LGN5809 - Genética Molecular

CONTROLE DA EXPRESSÃO GÊNICA EM PROCARIOTOS

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SUMÁRIO

- Diversidade de procariotos;
- Regulons x Estimulons;
- Controle do inicio da transcrição;
- Controle transcricional;
- Controle pós-transcricional;
- Próxima aula.

DIVERSIDADE DOS PROCARIOTOS

DIVERSIDADE DOS PROCARIOTOS

- Onde eles podem ser encontrados???

em quase
todos os

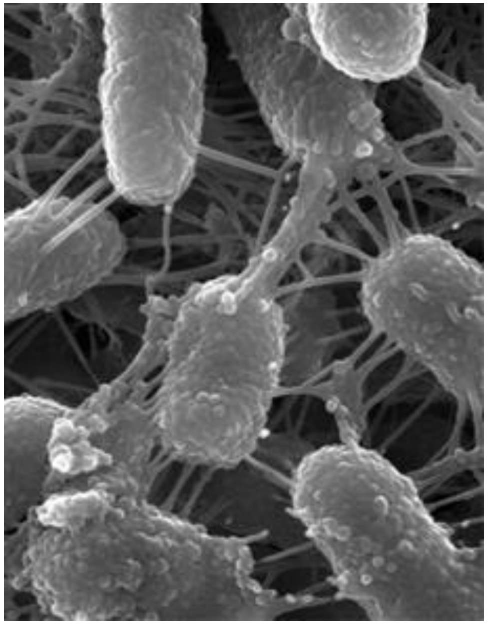
T elevadas (+110ºC)
T muito baixas

Alta variação na atividade metabólica – controle bem ajustado da expressão

O *lifestyle* INFLUÊNCIA…

Mycobacterium

endossimbionte



Pseudomonas

vida-livre

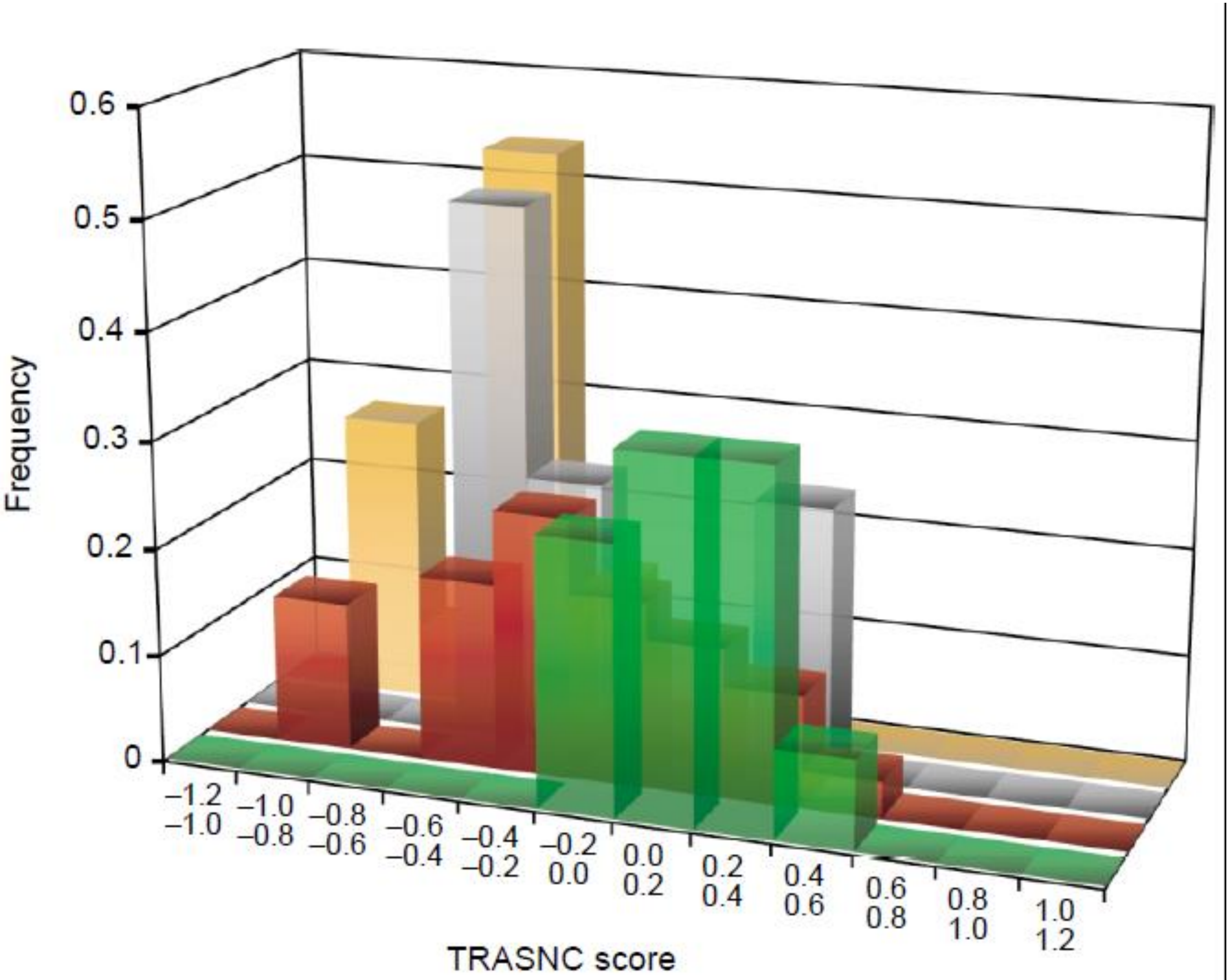
Qual dessas duas bactérias tem um sistema mais complexo de regulação gênica?

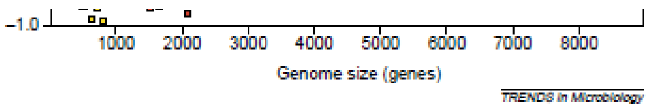
Transcription regulation and environmental adaptation in bacteria

Ildefonso Ca

[1]Computational Ge
Wellcome Trust Ge
[2]National Centre fo

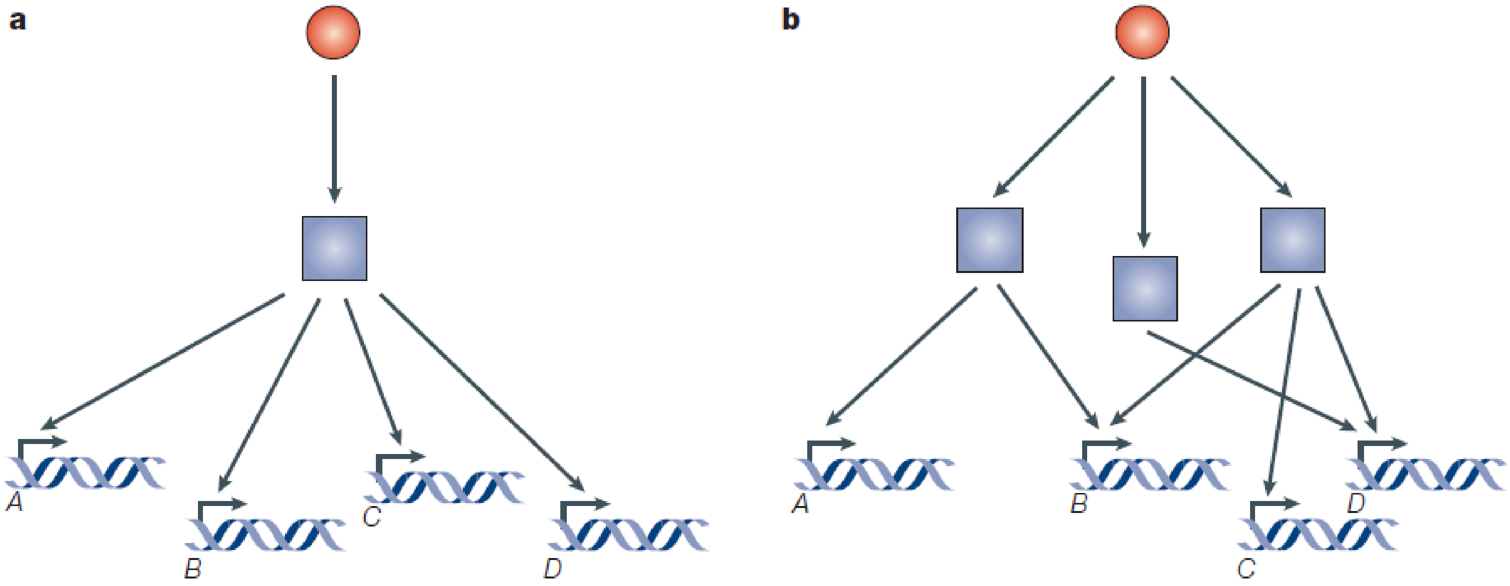
Lifestyle can be v an organism anc with other speci contribute to the assess how these we investigated t transcription-rela olism enzymes, a classified by life harbour more t smaller ones. In few exceptions) factors, beyond t genome size. Th ditions, gene ex gration have bee adaptation to env





Trends Microbiol. **11**, 248–253 (2003).

REGULON X ESTIMULON

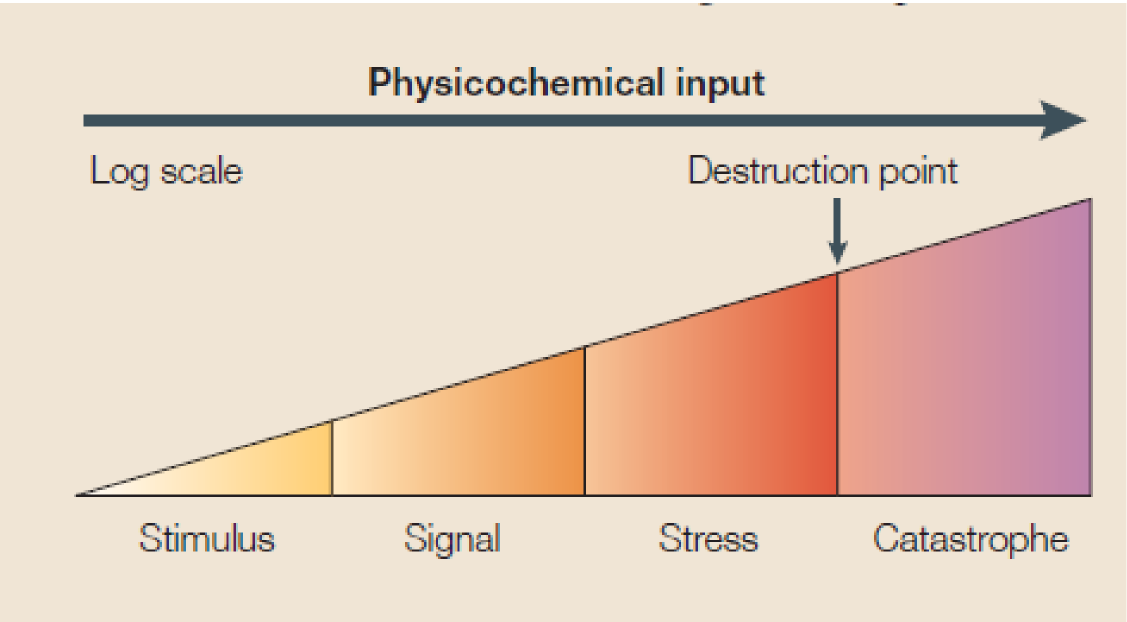


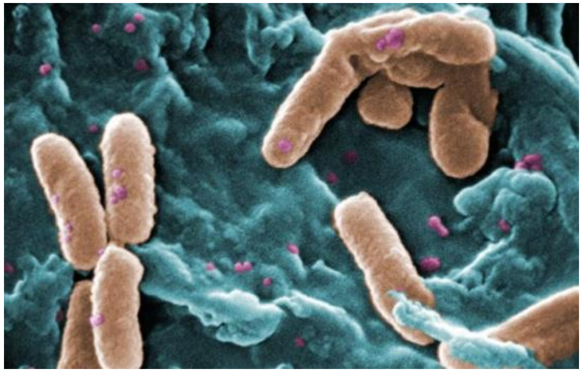
Regulon – conjunto de genes regulados por um determinado fator de transcrição

Estimulon – conjunto de genes regulado por um estímulo

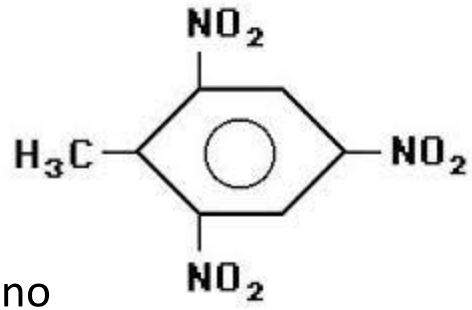
doi:10.1038/nrmicro1084

MAS O QUE SERIA UM ESTÍMULO?



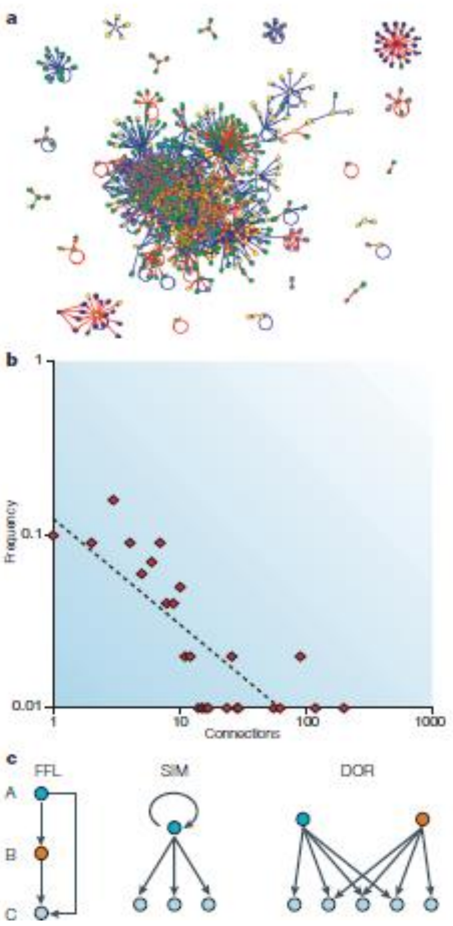


Pseudomonas sp. e derramamento de petróleo



Tolueno

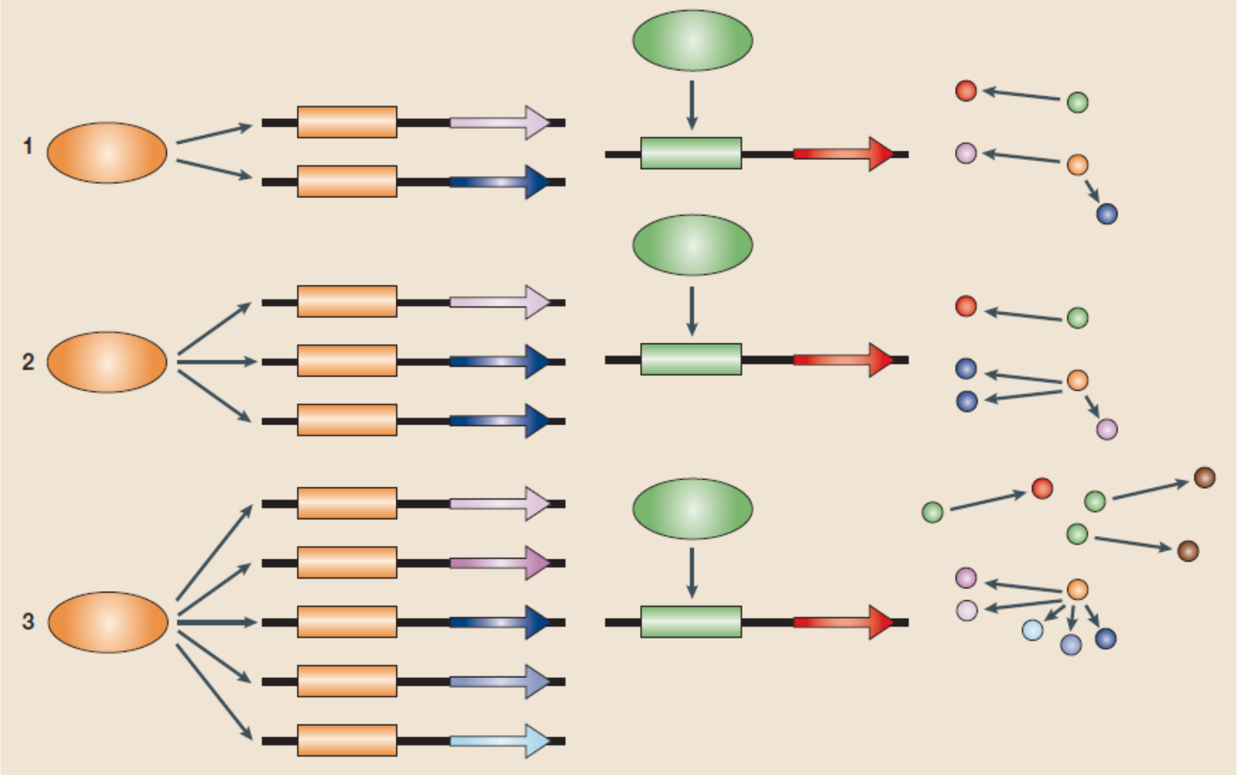
doi:10.1038/nrmicro1084



De acordo com dados experimentais - RegulonDB database (banco de operons e fatores regulatórios), o *incoming connectivity*, o número de fatores de transcrição que regulam um particular gene pode variarde 1 a 8 resultando em uma distribuição exponencial de possíveis *networks*

