

Regulation of *Clostridium difficile*

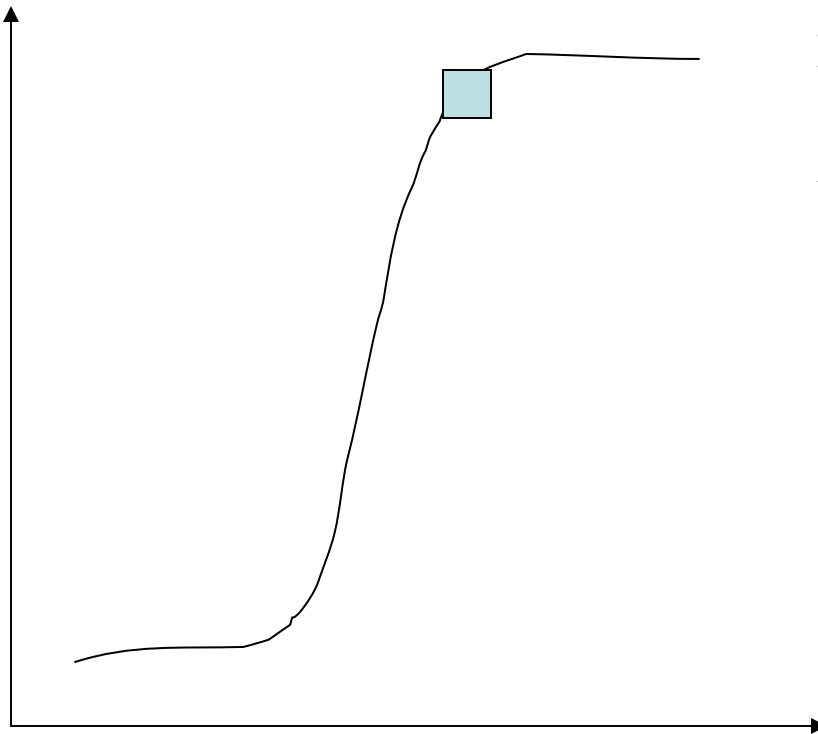
Toxin Gene Expression

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Relationships between *C. difficile* sporulation and pathogenesis:

- **Spores act as the reservoir of disease-causing organisms**
- **Germination in the GI tract is essential for pathogenesis**
- **Toxins A and B are only synthesized during stationary phase/sporulation**



Motility and chemotaxis
Secretion of degradative enzymes
