6. Figure Appendix

Table 1: UP-element abundance in σ^s-controlled promoters

I) σ^s-controlled promoters with putative full UP-element sites

	(AWWWWWWTTTT)	(AAAAAA	RNR)	-35	◄— 15-19bp —►	-10		+1
	AAAWWTWTTTT	**NNAAAA**	(N)NNN	**TTGACA**		**C**	**TATACT**	TATTTT	A/G
adhE(P1)	**TATCTAGTTGT**	**GCAAAA**	catg	CTAATG	TAGCCACCAAATCATA	**C**	**TACAAT**	TTATTA	A
gadY	**CTTATGTTTAT**	**AAAAAA**	atgg	**CTGATC**	TTATTTCCAGTAAAAG	**T**	**TATATT**	TAACTT	A
osmB(P1)	**ATTTGCAGTTT**	**GGCAAA**	tcat	CCGCTC	TAAGATGATTCCTGGT	**T**	**GATAAT**	TAAG	A
xthA	**TCAAATCACTT**	**AACAAC**	agg	CGGTAA	GCAACGCGAAATTCTG	**C**	**TACCAT**	CCACGC	A

II) σ^s-controlled promoters with putative proximal UP-element half-sites

				-35	◄— 15-19bp —►	-10		+1
		AAAAAA	**RNR**	**TTGACA**		**C**	**TATACT**	TATTTT	A/G
hmp	TGAAAAACACC	**AAAGAA**	**CCA**	**TTTACA**	TTGCAGGGCTATTTTTT	**A**	**TAAGAT**	GCATTT	G

III) σ^s-controlled promoters with putative distal UP-element half-sites

				-35	◄— 15-19bp —►	-10		+1
	AWWWWWWTTTT			**TTGACA**		**C**	**TATACT**	TATTTT	A/G
ada	**AATTAAAGCGC**	AAGATT	gttg	GTTTTT	GCGTGATGGTGACCGG	**G**	**CAGCCT**	AAAG	G
adhE(P2)	**AAAATTTGATT**	TGGATC	acg	**TAATCA**	GTACCCAGAAGTGA	**G**	**TAATCT**	TGCTTAC	G
aidB	**GAATGTTTTAG**	CAATCT	cttt	**CTGTCA**	TGAATCCATGGCAGTGA	**C**	**CATACT**	AATGGT	G
ansP(P1)	**GTGATAACTAT**	CATCGC	cagg	**ATGAAT**	AAACATTGTTCATG	**G**	**CAACTT**	ATAT	G
ansP(P2)	**ATAAAGAATAA**	TGGTGA	taa	**CTATCA**	TCGCCAGGATGAATAA	**A**	**CATTGT**	TCATGGC	A
appY	**TGTATTTAATT**	GGTTGT	tat	**TTGACT**	ACTATCAACTTGTTTTA	**A**	**TTTTAT**	GATAGGTGC	A
blc	**GTCCGAATTTT**	CGGACC	tttt	CTCCGC	TTTTCCTTGCTGTCAT	**C**	**TACACT**	TAGA	A
bolA(P1)	**GGTAAATATTT**	GTTGTT	aag	**CTGCAA**	TGGAAACGGTAAAAGCGG	**C**	**TAGTAT**	TTAAAG	G
cbpA(P2)	**TAACATATTCT**	GTGTTG	gcat	**ATGAAA**	TTTTGAGGATTACC	**C**	**TACACT**	TATA	G
cfa(P2)	**CGGTTTTTTCT**	GCGAGA	ttt	**CTCACA**	AAGCCCAAAAAGCGT	**C**	**TACGCT**	GTTTT	A
csgDEF	**ATTTAGTTACA**	TGTTTA	acac	**TTGATT**	TAAGATTTGTAATGG	**C**	**TAGATT**	GAAATC	A
csiD	**AAAACAATATG**	TCGCTT	ttg	**TGCGCA**	TTTTTCAGAAATGTAG	**A**	**TATTTT**	TAGATT	A
csiE(P1)	**CAACATTTCTG**	ATGATT	agca	**TTCCCT**	TCGCCATTTCCTTGA	**G**	**CAAACT**	TTAGCT	A
csiE(P2)	**ATGATTAGCAT**	TCCCTT	cgc	CATTTC	CTTGAGCAAACTTTAG	**C**	**TATTCT**	TATCAATT	A
dnaN(P1)	**GACATTCGTTT**	GCCGGG	cgaa	**GTGGCG**	TTCTTTATCGCCAAGCGT	**C**	**TACGAT**	CTAAC	G
fbaB	**AACATTTTTTC**	TGATGA	atc	**GAGCCA**	ACAGAAAACGCTGAAAAAA	**A**	**CATCCA**	AAAG	A
frdA	**TAAAAAAATCG**	ATCTGC	tcaa	**ATTTCA**	GACTTATCCATCAGA	**C**	**TATACT**	GTTGTA	C
ftsQ(P1)	**AGAAATTTTAC**	CGTCAA	tacg	**TATTCA**	ACCGTCCGGAACCTT	**C**	**TATGAT**	TATGT	G
gadX	**TAAATTTATTT**	ATCAAT	caat	**TTGACT**	TAAGAGGGCGGCGTG	**C**	**TACATT**	AATAAACA	G
hdeAB	**TTTGATATTTT**	CCATCA	acc	**ATGACA**	TATACAGAAAACCAGGTT	**A**	**TAACCT**	CAGT	G
htrE(P2)	**ATTGAATGAAT**	ATACAG	gga	**ATAATA**	ATTTCTATTTTATATT	**A**	**TTCCCT**	GTTTTA	A
mscL	**TTAATTAAATT**	CATTCC	tggc	**AGGAAA**	ATGGCTTAACATTTG	**T**	**TAGACT**	TATGGTT	G
mscS	**ATGAGAAATCT**	GTGATC	tat	**TTGGCA**	AAATTATGCTTTATTGT	**T**	**TACCCT**	TGTCAG	A
msyB	**CTGATTTTTCG**	CCTTTC	atac	**TTGCAA**	AAGCGGAGAATCAG	**C**	**TATCCT**	TTTCCCT	G
osmY	**CTTATGTTTTC**	GCTGAT	atcc	CGAGCG	GTTTCAAAATTGTGAT	**C**	**TATATT**	TAACAAA	G
proP(P2)	**GTTTGATTGTA**	CATTCC	ttaa	CCGGAG	GGTGTAAGCAAACCCG	**C**	**TACGCT**	TGTTAC	A
rraA	**AATTAACAATT**	GATGAT	tttg	**CCAACA**	GCCCACATAGCGCG	**A**	**TATACT**	GAAA	A
sra	**ATCAATTATGT**	GGTCAG	tggc	CAGCAC	CCTACGCTTTAAGGTG	**C**	**TATGCT**	TGATCG	G
talA(P1)	**ACACTGATGTT**	ACCTGC	ttaa	TCCAGC	AATACCATGCCTGTCTG	**C**	**TATGCT**	TTTT	T
uspB	**ATTGATAGTGG**	TTAACC	ttc	**TCGAAA**	AAAAACAACCTGATCTC	**C**	**TACACT**	ATCT	A
ytfK	**TAAAAAAAGTT**	ATACGC	gg	**TGGAAA**	CATTGCCCGGATAGT	**C**	**TATAGT**	CACTAA	G

IV) σ^s-controlled promoters with no putative UP-element sites

acnA(P1)	CCGTCGTTATT	CCAGAC	gac	**TGGCAA**	CTAACATCGCAGCAG	**C**	**AAGCCT**	TTATAG	A
aldB	GTCGTAAAGCT	GTTACC	gac	**TGGCGA**	AGATTTCGCCAGTCACGT	**C**	**TACCCT**	TGTTAT	A
artP(P3)	CCGACATTTAT	GCTCGC	cga	CCACCG	CCCCCGTTATTTTGTG	**C**	**TATGTT**	TATTGA	A

cfa(P2)	CGCGGTTTTTT	CTGCGA	gat	TTCTCA	CAAAGCCCAAAAAGCGT	C	TACGCT	GTTTT	A
cpxRA	GTGTAAAACAA	CGTAAA	gtca	TGGATT	AGCGACGTCTGATGAC	C	TAATTT	CTGCCTC	G
csgBA	AAAATACAACG	CGCGGG	tgag	TTATTA	AAAATATTTCCGCAGAC	A	TACTTT	CCATC	G
cyx	AATACCTCTGG	TCGTAG	agt	TTCAGG	ATAAAGAGGGAGATCTA	C	CATTAT	CGGGTT	A
dnaN(P2)	CGCTGCGCGAC	TTGCTG	gca	TTGCAG	GAAAAACTGGTCACCAT	C	GACAAT	ATTCA	G
dnaN(P4)	GCCGTAAGATC	GAGCAG	ttgc	GTGAAG	AGAGCCACGATATCAAA	G	AAGATT	TTTCAA	A
dps	AGTGTGATAGG	AACAGC	caga	ATAGCG	GAACACATAGCCGGTG	C	TATACT	TAATCTC	G
ecnB	TCCGAAAAATC	ATCAGA	ttc	CCATCA	TTTTTGGCGATGTTGT	C	TATTAT	TAATTT	G
esp	CATTACCAGAT	CTTGCT	tta	TTGATA	GTGAGCAGAGAGAGACT	G	CATTAT	TAATGAT	T
fic	TGCCCGCGCTT	CTGCTC	ttc	CGGCGT	AACCCGGATTTGCCGC	T	TATACT	TGTGG	G
gabD	AGATTTTGGGC	TCGTCG	ggga	TTCGCC	GGGTGCTGCAAAACCAT	C	TACGCT	CAGGACT	G
gadA	TTAAATTAAGC	CTGTAA	tgcc	TTGCTT	CCATTGCGGATAAATC	C	TACTTT	TTTATT	G
gadB	TAAACACGAGT	CCTTTG	cac	TTGCTT	ACTTTATCGATAAATC	C	TACTTT	TTTAAT	G
gadC	ACGAACCCGTT	TCGGGA	caa	TTTCCA	AAGTCTGTTCACTG	G	CATTAG	CAACGG	A
glgS(P2)	TGATCGGGGAC	CAATAT	att	TACGCA	CGTTATGTTTAAAGGCA	C	TACACT	GATTGGG	A
gor	CGGAGTAATTG	CAGCCA	ttg	CTGGCA	CCTATTACGTCTCGCG	C	TACAAT	CGCGGT	A
hchc(P2)	GCACTAAATCT	CTCCCC	gcc	ACCCCG	TACCTCTGATAATGGT	C	TAAAAT	CATTGA	A
himA(P4)	TTTATCCGAAT	GTAAGA	aag	TTGGCG	TAAATCAGGTAGTTGGC	G	TAAACT	TATTT	G
hyaAB	ATAAATCCACA	CAGTTT	gta	TTGTTT	TGTGCAAAAGTTTCA	C	TACGCT	TTATTAA	C
katE	CCGTTTCCAGA	ATAGTC	tcc	GAAGCG	GGATCTGGCTGGTGGT	C	TATAGT	TAGAGA	G
katG	GCATCCGTGGA	TTAATT	caa	TTATAA	CTTCTCTCTAACGCTGT	G	TATGCT	AACGCTA	A
osmB(P2)	CGAGCAGATTT	CACGGA	ata	ATTTCA	CCAGACTTATTCTTAG	C	TATTAT	AGTTAT	A
osmC(P2)	ATTCGGAATAT	CCTGCT	tat	CGTGCT	GTTTCTCACGTAGTCT	A	TAATTT	CCTTTTTA	A
osmE	AGCCGTTTCGT	TCACGG	gcc	TTGAAA	AAGCGCCCAATGTATT	C	CAGGCT	TATCTA	A
otsBA	TTGGCTGTTCT	TCCTTG	cca	ATGGCG	ACCCCCGTCACACTGT	C	TATACT	TACAT	G
pfkB(P2)	GATGGCAGGAA	CTGTCT	tca	AAAGCT	CCAATAAATCATATTG	T	TAATTT	CTTCACT	T
poxB	GCCTCCTTTCT	CTCCCA	tccc	TTCCCC	CTCCGTCAGATGAA	C	TAAACT	TGTTACC	G
pqi5(P2)	GGCAAAAGCAG	AAACTG	taa	AACGCA	GCAGTAGCAAACTAAG	C	TATAAA	TTGCAGC	G
proU(P1)	TACCCGCCAAA	TAGCTT	ttt	ATCACG	CAAATAATTTGTGGTGAT	C	TACACT	GATACT	C
rraA	TCAATTAACAA	TTGATG	att	TTGCCA	ACAGCCCACATAGCGCG	A	TATACT	GAAA	A
rsd	GAAATTTGCCC	GTTCCC	gat	ATGGCA	ATTCTCCCTTCGGCAA	C	CATAAT	TTTTGTTC	A
rssAB	CAGGTGCAACC	TTTTCA	cca	GACACA	TAAGGCTGCCAACATAGG	C	TATACT	CGACAGC	A
sodB	CAACAGGGTAA	GTTCAT	ctt	TTGTCT	CACCTTTTAATTTG	C	TACCCT	ATCC	A
sodC	ACTTTTAGGAA	TAGCCG	ccg	TTCAAA	AATGTGTCACTGGT	T	TATACT	TATTCA	G
ssrS(P2)	CGGACGATCTG	AACCAA	cggg	TTGCAA	GATCTGAAAGAACGCA	C	TAGAGT	CACAAAT	A
topA(P1)	CTGGTGGCAAG	AGCGCC	tta	CTGGCA	ACTTTGGATTTTGCATG	C	TAATAA	AGTTGC	G
treA	CGCAGAATGAG	ATTTCG	atc	ATGCAG	CTAGTGCGATCCTGAA	C	TAAGGT	TTTCTG	A
ybgA(P1)	CCTGCTACAAC	AGGATT	aac	TTCACA	AATATCATTTCTCAAGGT	C	TACACT	TACTCCT	G
ybgA(P2)	GCTATGGTTAG	AAACTA	cctg	ACGTCA	GTCCTTGCGGGGAGCAGG	C	TTTCGT	AAATTT	G
ybjP	TTGCTAAGCCT	TCGATC	tca	AAAGCA	TTATCAGACTGATACG	C	TATTAT	TGAAA	G
yehZXYW	TGCAACTGAAT	CCTTCC	gctc	AAGCTA	ACCCCGCCATTATCAA	C	TATGCT	TTTCTC	T
yggE(P1)	AAGTCATGAAG	CAAGGC	aga	TGGAAA	AATAAAACAGAGGCG	C	TAAGCT	TGCCTCC	A
yggE(P2)	TGATGCAGTCG	CCGTGG	ttg	CTGGCG	AGAGACGGTATTGC	T	CATGCA	CAAGC	C
yiaG	GAGCATGCCCT	GACTTC	acc	CCGCTG	TGTCTGCTTTTCCCGA	C	TATTCT	TAATGA	G