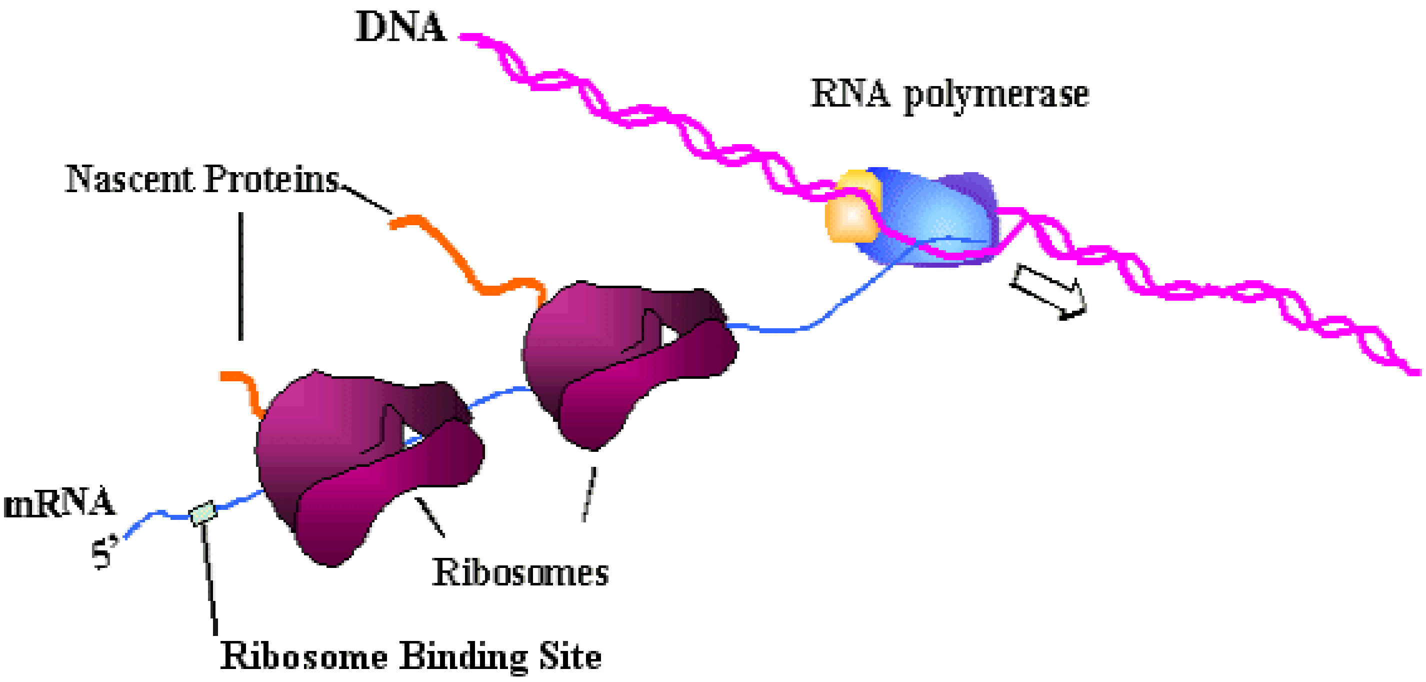
http://regulondb.ccg.unam.mx/

Principio de Mateus

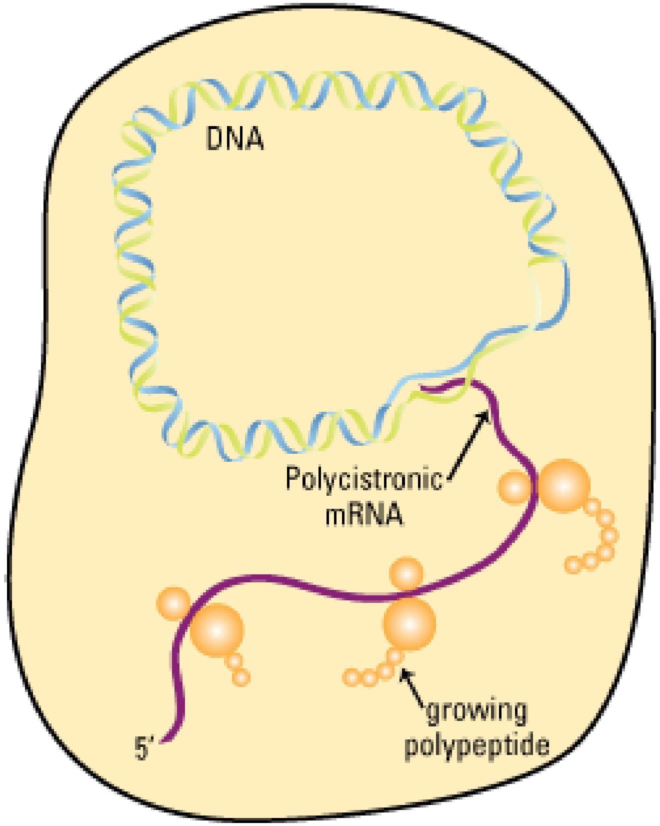


Pois a quem tem, mais se lhe dará, e terá em abundância; mas, ao que quase não tem, até o que tem lhe será tirado Mateus 13:12

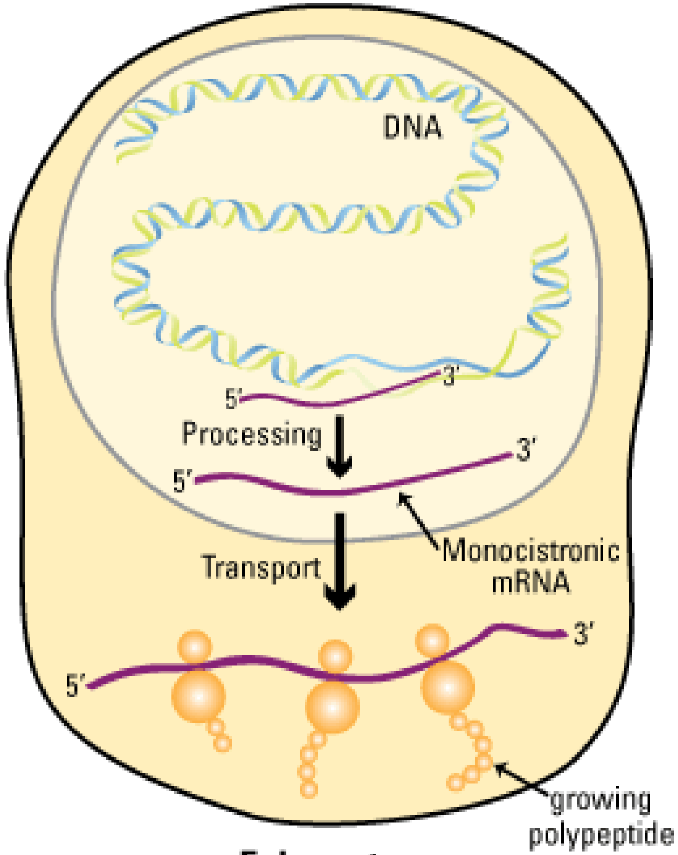
VALE A PENA LEMBRAR...



Transcrição e tradução simultâneas...Dai a importância do controle de expressão durante a transcrição!

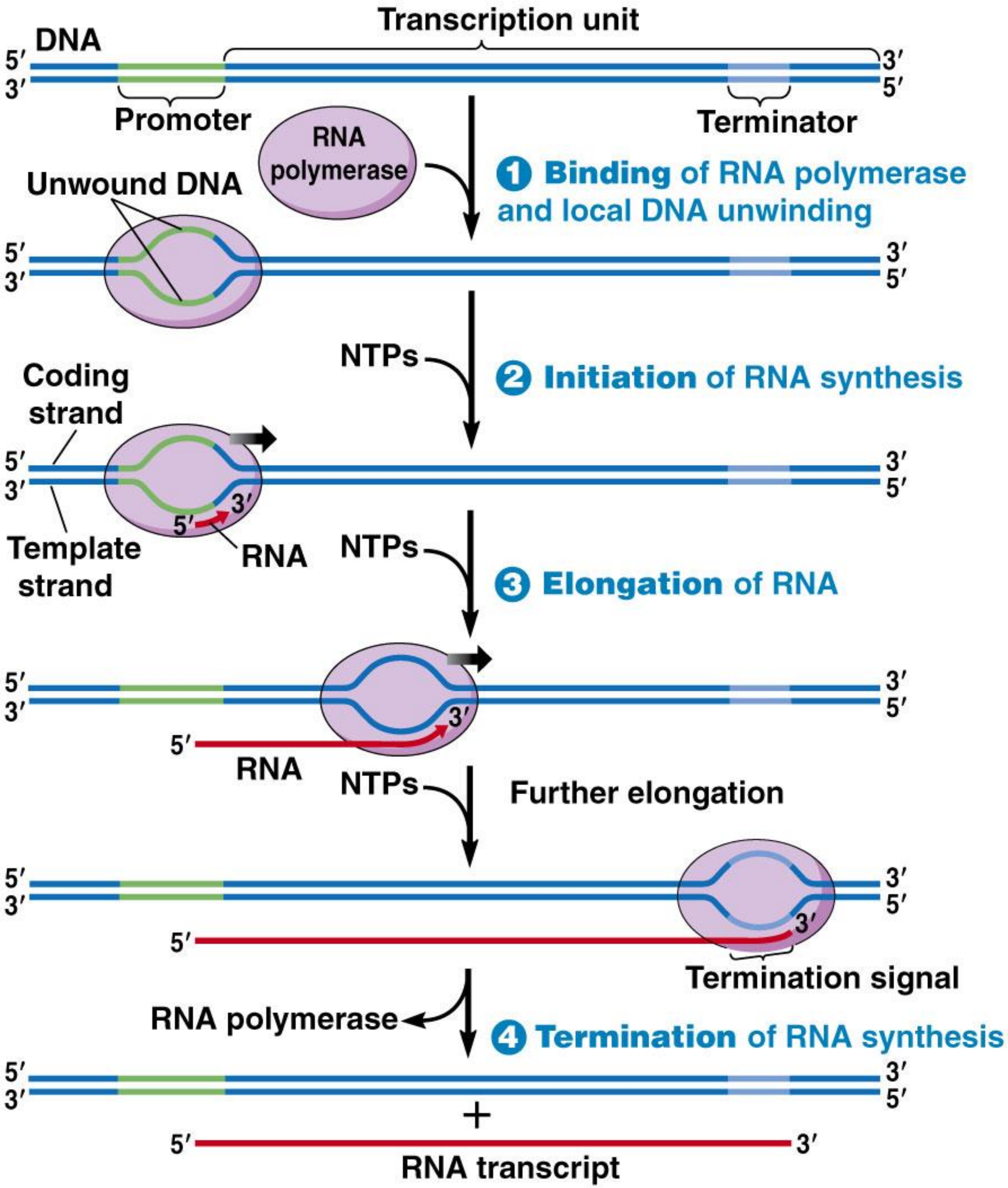


Prokaryotes



Eukaryotes

Estágio da transcrição

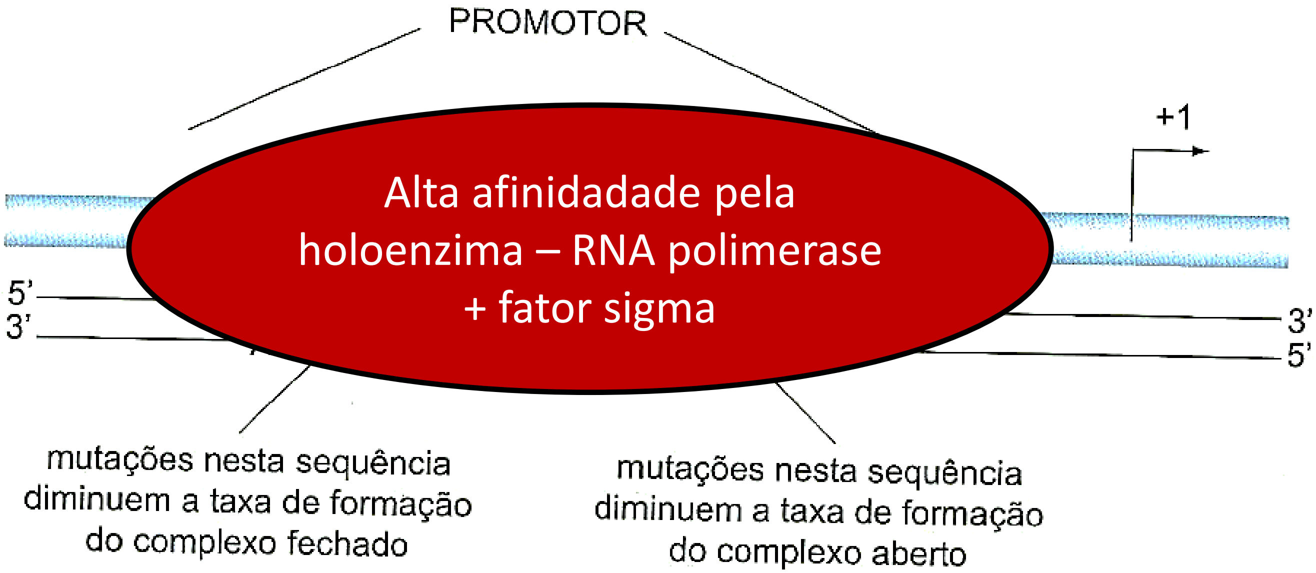


A REGULAÇÃO DA TRANSCRIÇÃO DEPENDE…

- Sequencia promotora
- Fatores de transcrição
- Pequenos ligantes
- Estrutura de dobradamento dos cromossomos

Todos juntos - ‘fine tuning’

DUAS REGIÕES DETERMINANTES PARA O INÍCIO TRANSCRIÇÃO



Alta afinidadade pela holoenzima – RNA polimerase + fator sigma

mutações nesta sequência diminuem a taxa de formação do complexo fechado

mutações nesta sequência diminuem a taxa de formação do complexo aberto

Sequências com menor afinidade mais precisamente reguladas

RNA POLIMERASE (RNAP)

Subunidade	Gene	Número	Função
Alfa	*rpoA*	2	Montagem estável da enzima. Reconhecimento do promotor Interação com fatores regulatórios
Beta	*rpoB*	1	Ligação dos ribonucleotideos
Beta’	*rpoC*	1	Ligação ao DNA
Omega	*rpoZ*	1	Montagme estável da enzima
Sigma	*rpoD, rpoS, rpoN …*	1	Ligação espefícia à sequência promotora

Evolution of multisubunit RNA polymerases in the three domains of life

Finn Werner and Dina Grohmann

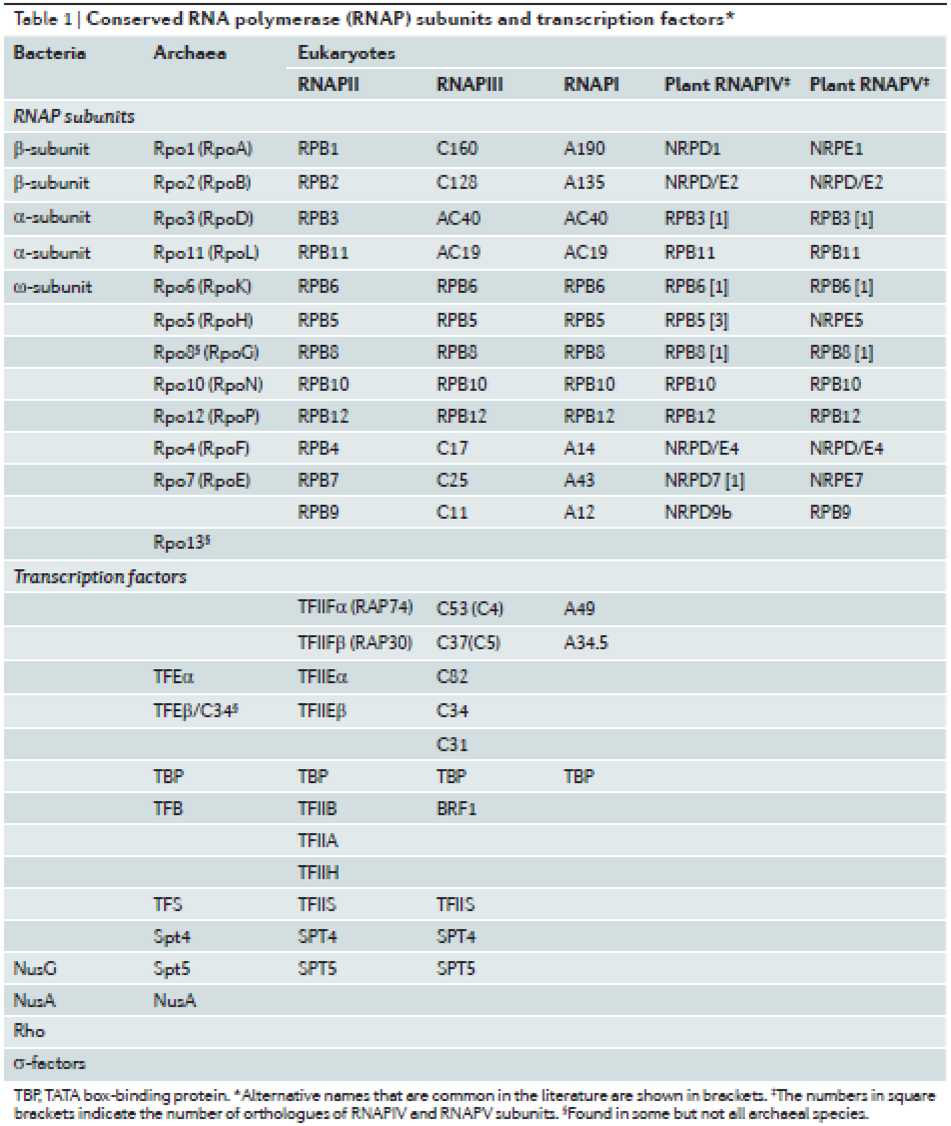
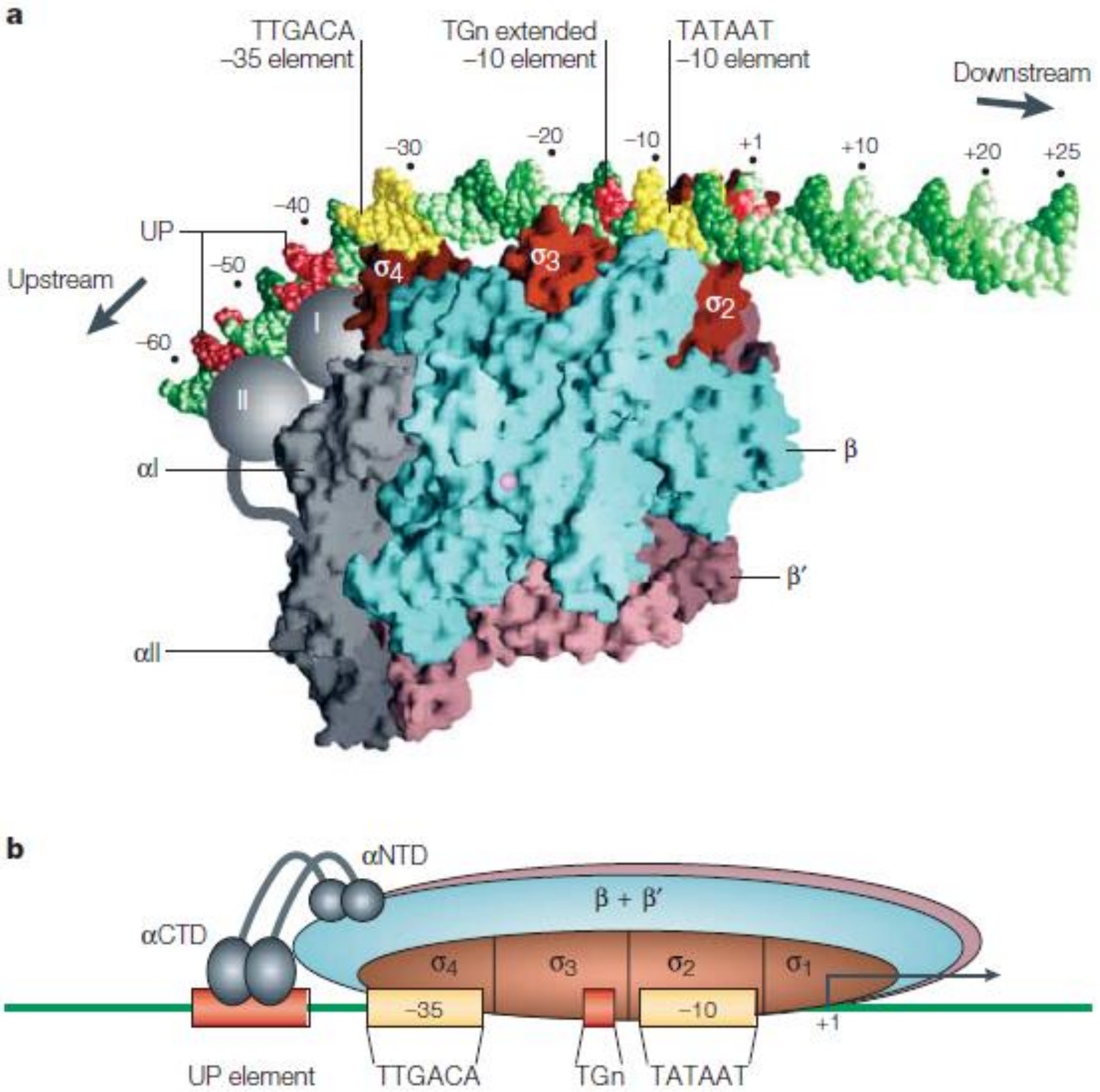


Table 1 | Conserved RNA polymerase (RNAP) subunits and transcription factors[*]

Bacteria	Archaea	Eukaryotes				
		RNAPII	**RNAPIII**	**RNAPI**	**Plant RNAPIV[‡]**	**Plant RNAPV[‡]**
RNAP subunits						
β-subunit	Rpo1 (RpoA)	RPB1	C160	A190	NRPD1	NRPE1
β-subunit	Rpo2 (RpoB)	RPB2	C128	A135	NRPD/E2	NRPD/E2
α-subunit	Rpo3 (RpoD)	RPB3	AC40	AC40	RPB3 [1]	RPB3 [1]
α-subunit	Rpo11 (RpoL)	RPB11	AC19	AC19	RPB11	RPB11
ω-subunit	Rpo6 (RpoK)	RPB6	RPB6	RPB6	RPB6 [1]	RPB6 [1]
	Rpo5 (RpoH)	RPB5	RPB5	RPB5	RPB5 [3]	NRPE5
	Rpo8[§] (RpoG)	RPB8	RPB8	RPB8	RPB8 [1]	RPB8 [1]
	Rpo10 (RpoN)	RPB10	RPB10	RPB10	RPB10	RPB10
	Rpo12 (RpoP)	RPB12	RPB12	RPB12	RPB12	RPB12
	Rpo4 (RpoF)	RPB4	C17	A14	NRPD/E4	NRPD/E4
	Rpo7 (RpoE)	RPB7	C25	A43	NRPD7 [1]	NRPE7
		RPB9	C11	A12	NRPD9b	RPB9
	Rpo13[§]					
Transcription factors						
		TFIIFα (RAP74)	C53 (C4)	A49		
		TFIIFβ (RAP30)	C37(C5)	A34.5		
	TFEα	TFIIEα	C82			
	TFEβ/C34[§]	TFIIEβ	C34			
			C31			
	TBP	TBP	TBP	TBP		
	TFB	TFIIB	BRF1			
		TFIIA				
		TFIIH				
	TFS	TFIIS	TFIIS			
	Spt4	SPT4	SPT4			
NusG	Spt5	SPT5	SPT5			
NusA	NusA					
Rho						
σ-factors						

TBP, TATA box-binding protein. [*] Alternative names that are common in the literature are shown in brackets. [‡] The numbers in square brackets indicate the number of orthologues of RNAPIV and RNAPV subunits. [§] Found in some but not all archaeal species.



