Modeling of gene regulation by DNA supercoiling in bacteria

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https://rforquet.wordpress.com

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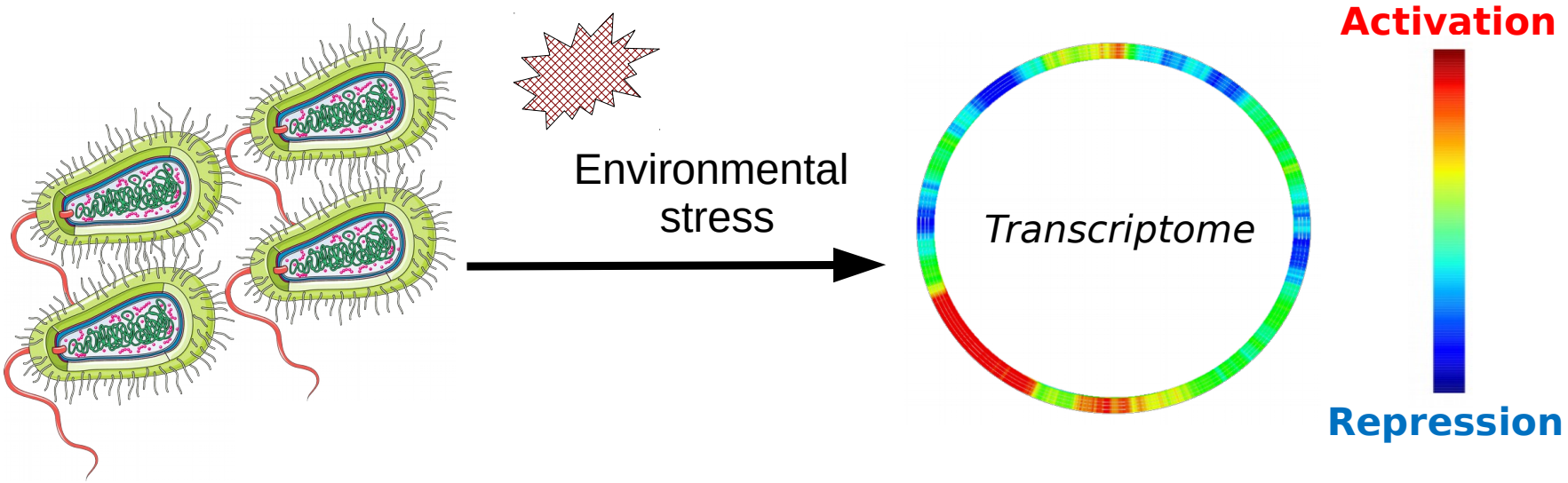
CRP Team - Chromatin and Regulation of bacterial Pathogenesis

Gene regulation in bacteria



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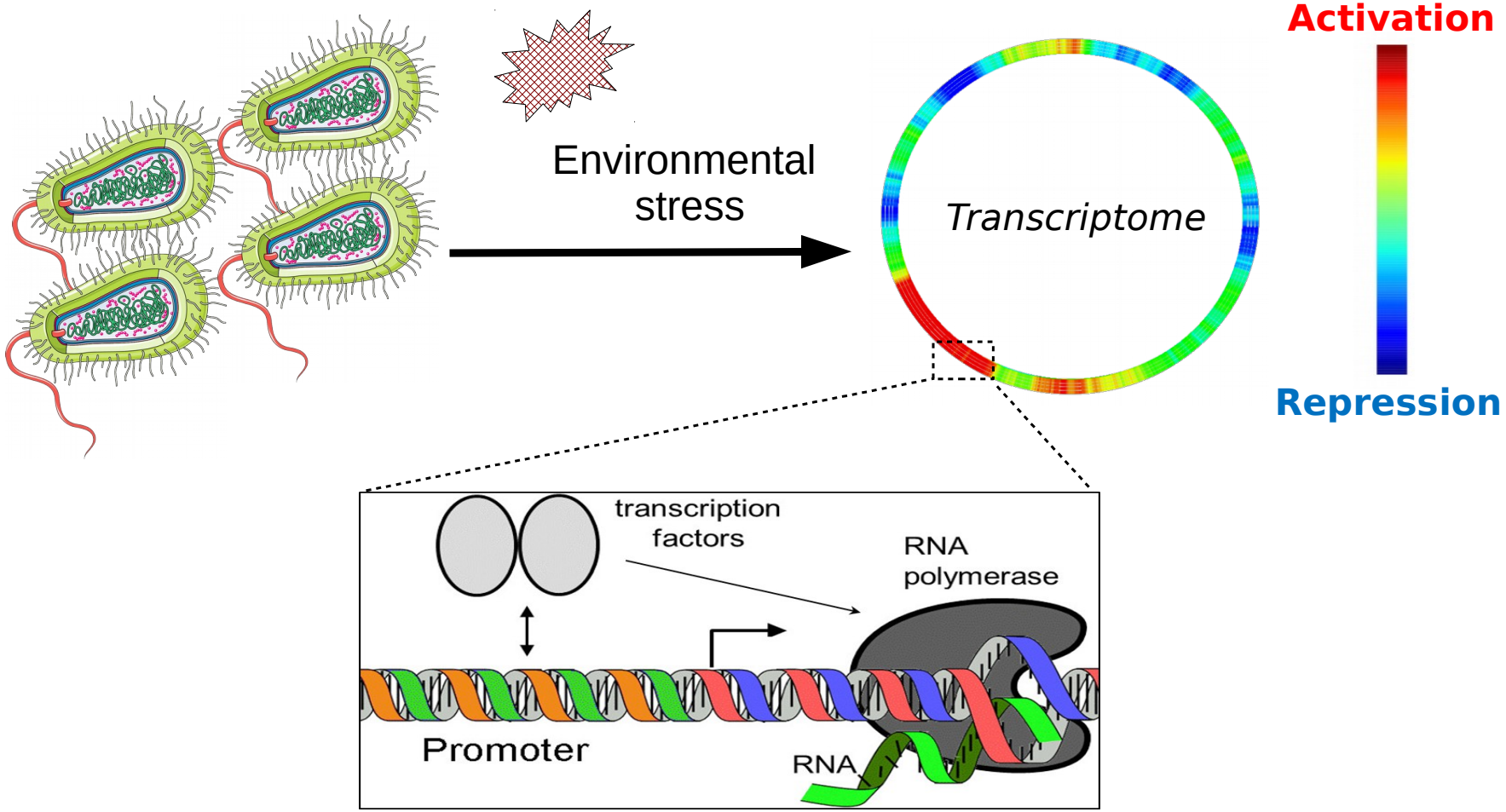

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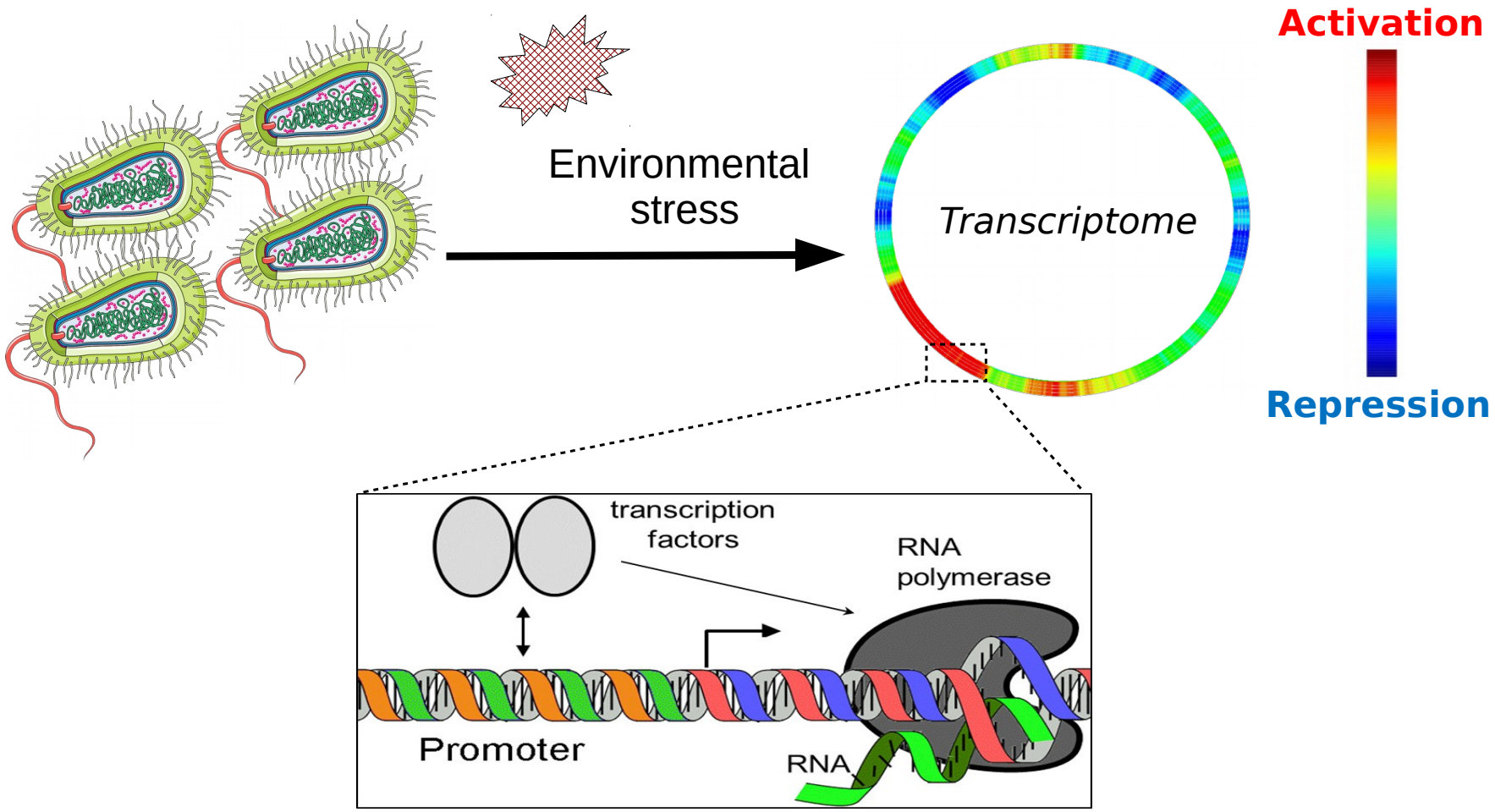
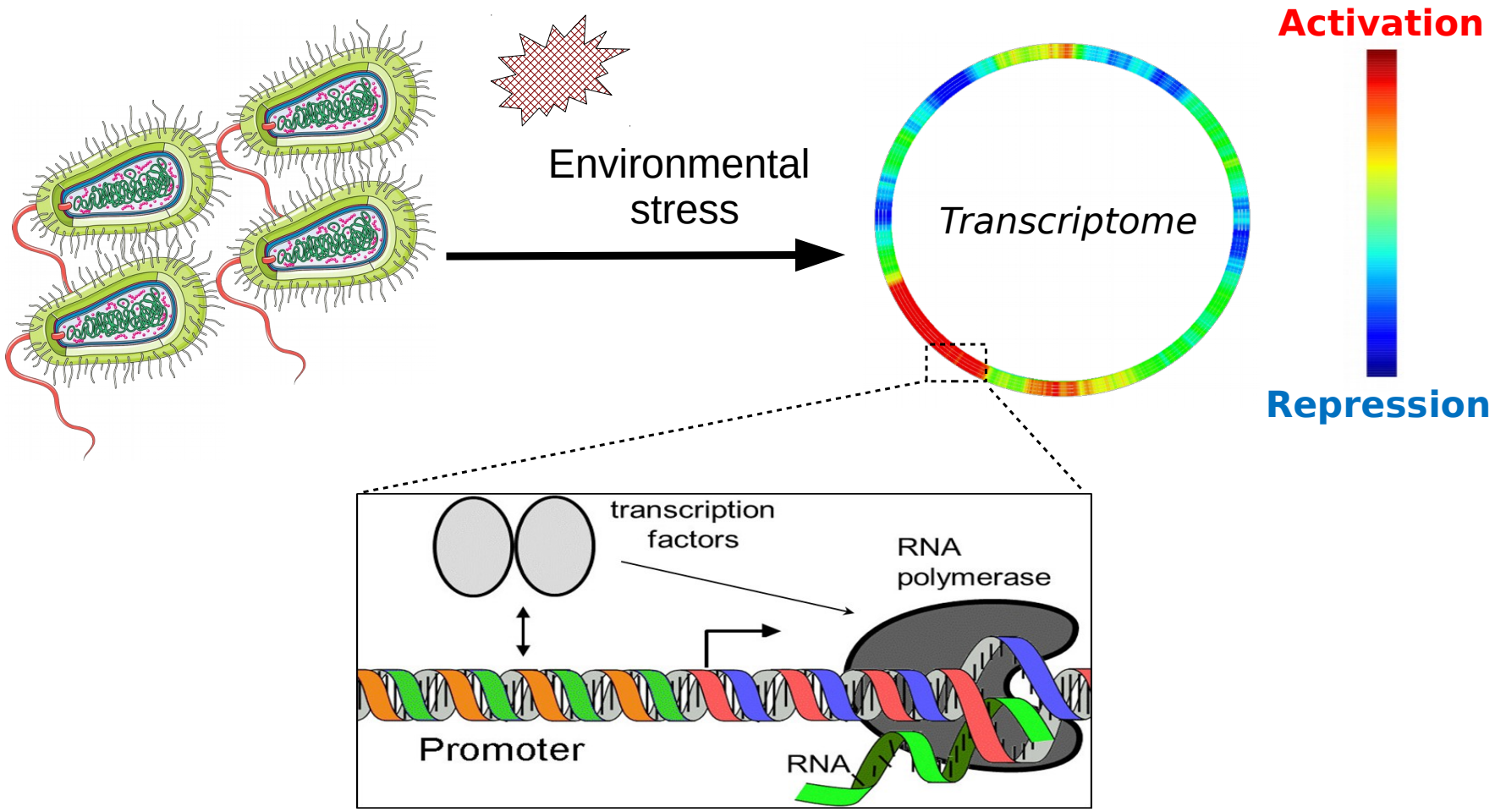


