas $E\sigma^s$ acquired a major role in the promoter expression, when the latter carried a distal UP-element sub-site. The same tendency was observed in in-vitro transcription experiments.

In contrast to that, all different synthetic promoters devoid of a –35 element showed enhanced activity when half or full UP-element sites were introduced to them, but retained the same selectivity towards any of the two RNAPs. Interestingly, a distal UP-element sub-site stimulated the activity of this set of promoters more than the presence of a proximal half-site did, in contrast to what is known for ribosomal promoters, known to carry "consensus-like" –35 elements. Proximal UP-element sub-sites have been shown to stimulate the expression of ribosomal promoters mainly by facilitating an αCTD-σ interaction (Ross et al., 2003). For our "-35-less" promoter constructs this interaction was defective, since the use of an alpha subunit allele that abolishes this interaction (αE261A) did not influence the $E\sigma^{70}$-derived transcription for any of our promoter constructs (see Fig. 17 in Figure Appendix). Thus, the defective α-σ interaction accounts for the small stimulatory effect that the proximal UP-element sub-site conferred to the activity of our "-35-less" promoter constructs.

The molecular mechanism that allows differential use of UP-element full and half-sites by $E\sigma^S$ and $E\sigma^{70}$

Despite the high degree of protein sequence similarity that σ^S and σ^{70} exhibit, the C-terminal region of region 4.2 seems to be highly divergent between the two sigmas. Since the σ^{70}-αCTD interface involves several residues located in the region 4.2 of σ^{70} (Chen et al., 2003; Ross et al., 2003), we decided to construct more "σ^{70}-like" variants of σ^S -by introducing single amino acid substitutions in corresponding residues of σ^S or making combinatory mutations- and to monitor their behaviour in promoters with different UP-element configurations. Consistently with the fact that the σ^{70}-αCTD interface is slightly different for transcriptional activator-dependent and -independent UP-element utilisation (Chen et al., 2003; Ross et al., 2003), different mutant alleles of σ^S were found to respond in a more "σ^{70}-manner" to the presence of various UP-element configurations in the synthetic promoter carrying a –35 element and *csiD*p (co-activated by CRP). In the former case, a σ^S variant, carrying exchanges in all negatively charged (or neutral) amino acids, that are located in the outer face of σ^S (and thus exposed to the bound αCTD to the proximal UP-element sub-

site), to the corresponding basic amino acids of σ^{70}, E308K+E315H+Q318R (designated also as "triple mutant"), could utilise better a promoter construct with a proximal sub-site, but it had partially lost its ability to profit from the presence of a distal UP-element sub-site in the promoter. On the other hand, the most prominent residue of sigma, that is part of the σ^{70}-αCTD interface in activator-dependent promoters, is conserved in σ^{S} and σ^{70} (R311 and R596 respectively). Therefore, it was not surprising that the deficiency of $E\sigma^{S}$ to utilise the *csiD*p carrying a full-UP element site was due to more indirect reasons. A σ^{S} variant, Q306E (E591, the counterpart residue of σ^{70}, is indirectly involved in –35 box recognition (Nickels et al., 2002), was shown to allow $E\sigma^{S}$ to utilise the various promoter constructs in a more "$E\sigma^{70}$-like" manner.

A model was built to explain the different binding/activation mode of $E\sigma^{70}$ and $E\sigma^{S}$ in promoters with proximal or distal UP-element sub-sites (Fig. 15). In a promoter with a proximal UP-element sub-site, only $E\sigma^{70}$ can support an interaction to the bound αCTD and thus activate the promoter (Fig. 15A). In a promoter carrying a distal UP-element sub-site, either both αCTDs are accommodated to the 11bp DNA binding-site (Benoff et al., 2002) or alternatively one αCTD binds to the consensus distal half-site and the other one to the downstream, non-consensus proximal sub-site (Gourse et al., 2000). Equilibrium exists between the two situations. This equilibrium can be probably shifted towards the latter configuration for $E\sigma^{70}$, due to the optimal α–σ interface, whereas the former situation might be preferred by $E\sigma^{S}$ for the exact opposite reasons (Fig. 15B). Transcriptional activation is only achieved through RNAP having both its αCTDs binding to the distal half-site consensus.