Transport of secondary nutrients
Intracellular catabolic pathways
Genetic competence
Antibiotic and toxin production

Sporulation

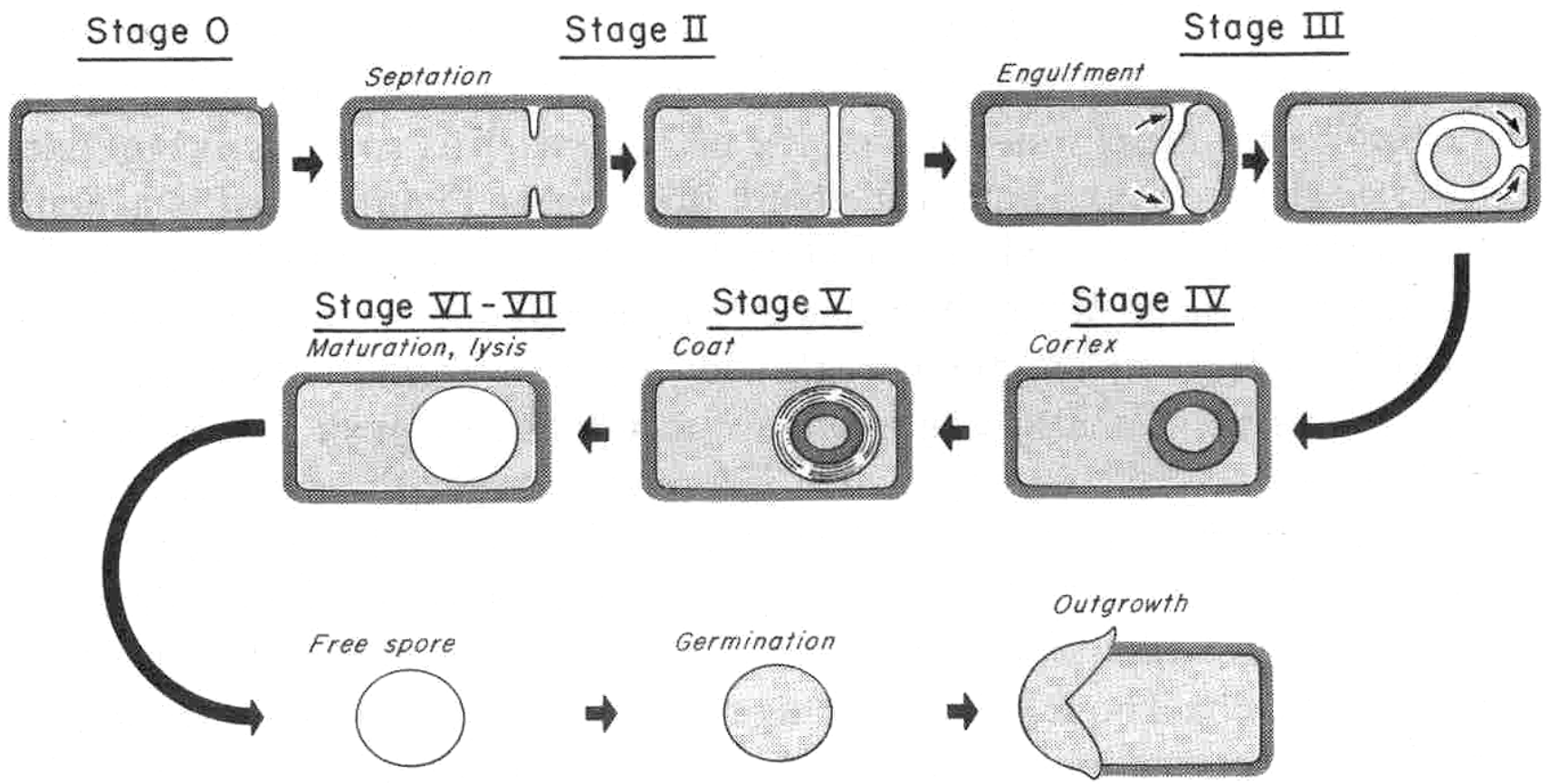
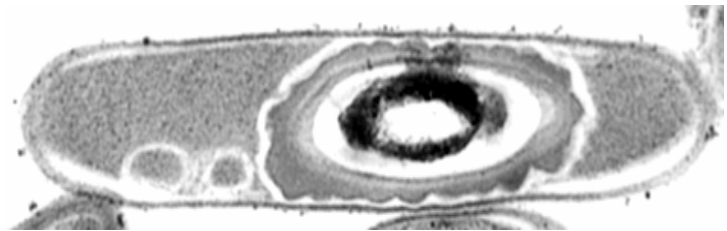
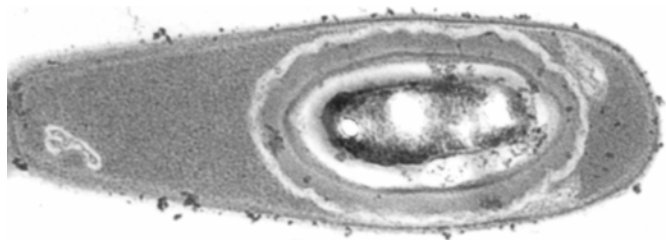
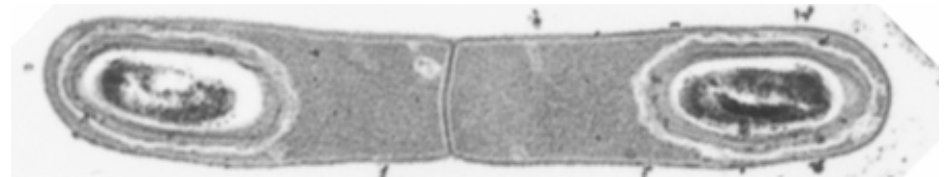
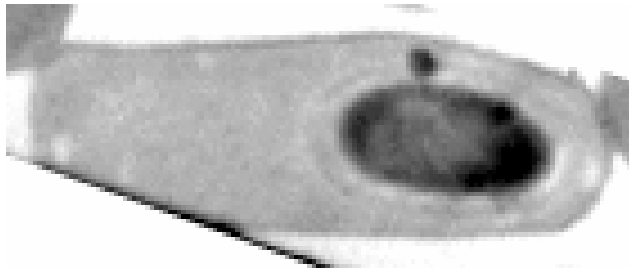
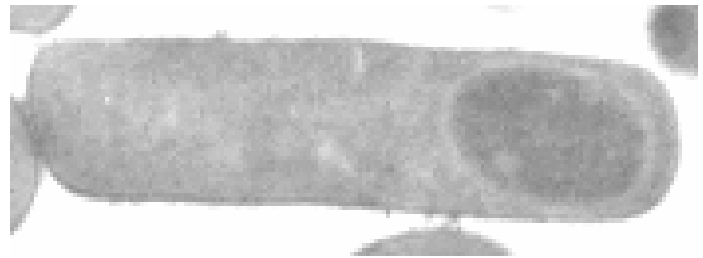
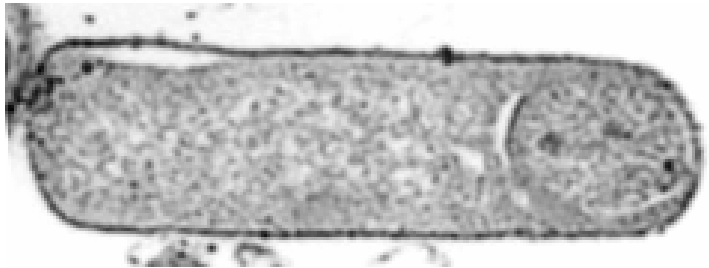
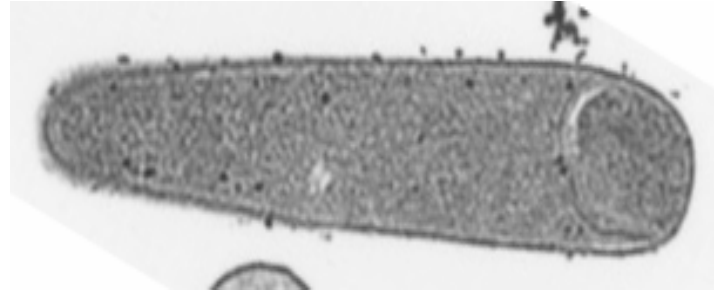
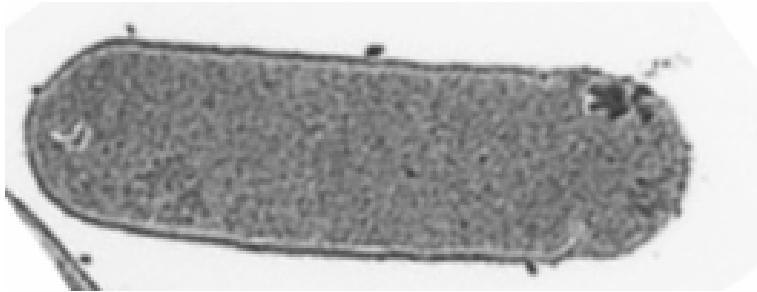