- *E.coli* $\Rightarrow \frac{1}{2}$ gene not regulated by any known TF, organisms almost devoid of them *e.g. Mycoplasma*, *Buchnera*

Gene regulation in bacteria

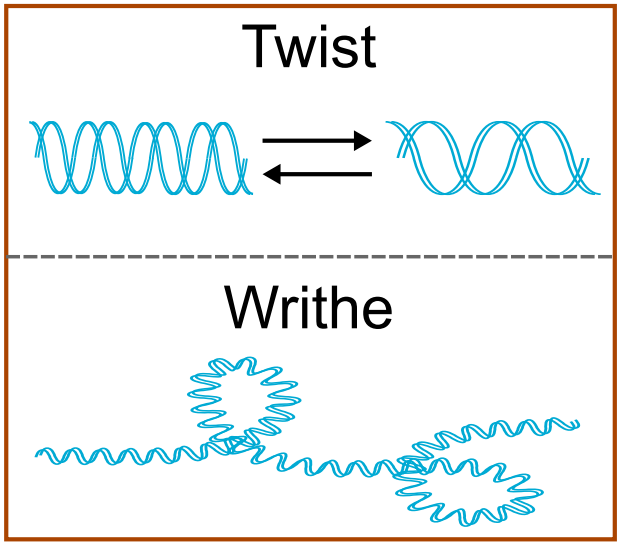


- *E.coli* $\Rightarrow \frac{1}{2}$ gene not regulated by any known TF, organisms almost devoid of them *e.g. Mycoplasma*, *Buchnera*
- Extensive regulation by DNA topology *i.e.* supercoiling (SC) ?

DNA supercoiling (*Martis, Forquet et al. 2019*)

What is DNA supercoiling ?

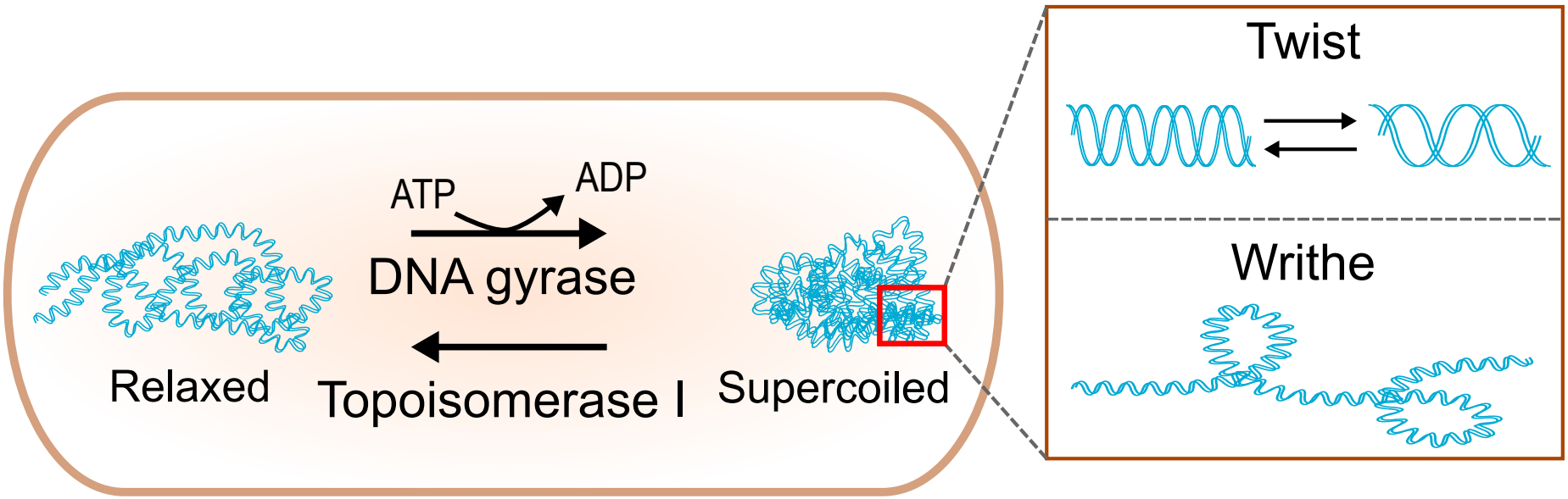
- Deformation of DNA double-helix resulting from **torsional stress**



DNA supercoiling (*Martis, Forquet et al. 2019*)

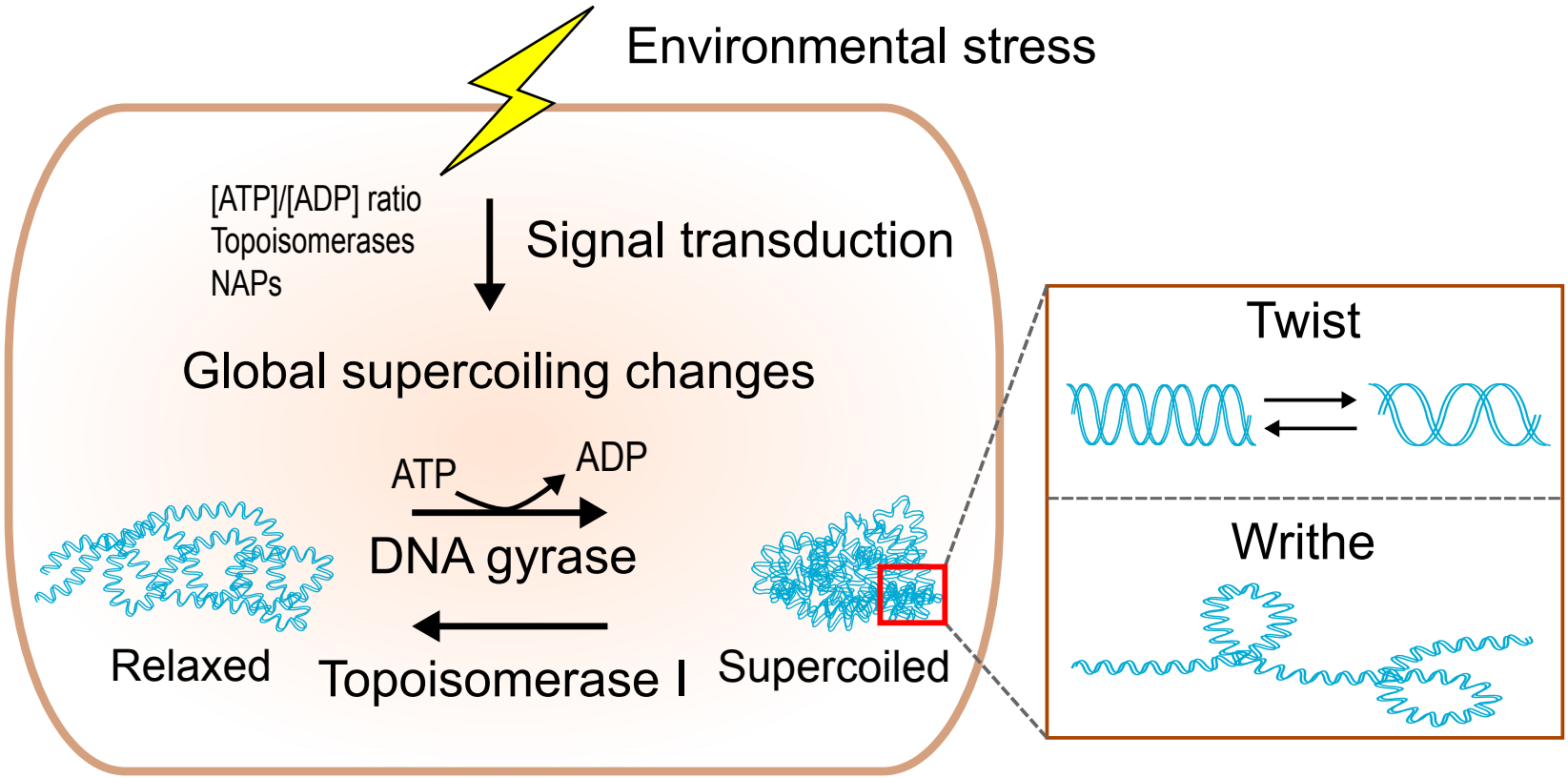
What is DNA supercoiling ?

- Deformation of DNA double-helix resulting from **torsional stress**
- Constant regulation by **topoisomerases** which are **essential** and **highly conserved**



DNA supercoiling (*Martis, Forquet et al. 2019*)