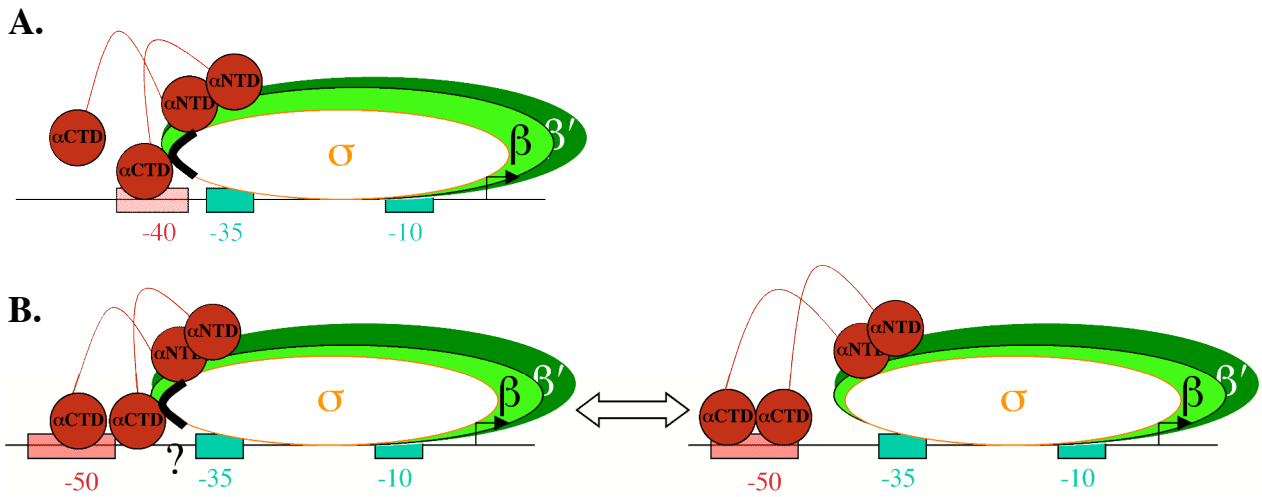

Fig. 15: Schematic representation of RNA polymerase binding to a promoter carrying either a proximal (**A**) or a distal (**B**) UP-element sub-site. In the former case (**A**), RNA polymerase gets stimulated by an αCTD-σ interaction; $E\sigma^{70}$, but not $E\sigma^{S}$, can support such an interaction. In the case of the promoter carrying a distal half UP-element site (**B**), RNAP can adopt two conformations; the first

(on the left panel) is favoured by $E\sigma^{70}$ and the second (on the right panel) is favoured by $E\sigma^{S}$. Only the second conformation leads to an active transcription initiation complex (see also text).

Further experiments were performed to test our model (Typas A, Sclavi B, Buckle M and Hengge R, in preparation), after the first paper was published. Using footprinting analysis, we observed that $E\sigma^{70}$ provided protection against DNase I attack to the DNA site between the consensus distal UP-element half site and the –35 hexamer, whereas $E\sigma^{S}$ offered no protection to the same site (see Fig. 18 in Figure Appendix). Such a footprint pattern is consistent with our hypothesis (Fig. 15B). In further DNase I footprint experiments, a strong hypersensitive site appeared at positions -39 and -38 of the template strand when $E\sigma^{70}$, but not $E\sigma^{S}$, bound to the promoter carrying a distal UP-element sub-site (see Fig. 19 in Figure Appendix). This indicated that the DNA directly upstream of the –35 gets strongly bent towards $E\sigma^{70}$, after the binding of the αCTDs (Campbell et al., 2002; Murakami et al., 2002), and might even wrap around $E\sigma^{70}$ (Davis et al., 2005; Rivetti et al., 2003), but probably not around $E\sigma^{S}$. The wrapping of upstream DNA around the holoenzyme might somehow defect transcriptional initiation in this promoter case (Shin et al., 2005).

UV laser photo-footprinting verified that the two RNAPs choose separate ways for initiating transcription from the same promoters (see Fig. 20 in Figure Appendix; Lee and Gralla, 2001), and that their binding-kinetics to the promoters with the various UP-elements configurations (measured by progression curves of protection or hypersensitivity appearance, which could most of the times be fitted to single exponential expressions) correspond perfectly to their in-vivo preference for them (see Table 2 in Figure Appendix). Interestingly, $E\sigma^{70}$ exhibited binding kinetics that could be fitted into a double exponential equation (for some of its signals), when recruited to the promoter carrying a distal UP-element half-site. A very fast (burst) initial phase could be observed for signals monitoring the promoter open complex formation, followed by a much slower secondary one. This might mean that $E\sigma^{70}$ rapidly adopts a dysfunctional conformation when bound to a promoter with a distal UP-element half-site, and then slowly resides to the productive one, which is different (and probably less efficient), though, than the $E\sigma^{S}$ conformation on the same promoter.