Figure 2 The stages of sporulation and germination.

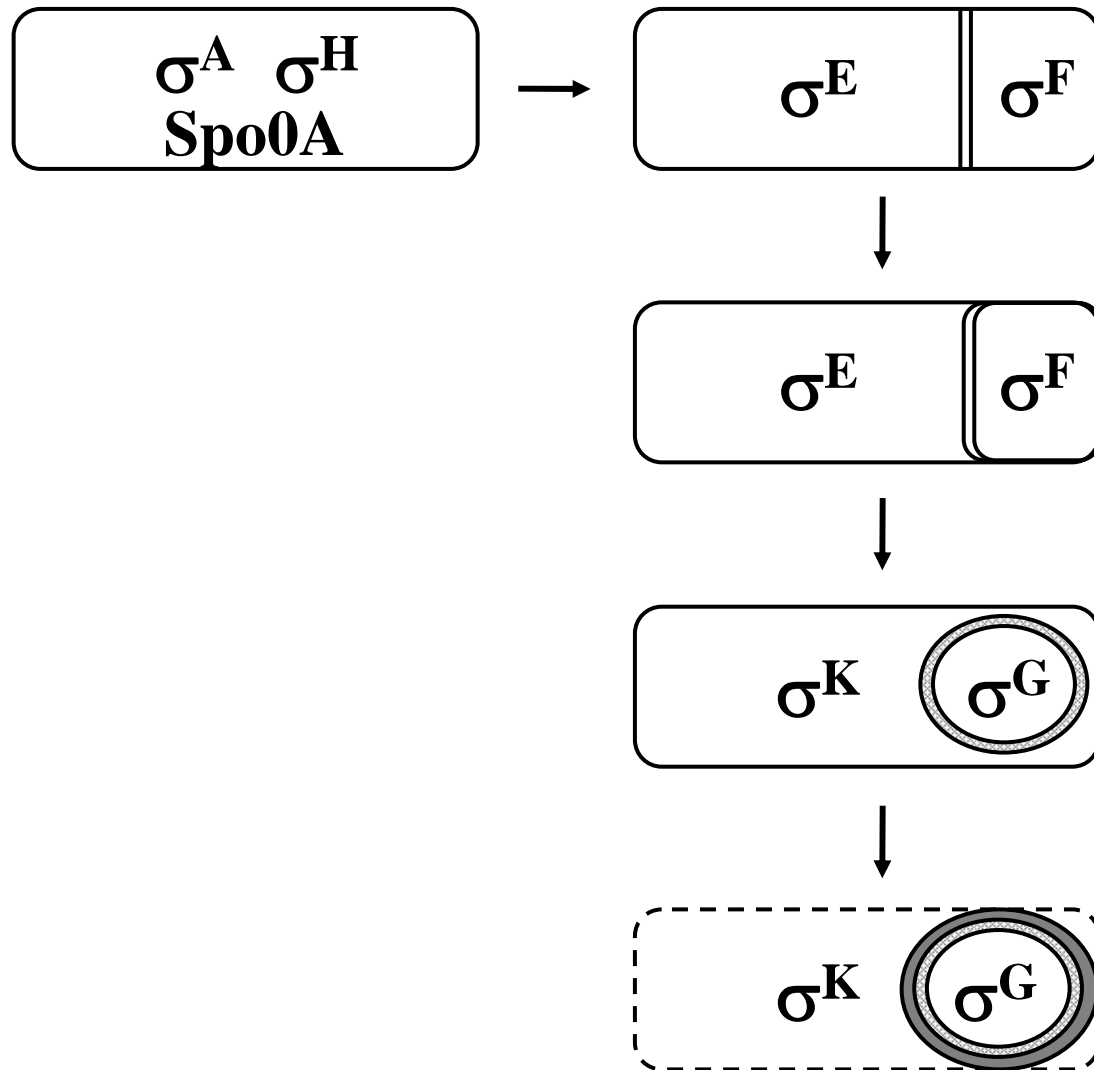


Transcriptional regulation during sporulation

Spo0A~P - a major transcription factor for early sporulation genes

RNA polymerase sigma factors - dissociable subunits that direct RNA polymerase to specific promoter sites

Regulation of Sporulation Gene Expression in *Bacillus subtilis*



RNA polymerase Sigma Factors of *C. difficile*

σ^{70} Family

Primary

σ^A , σ^A -like

Sporulation

σ^H , σ^F , σ^E , σ^G , σ^K

Alternative

Stress

σ^B

Toxin gene expression

TcdR

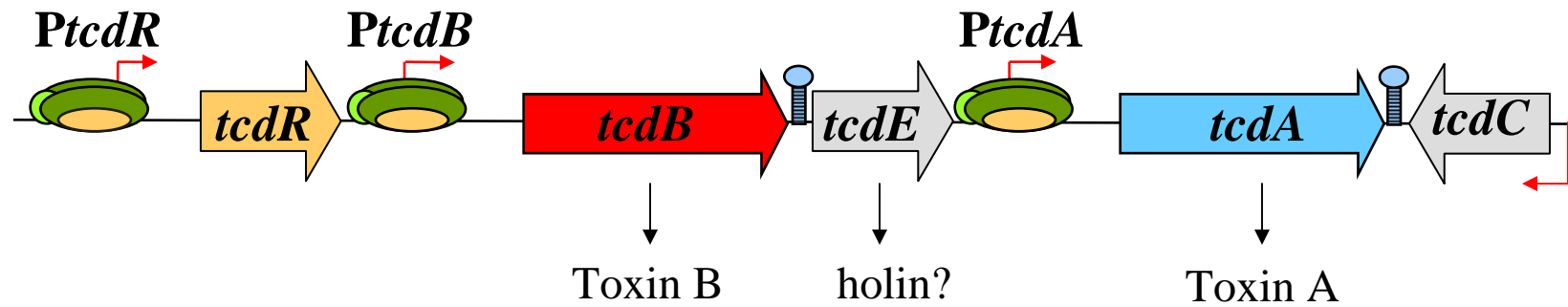
ECF

σ^W , σ^V , σ^X

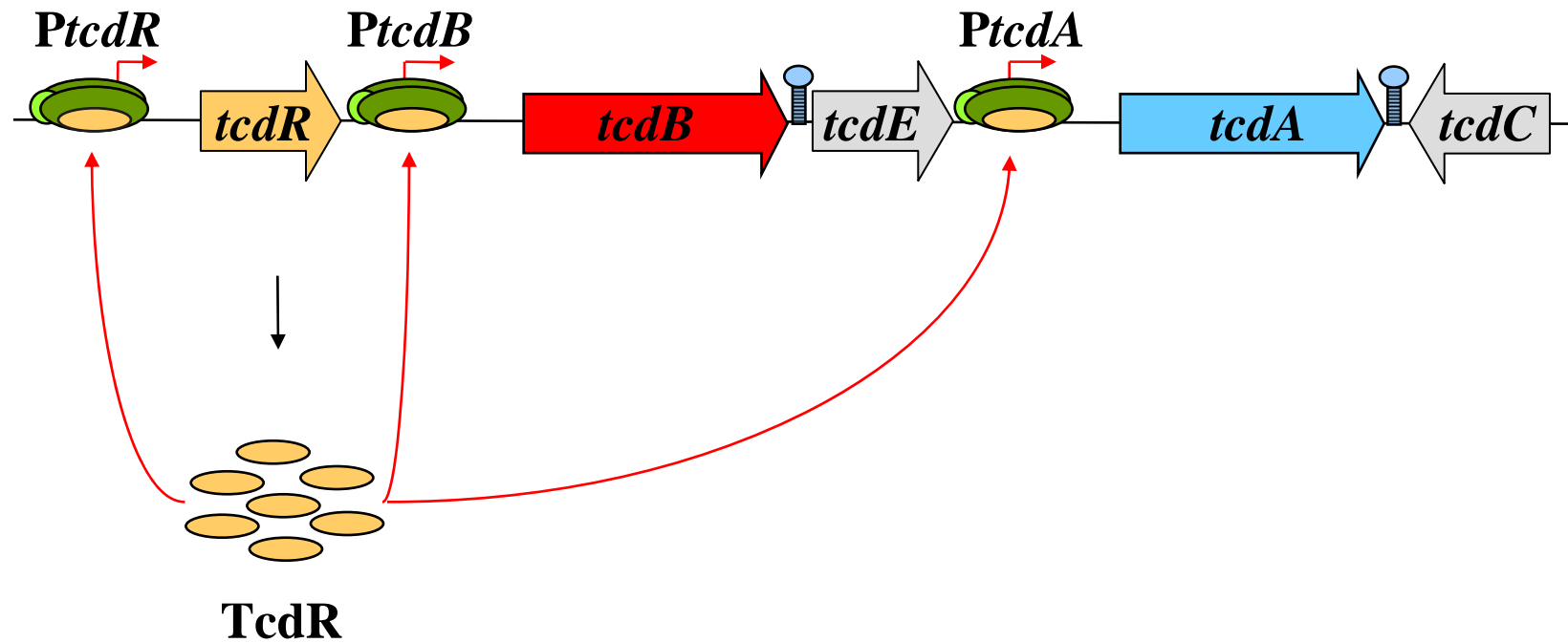
σ^{54} Family

σ^L

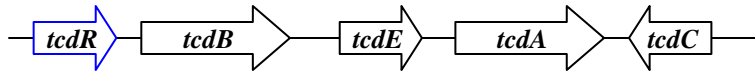
Toxin Gene Locus (PaLoc) in *C. difficile*