- A rapid physical sensor of changing environments in bacteria



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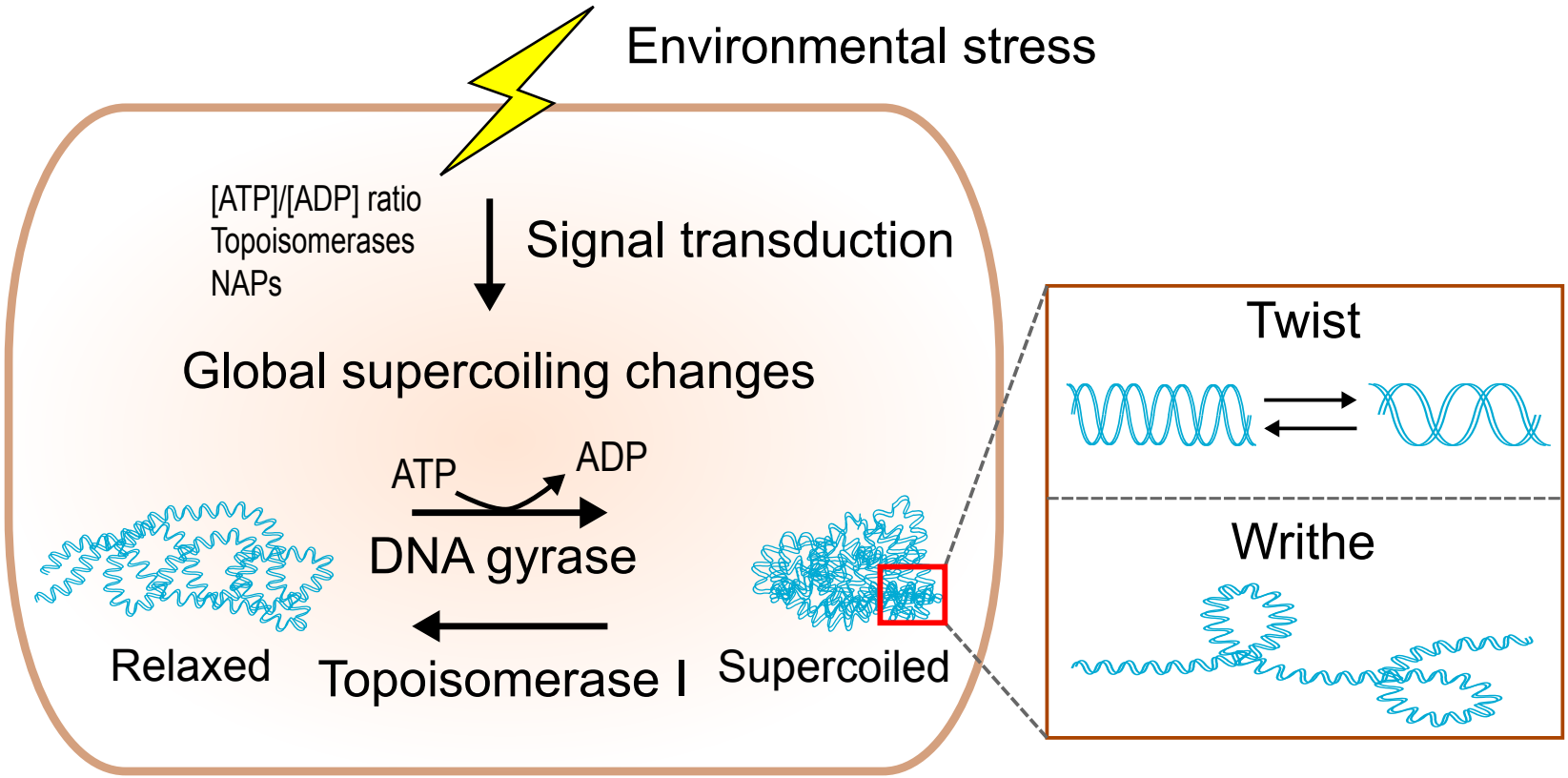
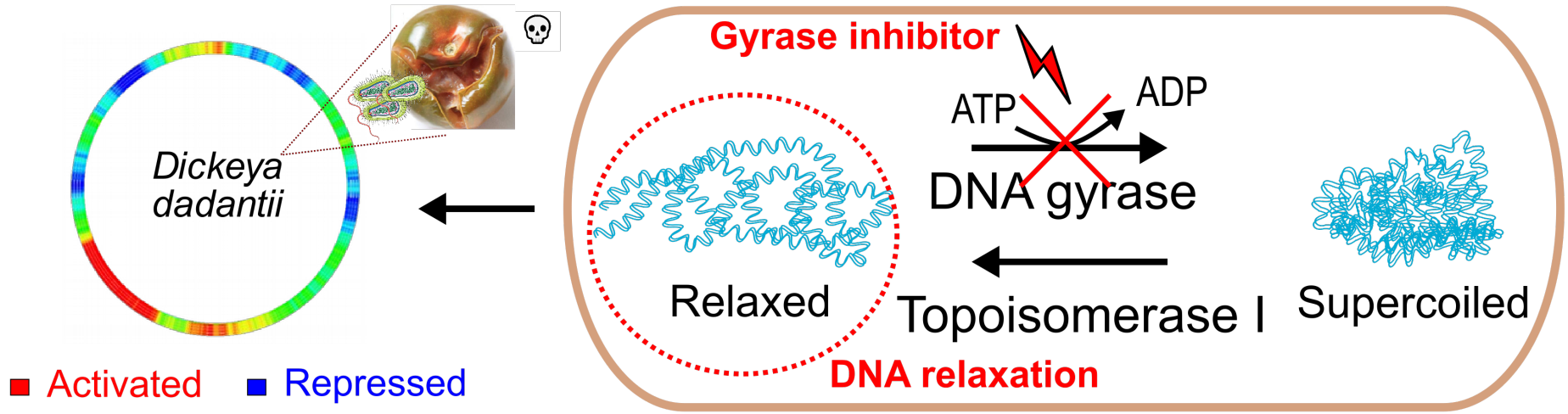


- Contribution to global gene expression reprogramming besides stress-specific response pathways ?

DNA supercoiling (*Martis, Forquet et al. 2019*)

- A global, complex (and ubiquitous ?) regulator in bacteria

Gene response to DNA relaxation



DNA supercoiling (*Martis, Forquet et al. 2019*)

- A global, complex (and ubiquitous ?) regulator in bacteria

Phylum	Species	SC change	Method	Genes significantly affected (% genome)
Proteobacteria	***Escherichia coli***	Rel (+)	Norfloxacin	613 (**15%**)
		Rel (+)	Novobiocin / pefloxacin	1957 (**48%**)
		Rel (+)	Genetic engineering	740 (**18%**)
		Rel (+)	Genetic engineering / norfloxacin / novobiocin	306 (**7%**)
	Salmonella typhimurium	Rel (+)	Genetic engineering	499 (**10%**)
	Dickeya dadantii	Rel (+)	Novobiocin	1461 (**32%**)
		Rel (+)		1212 (**27%**)
	Haemophilus influenzae	Rel (+)	Novobiocin / ciprofloxacin	640 (**37%**)
Firmicutes	***Streptococcus pneumoniae***	Rel (+)	Novobiocin	290 (**14.2%**)
		Hyp (-)	Seconeolitsin	545 (**27%**)
	Staphylococcus aureus	Rel (+)	Novobiocin	280 (**11 %**)
	Bacillus subtilis	Rel (+)		1075 (**24 %**)
Actinobacteria	***Streptomyces coelicolor***	Rel (+)	Novobiocin	121 (**1.5 %**)
	Mycobacterium tuberculosis	Rel (+)		Not provided
Tenericutes	***Mycoplasma pneumoniae***	Rel (+)	Novobiocin	469 (**43 %**)
Cyanobacteria	***Synechocystis***	Rel (+)	Novobiocin	Several genes
	Synechococcus elongatus	Rel (+)		Not provided

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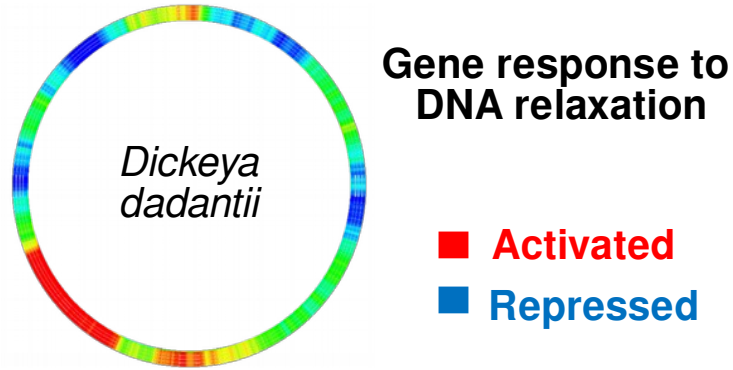
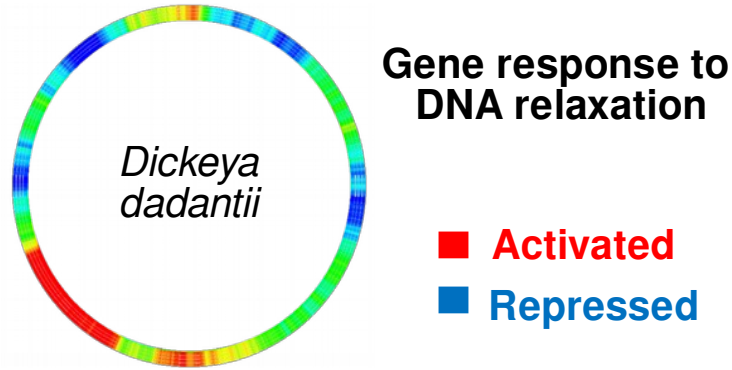
$\Rightarrow$ **No quantitative nor qualitative modeling**

Modeling of gene regulation by DNA supercoiling



Complexity of transcriptomic response to σ variation

Modeling of gene regulation by DNA supercoiling

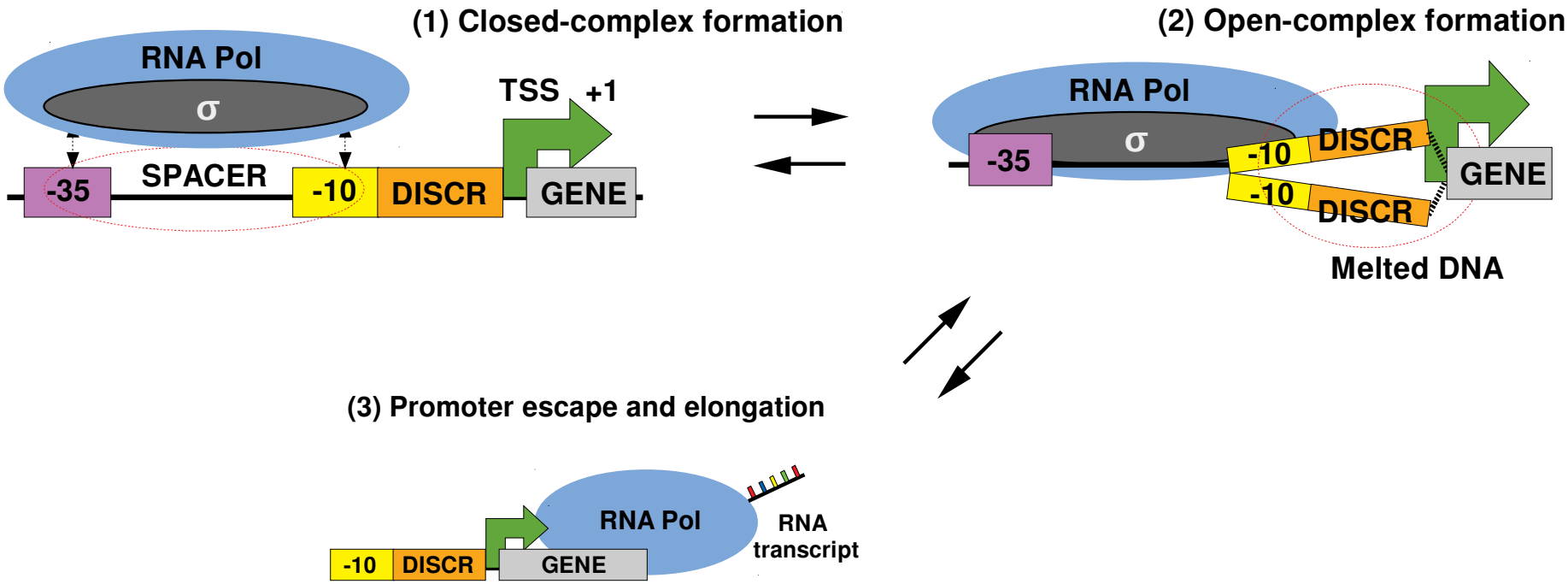


Complexity of transcriptomic response to σ variation

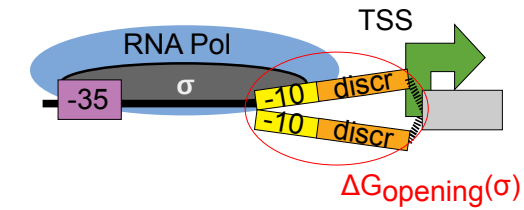
Hypothesis : promoter-specific response

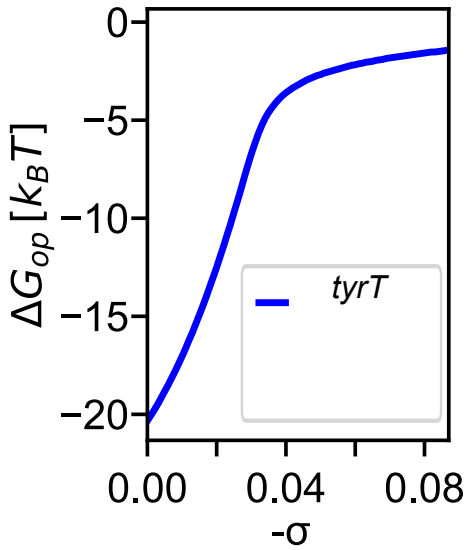
Regulation without regulators

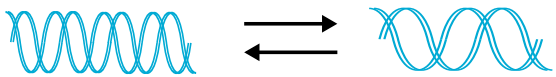
- Basal regulation of RNA Polymerase-DNA interaction during transcription initiation