Finally, we aimed to clarify whether the presence of half or full UP-element sites could influence promoter selectivity, even when those were located in more upstream sites (note that αCTD is tethered with a flexible linker to αNTD and RNAP, and therefore can reach further upstream DNA elements; Meng et al., 2000). Both in synthetic promoters possessing -35 hexamers or others being devoid of such elements, implanting UP-elements one turn of the

DNA helix upstream of their expected sites, changed the promoter activity but not its selectivity towards $E\sigma^S$ or $E\sigma^{70}$ (see Fig. 21 in Figure Appendix). Thus, only canonically situated half or full UP-elements sites influence the promoter selectivity.

4.2 Role of the spacer between the –35 and –10 regions in σ^S promoter selectivity in *Escherichia coli*

Typas A. and Hengge R. (2006), *Mol Microbiol*, **59**(3), 1037-51

A.T. and R.H. designed research, A.T. performed research and analysed data, A.T. and R.H. wrote the paper

Several pieces of evidence (see also the "goal setting" section) prompted us to reassess the conservation degree of –35 elements in σ^s-dependent promoters. A more careful alignment of all mapped σ^s-dependent promoters (for an updated version see Table 3 in Figure Appendix) revealed that the majority of them did carry putative –35 boxes, albeit not always optimally located towards the –10 region. Although σ^{70}-dependent promoters exhibit a clear preference for 17bp spacers (Mitchell et al., 2003), σ^s-dependent promoters turned out to be more flexible in this matter, and could encompass spacer lengths ranging from 15-19 bp in a relatively equal frequency. A set of experiments were planned and executed, aiming to tackle questions about the importance of -35 elements, and that of the spacing length between -35 and -10 boxes, in σ^s-dependent promoters.

Functional but non-optimally placed –35 elements are conserved in σ^S-dependent promoters because they increase $E\sigma^S$ promoter selectivity

In order to test whether the "misplaced" and/or relatively degenerate -35 elements predicted for natural σ^S-dependent promoters were truly functional, three of them were further tested. In all three cases, the putative –35 elements turned out to be functional (even in the case of *cfa* (P2), which had two putative overlapping –35 elements), adding up to previously reported analogous cases (Checroun et al., 2004; Jung et al., 1990; Lacour et al., 2002; Rosenthal et al., 2006; Wise et al., 1996).

A synthetic promoter that could be equally well transcribed by both $E\sigma^{70}$ and $E\sigma^S$ was chosen for further studying, in order to avoid the complex regulation that natural promoters frequently exhibit and to be able to draw more general conclusions (and not promoter-specific ones) about the role of the spacer length in promoter selectivity. Both in-vivo and in-vitro experiments showed that $E\sigma^S$ could cope relatively well, compared to $E\sigma^{70}$, with promoters carrying 1-2 bp longer or shorter spacers than the optimum, i.e. 17 bp. In contrast, $E\sigma^{70}$ had the highest contribution to promoter expression when the promoter carried optimally spaced core promoter elements. A promoter variant lacking the -35 element showed the strongest

$E\sigma^S$-selectivity, but had only very weak overall activity (at this point is worth mentioning that the non-optimally spaced promoters showed almost the same σ^S-selectivity as the "-35-less" promoter at 30°C, indicating that $E\sigma^S$-mediated promoter recognition/utilisation is more dependent on the presence of a -35 element at lower temperatures; see Fig. 22 in Figure Appendix). To conclude, "misplaced" –35 elements are conserved in natural σ^S-dependent promoters, because they enhance σ^S promoter selectivity.

A step-wise mutation analysis of a sub-optimally located -35 element revealed that a "misplaced" and relatively degenerate –35 element can result in pronounced σ^S selectivity and, at the same time, satisfying levels of promoter activity, in contrast to the dramatic reduction in promoter activity observed in the σ^S-selective "-35-less" synthetic promoter. In addition, this analysis verified that sub-optimally situated –35 elements are truly recognised and bound by RNAP.