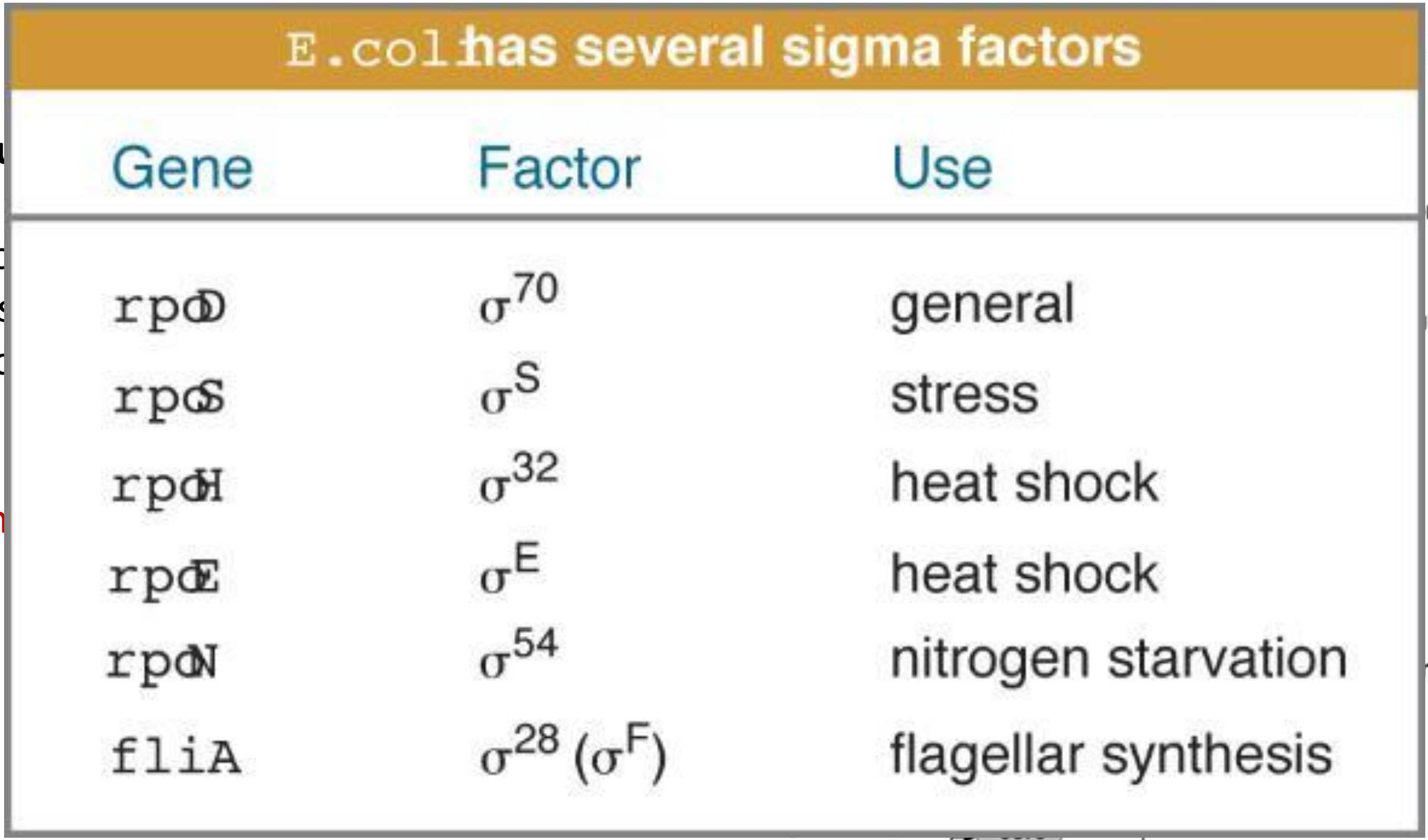
doi:10.1038/nrmicro787

FATORES SIGMA

Três fu

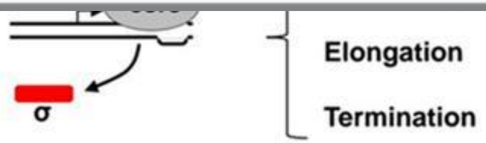
- Ga
  pro
- Pos
- Fac

A m



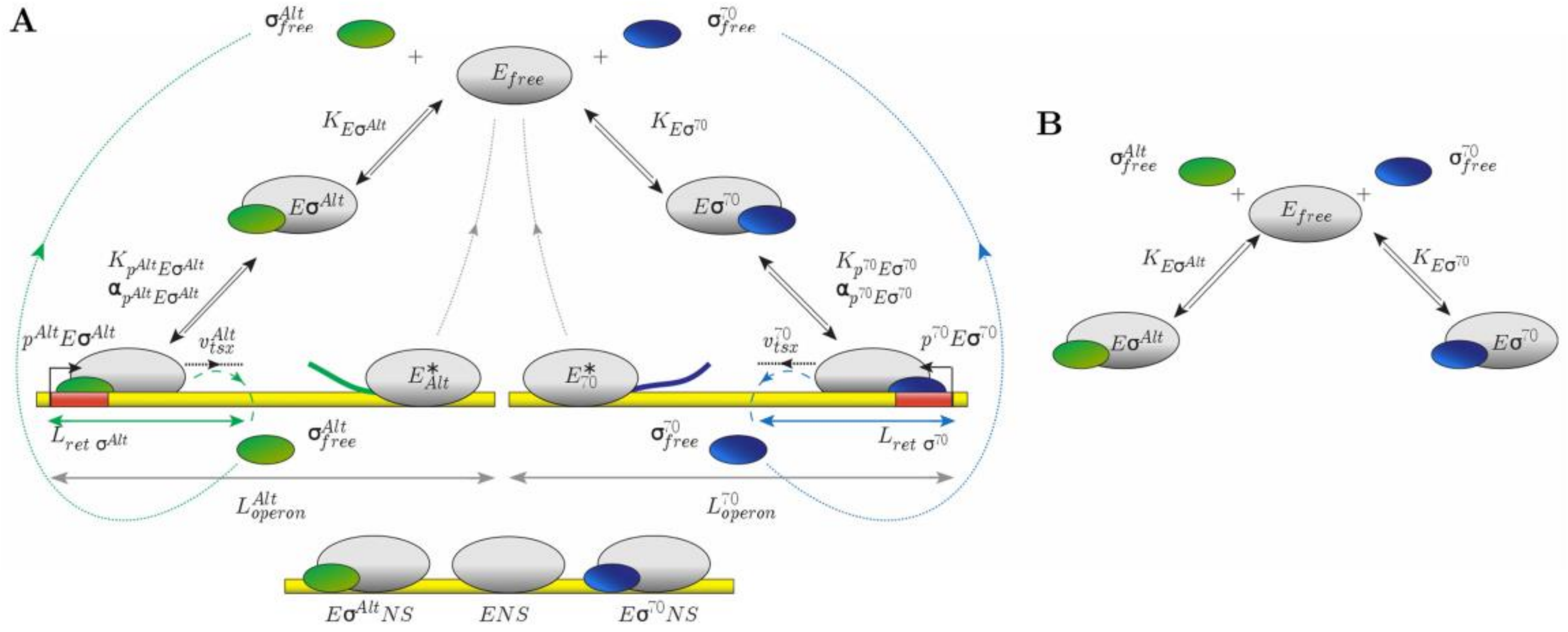
E.coli has several sigma factors

Gene	Factor	Use
rpoD	σ^{70}	general
rpoS	σ^{S}	stress
rpoH	σ^{32}	heat shock
rpoE	σ^{E}	heat shock
rpoN	σ^{54}	nitrogen starvation
fliA	σ^{28} (σ^{F})	flagellar synthesis



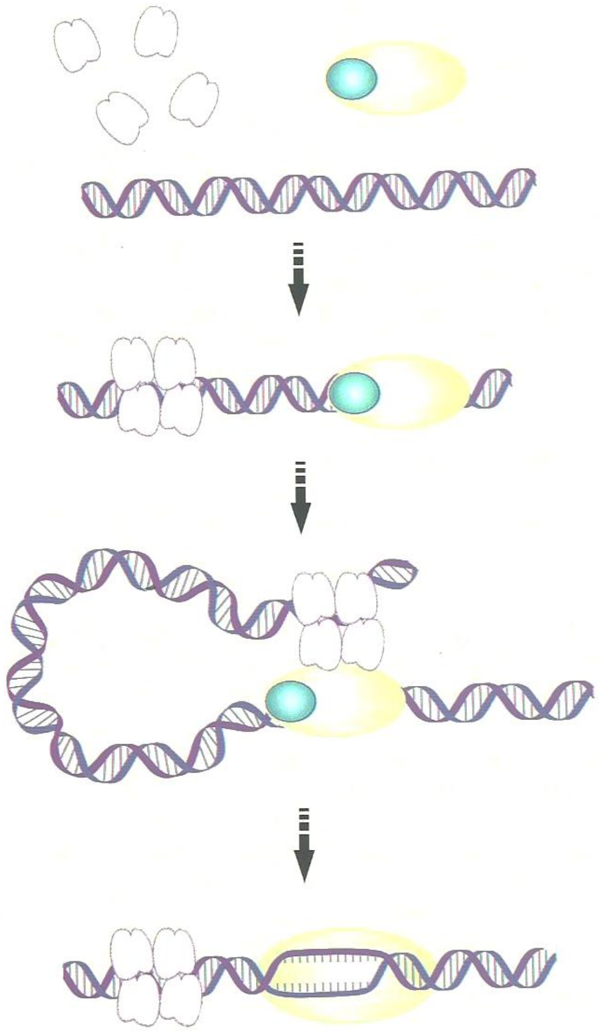
Número de RNA polimerase é limitado.. .4,000–5. 000 genes nas células gera competição!

Um gene pode ter mais de um promotor com afinidade diferentes por fatores sigmas!



doi:10.1371/journal.pcbi.1003845.g001

FATOR SIGMA 54...UM CASO ESPECIAL



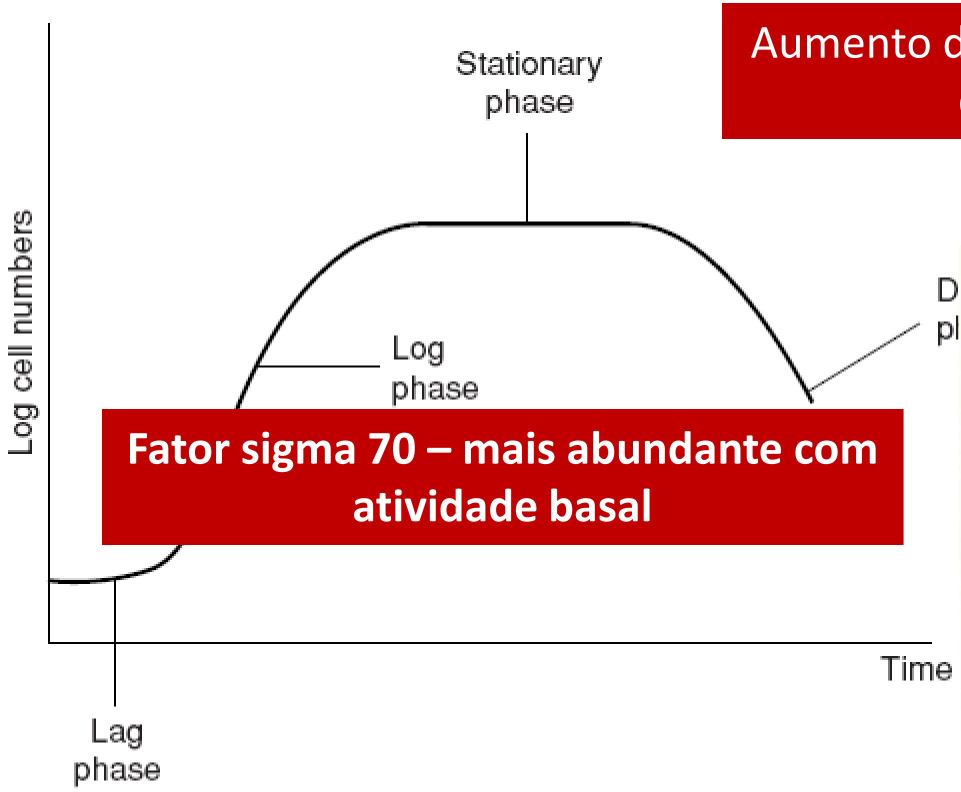
Responsável pela ativação de processos como: metabolismo de nitrogênio, fontes alternativas de carbono, quimiotaxia, formação de flagelos e fimbria, etc.

Se liga ao promotor mas não é capaz de abrir a fita de DNA – se liga -24 e -12
Ativado pela proteína IHF (*Integration host factor*) – 100 pb a montante.

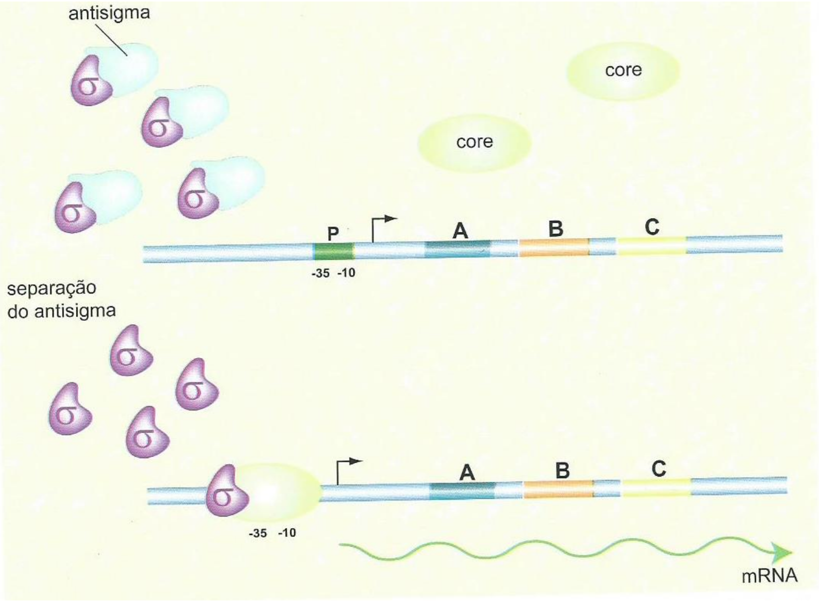
Qual vantagem?

E OS OUTROS FATORES SIGMA?