Table 2: Kinetics of complex formation of $E\sigma^{70}$ and $E\sigma^{S}$ on promoters with different UP-element configurations (experiments performed in collaboration with M. Buckle and B. Sclavi).

	Proximal UP-element sub-site K_{obs}		Distal UP-element sub-site K_{obs}	
DNA-site	$E\sigma^{70}$	$E\sigma^{S}$	$E\sigma^{70}$	$E\sigma^{S}$
-47	-	-	~3.5 min^{-1}	~3.5 min^{-1}
-34	++	~1 min^{-1}	~3.5 min^{-1}	~3.5 min^{-1}
-12	-	~1.5-2 min^{-1}	-	~3.5 min^{-1}
-6	++	~0.8 min^{-1}	~4/1 min^{-1}	~1 min^{-1}
-4	++	~2 min^{-1}	~4 min^{-1}	~3.5 min^{-1}
-2	++	~0.8 min^{-1}	~4/1 min^{-1}	~1 min^{-1}

UV irradiation (5 ns) was performed at different time intervals after the addition of 50nM RNAP to 1nM of supercoiled DNA. Time-course appearance and disappearance of different signals was monitored. Data fitting was carried out using the Origin version 5.0. The progression curves of appearance of protection/hyperesenstivity were fit individually to single or double exponential expressions:

$$y = L + (U-L)\, e^{-kt}$$

$$y = L + A\, e^{-kAt} + B\, e^{-kBt},\ U=L+A+B$$

A and B are the signal amplitudes. k, k_A and k_B are the observed, apparent rate constants (K_{obs}), which are presented in the table above. U and L represent the upper and lower limits from these fits respectively. In most cases, an expression containing a single exponential better described the results for each signal, and therefore only one rate constant is provided here, in the table. When, on the other hand, a double exponential expression described better the time-course appearance/disappearance of a signal, then two rate constants could be calculated (separated by a slash in the Table). Finally, in cases where the appearance/disappearance of a signals was completed faster that the first couple of secs (2-5 secs were the first time-points of our series of experiments), then rate constants could not be calculated, and therefore a "++" in the Table denotes that the events were extremely fast to be measurable. Note that each rate constant is calculated as an average of two or more independent experiments. Furthermore, by performing a series of promoter-binding, kinetics experiments with different $E\sigma^{S}$ concentrations, we could deduce that the rate constants of certain signals remained unchanged with increasing amounts of RNAP, whereas others increased proportionally to the amount of RNAP added. Thus, the former group of signals monitor the open complex formation of the holoenzyme (underlined in the Table), whereas the latter group represent the initial recruitment of RNAP to the promoter.

Table 3: mapped σ^s-dependent promoters of *E. coli* and other bacteria (in bold face; *Sty* stands for *Salmonella typhimurium*, *Pau* stands for *Pseudomonas aeruginosa*, *Pol* stands for *Pseudomonas oleovorans*, *Bbu* stands for *Borrelia burgdorferi* and *Avi* stands for *Azotobacter vinelandii*) grouped according to the existence/positioning of the –35 element. Existence of a –35 box was regarded relevant when it was present as three or more matches to the consensus hexamer TTGACA, in a location 15-19bp upstream of the –10 element. Note that some promoters contain overlapping putative –35 elements.

	-35	◄——17bp——►		-10		+1
	c**TTGACA**		**C**	**TATACT**	TATTTT	A/G
no –35 element						
ada	gGTTTTT	GCGTGATGGTGACCGG	**G**	**CAGCCT**	AAAG	G
adhE(P1)	gCTAATG	TAGCCACCAAATCATA	**C**	**TACAAT**	TTATTA	A
artP(P3)	aCCACCG	CCCCCGTTATTTTGTG	**C**	**TATGTT**	TATTGA	A
csiE(P2)	cCATTTC	CTTGAGCAAACTTTAG	**C**	**TATTCT**	TATCAATT	A
dps	aATAGCG	GAACACATAGCCGGTG	**C**	**TATACT**	TAATCTC	G
ecnB	cCCATCA	TTTTTGGCGATGTTGT	**C**	**TATTAT**	TAATTT	G
fic	cCGGCGT	AACCCGGATTTGCCGC	**T**	**TATACT**	TGTGG	G
hchA(P2)	cACCCCG	TACCTCTGATAATGGT	**C**	**TAAAAT**	CATTGA	A
katE	cGAAGCG	GGATCTGGCTGGTGGT	**C**	**TATAGT**	TAGAGA	G
lecA(*Pau*)	gGCGGTA	CTTCCTCGTTGCTGTG	**C**	**TTTGCT**	AACAGG	G
osmC(P2)	tCGTGCT	GTTTCTCACGTAGTCT	**A**	**TAATTT**	CCTTTTTA	A
osmY	cCGAGCG	GTTTCAAAATTGTGAT	**C**	**TATATT**	TAACAAA	G
pfkB(P2)	aAAAGCT	CCAATAAATCATATTG	**T**	**TAATTT**	CTTCACT	T
Pm(*Pau*)	cTATCTC	TAGAAAGGCCTACCCC	**T**	**TAGGCT**	TTATGC	A
pqi5(P2)	aAACGCA	GCAGTAGCAAACTAAG	**C**	**TATAAA**	TTGCAGC	G
proP(P2)	aCCGGAG	GGTGTAAGCAAACCCG	**C**	**TACGCT**	TGTTAC	A
pstS	cATATAA	CTGTCACCTGTTTGTC	**C**	**TATTTT**	GCTTCTC	G
spvA(*Sty*)	cACAGCA	GAAAAATAGCACATAA	**A**	**TAAACT**	CAATAT	A
sra	cCAGCAC	CCTACGCTTTAAGGTG	**C**	**TATGCT**	TGATCG	G
talA(P1)	tCCAGCA	ATACCATGCCTGTCTG	**C**	**TATGCT**	TTTT	T
treA	cATGCAG	CTAGTGCGATCCTGAA	**C**	**TAAGGT**	TTTCTG	A
ybjP	aAAAGCA	TTATCAGACTGATACG	**C**	**TATTAT**	TGAAA	G
yehZXYW	cAAGCTA	ACCCCGCCATTATCAA	**C**	**TATGCT**	TTTCTC	T
yiaG	cCCGCTG	TGTCTGCTTTTCCCGA	**C**	**TATTCT**	TAATGA	G
xthA	gCGGTAA	GCAACGCGAAATTCTG	**C**	**TACCAT**	CCACGC	A
15bp spacer						
adhE(P2)	g**TAATCA**	GTACCCAGAAGTGA	**G**	**TAATCT**	TGCTTAC	G
algD(*Avi*)	t**TTGGCA**	CGACATTTTATTGA	**C**	**TATAAT**	TCGGCCT	G
ansP(P1)	g**ATGAAT**	AAACATTGTTCATG	**G**	**CAACTT**	ATAT	G
cbpA(P2)	t**ATGAAA**	TTTTGAGGATTACC	**C**	**TACACT**	TATA	G
csgBA(P1)	a**TTAAAA**	ATATTTCCGCAGAC	**A**	**TACTTT**	CCATC	G
dnaN(P4)	g**AAGAGA**	GCCACGATATCAAA	**G**	**AAGATT**	TTTCAA	A
gadC	a**TTTCCA**	AAGTCTGTTCACTG	**G**	**CATTAG**	CAACGG	A
msyB	c**TTGCAA**	AAGCGGAGAATCAG	**C**	**TATCCT**	TTTCCCT	G
osmB(P2)	a**TTCACC**	AGACTTATTCTTAG	**C**	**TATTAT**	AGTTAT	A
poxB	c**TTCCCC**	CTCCGTCAGATGAA	**C**	**TAAACT**	TGTTACC	G
rraA	g**CCAACA**	GCCCACATAGCGCG	**A**	**TATACT**	GAAA	A
sodB	t**TTGTCT**	CACCTTTTAATTTG	**C**	**TACCCT**	ATCC	A
sodC	g**TTCAAA**	AATGTGTCACTGGT	**T**	**TATACT**	TATTCA	G
yggE(P2)	g**CTGGCG**	AGAGACGGTATTGC	**T**	**CATGCA**	CAAGC	C

16bp spacer

acnA(P1)	c**TGGCAA**	CTAACATCGCAGCAG	**C**	**AAGCCT**	TTATAG	A
alkS(*Pol*)	t**TTGCAC**	CACCGATCATGCCGA	**C**	**TACACT**	TAAGT	G
cfa(P2)	t**CTCACA**	AAGCCCAAAAAGCGT	**C**	**TACGCT**	GTTTT	A
csgBA(P2)	g**TTATTA**	AAAATATTTCCGCAG	**A**	**CATACT**	TTCCATC	G
csgDEF	c**TTGATT**	TAAGATTTGTAATGG	**C**	**TAGATT**	GAAATC	A
fbaB	g**CCAACA**	GAAAACGCTGAAAAA	**A**	**CATCCA**	AAAG	A
frdA	a**ATTTCA**	GACTTATCCATCAGA	**C**	**TATACT**	GTTGTA	C
ftsQ(P1)	g**TATTCA**	ACCGTCCGGAACCTT	**C**	**TATGAT**	TATGT	G
gadX	t**TTGACT**	TAAGAGGGCGGCGTG	**C**	**TACATT**	AATAAACA	G
hyaAB	a**TTGTTT**	TGTGCAAAAGTTTCA	**C**	**TACGCT**	TTATTAA	C
katG	t**ATAACT**	TCTCTCTAACGCTGT	**G**	**TATGCT**	AACGCTA	A
mscL	c**AGGAAA**	ATGGCTTAACATTTG	**T**	**TAGACT**	TATGGTT	G
ospF(*Bbu*)	**TTGTAT**	TTATTAGCTGTTGCG	**T**	**TAGACT**	TAAGTAT	T
otsBA	a**TGGCGA**	CCCCCGTCACACTGT	**C**	**TATACT**	TACAT	G
yciGFE-	t**TTGACT**	AATCGGTTTAACCAA	**C**	**TAATTT**	AATAGG	G
katN(*Sty*)						
yggE(P1)	a**TGGAAA**	AATAAAACAGAGGCG	**C**	**TAAGCT**	TGCCTCC	A
ytfK	g**TGGAAA**	CATTGCCCGGATAGT	**C**	**TATAGT**	CACTAA	G