The molecular mechanism enabling $E\sigma^S$ to use better than $E\sigma^{70}$ sub-optimally spaced promoters

Using a derivative of the original synthetic promoter we could establish that the presence of a C(-13) at the extended –10 promoter region is essential for the ability of $E\sigma^S$ to preferentially utilise promoters carrying longer (not shorter though) spacers than the optimum. $E\sigma^S$ directly contacts C(-13) through its K173 residue of σ^S (Becker and Hengge-Aronis, 2001), inducing, thus, a DNA bend that enables RNAP to reach more easily further upstream situated –35 boxes. Furthermore, a genetic analysis of region 4 of σ^S revealed that an α-helix at its very C-terminus allows $E\sigma^S$ to efficiently utilise promoters with sub-optimal spacer lengths (see also Fig. 23 in the Figure Appendix for the mutants that are not presented in the paper due to space limitations). This 5th α–helix of σ_4 is probably involved in the interaction of sigma with core RNAP (β flap subunit; Lambert et al., 2004; Vassylyev et al., 2002), which initiates a conformational change that adequately positions region 4.2 of sigma towards its DNA recognition element, i.e. the –35 hexamer (Campbell et al., 2002; Kuznedelov et al., 2002; Murakami et al., 2002). $\sigma^S{}_4$ has been previously reported to mediate a stronger interaction with the β flap subunit of core RNAP than $\sigma^{70}{}_4$ did (Kuznedelov et al., 2002) and, therefore, it is foreseeable that such an interaction allows $E\sigma^S$ to be more flexible in respect with –35 element recogntion.

A/T-richness of the spacer region serves as a an activity stimulating feature in σ^S-dependent promoters

Degenerate -35 elements or sub-optimal spacer lengths improve σ^S promoter selectivity, but come at a cost of reduced promoter activity. Therefore σ^S-dependent promoters carry conserved, activity stimulating features, which, at the same time, do not influence promoter selectivity, in order to compensate for losses in promoter activity that come hand in hand with the increased σ^S-selectivity. Such a feature are the A/T-rich stretches, centred directly downstream of the -35 element and/or directly upstream of the extended -10 element in σ^S-dependent promoters.

4.3 The –35 sequence location and the Fis-sigma factor interface determine σ^S selectivity of the *proP* (P2) promoter in *Escherichia coli*

Typas A., Stella S., Johnson R.C. and Hengge R., *Mol Microbiol* (in revision)

A.T. and R.C.J. designed research, A.T., S.S. and R.C.J. performed research and analysed data, A.T., R.C.J. and R.H. wrote the paper

proP, encoding a transporter for proline and glycine betaine in *Escherichia coli*, is a unique case, where Fis and $E\sigma^S$ synergistically activate a promoter (Xu and Johnson, 1995; Xu and Johnson, 1997). In addition, it is the only promoter reported, where Fis acts as a class II transcriptional activator (for more details about transcriptional activation mechanisms see Fig. 7 and the corresponding section in introduction). In all other cases known, Fis co-activates the promoter with $E\sigma^{70}$, after binding a class I activating DNA-site and contacting RNAP only through the α subunit. Since the class II activation mechanism usually involves a direct sigma-activator interaction, we aimed to determine whether Fis contacts σ^S in the *proP* (P2) promoter, apart from its previously reported interaction with αCTD (McLeod et al., 2002; McLeod et al., 1999). In addition we sought to find out whether the interface between sigma and Fis is only suited for σ^S, thereby, providing an explanation for the extreme preference of *proP* (P2) for $E\sigma^S$ and the lack of other known cases, where a class II positioned Fis co-activates a promoter with $E\sigma^{70}$. The majority of experiments required to clarify such matters were planned and performed by me, however Reid Johnson and his co-worker, Stefano Stella contributed useful suggestions and experimental data (the models of the Fis-$E\sigma^S$ *proP* (P2) transcriptional complex and the elucidation of the Fis residues involved in the Fis-$E\sigma^S$ interface).