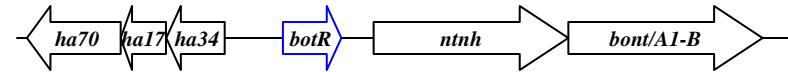
TcdR is a sigma factor for toxin gene transcription



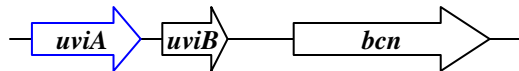
C. difficile Paloc locus



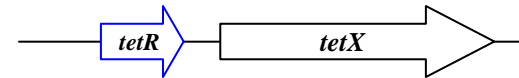
C. botulinum toxin locus

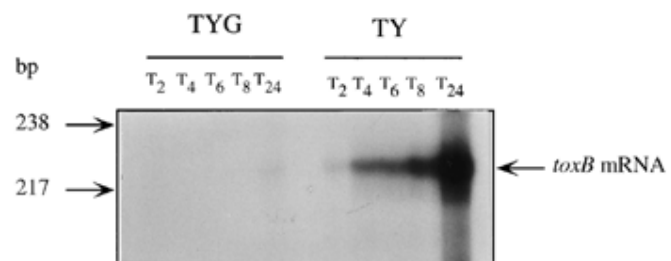
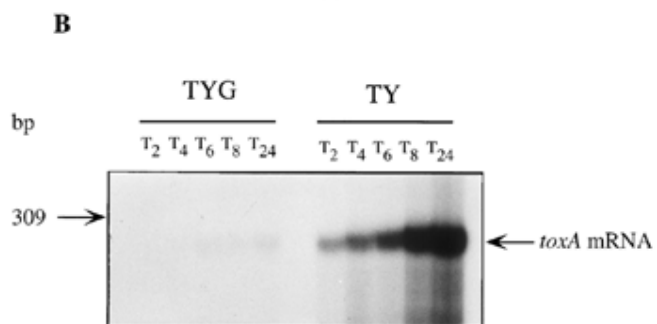
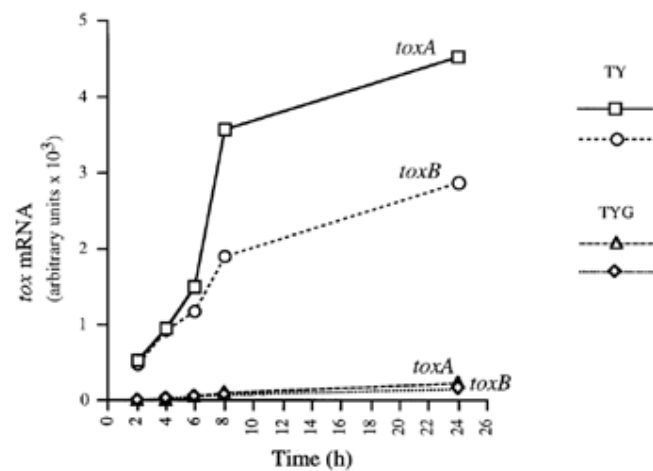
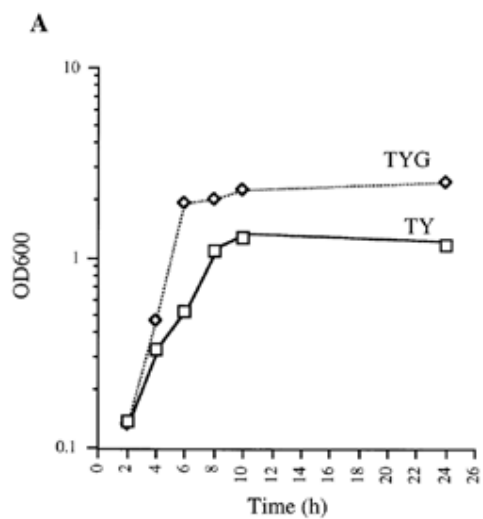


C. perfringens bacteriocin locus



C. tetani toxin locus





Questions:

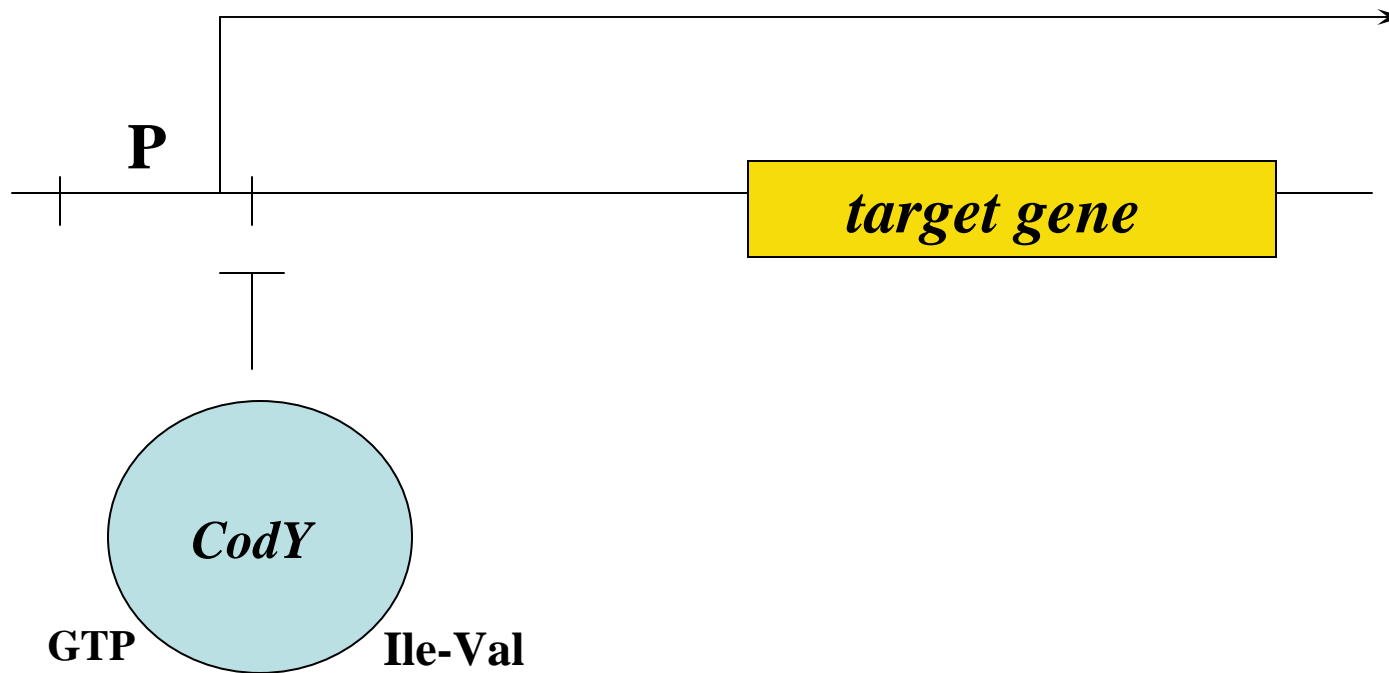
- 1) What is the metabolic signal for nutrient deprivation?**
- 2) What regulatory protein senses this signal?**
- 3) By what mechanism does this regulatory protein control expression of toxin genes?**

CodY protein



- a dimeric DNA binding protein first discovered in *Bacillus subtilis*.
- Helix-Turn-Helix region (HTH), located between residues 202-223, is required for DNA binding.
- represses mostly strongly in vivo in cells growing in a complex medium or a medium containing glucose and a **mixture of amino acids**.
- homologs are found in almost all low G+C gram-positive bacteria.