17bp spacer

acnA(P1)	g**CTGGCA**	ACTAACATCGCAGCAG	**C**	**AAGCCT**	TTATAG	A
alkS(*Pol*)	t**TTTGCA**	CCACCGATCATGCCGA	**C**	**TACACT**	TAAGT	G
ansP(P2)	a**CTATCA**	TCGCCAGGATGAATAA	**A**	**CATTGT**	TCATGGC	A
cpxRA	a**TGGATT**	AGCGACGTCTGATGAC	**C**	**TAATTT**	CTGCCTC	G
csiD	g**TGCGCA**	TTTTTCAGAAATGTAG	**A**	**TATTTT**	TAGATT	A
gabD	t**TCGCCG**	GGTGCTGCAAAACCAT	**C**	**TACGCT**	CAGGACT	G
gadA	c**TTGCTT**	CCATTGCGGATAAATC	**C**	**TACTTT**	TTTATT	G
gadB	c**TTGCTT**	ACTTTATCGATAAATC	**C**	**TACTTT**	TTTAAT	G
gadY	g**CTGATC**	TTATTTCCAGTAAAAG	**T**	**TATATT**	TAACTT	A
gor	g**CTGGCA**	CCTATTACGTCTCGCG	**C**	**TACAAT**	CGCGGT	A
htrE(P2)	a**ATAATA**	ATTTCTATTTTATATT	**A**	**TTCCCT**	GTTTTA	A
ogt(*Sty*)	g**TCGCTA**	AATGTGTTATCCCTGA	**C**	**TATCTT**	TTTAGG	A
osmB(P2)	a**ATTTCA**	CCAGACTTATTCTTAG	**C**	**TATTAT**	AGTTAT	A
osmE	c**TTGAAA**	AAGCGCCCAATGTATT	**C**	**CAGGCT**	TATCTA	A
otsBA	a**ATGGCG**	ACCCCCGTCACACTGT	**C**	**TATACT**	TACAT	G
spvR(*Sty*)	t**TGCACA**	TCAAAACATTTTTTCA	**G**	**GATTAT**	TCTGA	A
rsd(P1)	t**ATGGCA**	ATTCTCCCTTCGGCAA	**C**	**CATAAT**	TTTTGTTC	A
ssrS(P2)	g**TTGCAA**	GATCTGAAAGAACGCA	**C**	**TAGAGT**	CACAAAT	A

18bp spacer

aidB	t**CTGTCA**	TGAATCCATGGCAGTGA	**C**	**CATACT**	AATGGT	G
appY	t**TTGACT**	ACTATCAACTTGTTTTA	**A**	**TTTTAT**	GATAGGTGC	A
cfa(P2)	t**TTCTCA**	CAAAGCCCAAAAAGCGT	**C**	**TACGCT**	GTTTT	A
csgBA(P1)	g**TTATTA**	AAAATATTTCCGCAGAC	**A**	**TACTTT**	CCATC	G
cyx	t**TTCAGG**	ATAAAGAGGGAGATCTA	**C**	**CATTAT**	CGGGTT	A
dnaN(P2)	a**TTGCAG**	GAAAAACTGGTCACCAT	**C**	**GACAAT**	ATTCA	G
dnaN(P4)	t**GTGAAG**	AGAGCCACGATATCAAA	**G**	**AAGATT**	TTTCAA	A
esp	a**TTGATA**	GTGAGCAGAGAGAGACT	**G**	**CATTAT**	TAATGAT	T
gabD	a**TTCGCC**	GGGTGCTGCAAAACCAT	**C**	**TACGCT**	CAGGACT	G
glgS(P2)	t**TACGCA**	CGTTATGTTTAAAGGCA	**C**	**TACACT**	GATTGGG	A

himA(P4)	g**TTGGCG**	TAAATCAGGTAGTTGGC **G**	**TAAACT**	TATTT	G
hmp	a**TTTACA**	TTGCAGGGCTATTTTTT **A**	**TAAGAT**	GCATTT	G
katG	a**TTATAA**	CTTCTCTCTAACGCTGT **G**	**TATGCT**	AACGCTA	A
mscS	t**TTGGCA**	AAATTATGCTTTATTGT **T**	**TACCCT**	TGTCAG	A
rraA	t**TTGCCA**	ACAGCCCACATAGCGCG **A**	**TATACT**	GAAA	A
topA(Px1)	a**CTGGCA**	ACTTTGGATTTTGCATG **C**	**TAATAA**	AGTTGC	G
uspB	c**TCGAAA**	AAAAACAACCTGATCTC **C**	**TACACT**	ATCT	A

19bp spacer

aldB	c**TGGCGA**	AGATTTCGCCAGTCACGT**C**	**TACCCT**	TGTTAT	A
blc	t**TTCTCC**	GCTTTTCCTTGCTGTCAT**C**	**TACACT**	TAGA	A
bolA(P1)	g**CTGCAA**	TGGAAACGGTAAAAGCGG**C**	**TAGTAT**	TTAAAG	G
dnaN(P1)	a**GTGGCG**	TTCTTTATCGCCAAGCGT**C**	**TACGAT**	CTAAC	G
dnaN(P2)	c**ATTGCA**	GGAAAAACTGGTCACCAT**C**	**GACAAT**	ATTCA	G
fbaB	c**GAGCCA**	ACAGAAAACGCTGAAAAA**A**	**CATCCA**	AAAG	A
hdeAB	c**ATGACA**	TATACAGAAAACCAGGTT**A**	**TAACCT**	CAGT	G
proU(P1)	t**ATCACG**	CAAATAATTTGTGGTGAT**C**	**TACACT**	GATACT	C
rssAB	a**GACACA**	TAAGGCTGCCAACATAGG**C**	**TATACT**	CGACAGC	A
ybgA(P1)	c**TTCACA**	AATATCATTTCTCAAGGT**C**	**TACACT**	TACTCCT	G
ybgA(P2)	g**ACGTCA**	GTCCTTGCGGGGAGCAGG**C**	**TTTCGT**	AAATTT	G

Table 4: The Crl regulon in an *rpoS*$^-$ background (LB, OD_{578nm}=4, 30°C)

gene name	ID	Ratio of Medians	Function
crl	b0240	243,01	regulatory protein for curli, transcriptional regulator
paaA	b1388	0,381	subunit of putative phenylacetate-CoA oxygenase
paaB	b1389	0,461	subunit of putative phenylacetate-CoA oxygenase
paaD	b1391	0,425	subunit of putative phenylacetate-CoA oxygenase
paaF	b1393	0,293	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation
paaH	b1395	0,467	putative 3-hydroxyl-acyl-CoA dehydrogenase of phenylacetate degradation
paaK	b1398	0,385	phenylacetate-CoA ligase
yeeE	b2013	0,5	putative transport system permease protein
cysP	b2425	0,432	subunit of thiosulfate ABC transporter

RH90 (MC4100 *rpoS*::Tn10) and its isogenic *crl::cat* mutant (NT225) were grown in rich medium (LB) at 30ºC. Total RNA was extracted at an OD_{578} of 4.0 (i.e. during entry into stationary phase) and further processed for genome-wide microarray analysis. Genes with expression ratios in RH90 and its *crl* mutant derivative of >2-fold or <0.5-fold (average of three independent experiments) were considered relevant and are presented here.

Table 5: Putative promoter-proximal pausing sites in σ^s-dependent promoters in *E. coli*. In bold face are shown the putative pausing sites that satisfy the criteria set by Nickels *et al* (2004). Underlined are further "-10-like" elements, situated downstream of the transcriptional start, that fail one of the criteria set by Nickels *et al* (2004), but are nevertheless likely to be functional pausing sites (see also Fig. 29 for the cases of the *gadA* and the *bolA* promoters). Note that in some σ^s-dependent promoters with putative overlapping –35 elements, only one configuration is shown here.

	-35	←15-19bp→		-10		+1
	cTTGACA		C	TATACT	TATTTT	A/G
acnA(P1)	cTGGCAA	CTAACATCGCAGCAG	C	AAGCCT	TTATAG	Aactgtttgctgaagat
ada	gGTTTTT	GCGTGATGGTGACCGG	G	CAGCCT	AAA**G**	G**ctatcct**taa
adhE(P1)	gCTAATG	TAGCCACCAAATCATA	C	TACAAT	TTATTA	Actgttagctataat
adhE(P2)	gTAATCA	GTACCCAGAAGTGA	G	TAATCT	TGCTTAC	Gccacctggaagt
aidB	tCTGTCA	TGAATCCATGGCAGTGA	C	CATACT	AATGGT	Gactgccatt
aldB	cTGGCGA	AGATTTCGCCAGTCACGT	C	TACCCT	TGTTAT	Acctcacacc
ansP(P1)	gATGAAT	AAACATTGTTCATG	G	CAACTT	ATAT	Gacttttcat
ansP(P2)	aCTATCA	TCGCCAGGATGAATAA	A	CATTGT	TCATGG*C*	*Aacttatat*gact
appY	tTTGACT	ACTATCAACTTGTTTTA	A	TTTTAT	GATAGG**TG**C	**Aaagat**ggatt
artP(P3)	aCCACCG	CCCCCGTTATTTTGTG	C	TATGTT	TATTGA	Ataatgcgcttt
blc	tTTCTCC	GCTTTTCCTTGCTGTCAT	C	TACACT	TAGA	Aaaaaaacca
bolA(P1)	gCTGCAA	TGGAAACGGTAAAAGCGG	C	TAGTAT	TTAAAG	Ggatggatgacatct
cbpA(P2)	tATGAAA	TTTTGAGGATTACC	C	TACACT	TATA	Gga**g**t**tacctt**aca
cfa(P2)	tCTCACA	AAGCCCAAAAAGCGT	C	TACGCT	GTTTT	Aaggt*tctgat*ca
cpxRA	aTGGATT	AGCGACGTCTGATGAC	C	TAATTT	CTGCCTC	Ggaggtattta
csgBA(P1)	aTTAAAA	ATATTTCCGCAGAC	A	TACTTT	CCATC	Gtaacgcagcgtt
csgBA(P2)	gTTATTA	AAAATATTTCCGCAG	A	CATACT	TTCCATC	Gtaacgcagcgtt
csgDEF	cTTGATT	TAAGATTTGTAATGG	C	TAGATT	GAAATC	Agatgtaatccatt
csiD	gTGCGCA	TTTTTCAGAAATGTAG	A	TATTTT	TAGATT	Atggctacgaaat
csiE(P2)	cCATTTC	CTTGAGCAAACTTTAG	C	TATTCT	TATCAATT	Aatgcttatggga
cyx	tTTCAGG	ATAAAGAGGGAGATCTA	C	CATTAT	CGGGTT	Atttttctctctt
dnaN(P1)	aGTGGCG	TTCTTTATCGCCAAGCGT	C	TACGAT	CTAAC	Gtacgtgagct
dnaN(P2)	aTTGCAG	GAAAAACTGGTCACCAT	C	GACAAT	ATTCA	Gaagacggtggc
dnaN(P4)	gAAGAGA	GCCACGATATCAAA	G	AAGATT	TTTCAA	Atttaatcag
dps	aATAGCG	GAACACATAGCCGGTG	C	TATACT	TAATCTC	Gttaattact
ecnB	cCCATCA	TTTTTGGCGATGTTGT	C	TATTAT	TAATTT	Gctataggca
esp	aTTGATA	GTGAGCAGAGAGAGACT	G	CATTAT	TAATGAT	**Tg**g**taaagt**taat
fbaB	gCCAACA	GAAAACGCTGAAAAA	A	CATCCA	AAAG	Atg**g**a**aaaact**cg
fic	cCGGCGT	AACCCGGATTTGCCGC	T	TATACT	TGTGGC	Aaatggacacgtt
frdA	aATTTCA	GACTTATCCATCAGA	C	TATACT	GTTGTA	Cctataaa
ftsQ(P1)	gTATTCA	ACCGTCCGGAACCTT	C	TATGAT	TATGA	Ggcg**aagtat**ctct
gabD	tTCGCCG	GGTGCTGCAAAACCAT	C	TACGCT	CAGGACT	Gggcgagatga
gadA	cTTGCTT	CCATTGCGGATAAATC	C	TACTTT	TTTATT	Gcct*tcaaataaatt*t
gadB	cTTGCTT	ACTTTATCGATAAATC	C	TACTTT	TTTAAT	Gcgatccaat
gadC	aTTTCCA	AAGTCTGTTCACTG	G	CATTAG	CAACGG	Aaaa**tattgt**tct
gadX	tTTGACT	TAAGAGGGCGGCGTG	C	TACATT	AATAAACA	G**taatat**gtttat
gadY	gCTGATC	TTATTTCCAGTAAAAG	T	TATATT	TAACTT	Actgagagcacaaagt
glgS(P2)	tTACGCA	CGTTATGTTTAAAGGCA	C	TACACT	GATTGG**G**	A**aatact**gaaat
gor	gCTGGCA	CCTATTACGTCTCGCG	C	TACAAT	CGCGGT	Aatc**aacgat**
hchA(P2)	cACCCCG	TACCTCTGATAATGGT	C	TAAAAT	CATTGA	Agccacttgcgacg
hdeAB	cATGACA	TATACAGAAAACCAGGTT	A	TAACCT	CAGT	Gtc**gaaatt**gat
himA(P4)	gTTGGCG	TAAATCAGGTAGTTGGC	G	TAAACT	TATTT	Gacgtgtaccgc
hmp	aTTTACA	TTGCAGGGCTATTTTTT	A	TAAGAT	GCATTT	Gagatacatcaat
htrE(P2)	aATAATA	ATTTCTATTTTATATT	A	TTCCCT	GTTTTA	Att**aactct**atca
hyaAB	aTTGTTT	TGTGCAAAAGTTTCA	C	TACGCT	TTATTAA	Caa**tactttc**t
katE	cGAAGCG	GGATCTGGCTGGTGGT	C	TATAGT	TAGAGA	Gtttttgacc
katG	tATAACT	TCTCTCTAACGCTGT	G	TATGCT	AACGCTA	Acactgtagaggg
mscL	cAGGAAA	ATGGCTTAACATTTG	T	TAGACT	TATGGTT	Gtcggcttcat
mscS	tTTGGCA	AAATTATGCTTTATTGT	T	TACCCT	TGTCAG	Actgcccgtcataa
msyB	cTTGCAA	AAGCGGAGAATCAG	C	TATCCT	TTTCCCT	G**aaacct**catcaact
osmB(P2)	aTTCACC	AGACTTATTCTTAG	C	TATTAT	AGTTAT	Agagagcttacttc