Molecular models of Fis and $E\sigma^S$ bound to the *proP* (P2) promoter

Using the structure of *T.aquaticus* RNAP bound to a fork junction promoter fragment (Murakami et al., 2002), a model of Fis bound to DNA (Pan et al., 1996) and the crystal structure of the ternary *Taq* σ_4- λcI- DNA complex (Jain et al., 2004), we could deduce a model of Fis and region 4 of σ^S bound to the *proP* (P2) promoter. Fis and region $\sigma_4{}^S$ could be simultaneously accommodated to the *proP* (P2) promoter, only when $\sigma_4{}^S$ bound to a non-consensus -35 element, spaced 16 bp from the –10 hexamer, and Fis recognised its consensus DNA-determinant, centred at –41. When $\sigma_4{}^S$ was accommodated one bp further upstream, in an optimally positioned –35 region (17bp apart from its -10 counterpart), then it sterically

clashed with Fis. This result prompted us to examine more carefully the roles of i) the –35 element location/sequence, ii) the location of the Fis binding-site and iii) the Fis-σ^S interface, on the activation of the *proP* (P2) promoter.

Deciphering how the Fis-RNAP synergy is affected by the sequence/location of the -35 element and the location of the Fis binding-site at the *proP* (P2) promoter

Firstly, the role of a postulated alternative –35 element (GCGG; Lee and Gralla, 2004) in the expression of the *proP* (P2) promoter was evaluated. Abolishing or optimising this element had little influence on σ^s promoter selectivity and on the degree of Eσ^s-Fis collaboration. On the contrary implanting a canonical –35 element in the place where region 4 of sigma was predicted to bind by the modelling (16bp upstream of the –10 hexamer), had a modest increase in the ability of Eσ^{70} to utilise the *proP* (P2) promoter in concert with Fis.

However, Eσ^{70} and Fis co-activated the *proP* (P2) promoter more prominently when the promoter spacer length was increased to 17 bp. This increase in Eσ^{70}-Fis synergy was only topped by a promoter construct carrying both a canonical –35 element and a 17 bp spacer. This *proP* (P2) promoter variant, "-35/17bp", was the first to allow Eσ^{70} to acquire a predominant role in promoter activation. In contrast, Eσ^S retained a considerable contribution in the activity of the "-35/17bp" construct only in a *fis*$^-$ background, indicating that Eσ^S could still recognise better the core promoter elements, but could not be substantially recruited anymore to the promoter by Fis.

Shifting the Fis-binding site one bp closer to the –35 element completely abolished the ability of Fis to activate the promoter (independently of the RNAP present), probably due to steric hindrance. On the other hand, shifting the Fis-binding site away from the –35 element by 1 bp, increased the ability of Eσ^{70} to collaborate with Fis only when σ_4^{70} was allowed to also shift its binding site one bp upstream (using thus a 17 bp spacer towards the –10 box), and was not "locked" at a canonical –35 element, situated 16 bp upstream of the –10 hexamer. The ability of Eσ^S to collaborate with Fis was also slightly reduced at the two promoter variants, in which the Fis binding-site was relocated to –42.

To summarise, Fis binds at –41 at the *proP* (P2) promoter and sterically forces RNAP to recognise a 16bp spaced promoter with a non-consensus -35 element; this requirement can be successfully met by Eσ^S, but not Eσ^{70}. Fis can recruit Eσ^{70} to the promoter only when *proP* (P2) is transformed to an optimally spaced promoter, i.e. 17 bp, with a canonical –35 element.

Deciphering the molecular interface between Eσ^S and Fis

Substitutions of amino acids of Fis and σ_4^S, predicted to be located in their interface, were screened for their effect in Fis-mediated activation of the *proP* (P2) promoter. Two σ^S mutants (E308K and R311E) and two Fis mutants (T75A and N48C) exhibited specific defects on the ability of Fis to activate the *proP* (P2) promoter in concert with $E\sigma^S$ (see also Fig. 24 in Figure Appendix for results that were not included in the publication due to space limitation). Although one of the σ^S residues involved in the sigma-Fis interface is conserved in σ^{70} (R311/R596), the other one, E308, is only found in σ^S, since σ^{70} carries K593 at the equivalent position. Interestingly, a σ^{70} (K593E) allele co-operated much better with Fis than the wild-type σ^{70} did. Therefore, the Fis-RNAP interface seems to be better suited for $E\sigma^S$, without this meaning that the slightly worse Fis-$E\sigma^{70}$ interface is the limiting factor that hinders Fis from recruiting $E\sigma^{70}$ to the *proP* (P2) promoter (see above).