Modeling of supercoiling-assisted promoter opening







$\Delta G_{op}(\sigma, seq) \Rightarrow$ TwistDNA

2 known parameters : t°, $[salt]$

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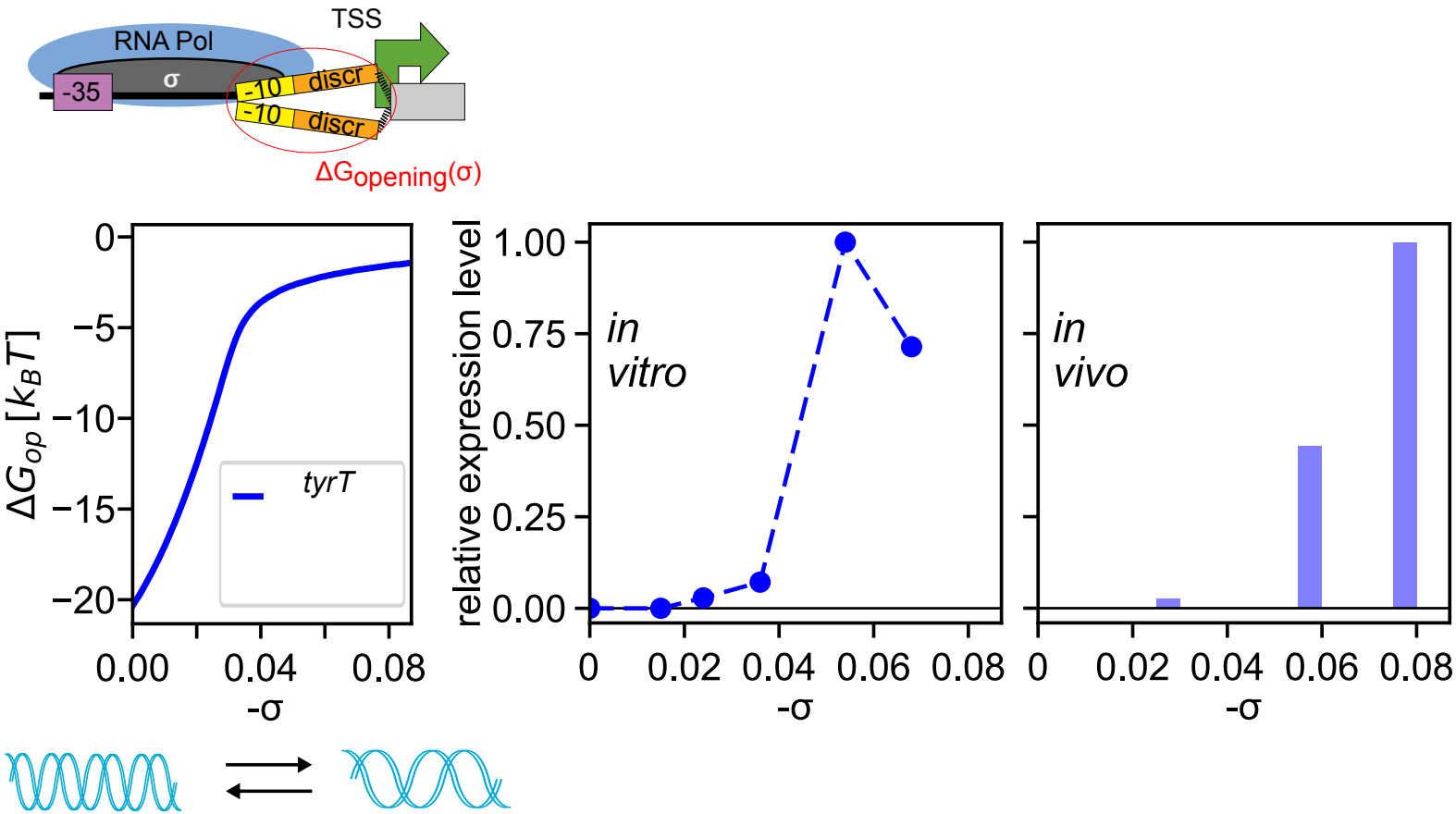

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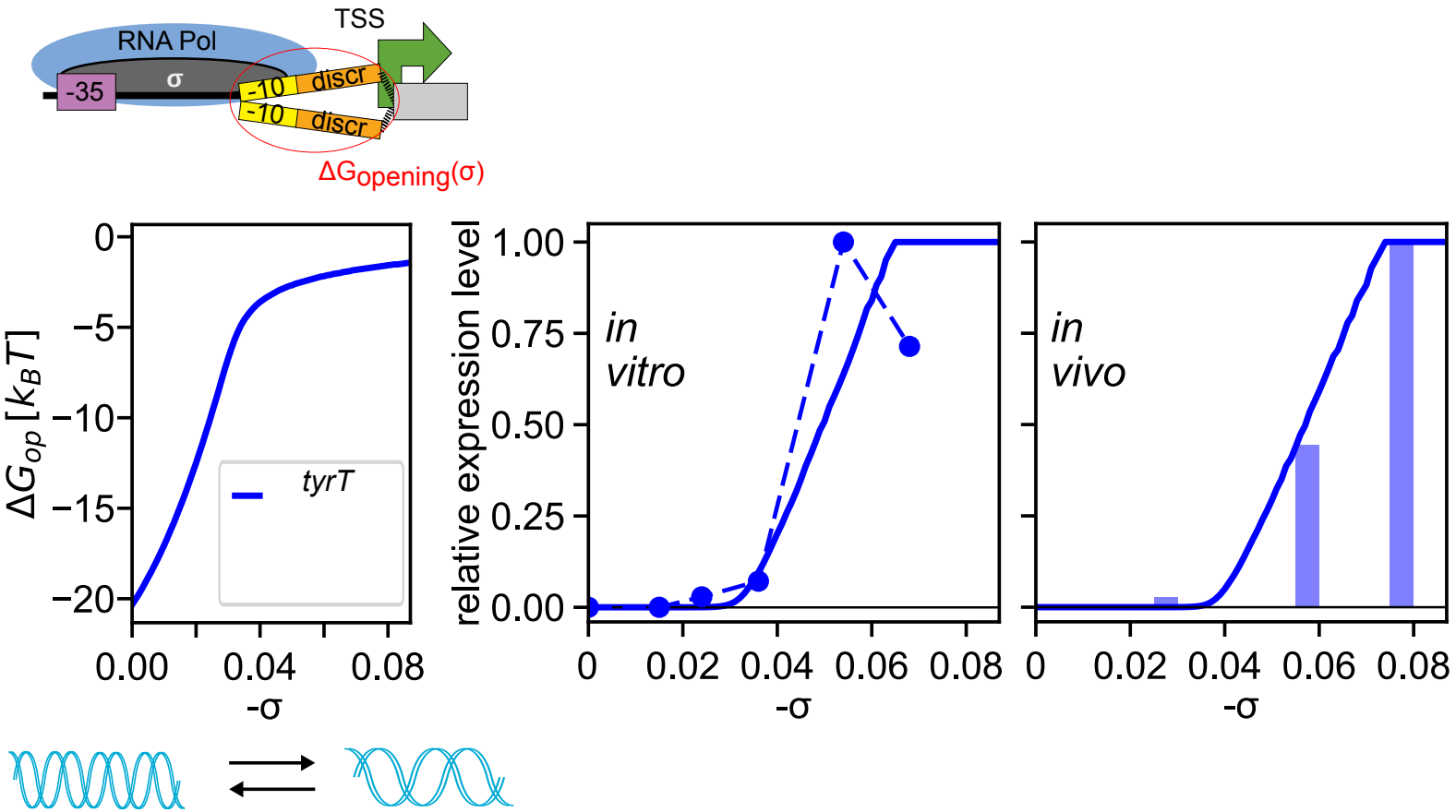

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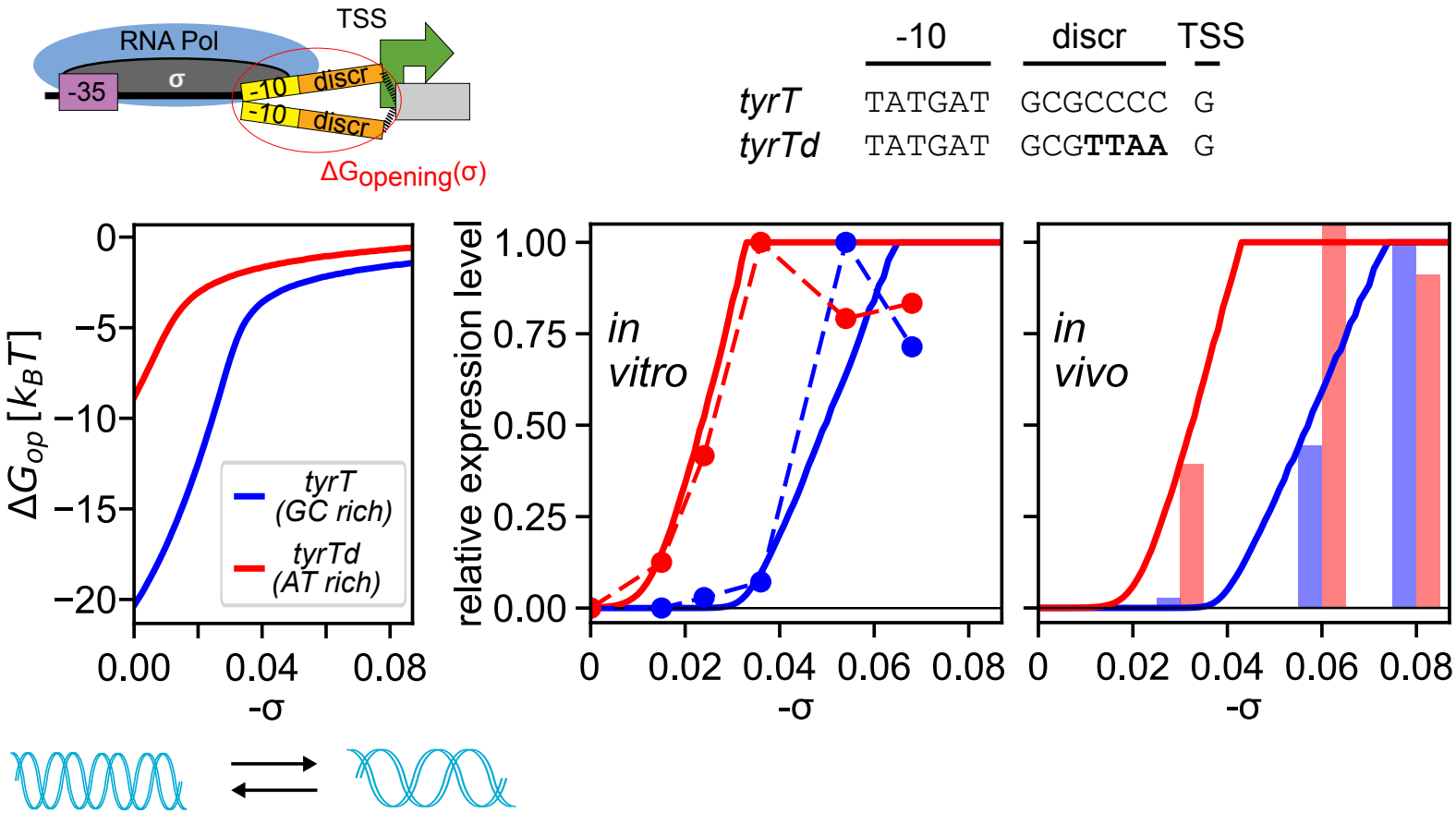
2 known parameters : $t^\circ, [salt]$

$$k(\sigma, seq) = k_0 \exp\left(\frac{\Delta G_{op}(\sigma, seq) + \Delta G_{RNAP}}{k_B T}\right)$$