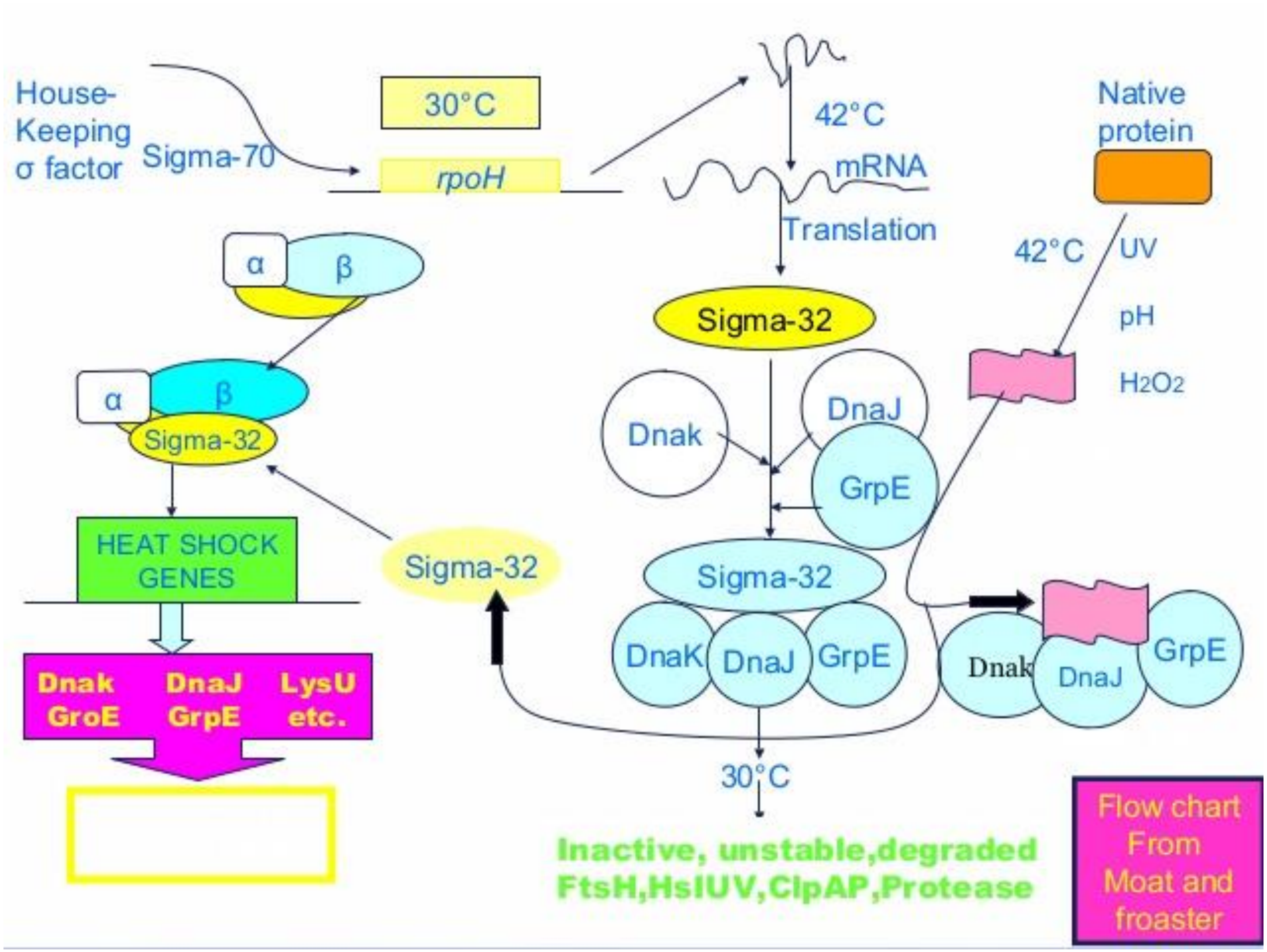
Aumento do fator Rsd e do RNA 6S – favorece genes dependentes de outros fatores



Fator sigma 70 – mais abundante com atividade basal



AUTORREGULAÇÃO – SIGMA 32

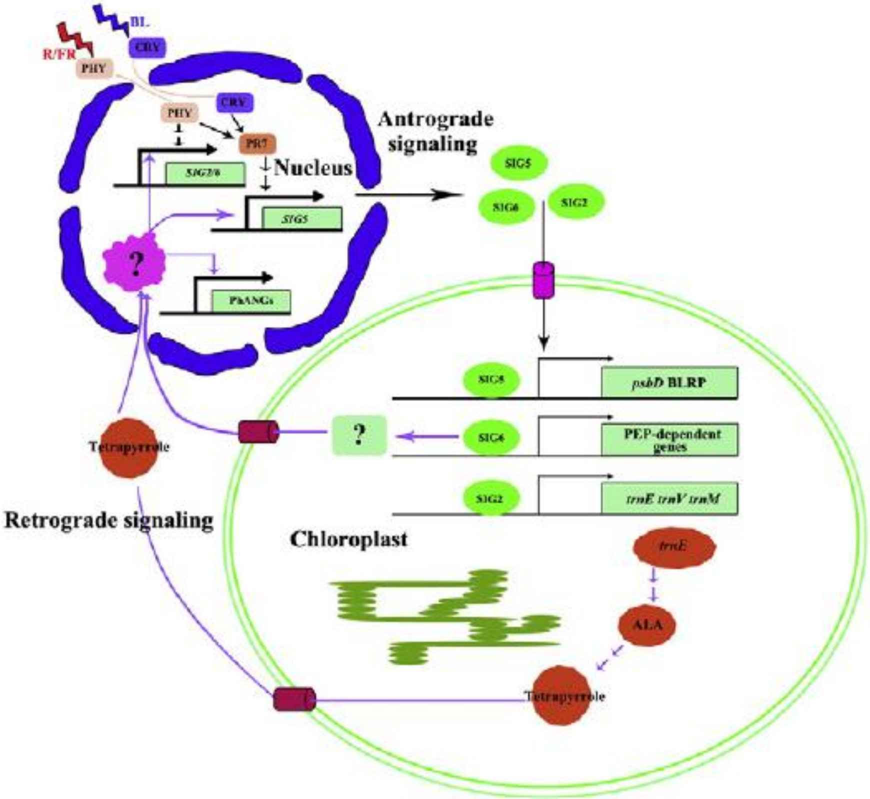


Review

Plastid sigma factors: Their individual functions and regulation in transcription☆

Wei Chi *, Baoye He, Juan Mao, Jingjing Jiang, Lixin Zhang

Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China



ATIVADORES X REPRESSORES X INDUTORES X CO-REPRESSORES

Fatores de transcrição ou **TF**s são proteínas que se ligam ao DNA de células eucarióticas para permitir que haja uma ligação entre a enzima RNA-polimerase e o DNA, permitindo assim a transcrição e a futura tradução. Qualquer proteína necessária para o início da transcrição, mas que não seja parte integrante do RNA polimerase

???

Usa-se o termo reguladores: proteínas que podem agir ativando ou reprimindo a expressão com a ajuda ou não de pequenas molecular não proteícas

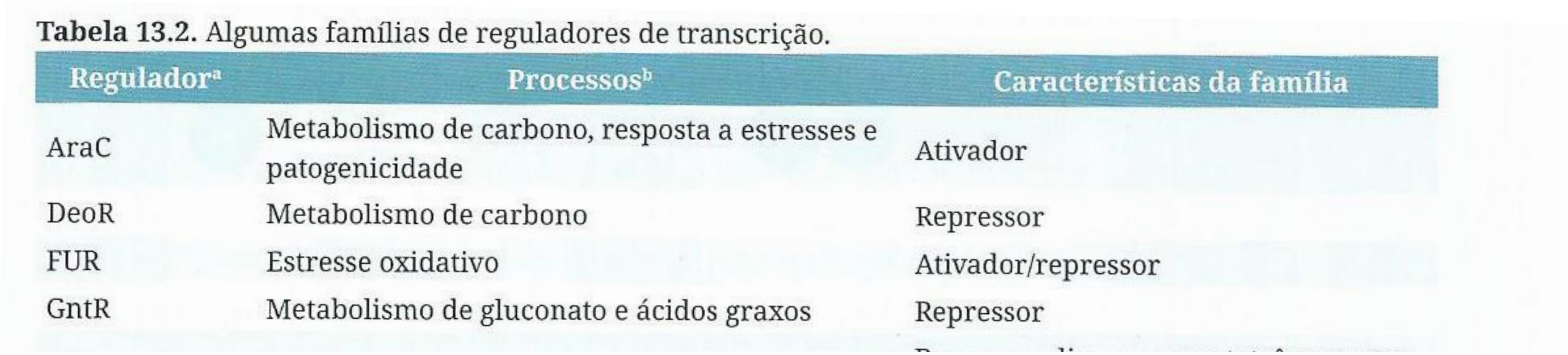
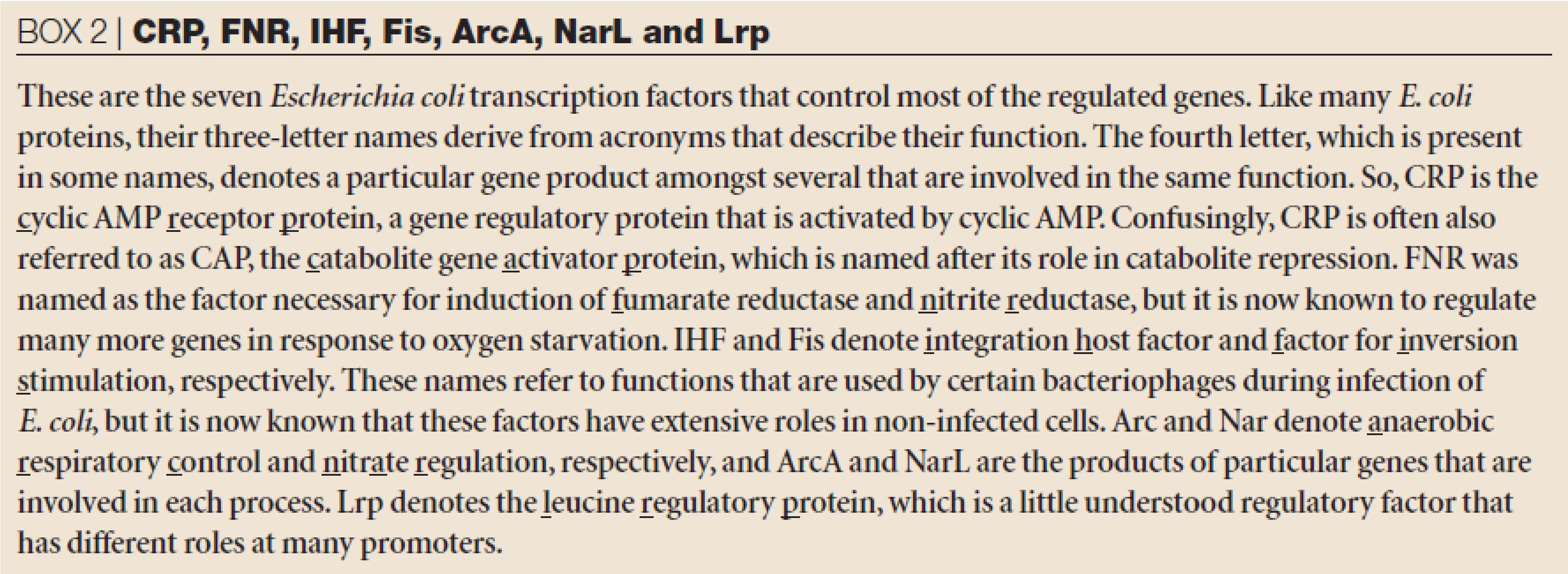


Tabela 13.2. Algumas famílias de reguladores de transcrição.

Regulador[a]	Processos[b]	Características da família
AraC	Metabolismo de carbono, resposta a estresses e patogenicidade	Ativador
DeoR	Metabolismo de carbono	Repressor
FUR	Estresse oxidativo	Ativador/repressor
GntR	Metabolismo de gluconato e ácidos graxos	Repressor

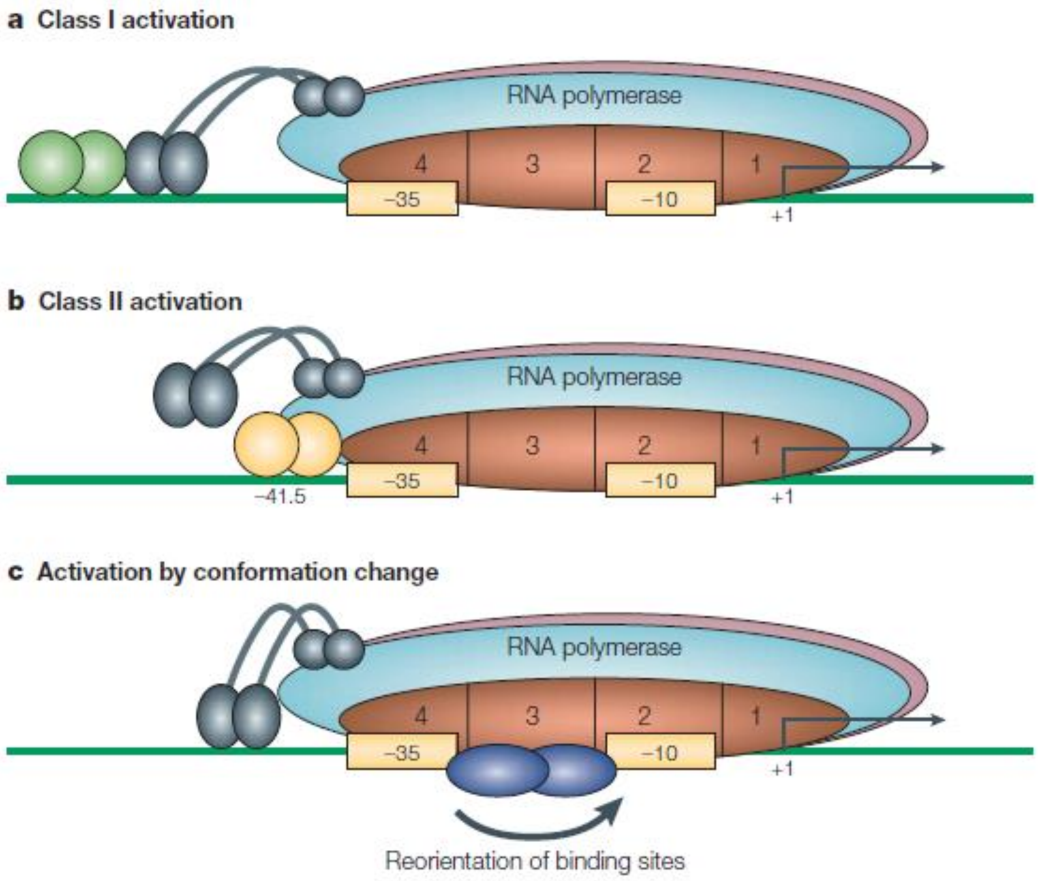


BOX 2 | **CRP, FNR, IHF, Fis, ArcA, NarL and Lrp**

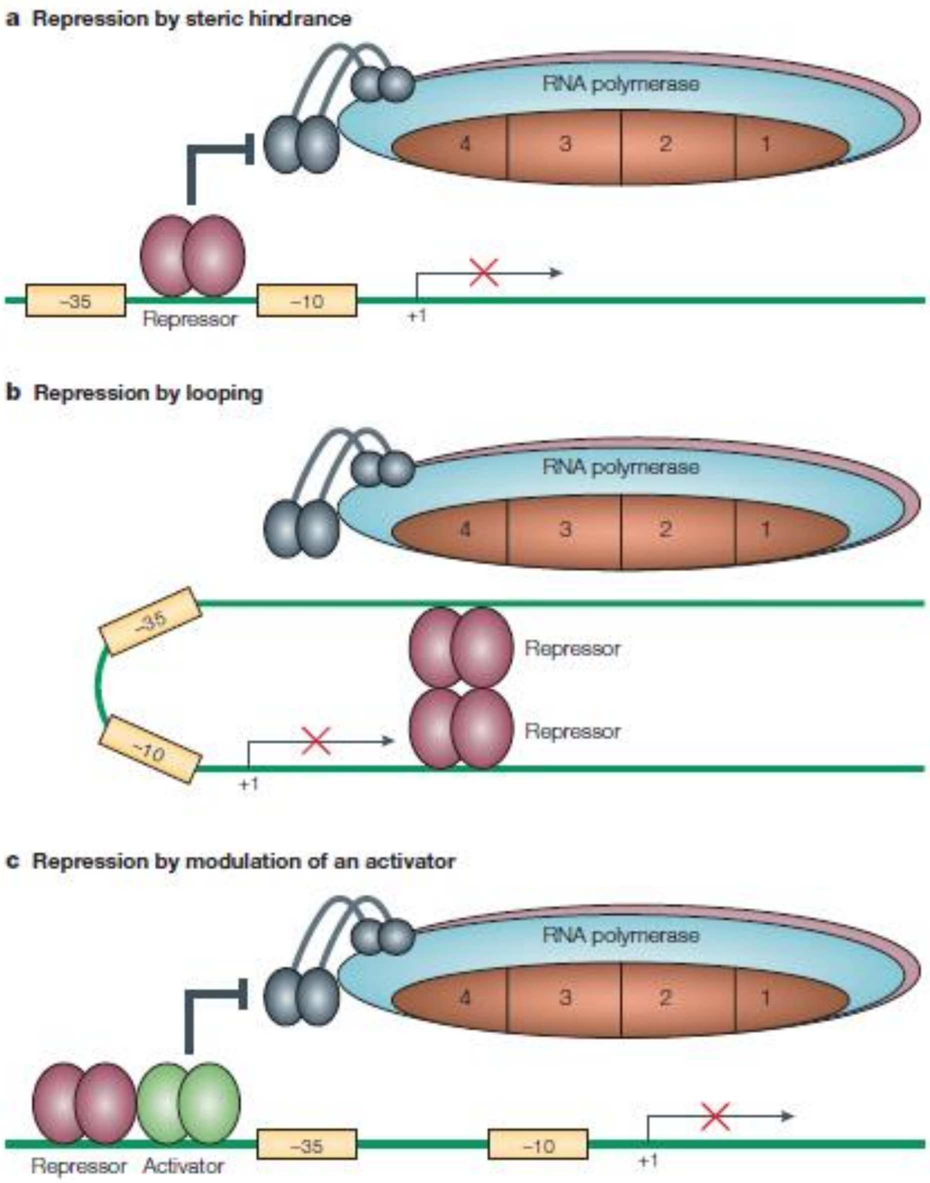
These are the seven *Escherichia coli* transcription factors that control most of the regulated genes. Like many *E. coli* proteins, their three-letter names derive from acronyms that describe their function. The fourth letter, which is present in some names, denotes a particular gene product amongst several that are involved in the same function. So, CRP is the cyclic AMP receptor protein, a gene regulatory protein that is activated by cyclic AMP. Confusingly, CRP is often also referred to as CAP, the catabolite gene activator protein, which is named after its role in catabolite repression. FNR was named as the factor necessary for induction of fumarate reductase and nitrite reductase, but it is now known to regulate many more genes in response to oxygen starvation. IHF and Fis denote integration host factor and factor for inversion stimulation, respectively. These names refer to functions that are used by certain bacteriophages during infection of *E. coli*, but it is now known that these factors have extensive roles in non-infected cells. Arc and Nar denote anaerobic respiratory control and nitrate regulation, respectively, and ArcA and NarL are the products of particular genes that are involved in each process. Lrp denotes the leucine regulatory protein, which is a little understood regulatory factor that has different roles at many promoters.

7 regulares de transcrição controlam mais de 50% do genes em *E. coli*!!!