CodY is activated additively by two different effectors



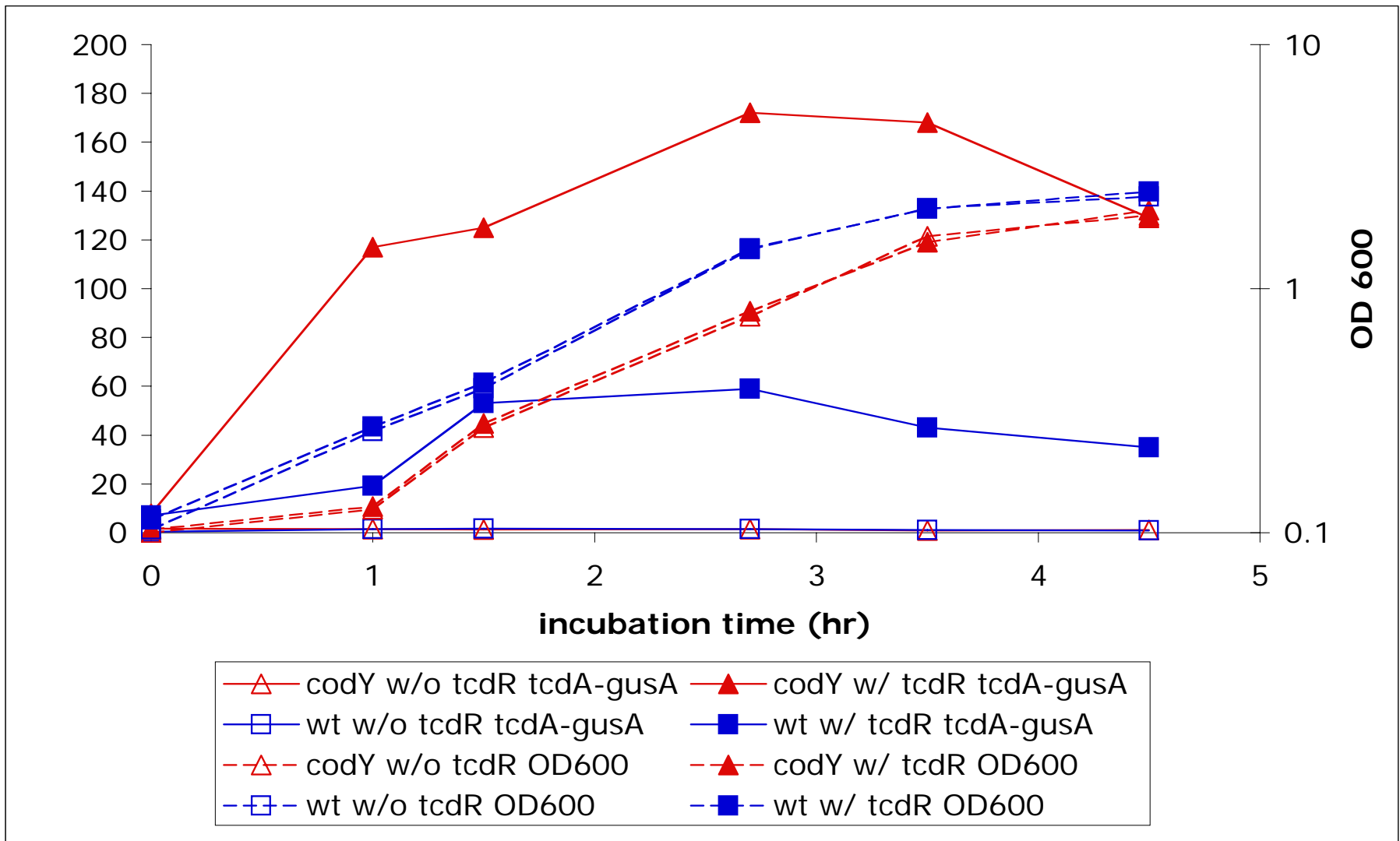
CodY Homologs

- Nearly ubiquitous in low G+C Gram-positive bacteria
- Strong conservation in putative motifs

Table 4. CodY HTH motifs

	<u>helix 1</u> <u>turn</u> <u>helix 2</u>
Bacillus subtilis	ASKIADRVGITRSVIVNALR
Bacillus stearothermophilus	ASKIADRVGITRSVIVNALR
Bacillus halodurans	ASKIADRVGITRSVIVNALR
Bacillus anthracis	ASKIADRVGITRSVIVNALR
Listeria innocua	ASKIADRVGITRSVIVNALR
Listeria monocytogenes	ASKIADRVGITRSVIVNALR
Staphylococcus epidermidis	ASKVADRVGITRSVIVNALR
Staphylococcus aureus	ASKVADRVGITRSVIVNALR
Clostridium perfringens	ASKIADKVGITRSVIVNALR
Clostridium difficile	ASKIADRVGITRSVIVNALR
Clostridium acetobutylicum	ASKIADKVGITRSVIVNALR
Clostridium botulinum	ASKIADKVGITRSVIVNALR
Desulfitobacterium hafniense	ASKIADRVGITRSVIVNALR
Carboxydotherrmus hydrogenoformans	ASKIADRVGITRSVIVNALR
Streptococcus equi	ASVIADRIGITRSVIVNALR
Streptococcus pyogenes	ASVIADRIGITRSVIVNALR
Streptococcus pneumoniae	ASVIADRIGITRSVIVNALR
Streptococcus mutans	ASVIADRIGITRSVIVNALR
Enterococcus faecalis	ASSIADEIGITRSVIVNALR
Lactococcus lactis	ASVIADKIGITRSVIVNALR