4.4 Stationary phase reorganisation of the *E. coli* transcription machinery by Crl protein, a fine-tuner of σ^s activity and levels

Typas A., Barembruch C. and Hengge R., *EMBO J* (in revision)

A.T. and R.H. designed research, A.T. and C.B. performed research, A.T. analysed data, A.T. and R.H. wrote the paper

Crl owns its name to earlier studies that identified it as an activator of genes responsible for curli fimbriae formation (Arnqvist et al., 1992; Olsen et al., 1989). Only years later, it was established that Crl exerts this effect by increasing the activity of $E\sigma^S$ at certain σ^S-dependent promoters, including that of the *csgBA* operon, which encodes the structural subunits of the curli organelles (Pratt and Silhavy, 1998). Recently, Crl was shown to directly interact with σ^S (and probably with $E\sigma^S$ too) and was proposed to act as a thermo-sensor protein that facilitates binding of $E\sigma^S$ to specific σ^S-dependent promoters at 30°C (Bougdour et al., 2004). I aimed to clarify how Crl affects the activity and/or the levels of σ^S, and what is its impact on global gene expression. Claudia Barembruch helped me in this project, by performing several co-immunoprecipitation, gel filtration and in-vivo degradation experiments. Alexandra Possling performed the in-vitro degradation experiments (see Fig. 28 in Figure Appendix), and therefore, she is also included in the author list in the revised manuscript version, which is currently in preparation.

Crl acts a fine-tuner of σ^S-mediated gene expression

A DNA microarray analysis revealed that the Crl regulon in stationary phase (rich medium, 30°C) is solely composed of a large sub-set of σ^S-controlled genes. The positive influence of Crl on these genes was significantly lower than that of σ^S, implying that Crl only fine-tunes the activity of σ^S. In an *rpoS*$^-$ background, Crl lost its regulatory role in stationary phase (see Fig. 25 in Figure Appendix and Table 4). Interestingly, we observed that the cellular levels of Crl did only slightly decrease at 37°C and thereby, it was unsurprising that Crl exhibited the same broad control over σ^S-dependent genes at this temperature (see Fig. 25 in Figure Appendix). In addition, it became apparent that Crl had an augmented role in σ^S-mediated gene expression when the cellular levels of the latter were low. When, in contrast, σ^S cellular levels were high, then the presence of Crl did not affect σ^S-dependent gene expression.

Crl acts on σ^S activity by affecting sigma factor competition for core RNAP

No common promoter motif could be found in the Crl-controlled genes identified in the microarray analysis, and, moreover, Crl had similar effects on a series of σ^S-dependent promoters carrying different σ^S selective features. Thus, Crl influenced σ^S-mediated gene expression independently of DNA *cis* acting elements. This observation, combined with the fact that the role of Crl on σ^S activity was more pronounced when σ^S cellular levels were low, implied that Crl exerted its effects at a level prior to $E\sigma^S$-mediated promoter recognition. To further clarify this issue, we assayed the role of Crl in σ^S-dependent transcription in-vitro. Crl clearly stimulated σ^S-derived transcription only when σ^S had to compete with the housekeeping σ^{70} for limiting amounts of core RNAP. When, on the other hand, σ^S alone was added to core RNAP in saturating amounts, Crl had only modest, if any, effects in σ^S-dependent transcription. This indicated that Crl mainly promotes $E\sigma^S$ formation, something that became even clearer after monitoring the levels of free or RNAP-bound σ^S and σ^{70} in stationary phase cells of *crl*$^+$ and *crl*$^-$ strains (see Fig. 26 in Figure Appendix).

Crl controls σ^S levels and activity by modulating the RssB-mediated proteolysis of σ^S

It has been previously shown that Crl somehow reduces intracellular σ^S levels in stationary phase, parallel with enhancing its activity (Pratt and Silhavy, 1998). We found out that increased σ^S cellular levels are present all along the growth curve of a *crl*$^-$ mutant strain. The reason is that *rssB*, encoding the targeting factor of σ^S to the ClpXP degradation machinery, is down regulated in a *crl*$^-$ background, as any other σ^S-dependent gene. Lower levels of RssB lead to decreased proteolysis rates of σ^S, and thus higher σ^S intracellular levels.

However, when we uncoupled the expression of *rssB* from its σ^S/Crl-dependent promoter, then the direct role of Crl on σ^S degradation was unmasked, i.e. the presence of Crl increased σ^S stability and, thereby, higher σ^S cellular levels and activity could be detected (see also Fig. 27 in Figure Appendix for further evidence that was omitted from the paper, due to space limitations). Since Crl favours σ^S on its competition with σ^{70} for limiting amounts of core RNAP, it, therefore, leads to increased $E\sigma^S$ formation (see also Fig. 16). By facilitating $E\sigma^S$ formation, Crl protects σ^S from degradation (Zhou et al., 2001).