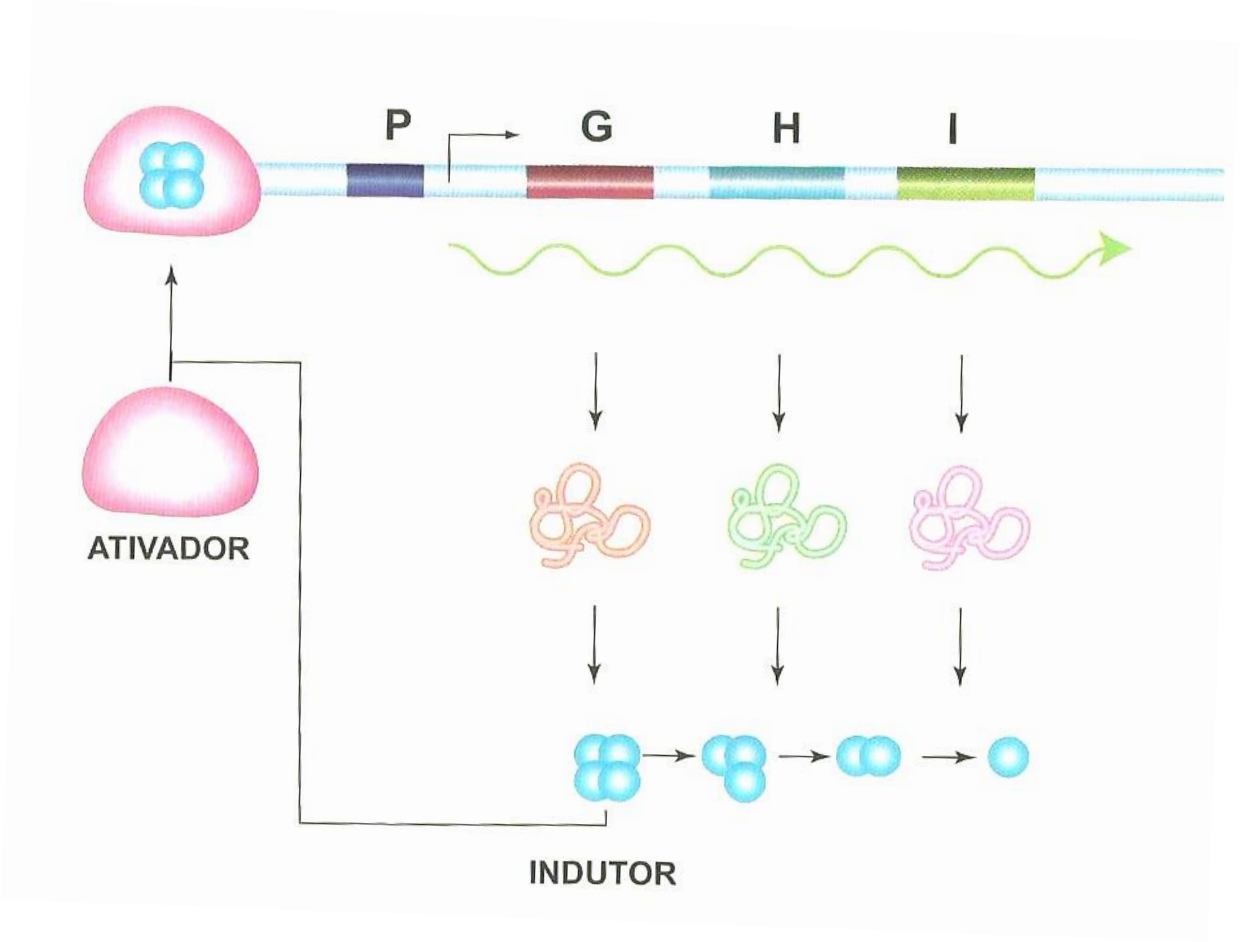
CLASSES DE ATIVIDORES



CLASSES DE REPRESSORES

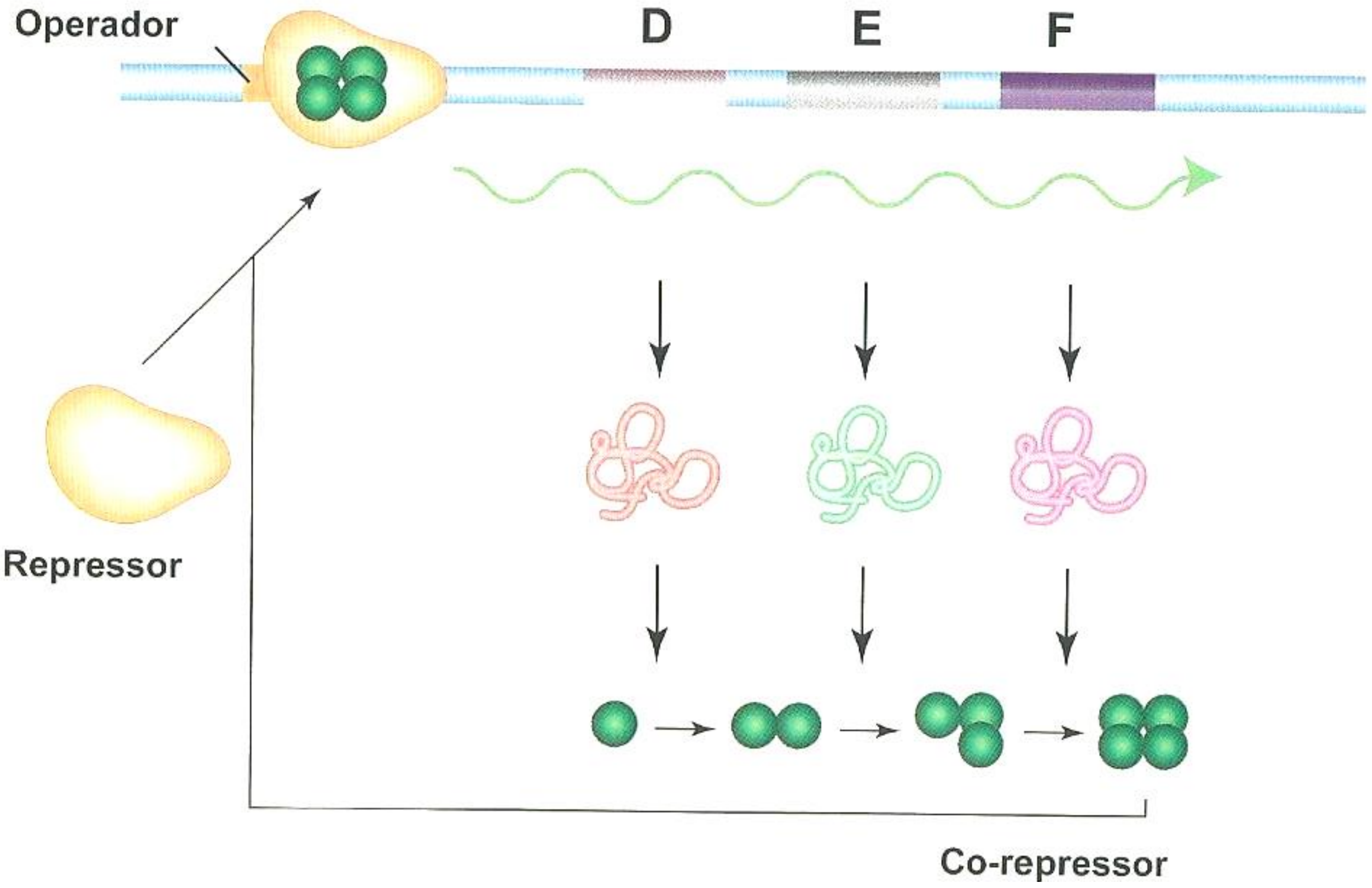


CONTROLE DO METABOLISMO



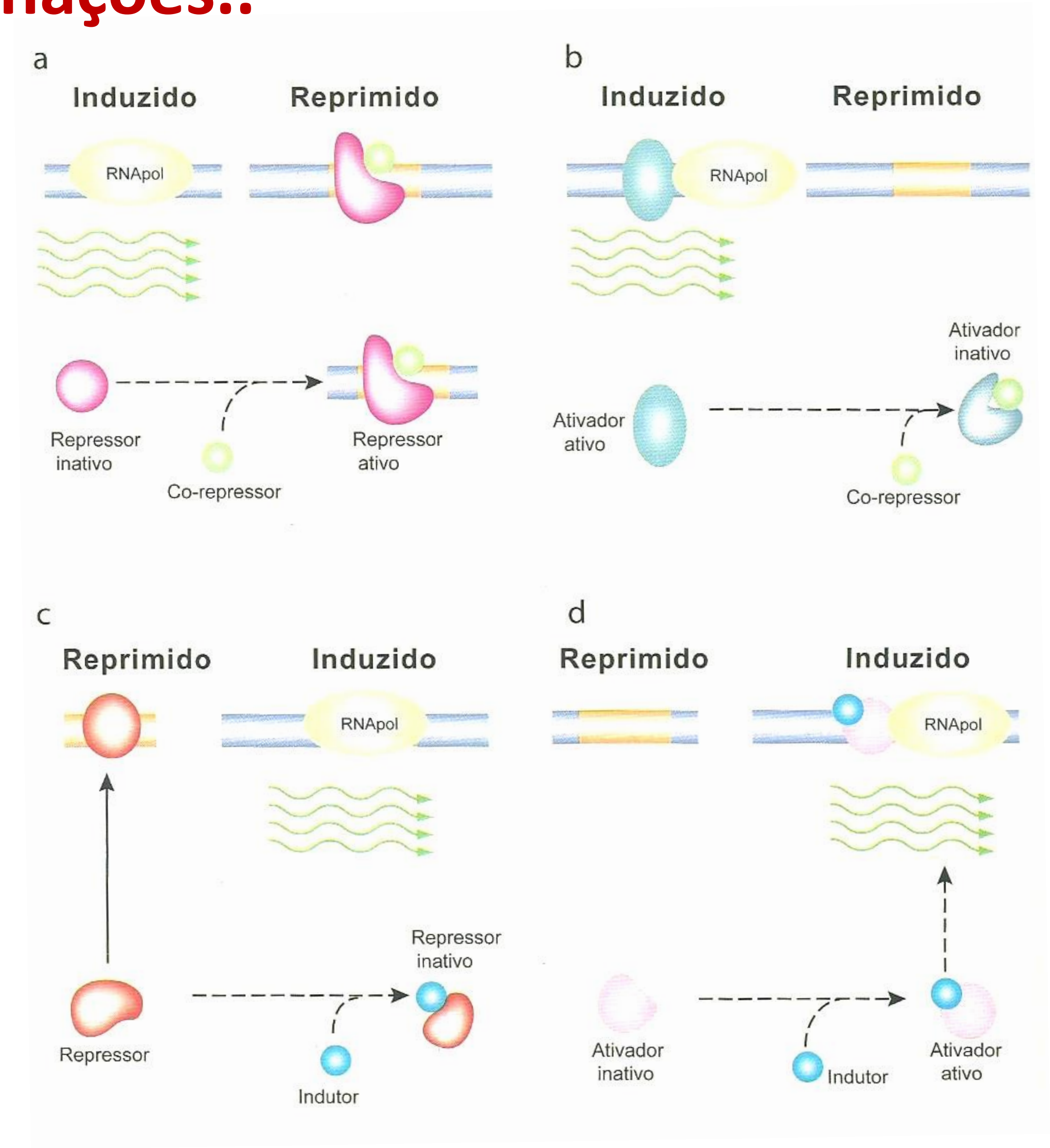
Importante em processos catabólicos!

CONTROLE DO METABOLISMO



Importante em processos anabólicos!

Possíveis combinações..



a – entrada de um repressor
b – saída de um ativador
c - saída de um repressor
d – ligação de um ativador

Co-dependência de um ou mais ativadores/repressores: mais sofisticado é o mecanismo

Real “fine tuning’

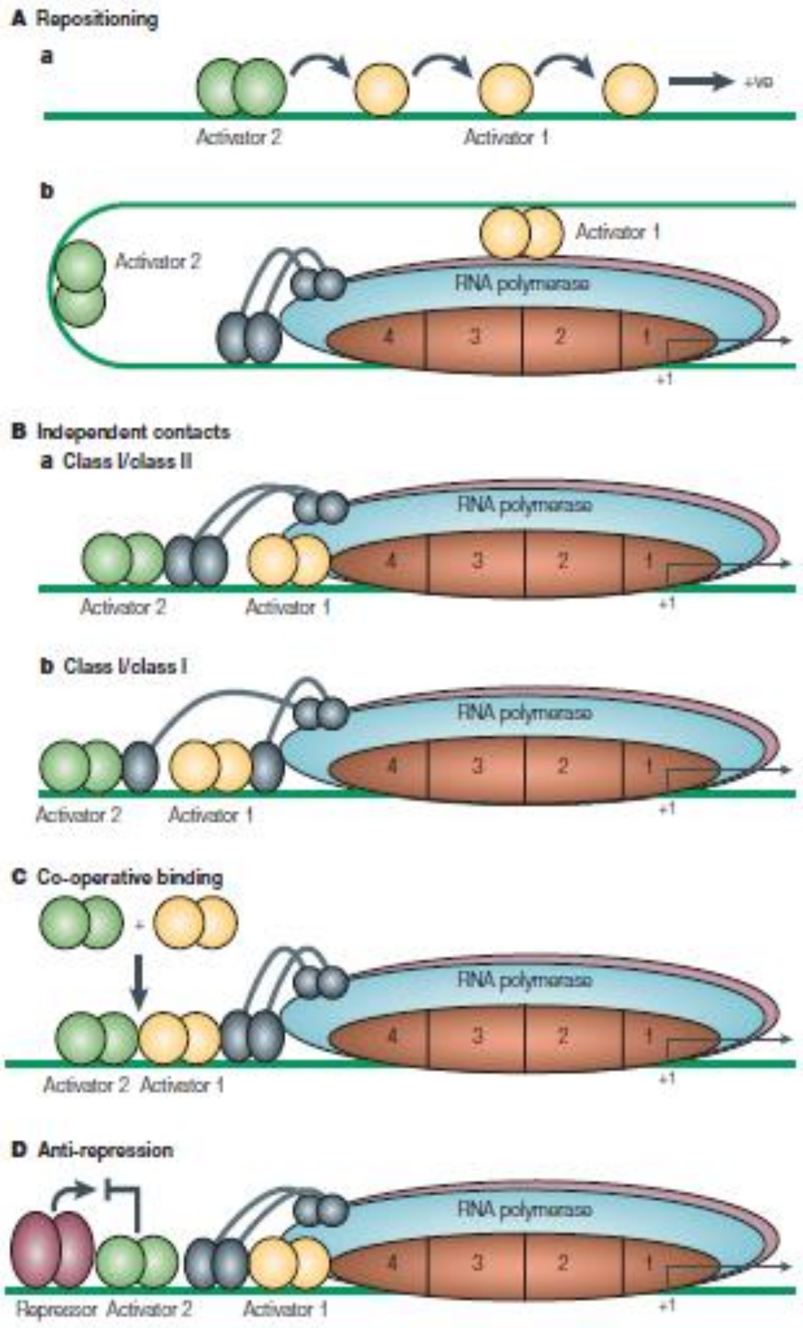
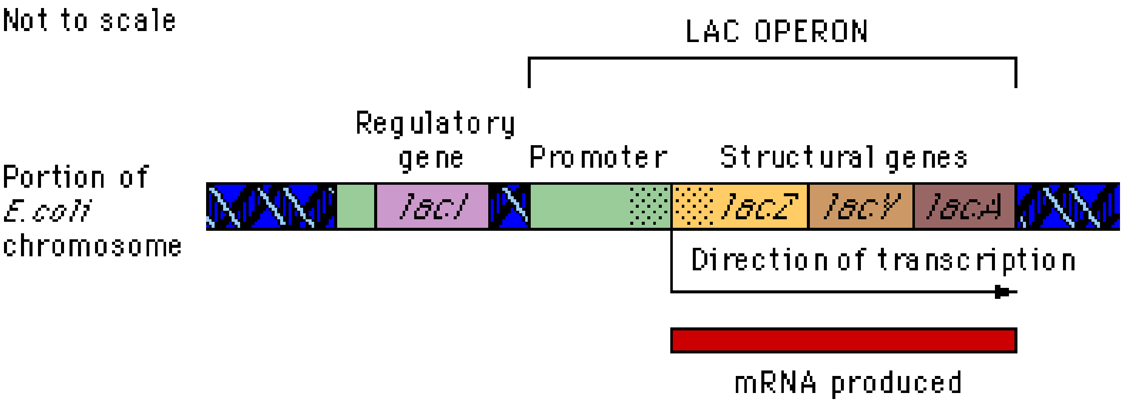
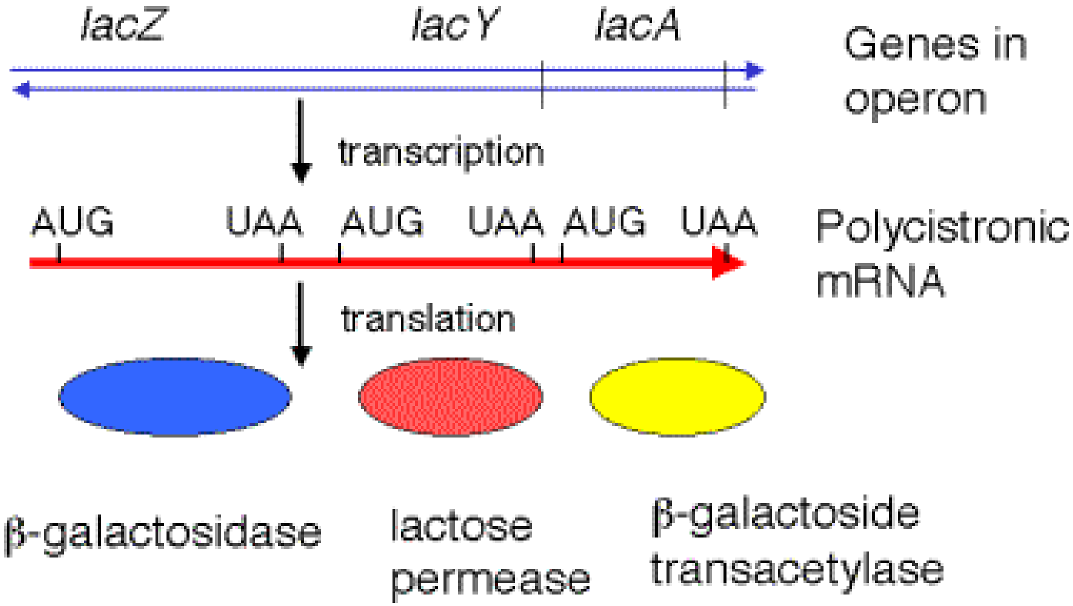