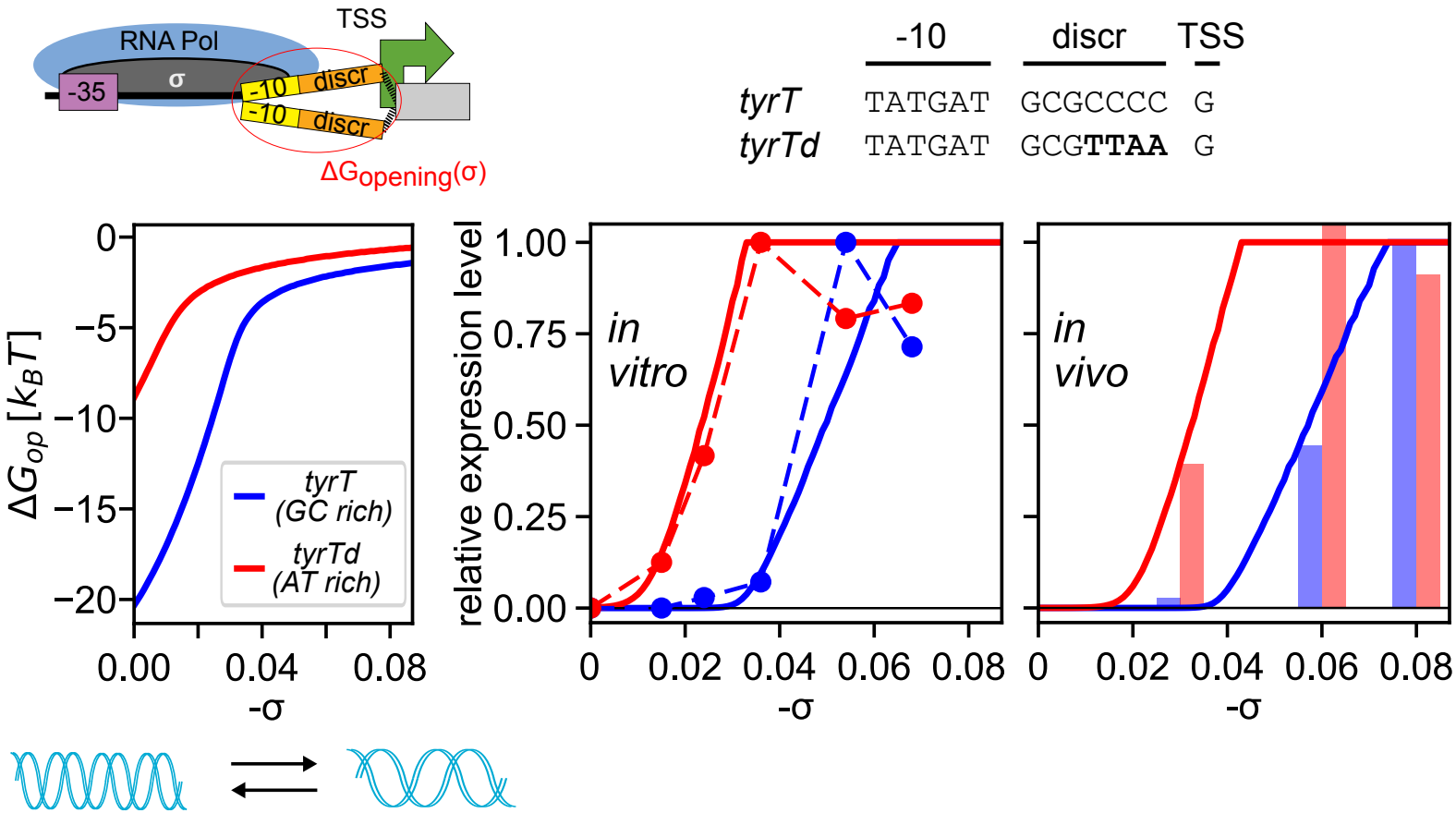
$$\Delta G_{RNAP} = 3.5 k_B T$$

Modeling of supercoiling-assisted promoter opening



- Regulation *i.e.* selective repression of promoters ? $\Rightarrow$ discriminator sequence

Modeling of supercoiling-assisted promoter opening



- Regulation *i.e.* selective repression of promoters ? $\Rightarrow$ discriminator sequence
- Relaxation *e.g.* σ = -0.06 to -0.04 $\Rightarrow$ much stronger repression of GC-rich discriminators

In vivo model validation on individual promoters

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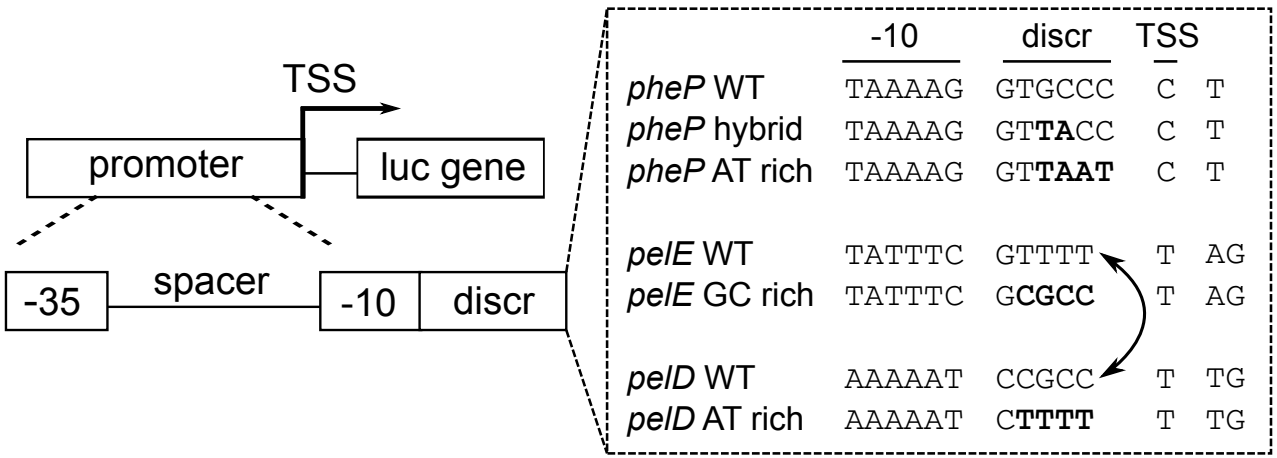
- Plasmid transformation in *E. coli*, monitoring of growth and expression



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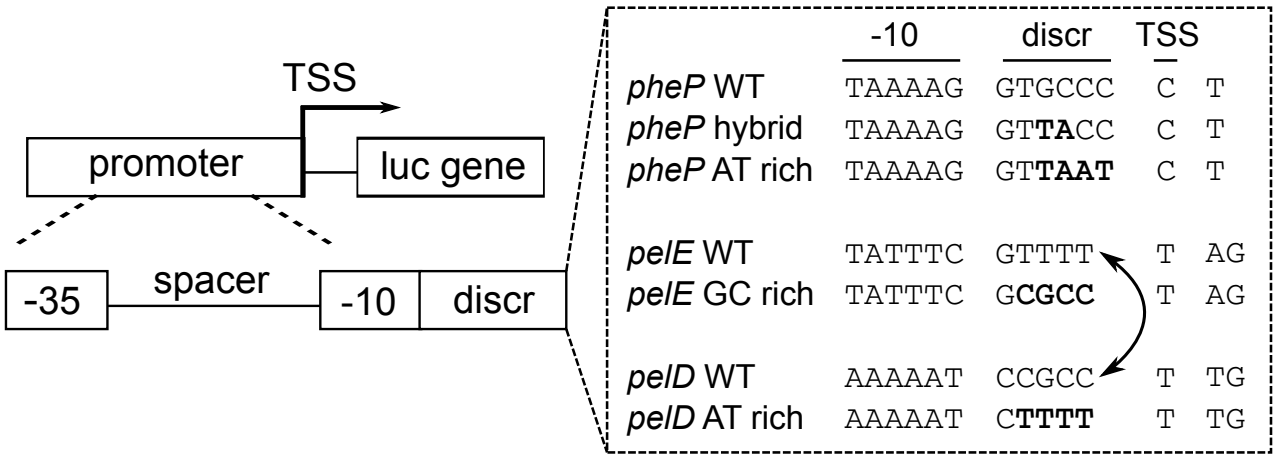
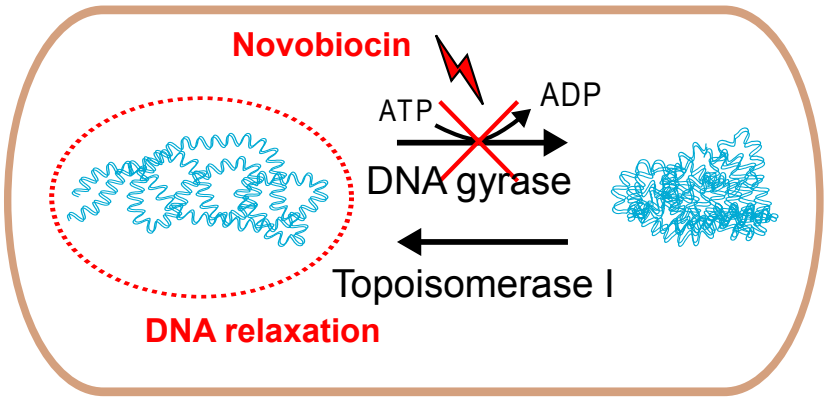
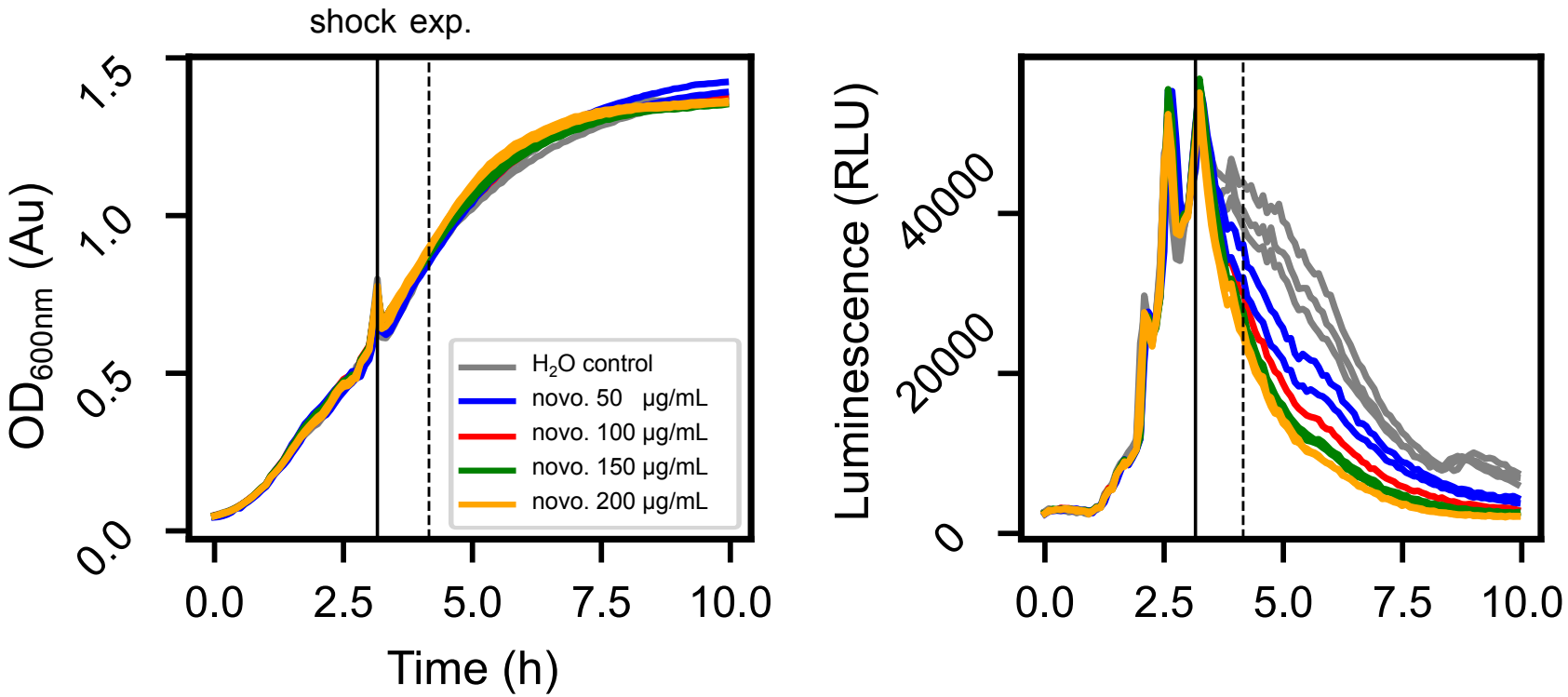


- Gene response to novobiocin-induced relaxation consistent with predictions ?