osmC(P2)	tCGTGCT	GTTTCTCACGTAGTCT	A	TAATTT	CCTTTTTA	Agcccacag
osmE	cTTGAAA	AAGCGCCCAATGTATT	C	CAGGCT	TATCTA	A**cacgct**gat
osmY	cCGAGCG	GTTTCAAAATTGTGAT	C	TATATT	TAACAAA	Gtgatgacatttct
otsBA	aATGGCG	ACCCCCGTCACACTGT	C	TATACT	TACAT	Gtctgtaaag
pfkB(P2)	aAAAGCT	CCAATAAATCATATTG	T	TAATTT	CTTCACT	Ttccgctgattc
poxB	cTTCCCC	CTCCGTCAGATGAA	C	TAAACT	TGTTACC	Gttatcacatt
pqi5(P2)	aAACGCA	GCAGTAGCAAACTAAG	C	TATAAA	TTGCAGC	Gcgaactggag
proP(P2)	aCCGGAG	GGTGTAAGCAAACCCG	C	TACGCT	TGTTAC	A**gagatt**gcat
proU(P1)	tATCACG	CAAATAATTTGTGGTGAT	C	TACACT	GATACT	C**tg**ttg**cattat**t
pstS	cATATAA	CTGTCACCTGTTTGTC	C	TATTTT	GCTTCTC	Gtagccaacaaacaat
rraA	tTTGCCA	ACAGCCCACATAGCGCG	A	TATACT	GAAA	Atctcgcagcaact
rsd(P1)	tATGGCA	ATTCTCCCTTCGGCAA	C	CATAAT	TTTTGTTC	Atggctgacga
rssAB	aGACACA	TAAGGCTGCCAACATAGG	C	TATACT	CGACAGC	Actaccacaggg
sodB	tTTGTCT	CACCTTTTAATTTG	C	TACCCT	ATCCAT	Acg**cacaat**aagg
sodC	gTTCAAA	AATGTGTCACTGGT	T	TATACT	TATTCA	Ggaatg**cacaat**g
sra	cCAGCAC	CCTACGCTTTAAGGTG	C	TATGCT	TGATCG	Gcaacc**taattt**
ssrS(P2)	gTTGCAA	GATCTGAAAGAACGCA	C	TAGAGT	CACAAAT	Actg**aacagt**tggt
talA(P1)	tCCAGCA	ATACCATGCCTGTCTG	C	TATGCT	TTTTT	Gatgcgtttagcgaa
topA(Px1)	aCTGGCA	ACTTTGGATTTTGCATG	C	TAATAA	AGTTGC	Gtatcggattttat
treA	cATGCAG	CTAGTGCGATCCTGAA	C	TAAGGT	TTTCTG	Atacttgaataccgt
uspB	cTCGAAA	AAAAACAACCTGATCTC	C	TACACT	ATCT	Atagagccgctcgtatgtt
ybgA(P1)	cTTCACA	AATATCATTTCTCAAGGT	C	TACACT	TACTCCT	Gtaaaccgctcag
ybgA(P2)	gACGTCA	GTCCTTGCGGGGAGCAGG	C	TTTCGT	AAATTT	Gtcctgctacaa
ybjP	aAAAGCA	TTATCAGACTGATACG	C	TATTAT	TGAAA	G**g**a**tatcat**tattat
yehZXYW	cAAGCTA	ACCCCGCCATTATCAA	C	TATGCT	TTTCTC	Ttaattcgctg
yggE(P1)	aTGGAAA	AATAAAACAGAGGCG	C	TAAGCT	TGCCTCC	Agaggtcctgaatt
yggE(P2)	gCTGGCG	AGAGACGGTATTGC	T	CATGCA	CAAGC	Cttgttcagttagg
yiaG	cCCGCTG	TGTCTGCTTTTCCCGA	C	TATTCT	TAATGA	Gcttcgatgcaatt
ytfK	gTGGAAA	CATTGCCCGGATAGT	C	TATAGT	CACTAA	Gcat**taaaat**tt
xthA	gCGGTAA	GCAACGCGAAATTCTG	C	TACCAT	CCACGC	Actctttatctgaat

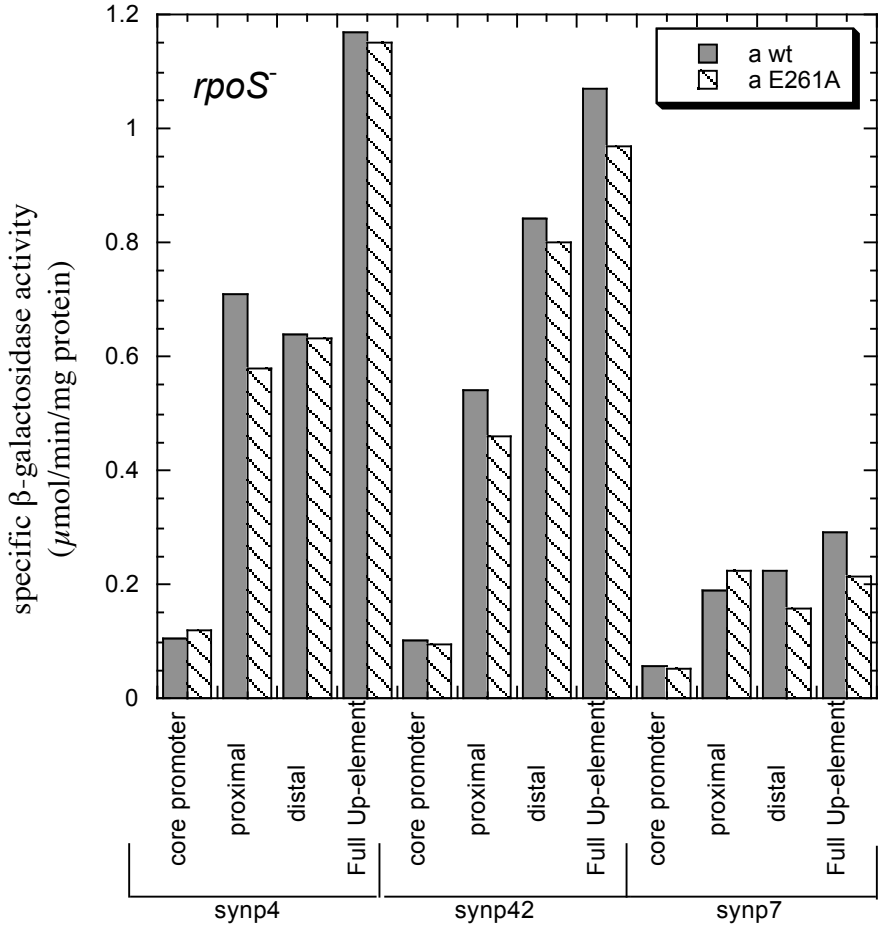


Fig. 17: An αCTD-σ interaction cannot be mediated in promoters lacking a -35 element, no matter of the kind of UP-element configuration present in front of them. The presence of an α subunit mutant (E261A), known to defect the α-σ interaction (Ross *et al*, 2005), does not influence the expression of a series of synthetic promoters lacking a –35 element and carrying different UP-element sites (for the exact sequences of the different synthetic promoters see the corresponding paper in the appendix).

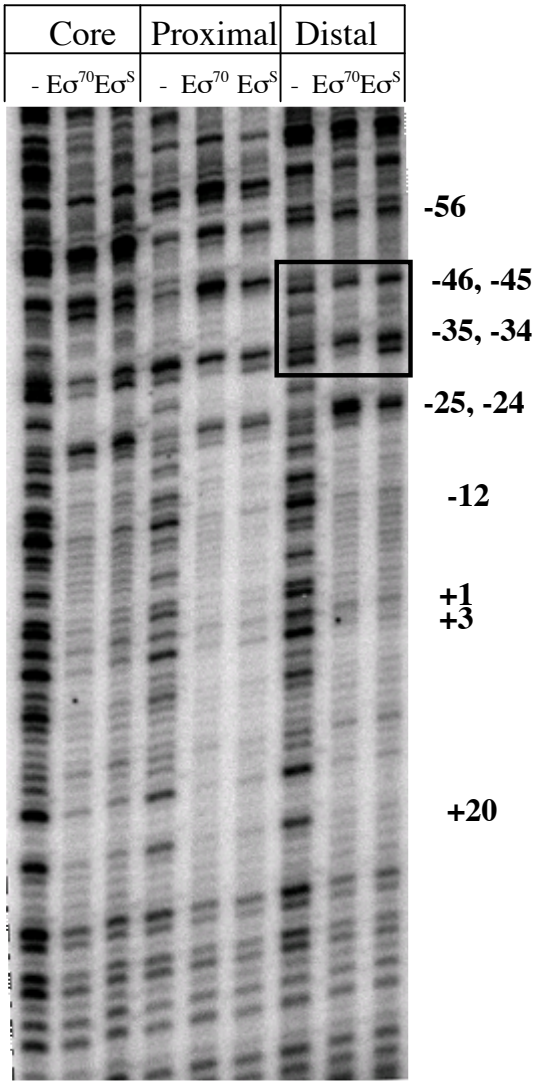


Fig. 18: DNase I footprinting of $E\sigma^{70}$ and $E\sigma^{S}$ complexes at different variants of the synthetic promoter synp213 (see Typas A and Hengge R, 2005 for more details): lanes 1-3, core synp213 promoter, without an UP-element site; lanes 4-6, synp213 with a proximal UP-element sub-site; and lanes 7-9, synp213 with a distal half UP-element site. The digestion patterns of the **non-template strand** were revealed after primer extension (for primer used and more experimental details, see Typas A and Hengge R, 2006) in order to be able to monitor both linear and supercoiled DNA (the picture shown here is with linear DNA). Protection patterns extend for both holoenzymes between, approximately, –60 and +20 in all promoter constructs. Considerable differences between the $E\sigma^{70}$ and $E\sigma^{S}$ footprints can be seen: i) in the spacer region between the –10 and –35 hexamers (different intensity of the hypersensitive sites at –25 and -24), ii) in the –35 element (different degree of protection by the two RNAPs), and iii) one turn of the helix upstream of the –35 element (different intensity of the hypersensitive sites either at –46 or at –45). It is also apparent that, in the "Distal" promoter variant (carrying a distal half UP-element site), only $E\sigma^{70}$ protects the region marked with a box, between the –35 element and the Distal UP-element sub-site (the latter is located in the region between –55 and –45). This result supports the model proposed in Fig. 15.B.