Figure 5 | **Mechanisms of promoter co-dependence on two activator proteins**

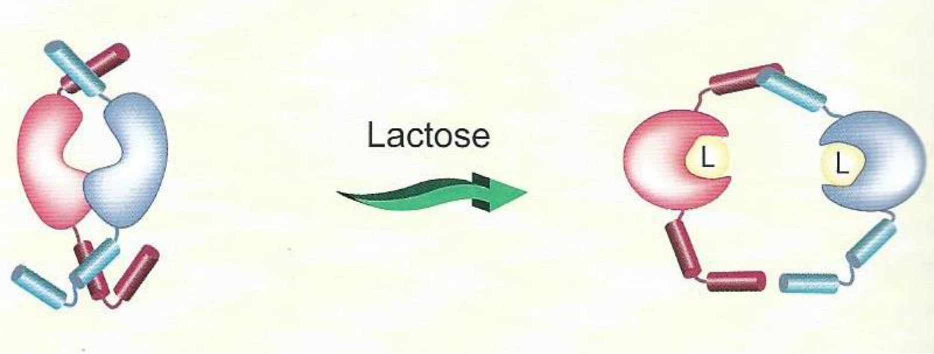
SISTEMA LACZ – SISTEMA MODELO…





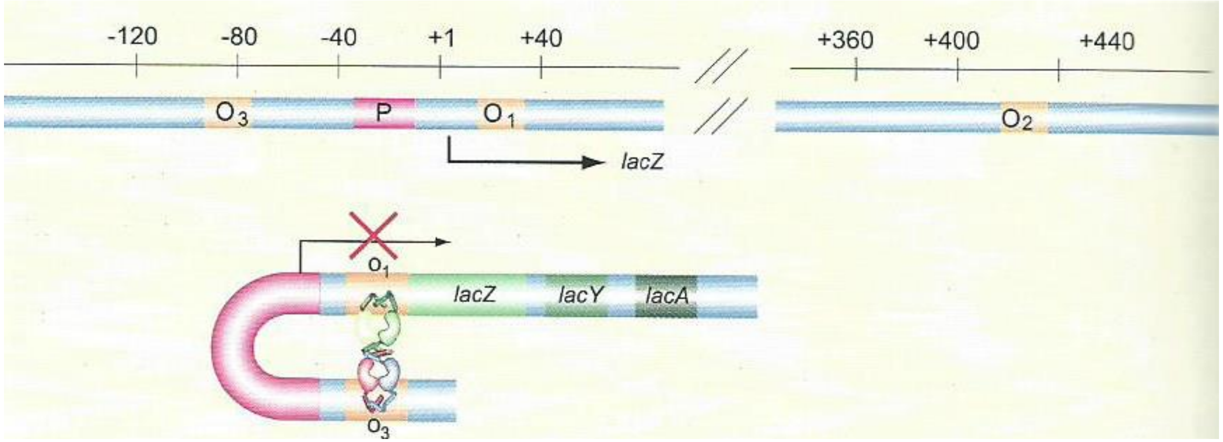
ECONOMIA É TUDO…

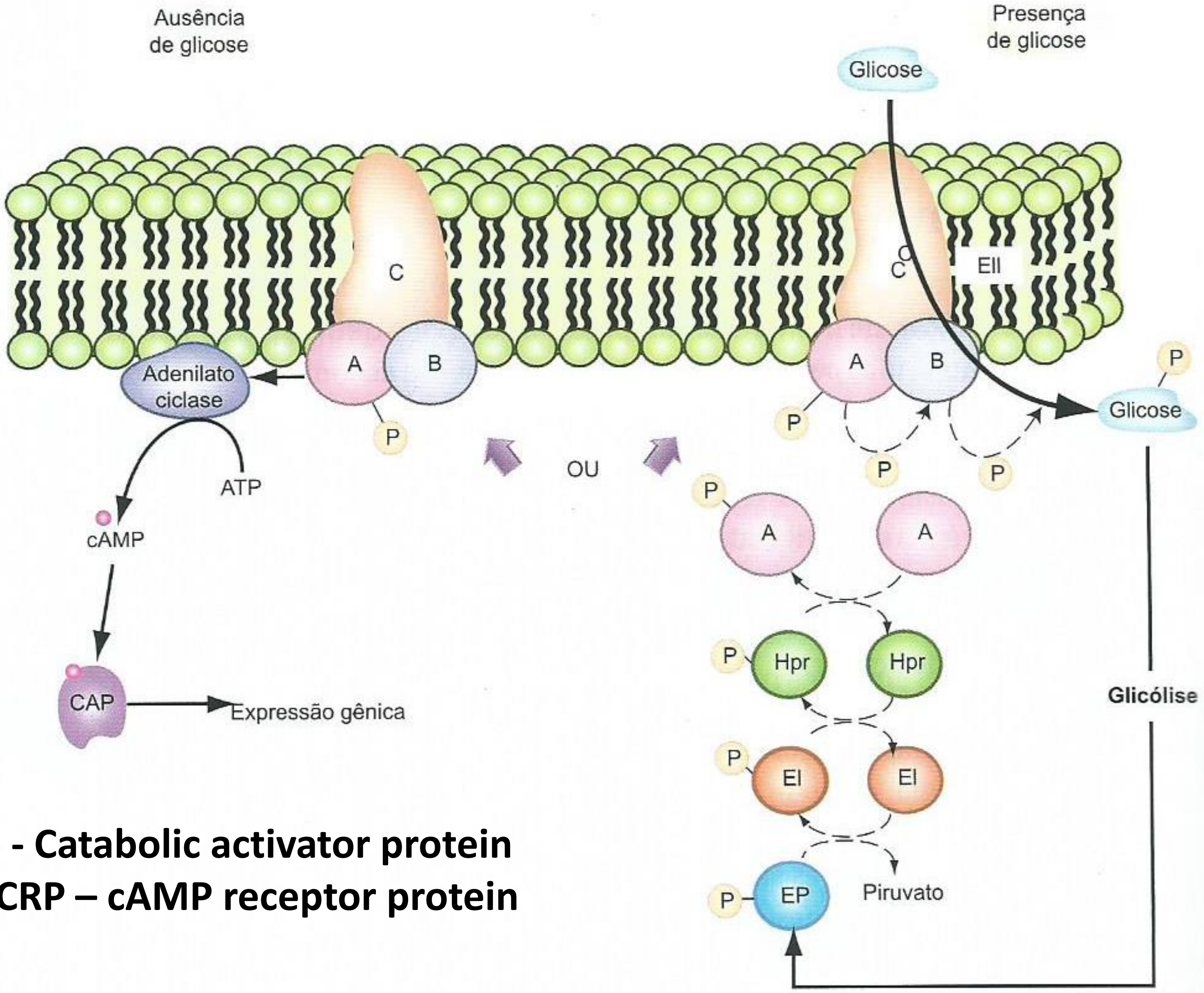
LacI



Tetrâmero com domínio
aminoterminal - HTH
Parte C terminal - tetramerização

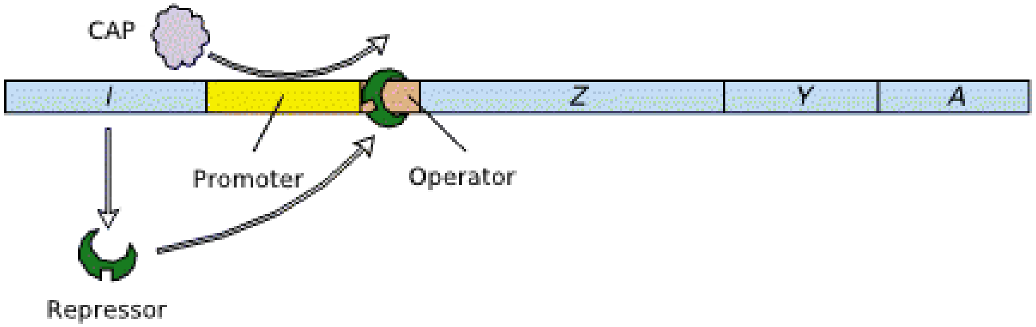
A logistica é perfeita!!



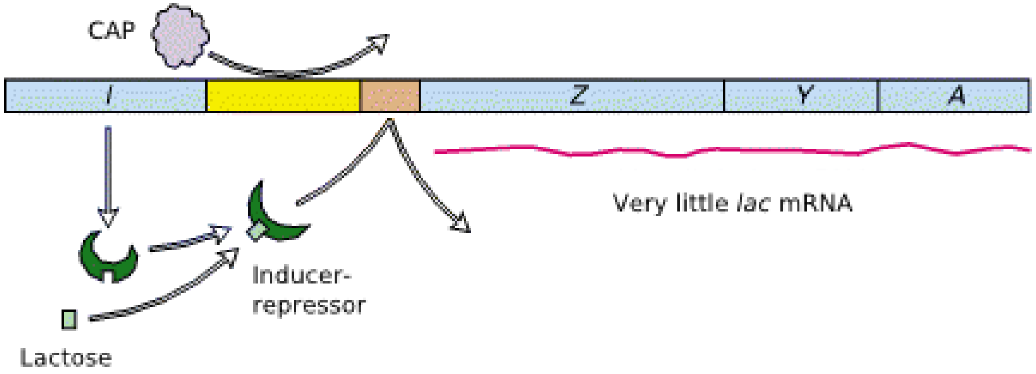


CAP - Catabolic activator protein
ou CRP – cAMP receptor protein

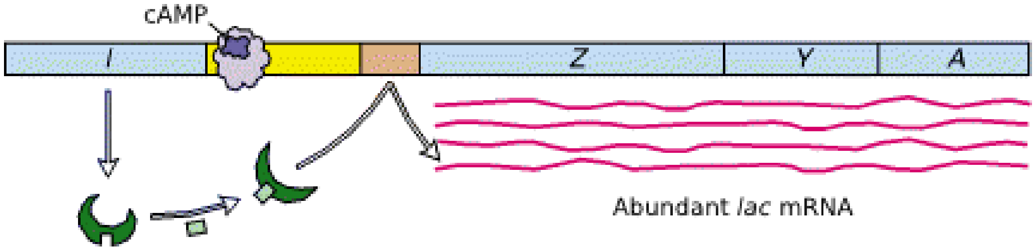
(a) Glucose present, lactose absent



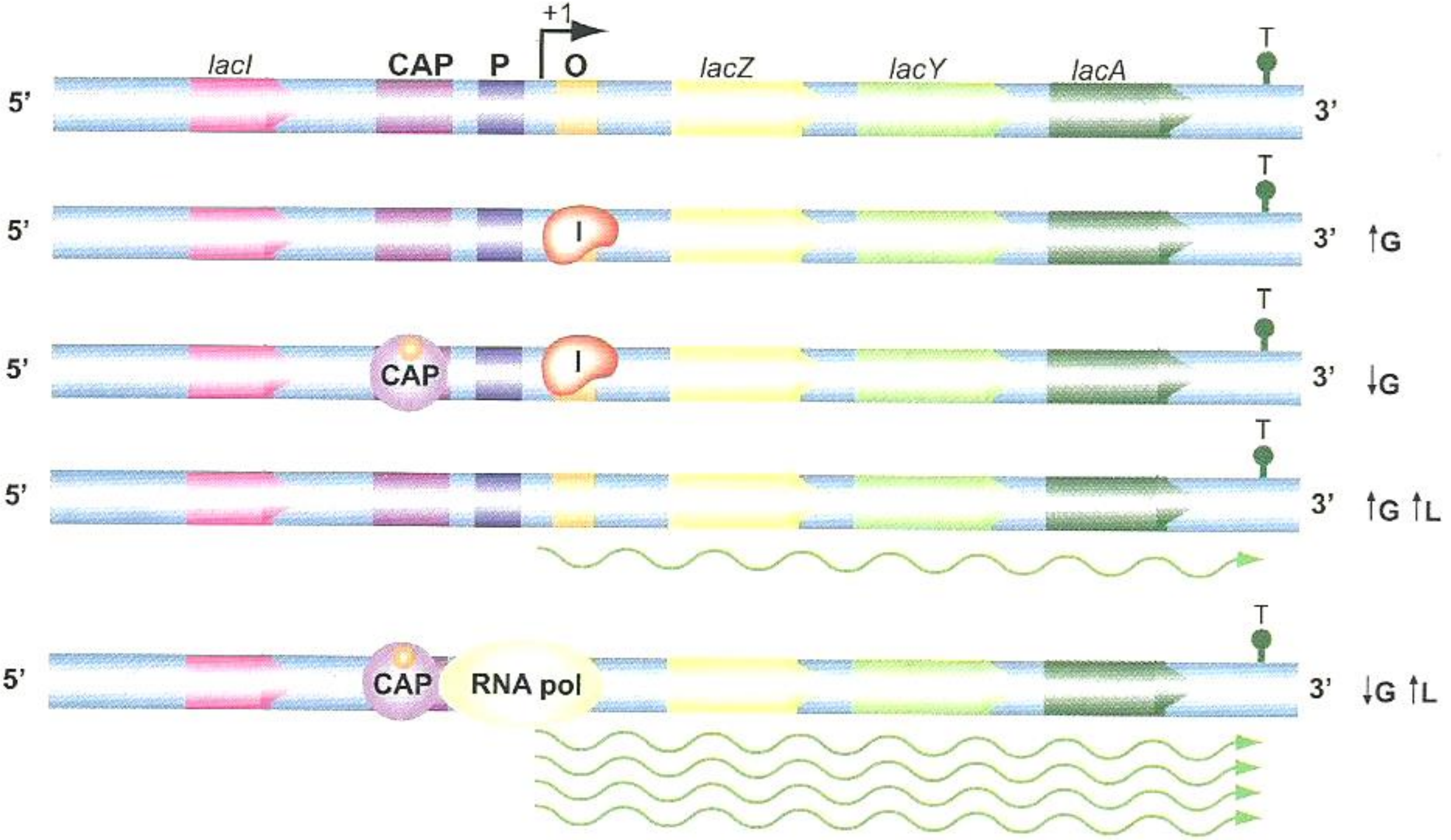
(b) Glucose present, lactose present



(c) Glucose absent, lactose present



SÃO DOIS SISTEMAS INDEPENDENTES DE CONTROLE!



Bacterial nucleoid-associated proteins, nucleoid structure and gene expression

Shane C. Dillon and Charles J. Dorman

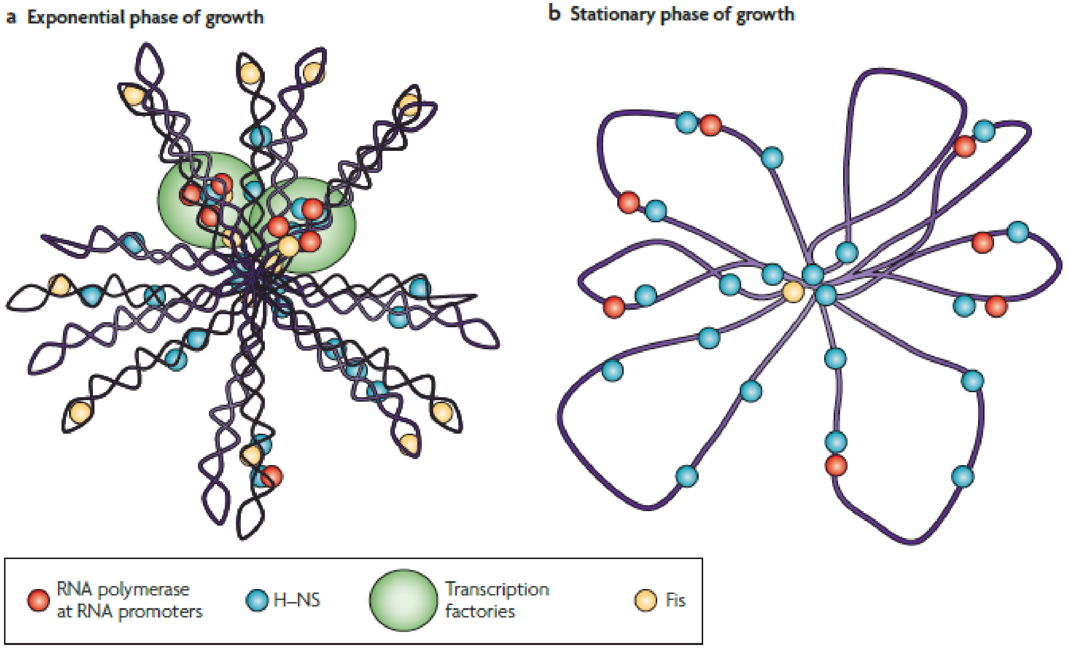
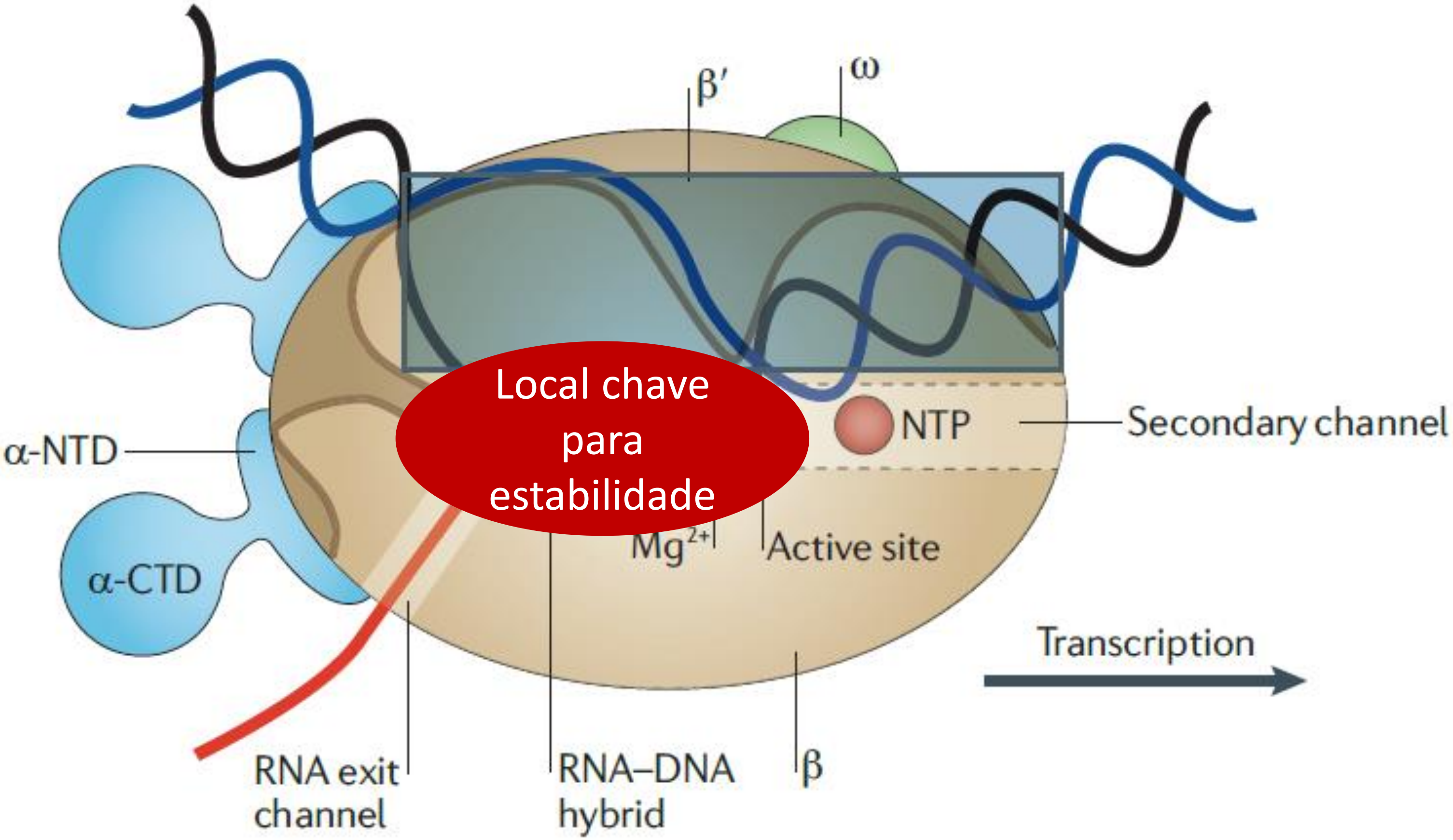


Figure 1 | **Nucleoid-associated proteins and DNA supercoiling influence nucleoid structure. a** | The folded chromosome is organized into looped domains that are negatively supercoiled during the exponential phase of growth. In this phase, the abundant nucleoid-associated proteins histone-like nucleoid-structuring protein (H-NS) and factor for inversion stimulation (Fis) bind throughout the nucleoid and are associated with the seven ribosomal RNA operons. As shown here in two cases, these are organized into superstructures called transcription factories. **b** | In stationary phase the rRNA operons are quiescent and Fis is almost undetectable. The chromosome has fewer looped domains, and those that are visible consist of relaxed DNA.