In vivo model validation on individual promoters

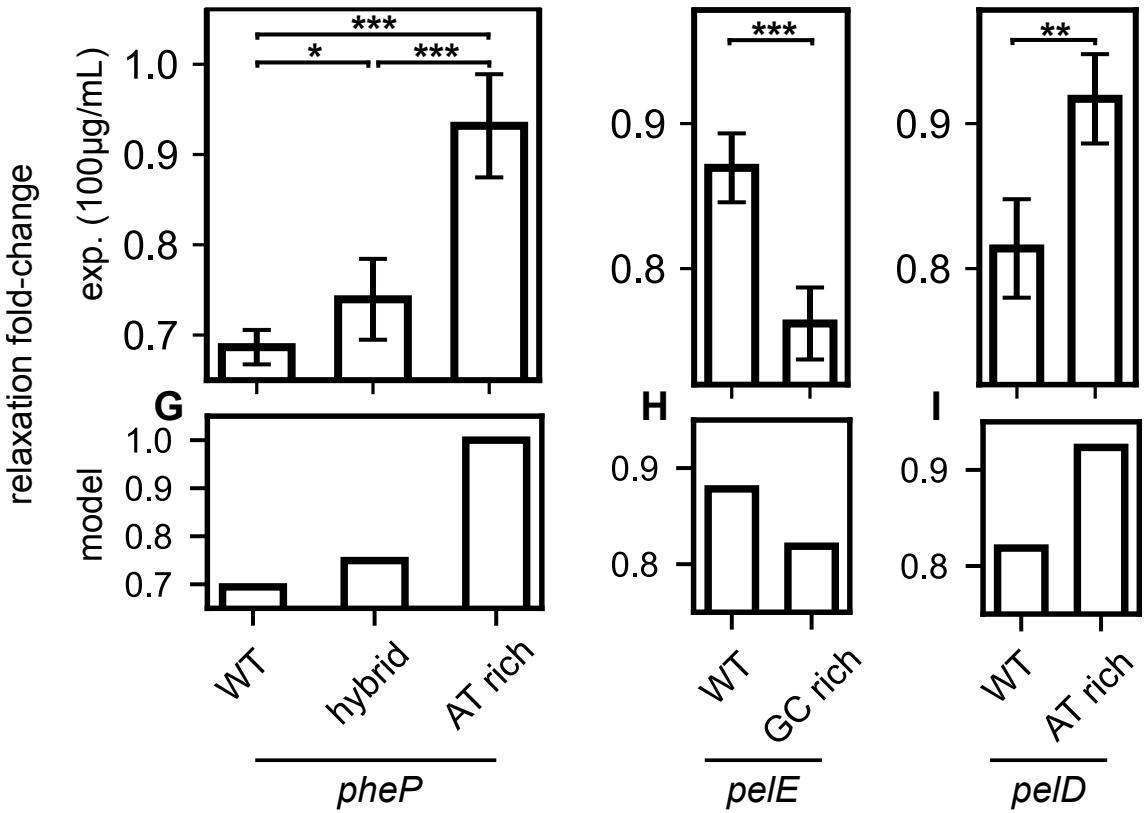
- *pheP* hybrid strain in LB rich medium



- exp. : time point used to compare the expression variation among promoters

In vivo model validation on individual promoters

- Relaxation response of promoters with mutated discriminators match the predictions



	-10	discr	TSS	
pheP WT	TAAAAG	GTGCCC	C	T
pheP hybrid	TAAAAG	GT**TA**CC	C	T
pheP AT rich	TAAAAG	GT**TAAT**	C	T
pelE WT	TATTTC	GTTTT	T	AG
pelE GC rich	TATTTC	G**CGCC**	T	AG
pelD WT	AAAAAT	CCGCC	T	TG
pelD AT rich	AAAAAT	C**TTTT**	T	TG

In vivo model validation on individual promoters

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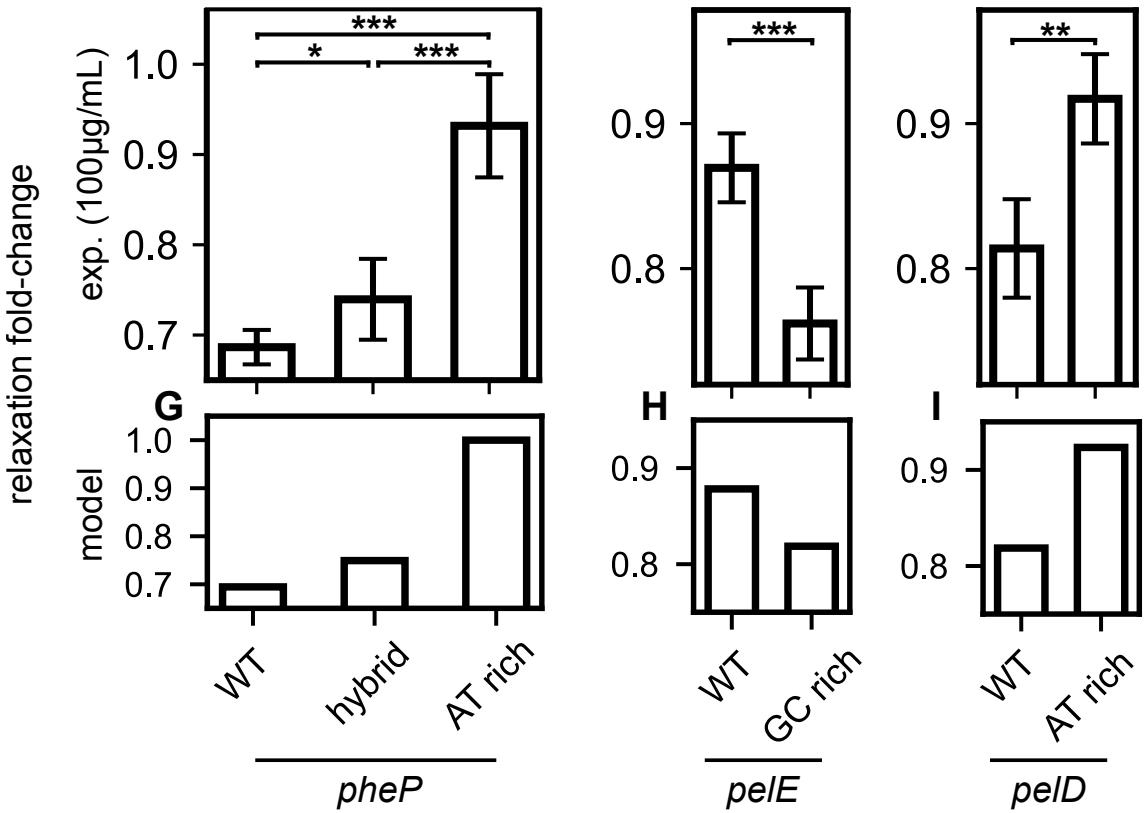

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⇒ **Selective repression depending on discriminator sequences**

⇒ **Global regulation mode ?**

In vivo model validation at the genome scale

Analysis of whole-genome expression data

- Transcriptome under DNA relaxation
- Map of all promoters with sigma factors, discriminator and genes

In vivo model validation at the genome scale

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- Transcriptome under DNA relaxation
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Predictions and confrontation to experimental data

$$k(\sigma, seq) = k_0 \exp\left(\frac{\Delta G_{op}(\sigma, seq) + \Delta G_{RNAP}}{k_B T}\right)$$

- Expression rates before/after DNA relaxation
- Normalization step to mimic transcriptomic protocol

In vivo model validation at the genome scale

Prediction : relaxation activates AT rich discriminators and represses GC rich ones

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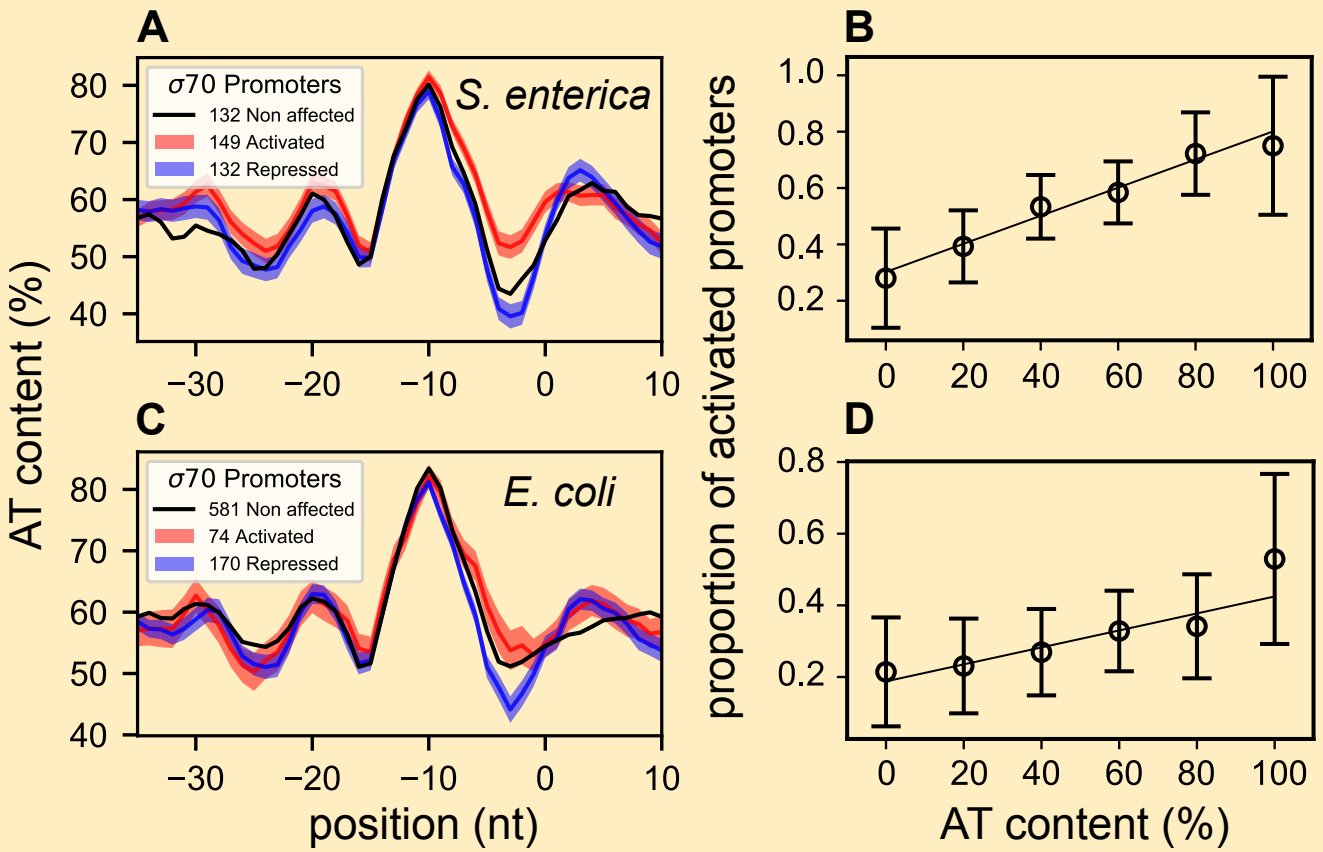
An ubiquitous regulation mode ?