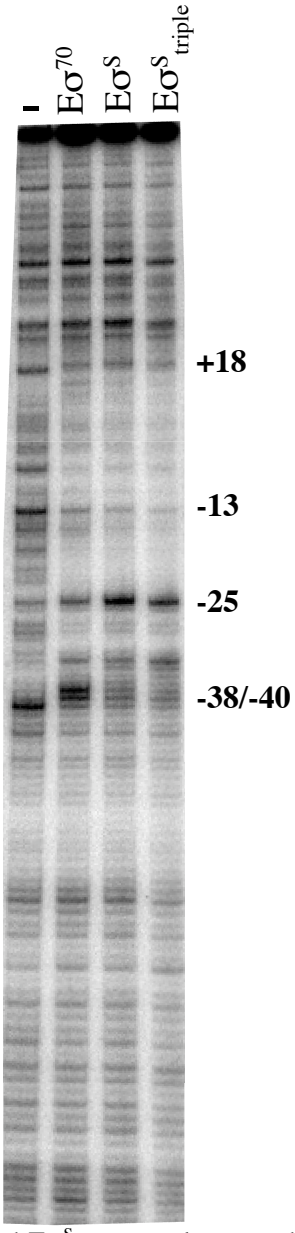


Fig. 19: DNase I footprinting of $E\sigma^{70}$, $E\sigma^{S}$ and $E\sigma^{S}_{triple}$ complexes at the synp213 derivative carrying a distal half UP-element site (Typas A and Hengge R, 2005). σ^{S}_{triple} is a σ^{S} mutant that carries three amino acid substitutions, E308K+E315H+Q318R, and behaves more like the housekeeping σ^{70} in respect with UP-element utilisation. The digestion patterns of the **template strand** were revealed after primer extension (for ^{32}P-labelled primer used and more experimental details, see Typas A and Hengge R, 2006) in order to be able to monitor both linear and supercoiled DNA (the picture shown here is with linear DNA). Only when $E\sigma^{70}$ bound to the promoter region, did hypersensitive sites at –39 and –38 appear. In addition, the footprint pattern of $E\sigma^{S}_{triple}$ is in between of those of $E\sigma^{70}$ and $E\sigma^{S}$, at least in respect with some aspects, i.e. intensity of the hypersensitive site at –25.

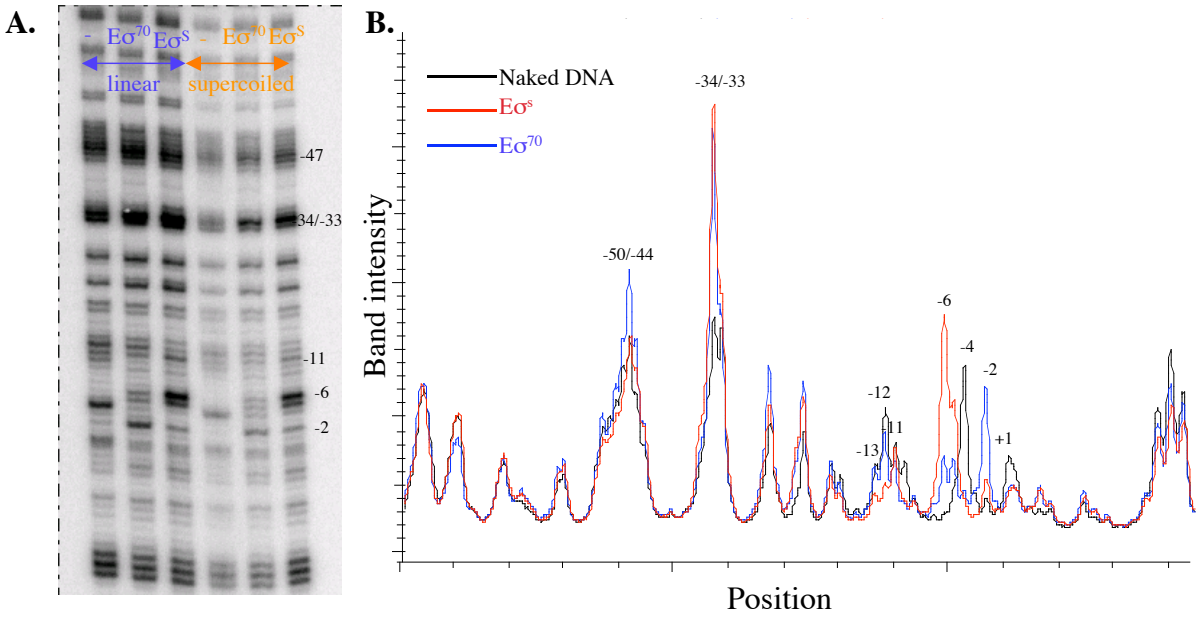


Fig. 20: $E\sigma^{70}$ and $E\sigma^{S}$ adopt different conformations in their transcription bubbles (experiments performed in collaboration with B. Sclavi and M. Buckle).

A. UV laser photo-footprinting of $E\sigma^{70}$ and $E\sigma^{S}$ complexes at the derivative of the synthetic promoter, synp213, carrying a distal half UP-element site (Typas A and Hengge R, 2005). Protein-DNA (linear/supercoiled) complexes were formed for 20min at 37°C, prior to irradiation by high intensity UV light. Samples were irradiated with a simple rapid (5ns) pulse of high intensity UV light (266nm) emitted by an NdYAG laser (DCR-11 spectra physics; more experimental details for the method can be found at Pemberton *et al*, 2002). Primer extension was then performed on the irradiated DNA, using an adequate ^{32}P-labelled primer (Typas A and Hengge R, 2006), in order to visualise the photo-modification of the **non-template strand**. Changes in UV photo-reactivity of the promoter DNA, after incubation with either of the holoenzymes, can be observed at several positions. Appearance of hypersensitivity at nuclotides -6 and –2 reflects the open complex formation of the holoenzymes, as determined by kinetic experiments (see also Table 2), whereas hypersensitivity at positions –47 (in the middle of the hypersensitive bands appearing in between –50 and -44, the heart of the distal UP-element half site) and –34, and protection at nucleotides –12 and -4 are due to initial recruitment of the holoenzymes to the promoter.

B. A normalised densitometric scan of the UV photo-reactivity of the non-template strand (linear DNA) in the absence (black line) or presence of holoenzymes (red line for $E\sigma^{S}$ and blue for $E\sigma^{70}$). Differences in the UV laser photo-footprinting of $E\sigma^{70}$ and $E\sigma^{S}$ reflect the discrepant mode of binding and melting of the promoter by the two holoenzymes.

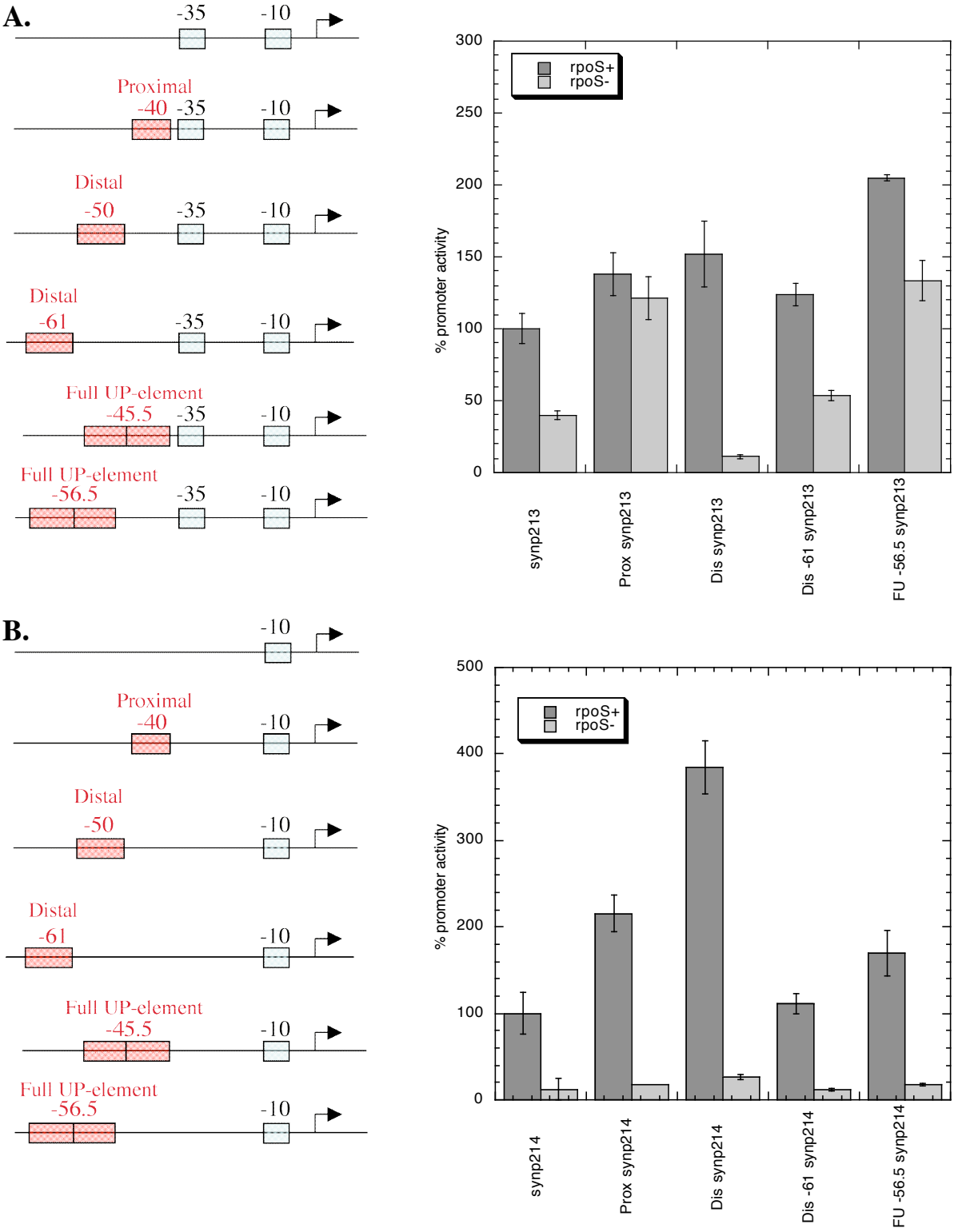


Fig. 21: Role of canonically and non-canonically positioned UP-elements in Eσ^S-dependent transcription. **A.** On the left can be seen a schematic representation of synp213 derivatives with different UP-element configurations (for more information about synp213 see Typas and Hengge, 2005). On the right is plotted the promoter activity (measured by *lacZ* reporter fusions) of the various constructs in relation with that of the core promoter (without UP-element), which is set as 100%. Cells were grown in LB medium and specific β-galactosidase activities were measured along the growth curve. Here are compared and presented the promoter activities during middle stationary phase (4-8 hours after entry in to stationary phase, when the β-galactosidase activities have reached their maximum). Average values of more than 3 experiments are shown. Error bars indicate standard deviations from all these experiments. Only the promoter variant, carrying a canonically positioned

distal UP-element sub-site, exhibits increased σ^S selectivity. The construct bearing a distal UP-element sub-site, centred one turn of the DNA helix upstream of its canonical location, shows similar promoter activity and selectivity as synp213. On the contrary, the presence of an "upstream" full UP-element site (centred at -56.5) causes an increase in promoter activity and in σ^{70} promoter selectivity, similarly to the effects observed with an optimally positioned full UP-element site. Note though that the relative promoter activity of the synp213 derivative, carrying an optimally-positioned full UP-element site, is omitted here for presentational reasons; its activity is more than 10-fold higher than that of the core synp213 promoter and if plotted then it becomes difficult to see the differences in the promoter activity of the rest of the constructs. As shown in Typas and Hengge (2005), this promoter construct is almost entirely used by $E\sigma^{70}$. **B.** The same organisation as panel A, but here are presented the results of the synp214 derivatives with different UP-element configurations (synp214 is the "-35-less" promoter variant of synp213; see Typas and Hengge, 2005). Cells were grown, specific β-galactosidase activities were measured and data were calculated and presented as in panel A. Similarly to optimally positioned UP-element sites, non-canonically positioned UP-element sites did not change the promoter selectivity. The synp214 derivative, carrying an optimally positioned, full UP-element site, is omitted again for the same reasons mentioned above.