SIGMA NÃO ESTÁ MAIS PRESENTE

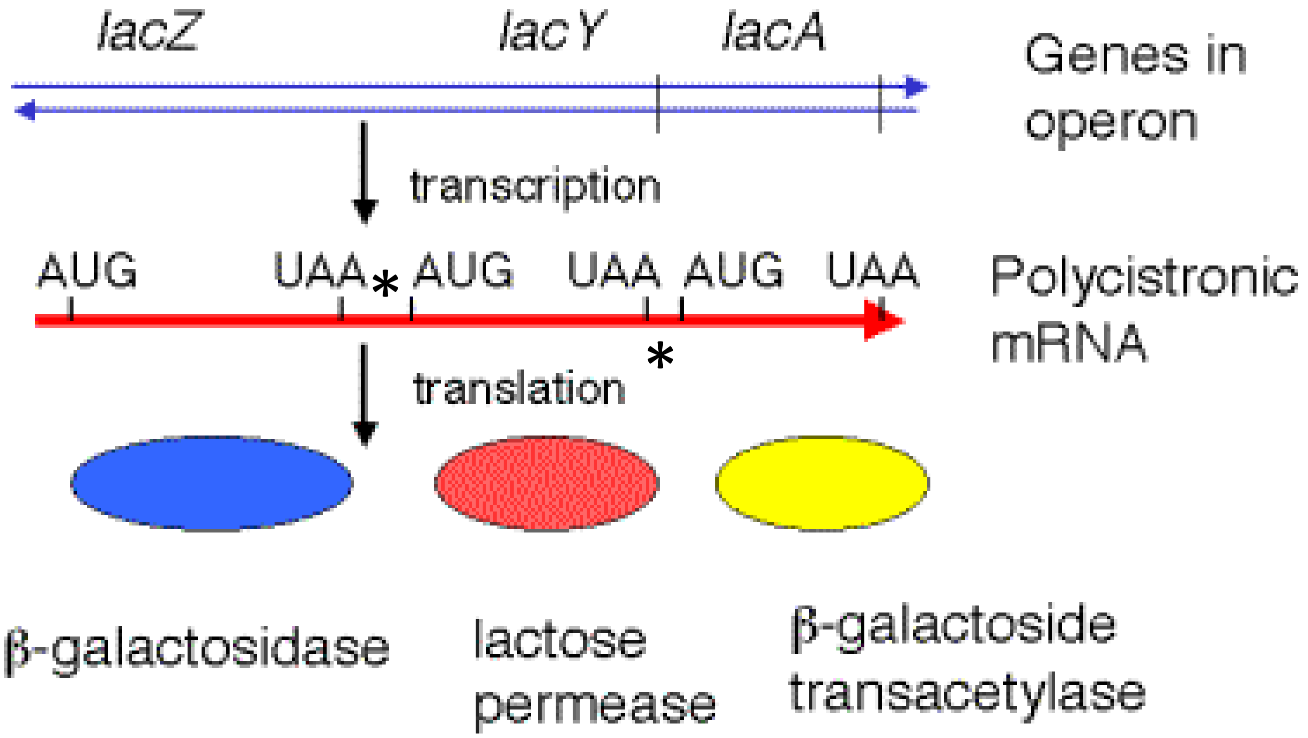


Local chave para estabilidade

Precisa manter a estabilidade do complexo de elongação

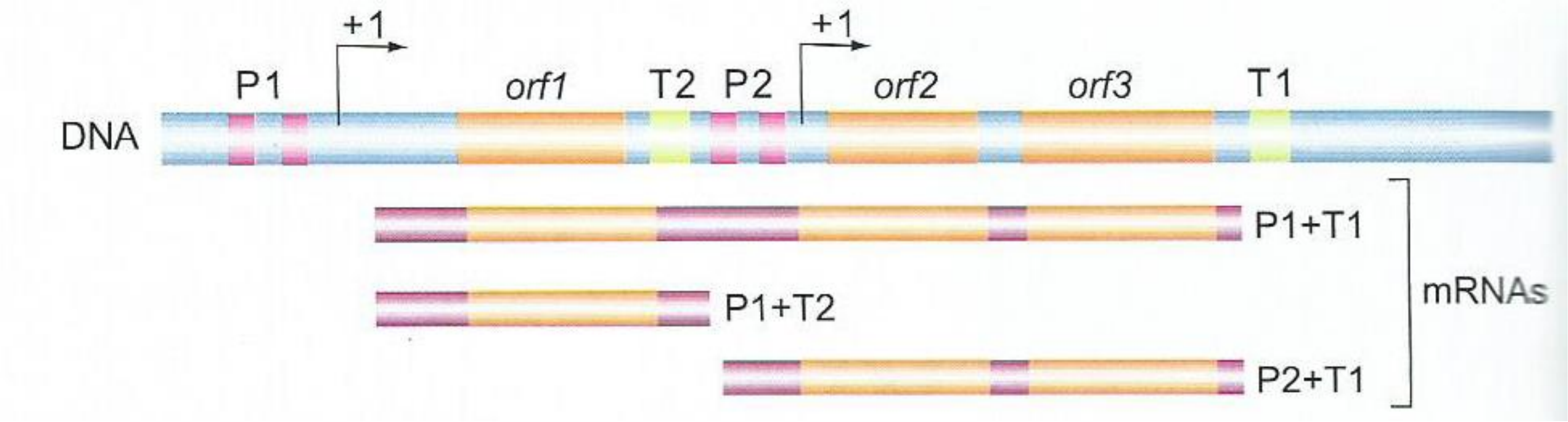
doi: 10.1038/nrmicro2560

GENES BACTERIANOS EM OPERON



* Sitios de parada: ocorre *check points*, início da tradução...

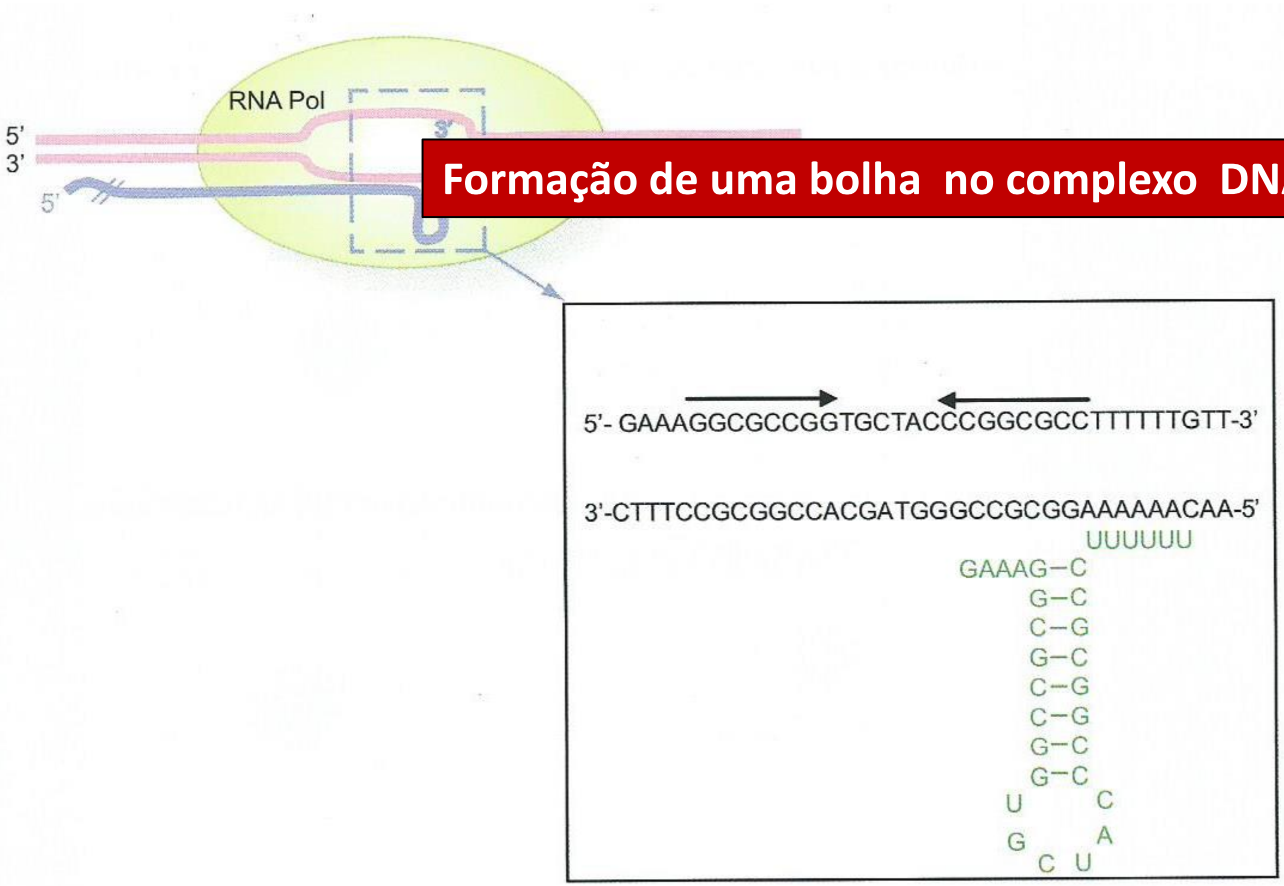
MAS COMO A RNA POLIMERASE SABE QUANDO PARAR DE TRANSCREVER?



Qual(s) desses mRNA é o correto?

Comum haver vários pontos de terminação!!!

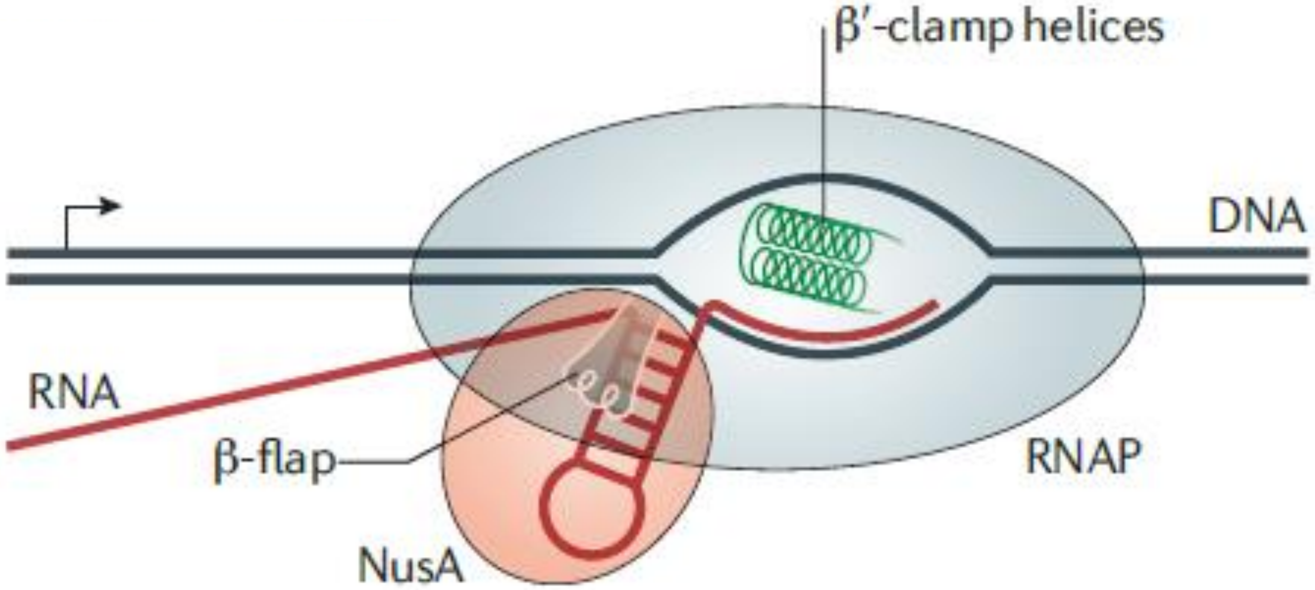
TERMINAÇÃO INTRINSECO



Formação de uma bolha no complexo DNA-RNA

Não é vital mas pode ser auxiliado por proteínas: exemplo NusA

Características:



E. Coli - 80% da terminação é intrinseca…sequência canônica facilmente detectável – ricas em CG

Algumas bactérias e várias arquéias não apresentam nenhuma sequência intrínseca de terminação!

doi: 10.1038/nrmicro2560

JOURNAL OF BACTERIOLOGY, Nov. 2009, p. 7102–7108
0021-9193/09/$12.00 doi:10.1128/JB.00982-09

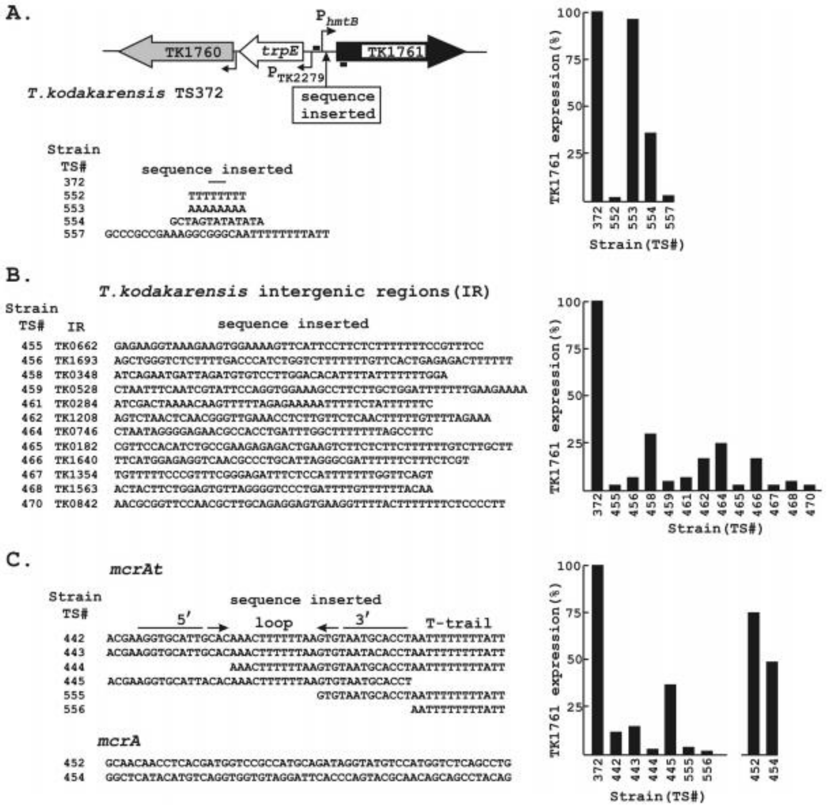
Vol. 191, No. 22

Archaeal Intrinsic Transcription Termination In Vivo▿

Thomas J. Santangelo, L'ubomíra Cubonová, Katherine M. Skinner, and John N. Reeve*
Department of Microbiology, Ohio State University, Columbus, Ohio 43210

Received 25 July 2009/Accepted 31 Augu

***Thermococcus kodakarensis* (formerly *Thermococcus kodakaraensis*) s
thetic and natural DNA sequences, predicted to function as archaea
positioned between a constitutive promoter and a β-glycosidase-encodi
of the reporter gene was almost fully inhibited by the upstream pr
reduced >70% by archaeal intergenic sequences that contained olig
sequence (t_{mcrA}) that conforms to the bacterial intrinsic terminator m
but this required only the oligo(T) trail sequence and not the inverted-
were amplified from each *T. kodakarensis* strain, and transcription in v
was terminated by sequences that reduced TK1761 expression in vivo. T
on these linear templates, including at a 5′-AAAAAAAA (A_8) sequence
in vivo. When these sequences were transcribed on supercoiled pla
almost exclusively at oligo(T) sequences. The results provide the f
intrinsic termination of archaeal transcription and confirm that arch
ulated by oligo(T) sequences and is different from the RNA hairpin
intrinsic bacterial termination.**



Strain TS#	sequence inserted
372	—
552	TTTTTTTT
553	AAAAAAAA
554	GCTAGTATATATA
557	GCCCGCCGAAAGGCGGGCAATTTTTTTTATT

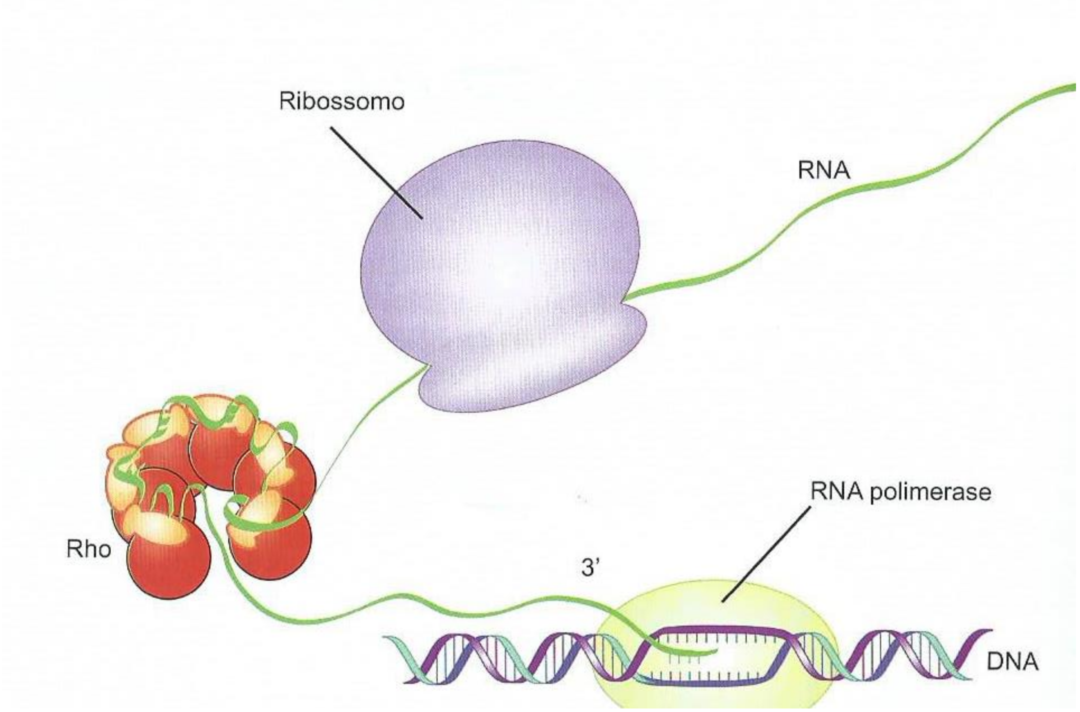
Strain TS#	IR	sequence inserted
455	TK0662	GAGAAGGTAAAGAAGTGGAAAAGTTCATTCCTTCTCTTTTTTTCCGTTTCC
456	TK1693	AGCTGGGTCTCTTTTGACCCATCTGGTCTTTTTTTGTTCACTGAGAGACTTTTTT
458	TK0348	ATCAGAATGATTAGATGTGTCCTTGGACACATTTTATTTTTTTGGA
459	TK0528	CTAATTTCAATCGTATTCCAGGTGGAAAGCCTTCTTGCTGGATTTTTTTGAAGAAAA
461	TK0284	ATCGACTAAAACAAGTTTTTAGAGAAAAATTTTTCTATTTTTTC
462	TK1208	AGTCTAACTCAACGGGTTGAAACCTCTTGTTCTCAACTTTTTGTTTTAGAAA
464	TK0746	CTAATAGGGGAGAACGCCACCTGATTTGGCTTTTTTTAGCCTTC
465	TK0182	CGTTCCACATCTGCCGAAGAGAGACTGAAGTCTTCTCTTCTTTTTTGTCTTGCTT
466	TK1640	TTCATGGAGAGGTCAACGCCCTGCATTAGGGCGATTTTTTCTTTCTCGT
467	TK1354	TGTTTTTCCCGTTTCGGGAGATTTCTCCATTTTTTTGGTTCAGT
468	TK1563	ACTACTTCTGGAGTGTTAGGGGTCCCTGATTTTGTTTTTTACAA
470	TK0842	AACGCGGTTCCAACGCTTGCAGAGGAGTGAAGGTTTTACTTTTTTTCTCCCCTT