This finding brought up the question whether Crl could *directly* compete with RssB for binding to σ^S. A series of in-vitro experiments ruled out such a scenario, even when Crl was present in significant excess over RssB (as the in vivo situation) in the assays. Consistently, in-vitro degradation experiments showed that Crl was unable to directly protect σ^S from

degradation, but could do so in the presence of core RNAP (see Fig. 28 in Figure Appendix). Thus, Crl can affect partitioning of σ^S between RssB and RNAP in favour of the latter and thus rescues σ^S from proteolysis (see also Fig. 16).

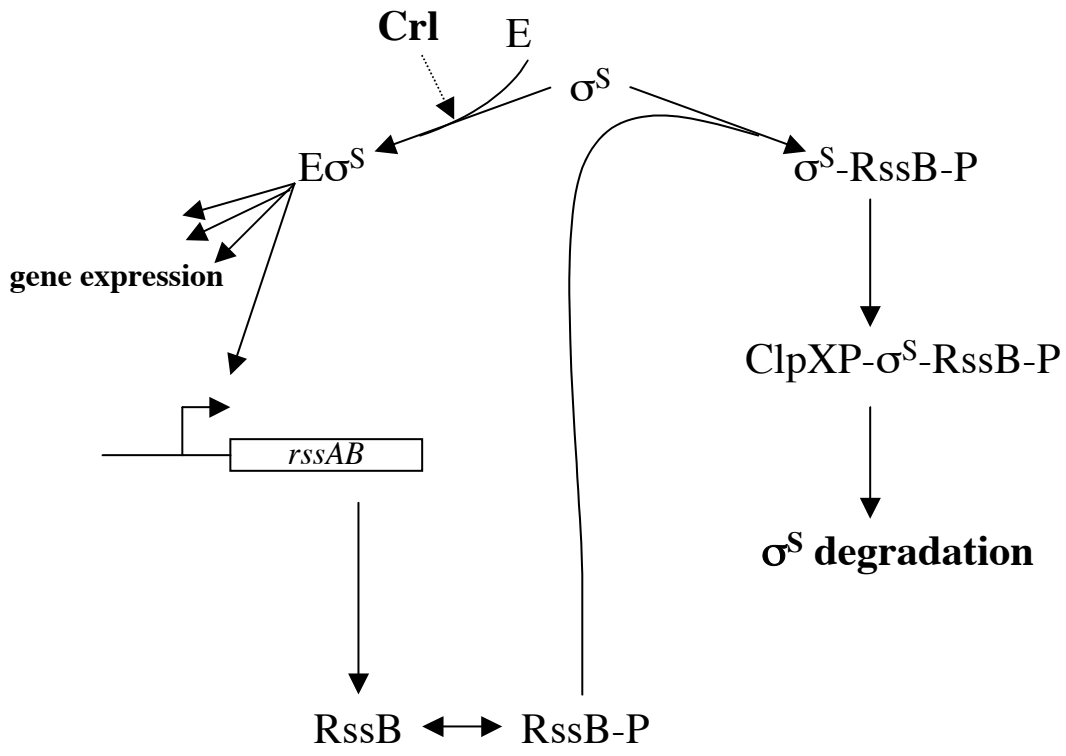

Fig. 16: The role of Crl in σ^S degradation. On one hand, Crl directly protects σ^S from degradation by ushering it to RNAP. On the other hand, it enhances σ^S degradation by stimulating the expression of its recognition factor, RssB. The latter effect is dominant. Only when a Crl/σ^S-independent promoter controls the expression of *rssB*, can the positive influence of Crl on σ^S stability be detected.

4.5 The molecular basis of selective promoter activation by the σ^S subunit of RNA polymerase

Typas A., Becker G. and Hengge R., submitted to *Mol Microbiol* (invited MicroReview)

The current state of the art on σ^S promoter selectivity is summarised in this paper. Several issues are discussed, involving *cis*- and *trans*- acting features that make a promoter σ^S selective, their mechanism of action, auxiliary factors that help σ^S to compete with the predominant σ^{70} for limiting amounts of core RNAP, and sigma factor co-evolution. In the Figure Appendix (Fig. 29 and Table 5) unpublished data can be found - cited on the MicroReview - about pausing of $E\sigma^S$ on promoter-proximal "-10-like" elements.