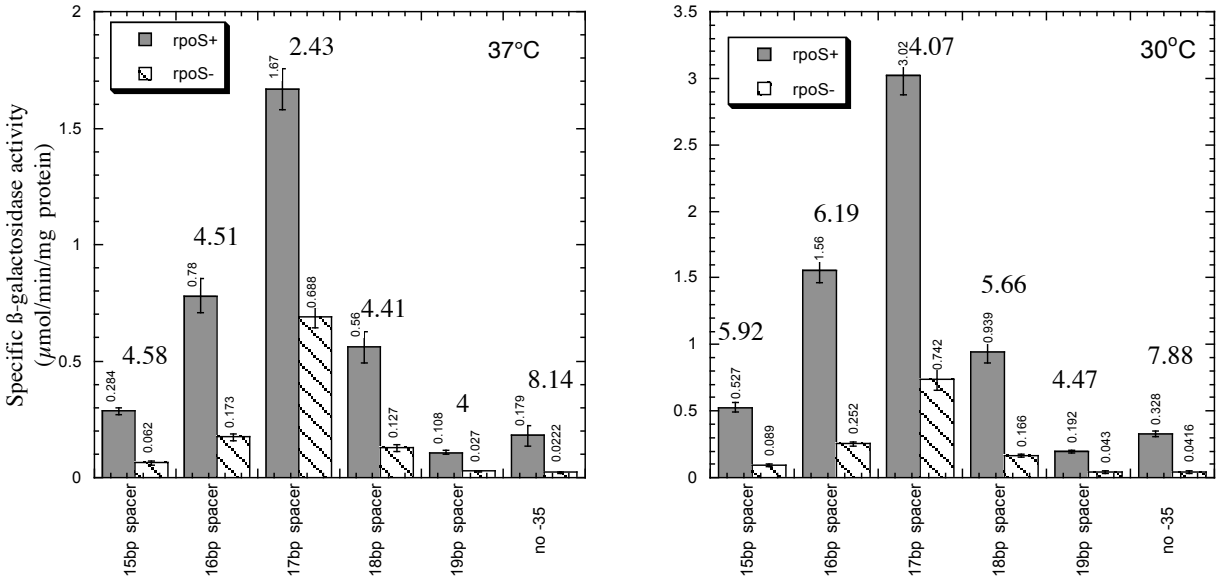


Fig. 22: Effects of changing the spacer length between –10 and –35 elements in the activity of the synthetic promoter, synp213, at 37°C and 30°C. Cells were grown in LB medium (30°C/37°C) and specific β-galactosidase activities were measured along the growth curve. Here are presented the promoter activities during middle stationary phase (4-8 hours after entry in to stationary phase, when the β-galactosidase activities have reached their maximum). Average values of more than 4 experiments are shown. Error bars indicate standard deviations from all these experiments. Above the bars are provided the ratios between $E\sigma^{70}$+$E\sigma^{S}$-mediated transcription and $E\sigma^{70}$-mediated transcription, which reflect the σ^{S}-selectivity of each promoter construct. It is apparent that the presence of a -35 element is more important for $E\sigma^{S}$-derived transcription at 30°C.

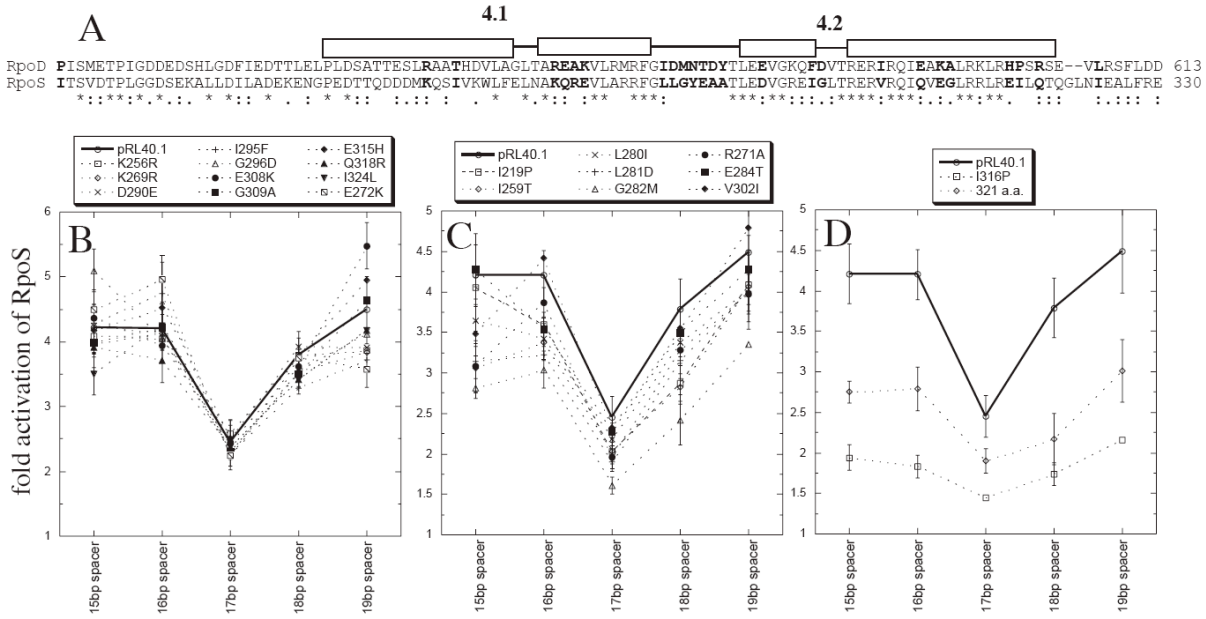


Fig. 23: Role of specific amino acids of σ^s, mostly situated in region 4, in its ability of utilizing non-optimal spaced promoters. **A.** Alignment of region 4 of σ^s (RpoS) with the corresponding region of σ^{70} (RpoD). Single amino acid substitutions of σ^s to the corresponding residue of σ^{70} used here are shown in bold. The positions of the helixes in region 4 derive from current structural data. **B-D.** Effects of the different σ^s variants in Eσ^s aptitude to trigger transcriptional activation from promoters with different spacer lengths (15-19bp). The mutants are divided in 3 different groups: **B.** no effect, **C.** general defect of σ^s activation but no change in its preference for non-optimal spacers, **D.** general defect of σ^s activation and partial alleviation of its preference for non-optimal spacers. Expression of the synthetic promoter synp213 is determined in *rpoS*$^-$ background, in which σ^s wild-type (pRL40.1) and its variants are expressed under the p_{tac} control from a plasmid. The ratio between the promoter activity in the presence of *rpoS* or any of its variants, and the absence of *rpoS*, is plotted against the different spacer lengths. Cells were grown in LB medium and specific β-galactosidase activities were measured during middle stationary phase (4-8 hours after entry in to stationary phase, when the activities remained constant). Average values of more than 3 experiments are shown. Error bars indicate standard deviations from all these experiments

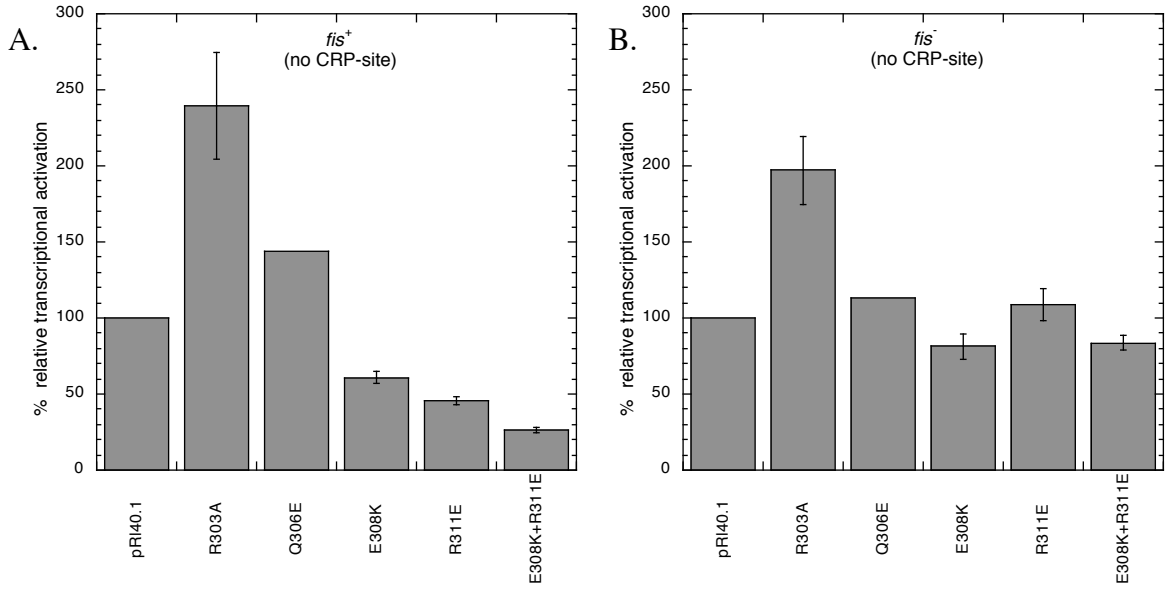


Fig. 24: Amino acid residues of σ^S_4 affecting the σ^s-Fis interplay at a *proP* (P2) promoter variant that has defected the CRP binding-site (centred at -121.5). Promoter activities originating from the different σ^s variants are presented relative to the activity generated by the wild-type σ^s (set as 100%), both in the presence (**A**) and absence (**B**) of Fis. Promoter activity of *proP* (P2) was determined in an *rpoS*$^-$ background, in which wild-type σ^s and its variants were expressed under the P_{tac} control from a plasmid (in the absence of inducer, as this results in σ^s levels comparable to those in wild-type strains). Cells were grown in LB medium and specific β-galactosidase activities were measured at the onset of stationary phase (*fis*$^+$) and after 3-4h in stationary phase (*fis*$^-$), when the *proP* (P2) promoter activity reached its peak. Average values and standard deviations of more than three experiments are shown.