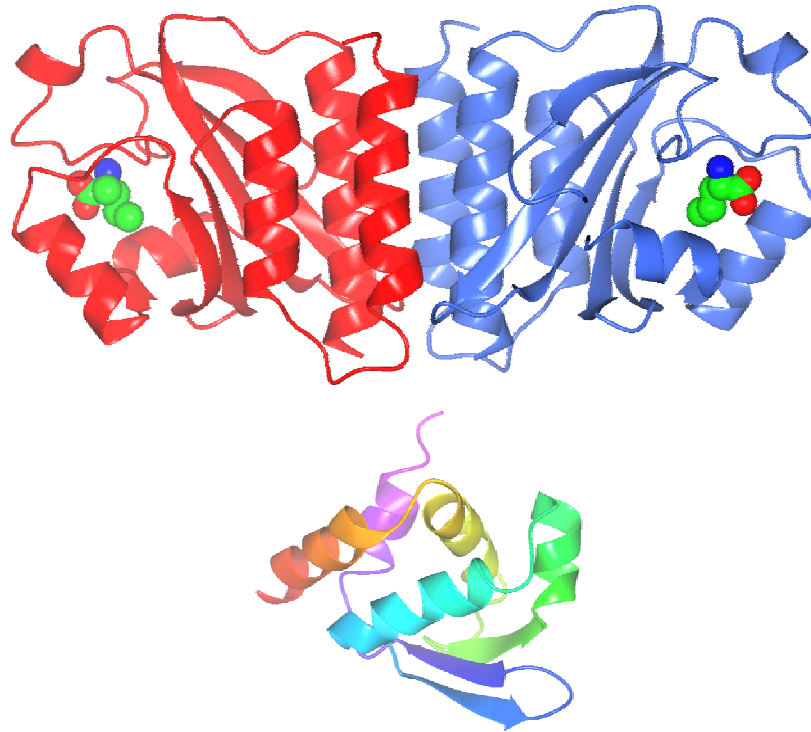
CodY represses *tcdA-gusA* expression in *B. subtilis*



Comparison of putative GTP binding motifs in CodY homologs.

Small GTPases	G1	G3	G4
Consensus sequences	GXXXGKT	DXXG	NKXD
	A S		TQ
CodY			
<i>B. subtilis</i>	GGERLGTL	DRVG	VLNNKFL
<i>B. stearothermophilus</i>	GGERLGTL	DRVG	VLNDKFL
<i>B. halodurans</i>	GGQRLGTL	DRVG	VLNDKFL
<i>B. anthracis</i>	GGERLGTL	DRVG	VLNDKFL
<i>B. cereus</i>	GGERLGTL	DRVG	VLNDKFL
<i>S. aureus</i>	GGERLGTL	DRVG	VKKEKFL
<i>S. epidermidis</i>	GGERLGTL	DRVG	VKKDKFL
<i>L. innocua</i>	GGERLGTL	DRVG	VLNDKFL
<i>L. monocytogenes</i>	GGERLGTL	DRVG	VLNDKFL
<i>C. hydrogenoformans</i>	GGVRLGTL	DRVG	ILNDYFL
<i>D. hafniense</i>	GGERVGTL	DRVG	DLNDYLL
<i>S. pneumoniae</i>	SGIRLGSL	DRIG	VLISDIF
<i>S. equi</i>	GGMRLGTL	DRIG	VINEGIF
<i>S. mutans</i>	GGMRLGSL	DRIG	VINEGIF
<i>S. pyogenes</i>	GGMRLGSL	DRIG	VINEGIF
<i>E. faecalis</i>	AGKRLGTL	DEIG	VLNQQFI
<i>L. lactis</i>	SGMRLGTF	DKIG	VLNTGLF
<i>C. difficile</i>	SGQRLGTL	DRVG	ILNDKLT
<i>C. perfringens</i>	NGDRLGTL	DKVG	ILNEKLM
<i>C. acetobutylicum</i>	NRERLGTL	DKVG	ILNDRLL
<i>C. botulinum</i>	NRERLGTL	DKVG	ILNDKLL

Crystal Structure of CodY with Ile



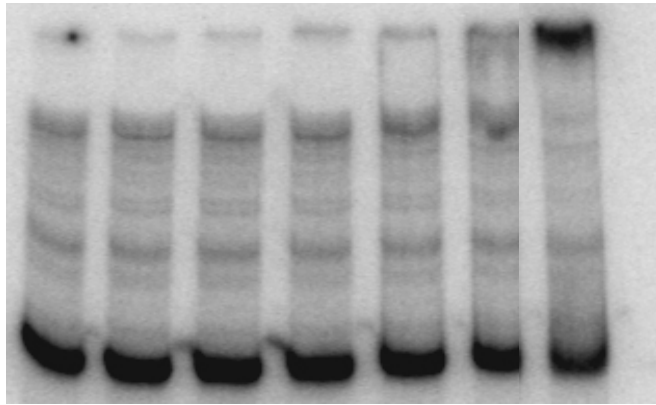
V. Levnikov
E. Blagova
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Hydrophobic Pocket Residues of CodY

	61	62	65	71	72	75	97	98	99	100
Bsu	R	M	M	F	P	Y	A	F	P	V
Ban	R	M	M	F	P	Y	A	F	P	V
Bli	R	M	M	F	P	Y	A	F	P	V
Lmo	R	M	M	F	P	Y	A	F	P	I
Efa	R	I	M	F	P	Y	A	F	P	F
Sau	R	I	M	I	P	Y	V	F	P	P
Sep	R	I	M	I	P	Y	V	F	P	P
Spy	R	V	F	F	P	Y	I	F	P	V
Sag	R	V	F	F	P	Y	I	F	P	V
Smu	R	V	F	L	P	Y	I	F	P	I
Sth	R	V	F	L	P	Y	I	F	P	V
Spn	R	V	F	F	P	Y	I	F	P	I
Lla	R	V	F	L	P	Y	I	F	P	E
Cdi	V	I	E	F	S	Y	I	F	P	E
Cpe	I	M	K	F	P	Y	V	F	E	G
Cte	T	V	K	F	P	Y	A	F	P	F

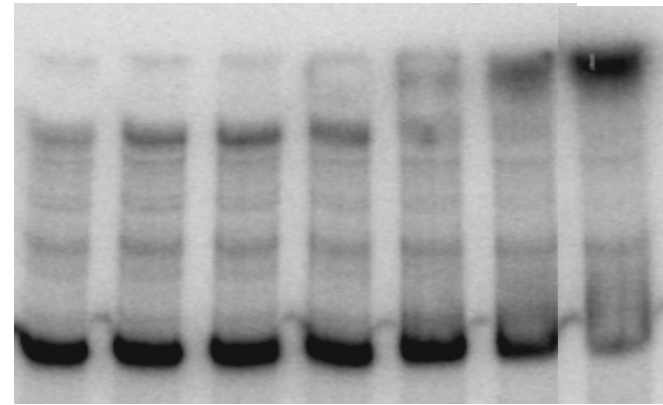
C. difficile CodY binds *tcdR* promoter

No effectors



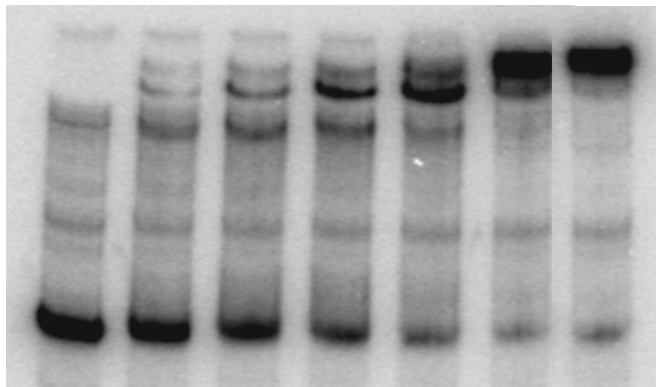
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2 mM GTP