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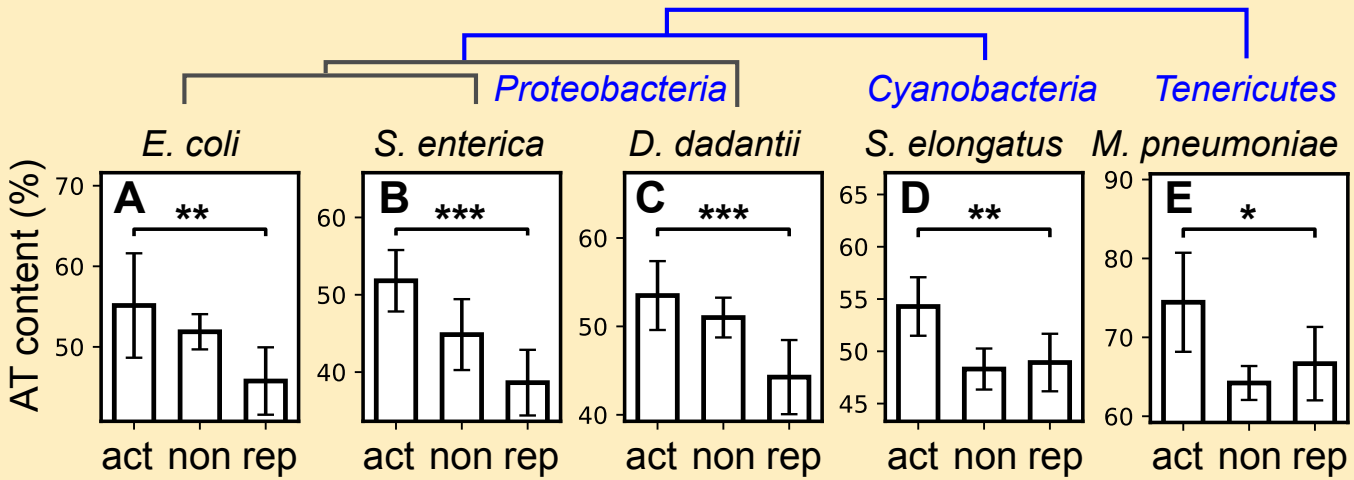

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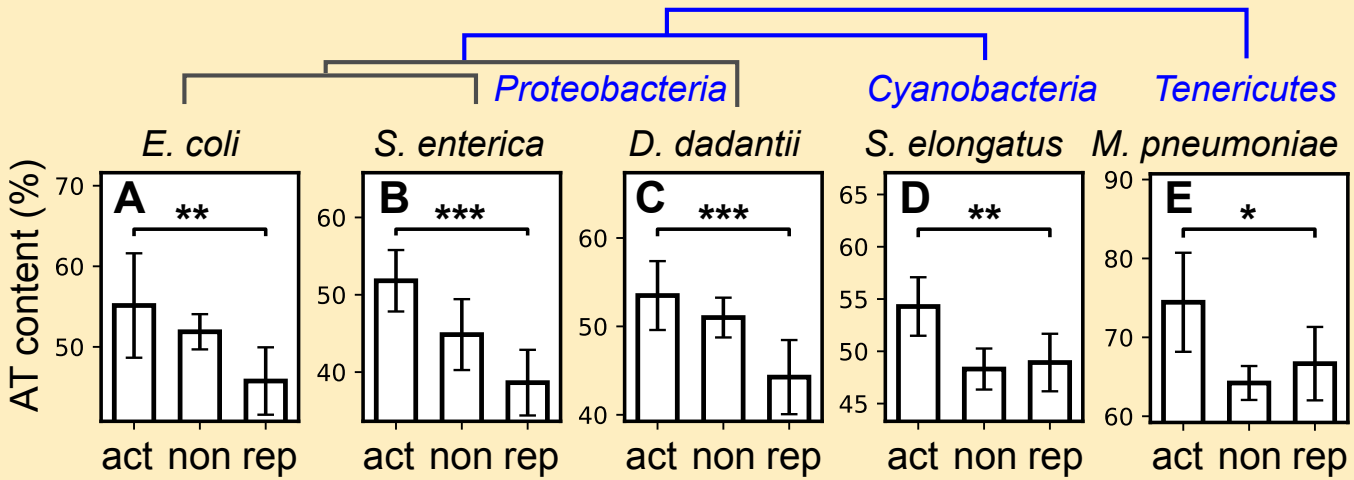

- Contribution to the entire genomic response to relaxation ?
- Proportion of accurately predicted promoters (sensitivity) and comparison to a random model $\Rightarrow$ up to 15% predictability gain

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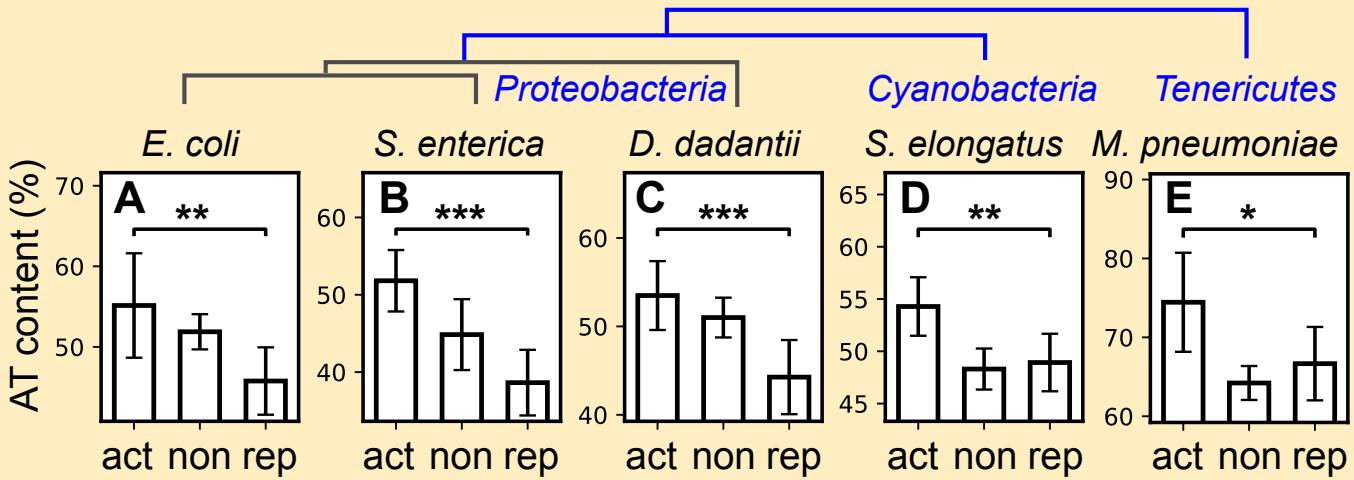

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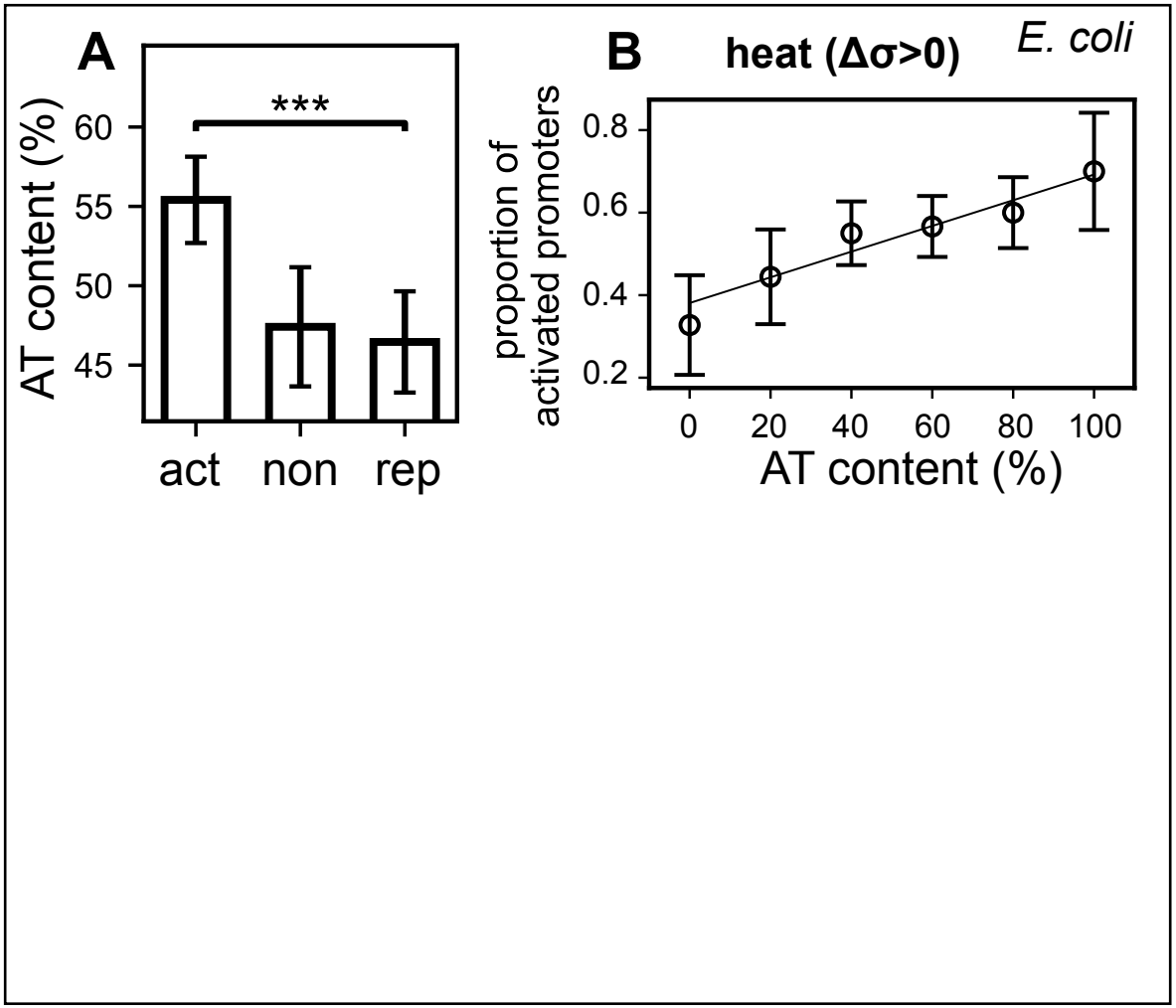
Limitations : promoters annotated with poor resolution, ≠ drug and protocol used, local SC levels ≠ global SC level of the chromosome, gene regulation at ≠ transcription steps

In vivo model validation in stress response



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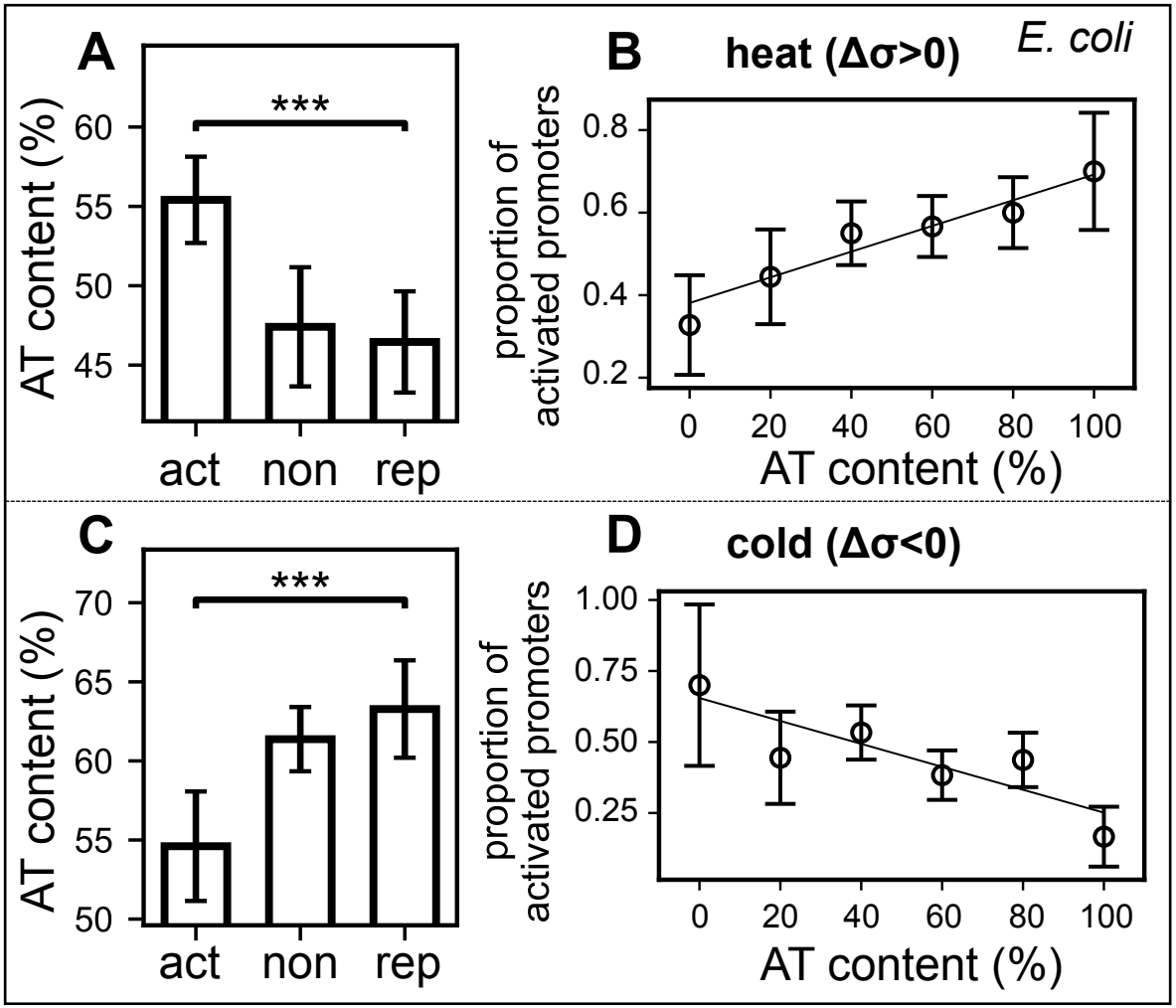