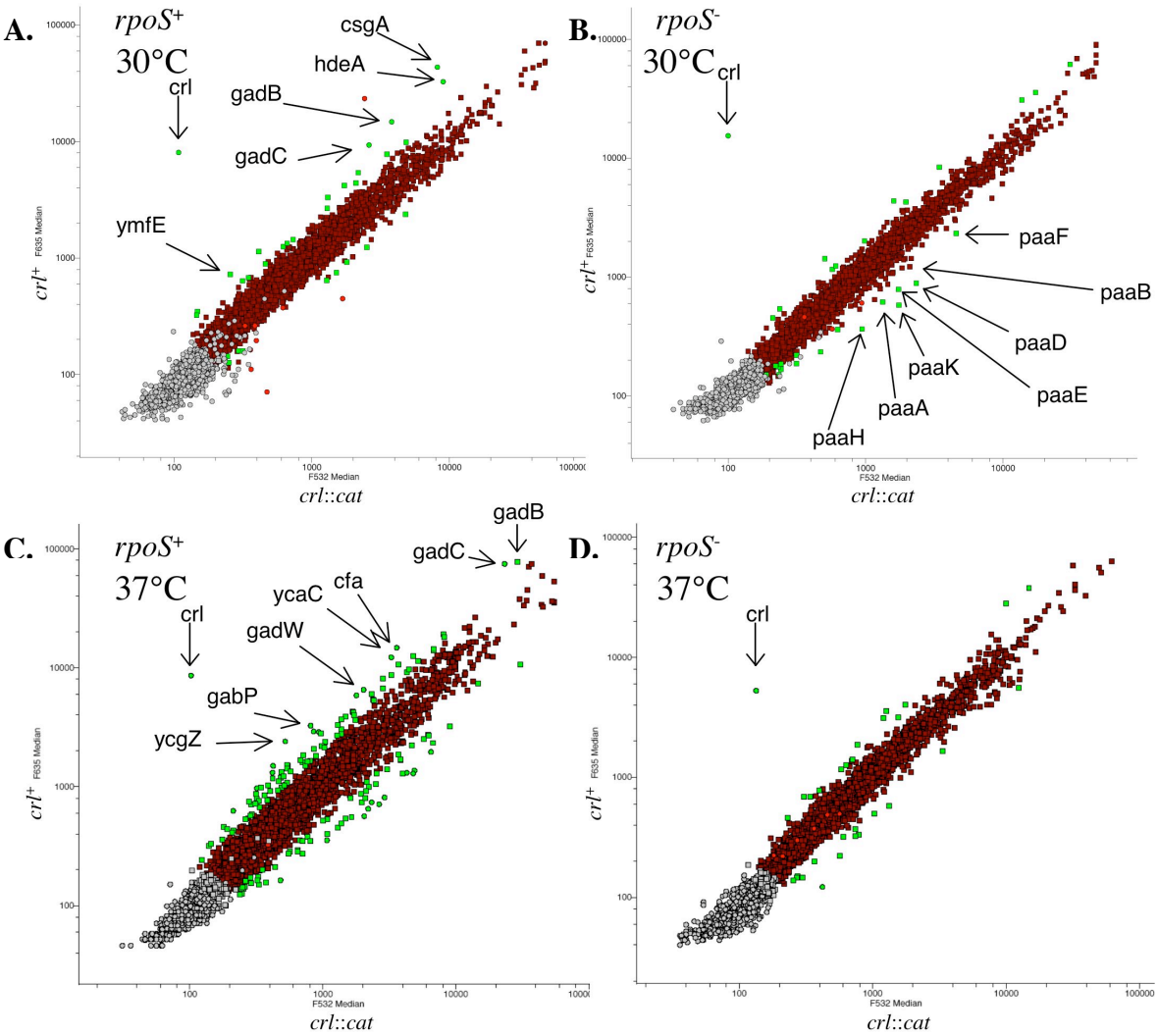


Fig. 25: Comparison of genome-wide gene expression in *crl*$^+$ and *crl*::*cat* strains (MC4100 and NT190, respectively; panel **A**), and in *rpoS*::Tn*10* and *rpoS*::Tn*10 crl*::*cat* strains (RH90 and NT225, respectively; panel **B**), during entry into stationary phase in LB medium at 30 °C. RNA was prepared after harvesting cells that had reached an OD_{578nm} of 4 . Cy3- and Cy5-labeled cDNAs were generated from these RNA preparations and were analysed by whole-genome microarray analysis. Here are shown their normalised intensities, as scatter plots, in two representative experiments (MC4100 versus NT190 in panel **A**, and RH90 versus NT225 in panel **B**). Each set of microarray analysis was repeated three times, and the average ratios of expression of Crl-controlled genes were extracted (both in *rpoS*$^+$ and *rpoS*$^-$ background; for the latter see Table 4). In panels **C** and **D** can be seen the normalised intensities, as scatter plots, in similar experiments performed at 37°C (MC4100 versus NT190 in panel **C**, and RH90 versus NT225 in panel **D**). Note that the set of experiments at 37°C was only performed once.

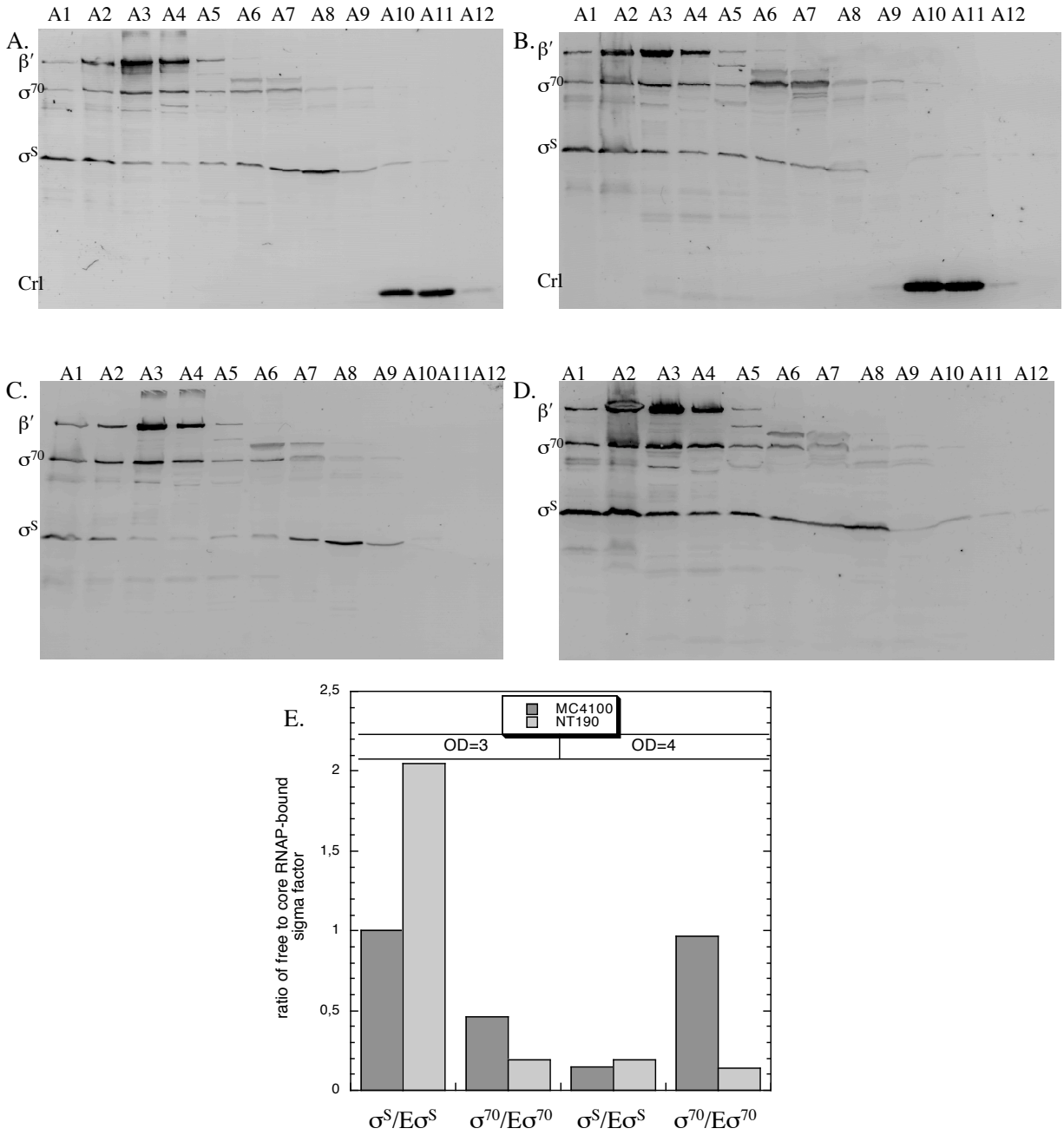


Fig. 26: Crl supports $E\sigma^S$ formation in stationary phase, in the expense of $E\sigma^{70}$ (experiments performed in collaboration with C. Barembruch). Whole-cell lysates from wild-type (panels **A** and **B**; MC4100) and *crl*⁻ (panels **C** and **D**; NT190) stationary phase cells (OD_{578nm}=3 in panels **A** and **C** and OD_{578nm}=4 in panels **B** and **D**), grown in LB at 30°C, were fractionated by gel filtration. Fractions were subsequently analysed by SDS-PAGE, and were visualised by immunoblots, using monoclonal antibodies against the σ^S, σ^{70} and β′ subunits of RNAP and a polyclonal antibody against Crl. As indicated by parallel experiments using purified proteins (data not shown), σ^S co-eluted with RNAP mostly in fractions A1 and A2 ($E\sigma^S$), and was also recovered in later fractions (A7-A9) with no traces of core subunits (free σ^S). On the other hand, when σ^{70} was part of the RNAP assembly ($E\sigma^{70}$), it eluted between fractions A2 and A4, whereas when it was free, it eluted mostly in fractions A6 and A7. Note that Crl always eluted where its free form would be expected (A10-A11). In panel **E** are presented the results of the quantification performed for the four western blots, using the IMAGE GAUGE software. The ratio of free to bound sigma factor was calculated for both σ^S and σ^{70} in the different genetic backgrounds and the different growth stages (bound σ^S: A1-A3; free σ^S: A7-A9; bound σ^{70}: A2-A4; free σ^{70}: A6-A8). More σ^S is free (hence unable to find free core RNAP to bind) in the *crl* mutant strain, during the early stages of transition into stationary phase in rich medium (LB),

whereas upon progression to later stages of stationary phase (OD_{578nm}=4), the presence of Crl has a smaller impact in $E\sigma^S$ formation (by then, most σ^S is, anyways, in complex with RNAP). On the contrary, the presence of free σ^{70} significantly increases upon progression into stationary phase, only when Crl is present in the cell. Note, however, that the presence of enhanced amounts of $E\sigma^{70}$ in the *crl* mutant strain at OD_{578nm}=4 do not cause any stimulation of $E\sigma^{70}$-mediated transcription, as seen by the microarray analysis.

Experimental conditions: Strains were grown in LB medium until they reached different stages of stationary phase. 450 ml of the cells was harvested and resuspended in 10 ml buffer (10 mM Tris-HCl pH 7.8, 0.1 mM DTT, 0.1 mM EDTA, 200 mM NaCl). Crude cell extracts were obtained using a French Pressure Cell. The extracts were subsequently centrifuged for 15 min at 16,000 rpm (using a Sorvall SS34). A total of 100 μl of the supernatant was applied to a gel filtration column (Superdex 200 10/300 GL). Elution with reconstitution buffer was performed at a flow rate of 0.5 ml/min at room temperature, gathering fractions of 1 ml. The proteins in the elution fractions were precipitated with aceton, analysed then by SDS-PAGE (12% acrylamide), electroblotted onto PVDF membranes and, finally, detected with specific antibodies. Either polyclonal sera against Crl, or monoclonal antibodies against the σ^S, σ^{70} and β' (Neolcone), and a Cy2-conjugated goat anti-rabbit IgG, plus a Cy2-conjugated goat anti-mouse IgG (both from Dianova), were used for protein visualisation.

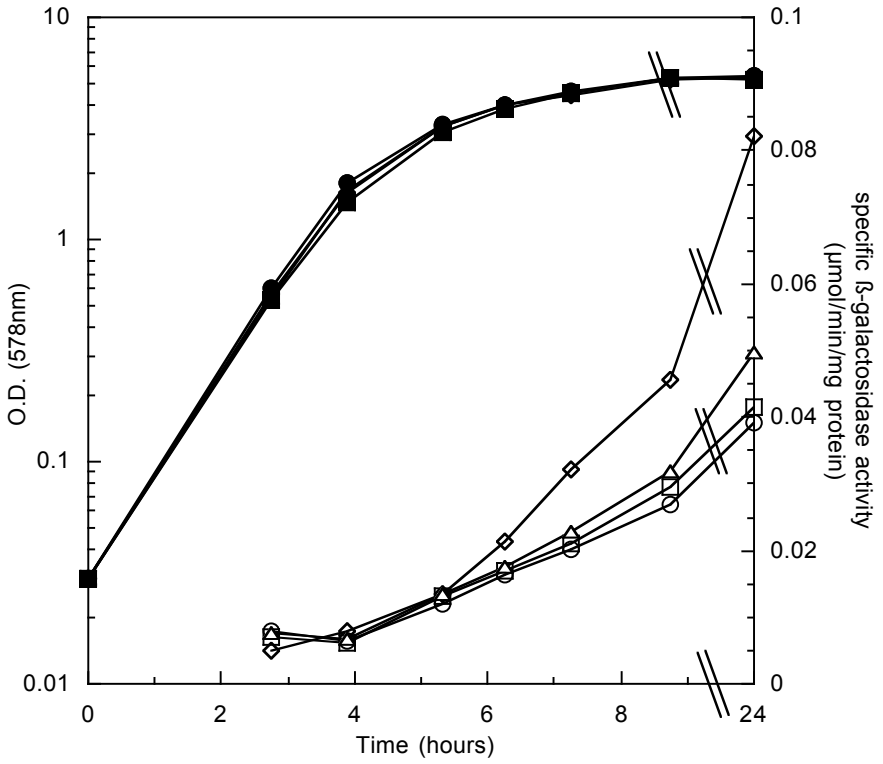


Fig. 27: Increased RssB levels severely impair $E\sigma^S$ activity, especially in a *crl*⁻ background. Expression of a single-copy synp9::*lacZ* protein fusion (synp9 is a strongly σ^s-dependent synthetic promoter) was determined in *rssB*⁻ *rpoS*⁺ *crl*⁺ (diamonds), *rssB*⁻ *rpoS*⁺ *crl*⁻ (triangles), *rssB*⁻ *rpoS*⁻ *crl*⁺ (squares) and *rssB*⁻ *rpoS*⁻ *crl*⁻ (circles) backgrounds, with RssB being ectopically expressed from pMP8, under the control of the p_{tac} promoter (no inducer present; RssB levels are even without inducer higher than physiological). Cells were grown in LB medium and optical densities (closed symbols) and specific β-galactosidase activities (open symbols) were measured along the growth curve. $E\sigma^s$-mediated expression is severely defected due to a dramatic decrease in σ^S cellular levels, caused by the increase in RssB expression (see also Figs 5 and S2 in the "Stationary phase reorganisation of the *E.coli* transcription machinery by Crl protein, a fine-tuner of σ^s activity and levels" paper; σ^S is only detectable when cells have reached an optical density higher than 4 and only in a *crl*⁺ background, in consistence with the genetic background and the time-point, in which a stimulation in $E\sigma^s$-mediated expression is observed here). Thus, increased cellular levels of RssB make the presence of Crl absolutely necessary for $E\sigma^S$-dependent transcription.