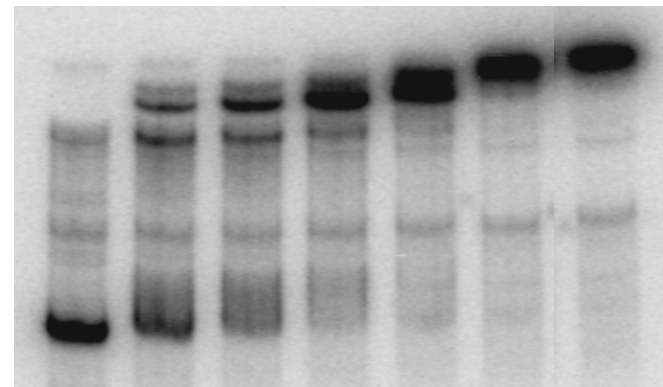
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10 mM ILV



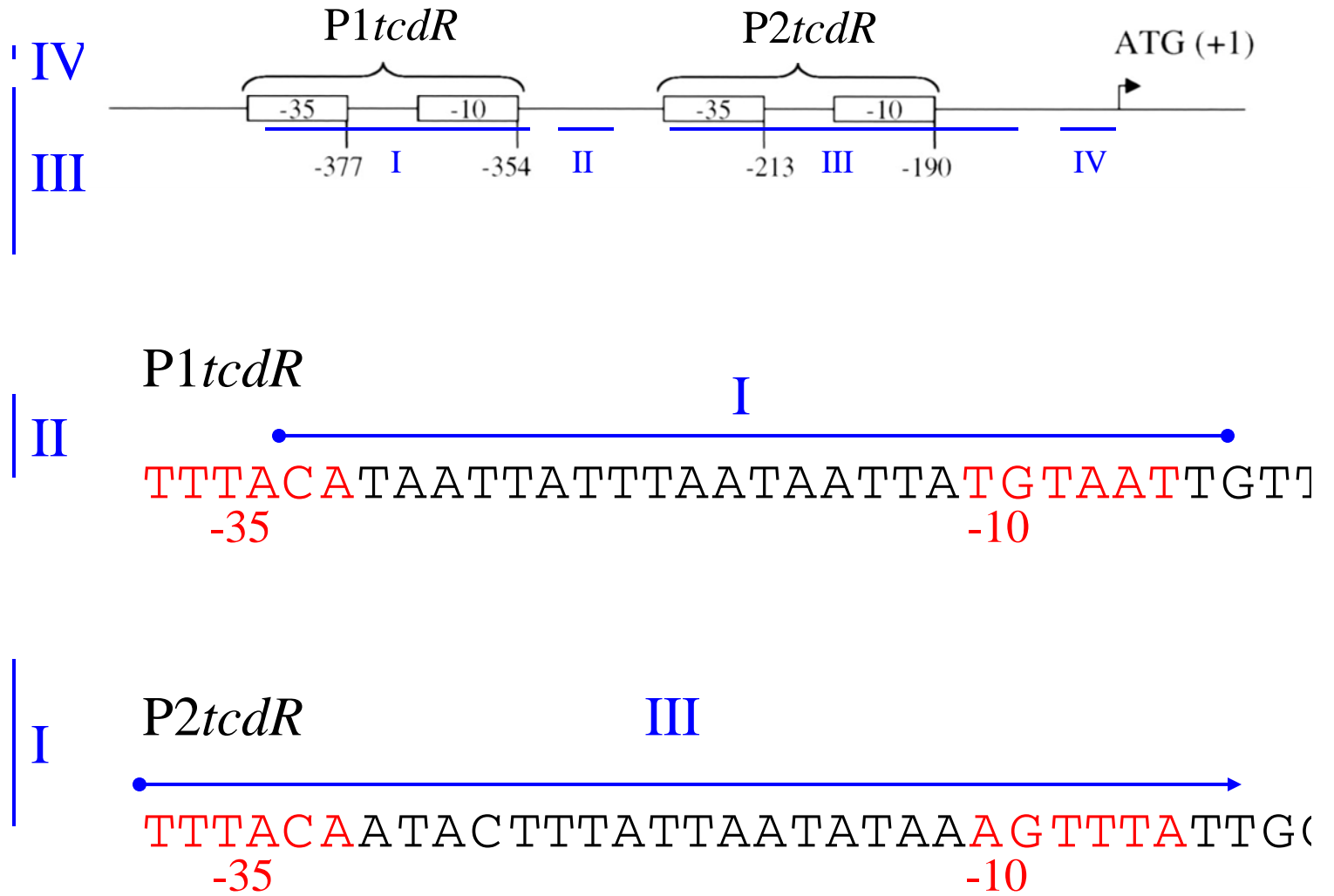
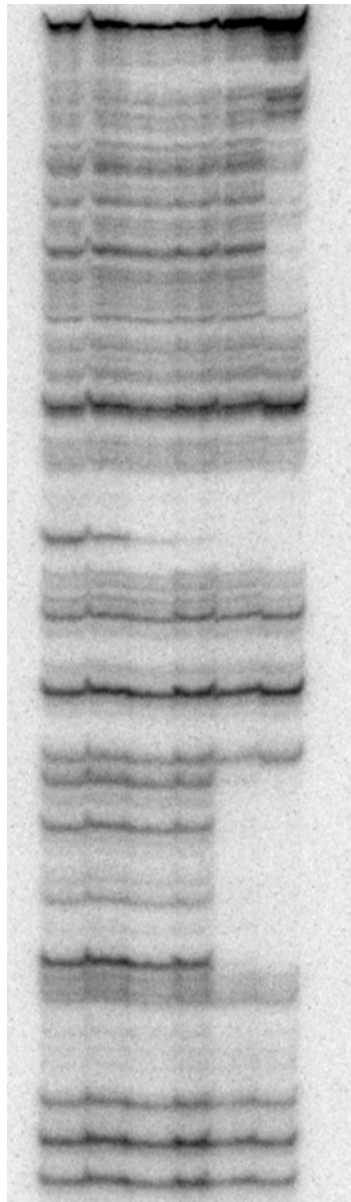
0 20 40 100 200 300 500