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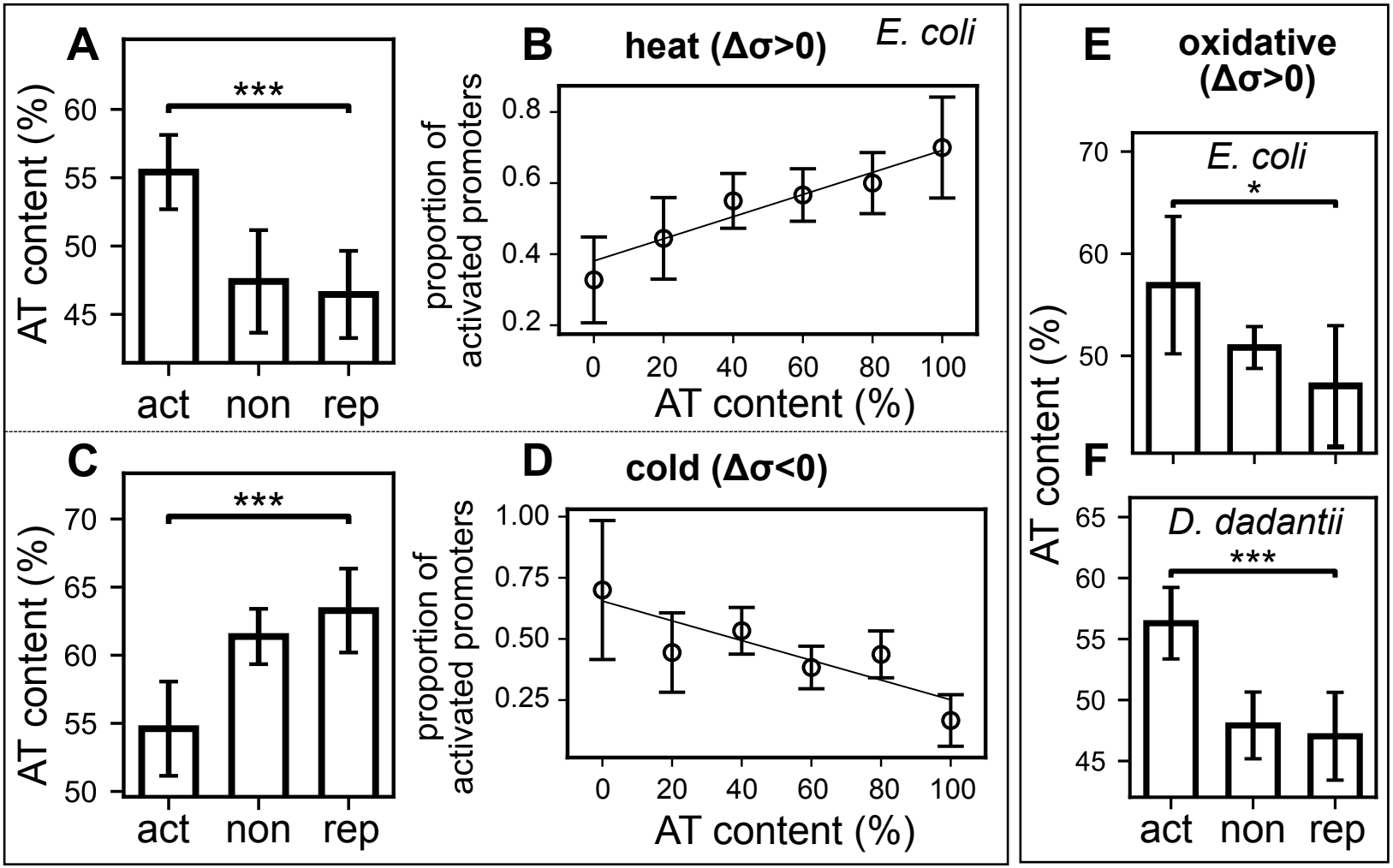

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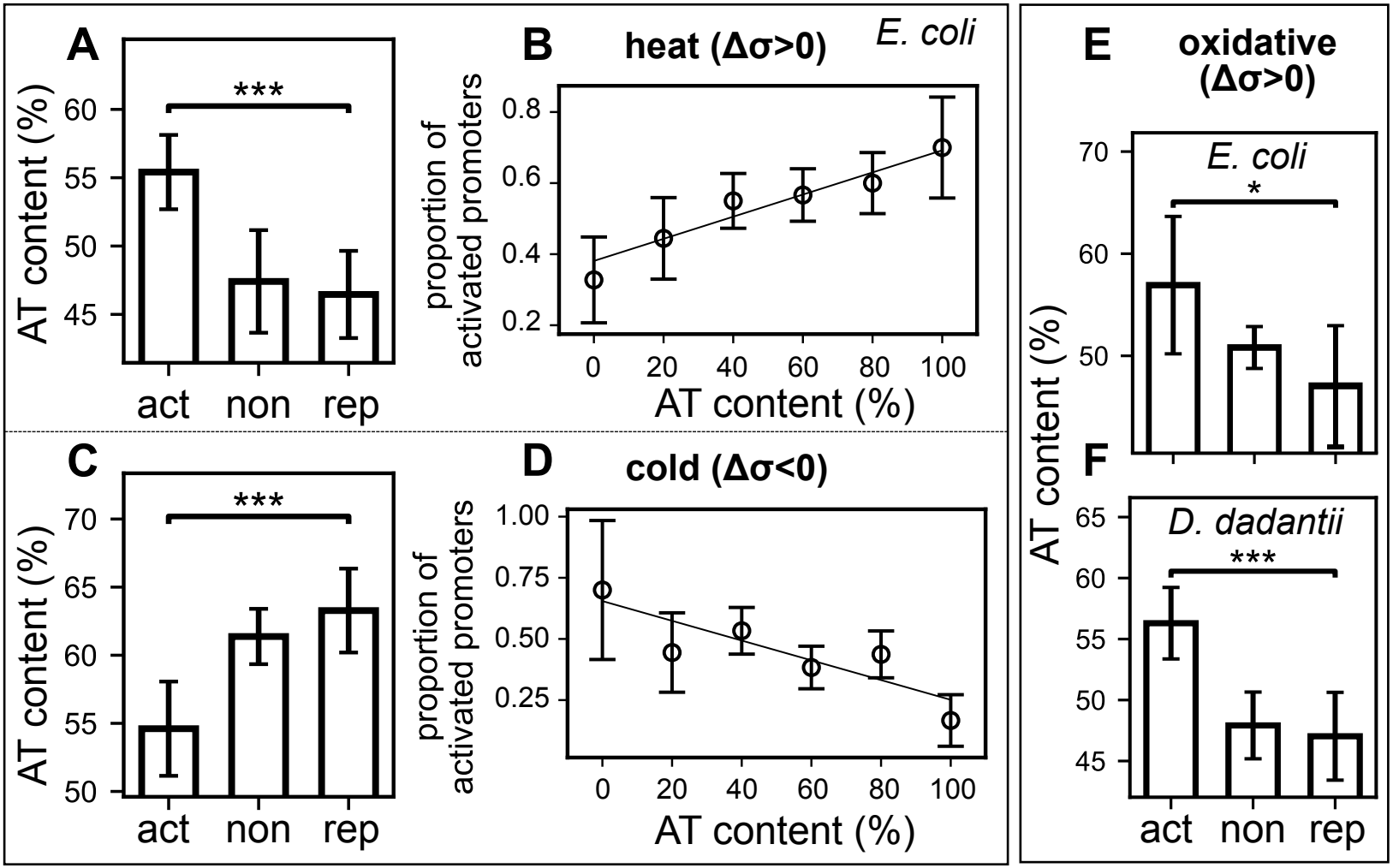


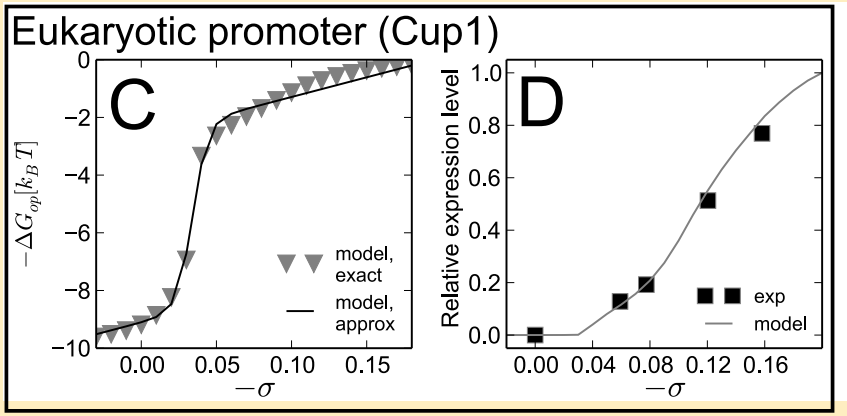
- Up to 10% predictability gain
- The selective activation/repression of promoters by stress conditions is controlled by changes in DNA SC depending on discriminator sequences

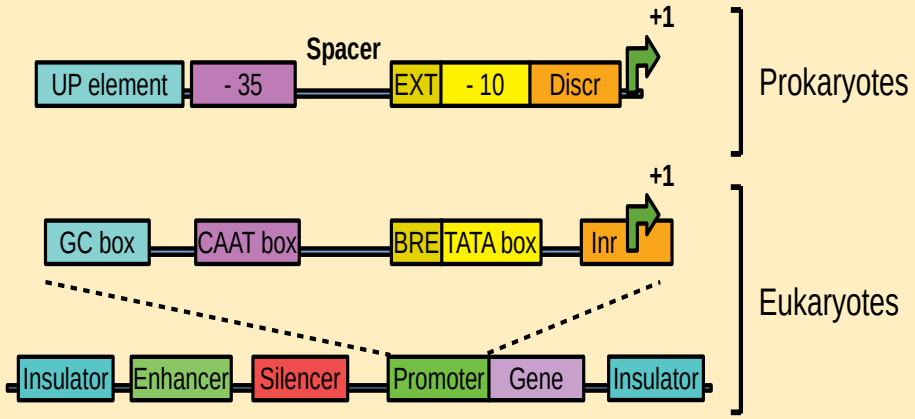
Conclusion

- DNA Supercoiling, by modulating RNA Polymerase-DNA interaction, constitutes a global, complex and ubiquitous mode of gene regulation, based on fundamental mechanical properties of DNA

In eukaryotes ?

- Open-complex formation : (1) ATP-dependent melting by TFIIH (optional ?) (2) local negative DNA supercoiling provided by transcription or nucleosomes
- Similarities among eukaryotic and bacterial promoters in spite of evolutionary distances





Leblanc 2000, Meyer 2014

Merci pour votre attention