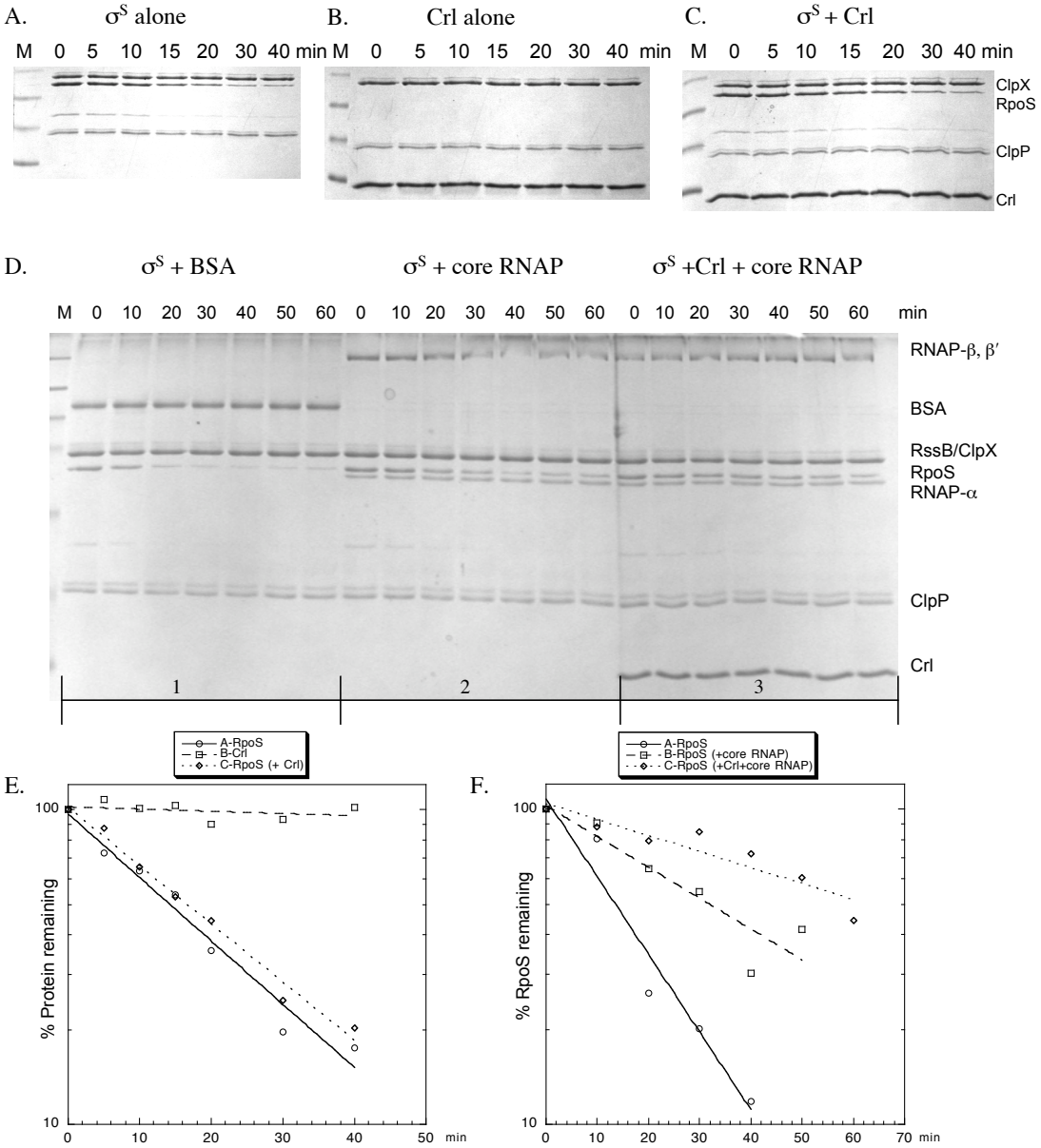
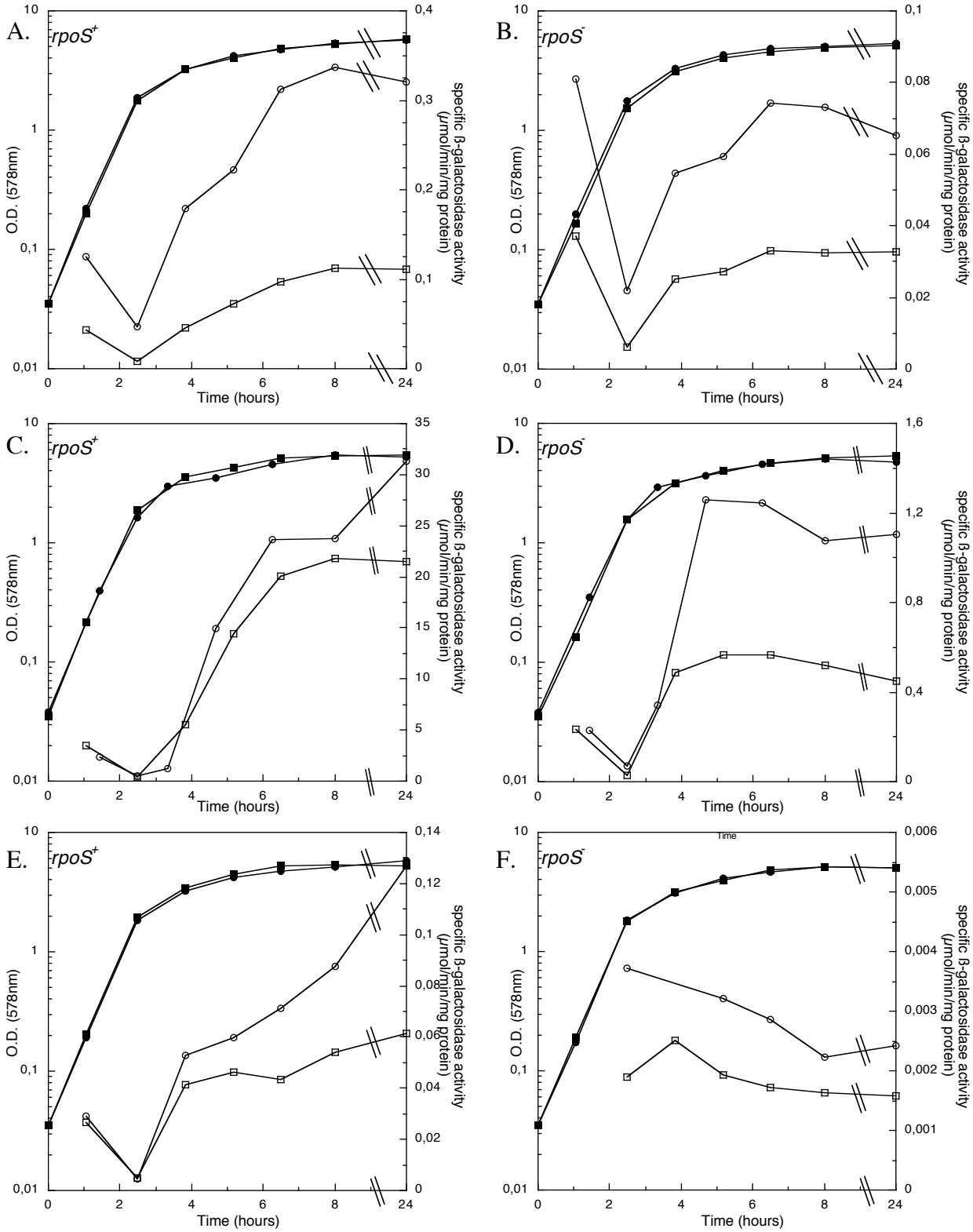


Fig. 28: Crl protects σ^S from degradation, only in the presence of core RNAP (experiments performed by A. Possling). In-vitro degradation of σ^S (panels **A**, **B** and **D**) in reaction mixtures containing 2μM RpoS, 0,2μM RssB, 0,2μM reconstituted ClpXP, 5mM ATP, 10mM acetyl phosphate and where applicable 4μM Crl (**C** and **D**3), 0,29μM core RNAP (**D**2 and **D**3) or 2μM BSA (**D**1). The mixtures were incubated at 30°C in buffer A (20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 140 mM KCl, 1 mM DTT, 0.1mMEDTA, 0.005% Triton X-100 and 5% glycerol [v/v]) for different time periods (stated above each lane), and were stopped with addition of SDS loading buffer, in order to be subsequently separated by SDS-PAGE and visualised by Coomasie staining. When Crl and σ^S were both included in the reaction, then they were separately pre-incubated for 10 min at room temperature, prior to their addition to the mixture. In panel **B** is presented a control in-vitro degradation assay of Crl, using the same conditions and reagents as for σ^S (note that Crl was also stable in an in-vitro degradation assay in which RssB was omitted; data not shown). Panels **E** and **F** depict densitometric quantifications of the data presented in **A**-**C** and **D**1-3 respectively. The intensity of bands representing σ^s (or Crl) was calculated relative to the intensity of bands representing a stable protein that was always present in the assay, i.e. ClpX. Each experiment was repeated two or three times with highly reproducible results; here is shown a representative of those experiments. The half-life of σ^S is 14.5 min (±1.2) in the absence of Crl, 15 min (±2) in its presence (2-fold excess), 34 min (±3) in the presence of sub-stoichiometric amounts of core RNAP (1:7 molecular ratio), and 57.5 min (±3.5) in the presence of both Crl and core RNAP. Note that the presence of BSA in the mixture did not influence the degradation rates of σ^S.



G.

	-10		+1 pause
bolA(P1)	CTAGTAT	TTAAAG	Ggatggatgacatct
	CTAGTAT	TTAAAG	Ggatg**C**a**A**gacatct
gadA	CTACTTT	TTTATT	Gccttcaaataaattt
	CTACTTT	TTTATT	Gccttcaaa**CC**aattt
gadX	CTACATT	AATAAACA	Gtaatatgtttat
	CTACATT	AATAAACA	G**AT**ata**A**gtttat

Fig. 29: Effect of mutating putative pausing sites, which resemble a -10 recognition element and are situated directly downstream of +1, on the expression of the σ^S-dependent promoters *bolA* (**A**, **B**), *gadA* (**C**, **D**), and *gadX* (**E**, **F**). Expression of single-copy *lacZ* protein fusions, carrying either the wild-type promoter (squares), or its promoter derivative with a defected promoter-proximal pausing site (circles), was determined in *rpoS*$^+$ (left panels; **A**, **C** and **E**) and *rpoS*$^-$ (right panels; **B**, **D** and **F**) backgrounds. Cells were grown in LB medium and optical densities (closed symbols) and specific β-gal activities (open symbols) were measured along the growth curve. Note that the scale of the β-galactosidase axes changes significantly for each gene in *rpoS*$^+$ and *rpoS*$^-$ background, as all of them are strongly σ^s-dependent. Below the six plots, in panel **G**, is presented the promoter sequence of the three genes with the putative "-10-like" pausing sites underlined, and the mutations introduced for destroying them in bold, capital face. Only the pausing site found in *gadX* satisfies the criteria set by Nickels *et al* (2004); the other two pausing sites in *gadA* and *bolA* (P1) were predicted to be functional by their close similarity to a -10 element (although they failed to have conserved the three essential nucleotides of a –10 element, **TA**taa**T**, they had either a 4/6 match to the consensus of 10 element or they had only a 3/6 match to the -10 element consensus sequence and an additional extended -10 element). In addition, *gadA* had two putative promoter-proximal pausing sites; the first is situated directly upstream from that depicted in panel **G** (**t**ca**aat**), but mutating it, did not influence the expression of *gadA*. Note that $E\sigma^{70}$+$E\sigma^S$-mediated transcription and $E\sigma^{70}$-mediated transcription are inhibited to a different extent by the presence of a pausing site in the *gadA* and *gadX* promoters.