2 mM GTP + 10 mM ILV

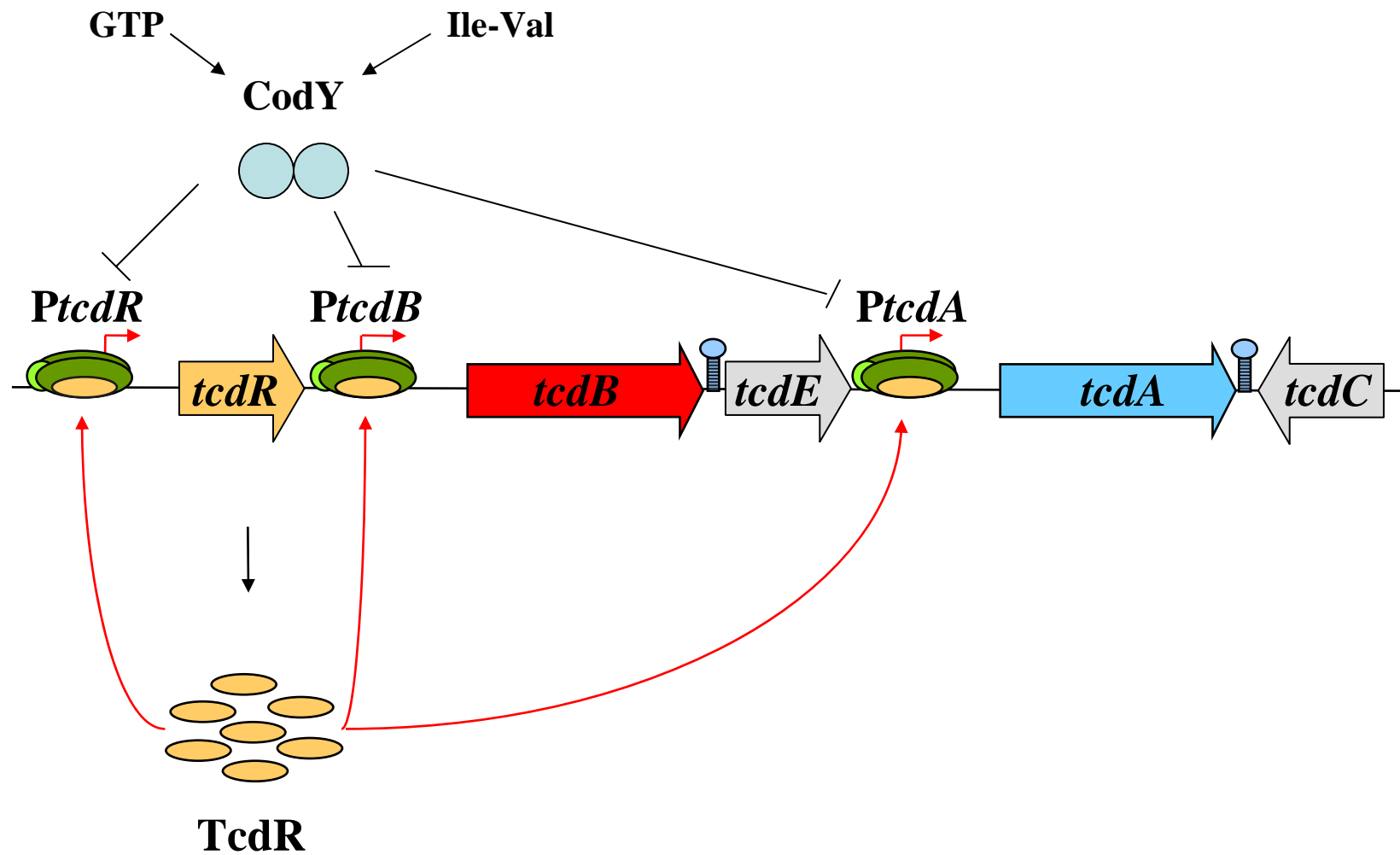


0 20 40 100 200 300 500

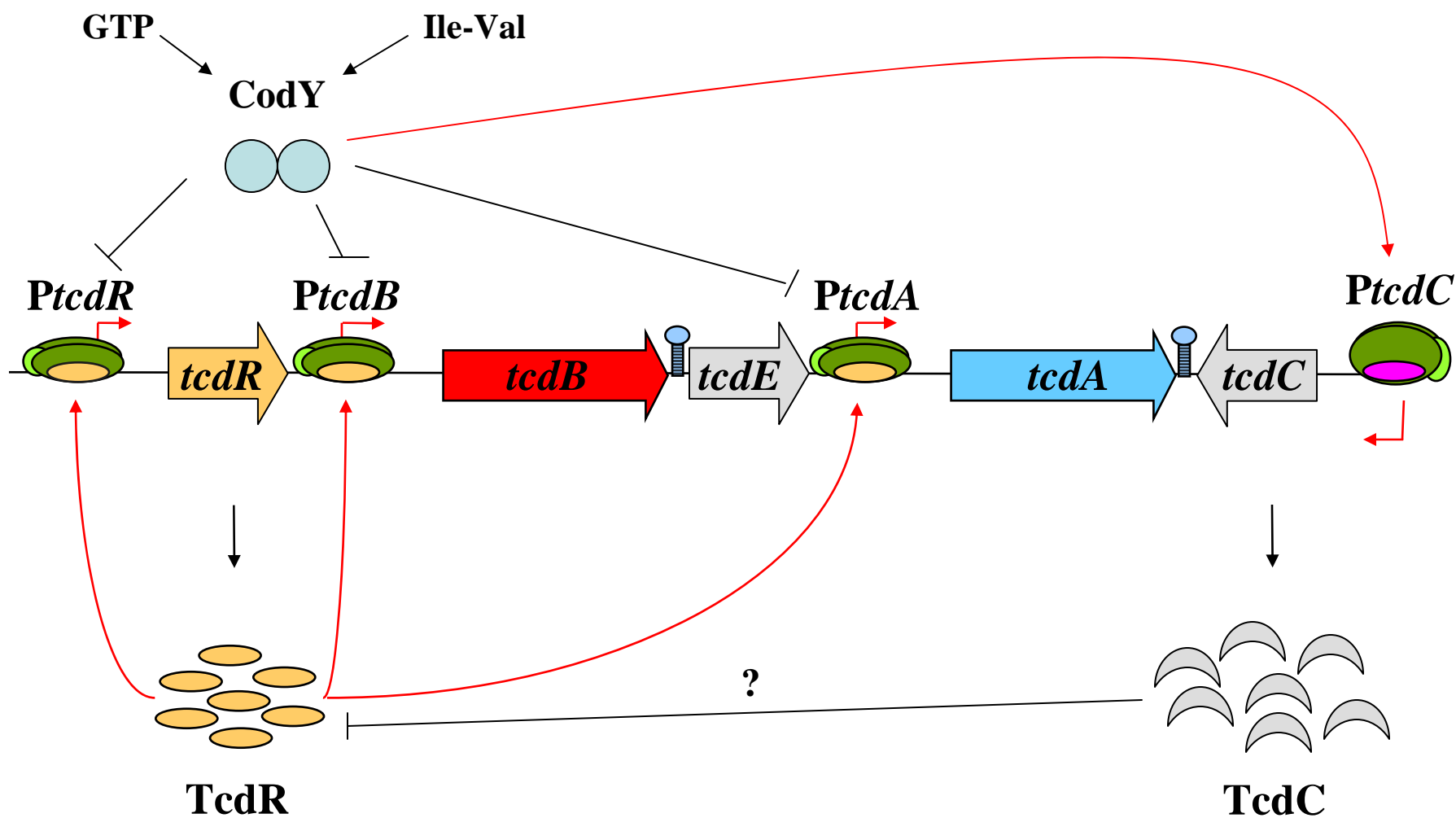
0 10 20 40 100 400 nM



A Model for Toxin Regulation in *C. difficile*



A Model for Toxin Regulation in *C. difficile*



Acknowledgements

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