Strain TS#	sequence inserted
442	ACGAAGGTGCATTGCACAAACTTTTTTAAGTGTAATGCACCTAATTTTTTTTATT
443	ACGAAGGTGCATTGCACAAACTTTTTTAAGTGTAATACACCTAATTTTTTTTATT
444	AAACTTTTTTAAGTGTAATGCACCTAATTTTTTTTATT
445	ACGAAGGTGCATTACACAAACTTTTTTAAGTGTAATGCACCT
555	GTGTAATGCACCTAATTTTTTTTATT
556	AATTTTTTTTATT
452	GCAACAACCTCACGATGGTCCGCCATGCAGATAGGTATGTCCATGGTCTCAGCCTG
454	GGCTCATACATGTCAGGTGGTGTAGGATTCACCCAGTACGCAACAGCAGCCTACAG

TERMINAÇÃO DEPENDENTE DE PROTEÍNAS

Sem sequência canônica

Três principais fatores:
NusA
Tau
Rho – comum e amplamente conservado em todas bactérias



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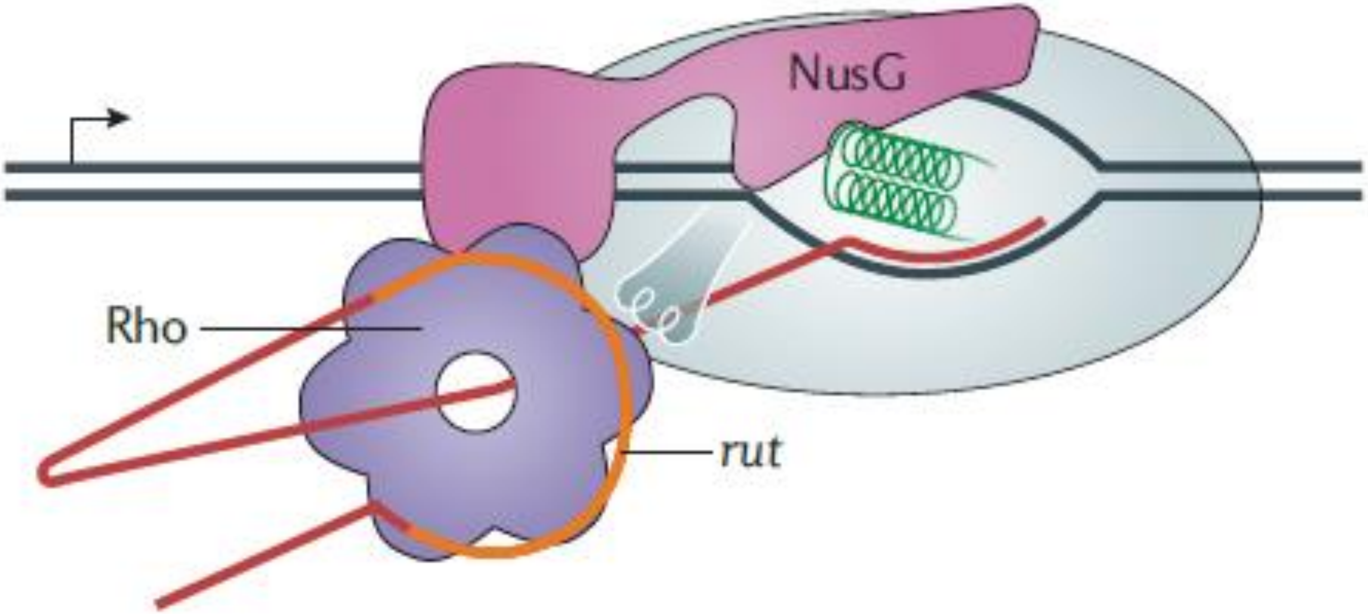
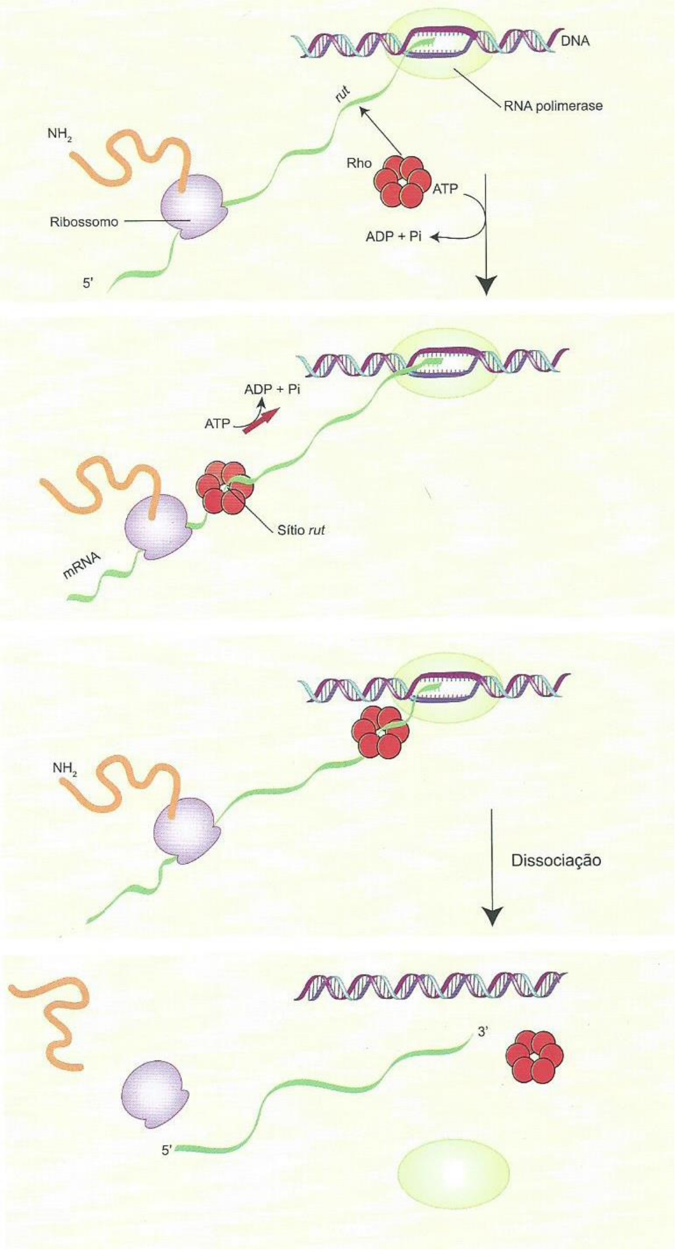


Relacionada:
Regulação de antisense
Degradação de RNA não traduzido
Evitar transcrição e replissomos (enzimas auxiliares…)

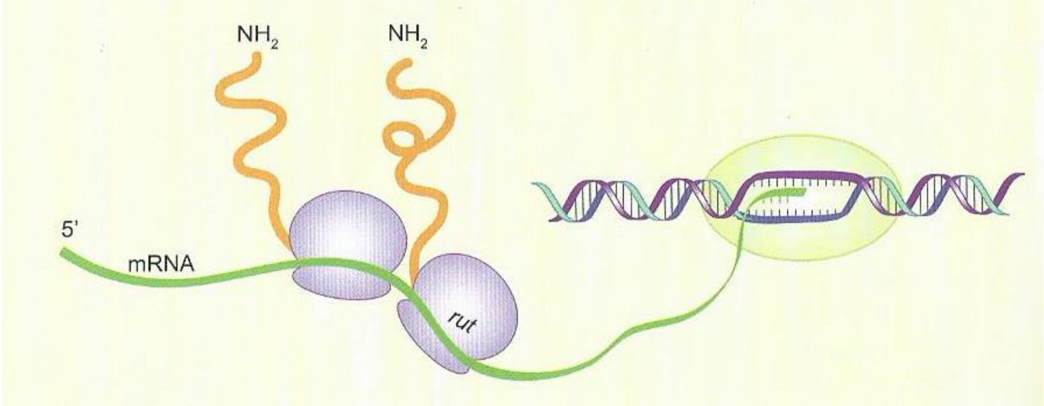
Se liga somente em RNA livre de ribossomos e usa ATP para se deslocar até o RNAP
Pode estar acoplado a outros sistemas… solta RNA em sítios de paradas…

doi: 10.1038/nrmicro2560

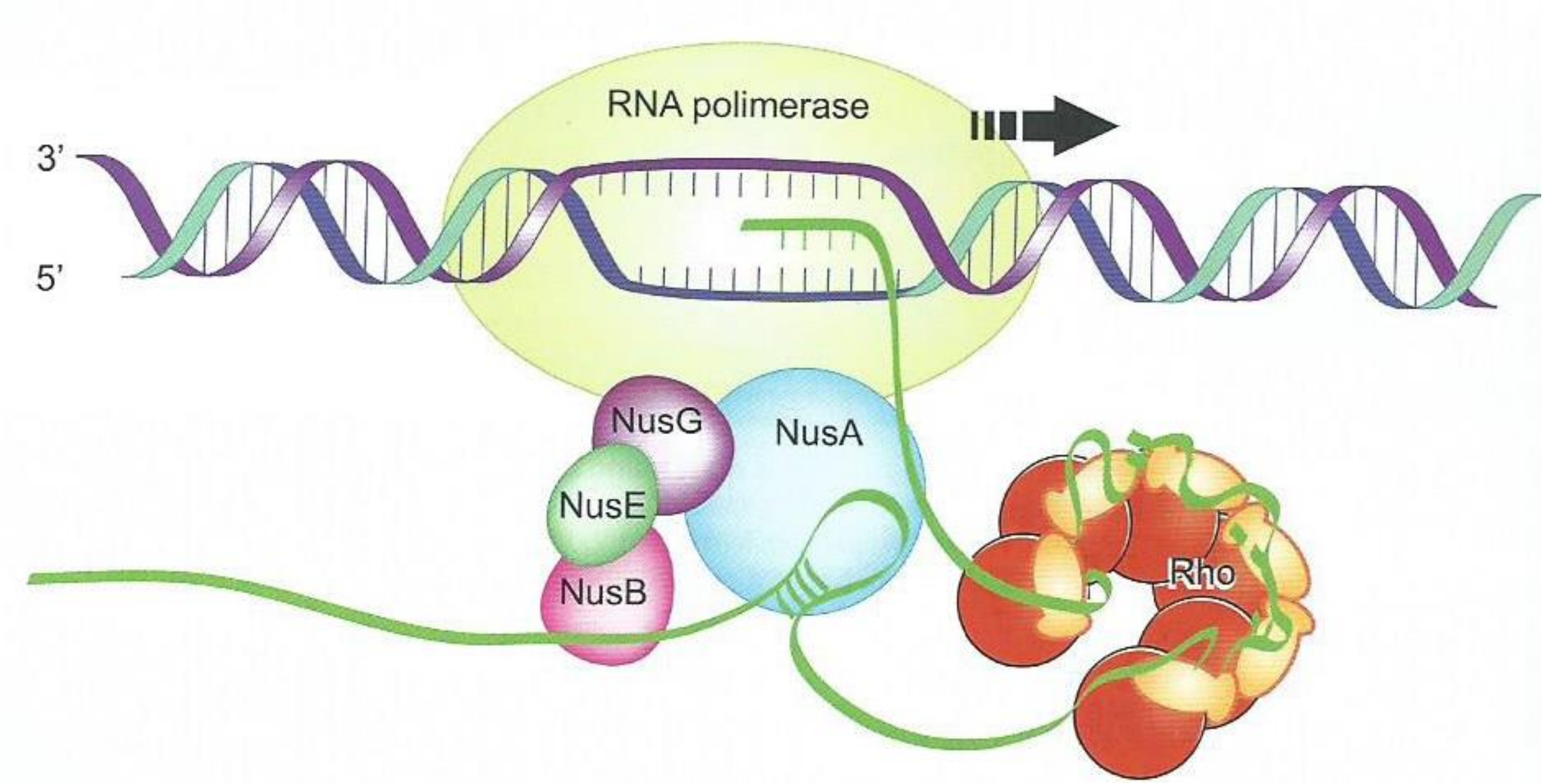


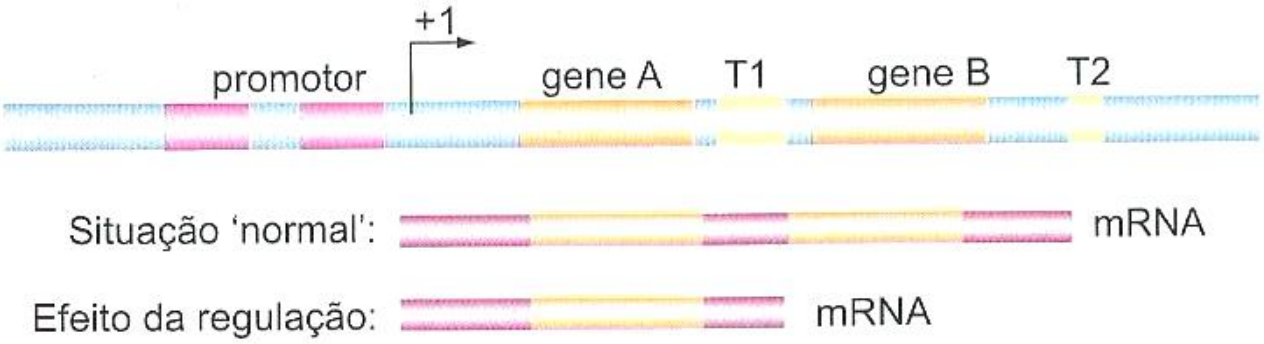
Sitios *rut* – aproximadamente 30 pb, ricas em pirimidinas...difícil reconhecer...sequencia não canônica!

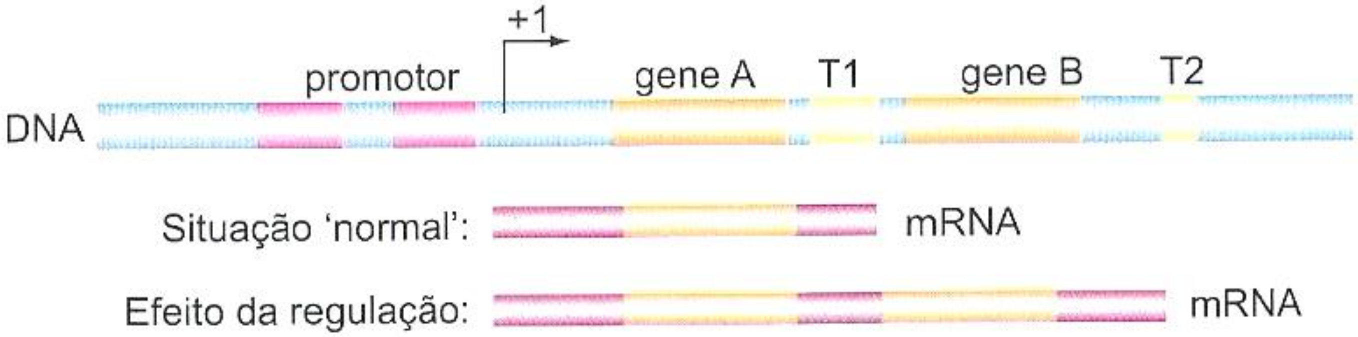
A presença de muitos ribossomos podem atrapalhar a terminação....



Rho É AUXILIADA POR OUTRAS PROTEÍNAS





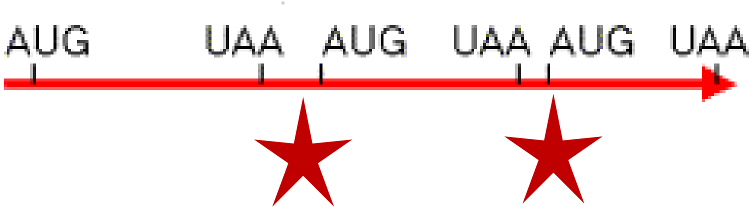


Qual está correto?

Antiterminação

Situação normal : terminação prematura

Sinal causa: **não terminação**



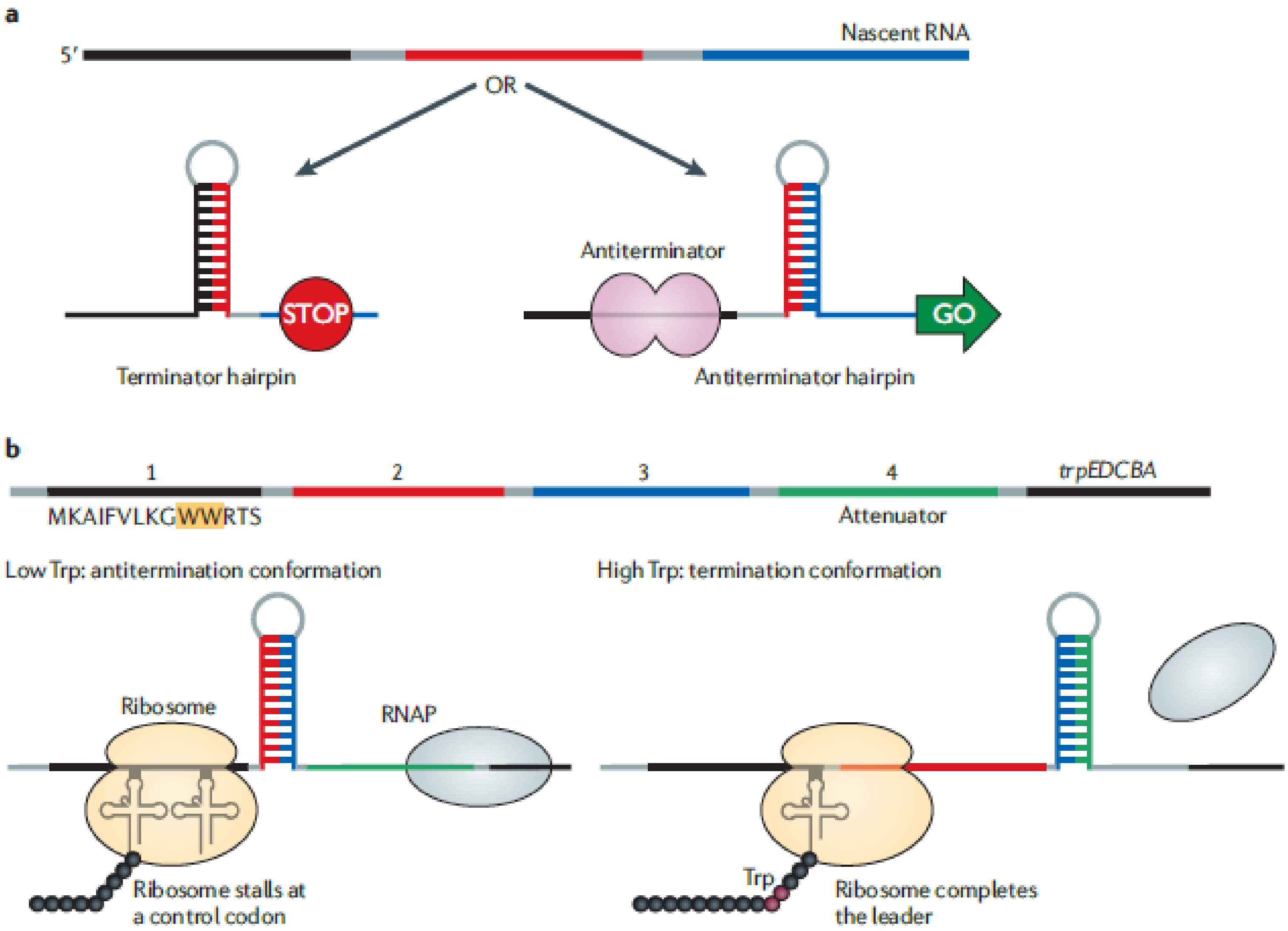
Sítios de parada

Atenuação

Situação normal: não teminação

Sinal causa: **terminação prematura**

ANTITERMINAÇÃO X ATENUADOR

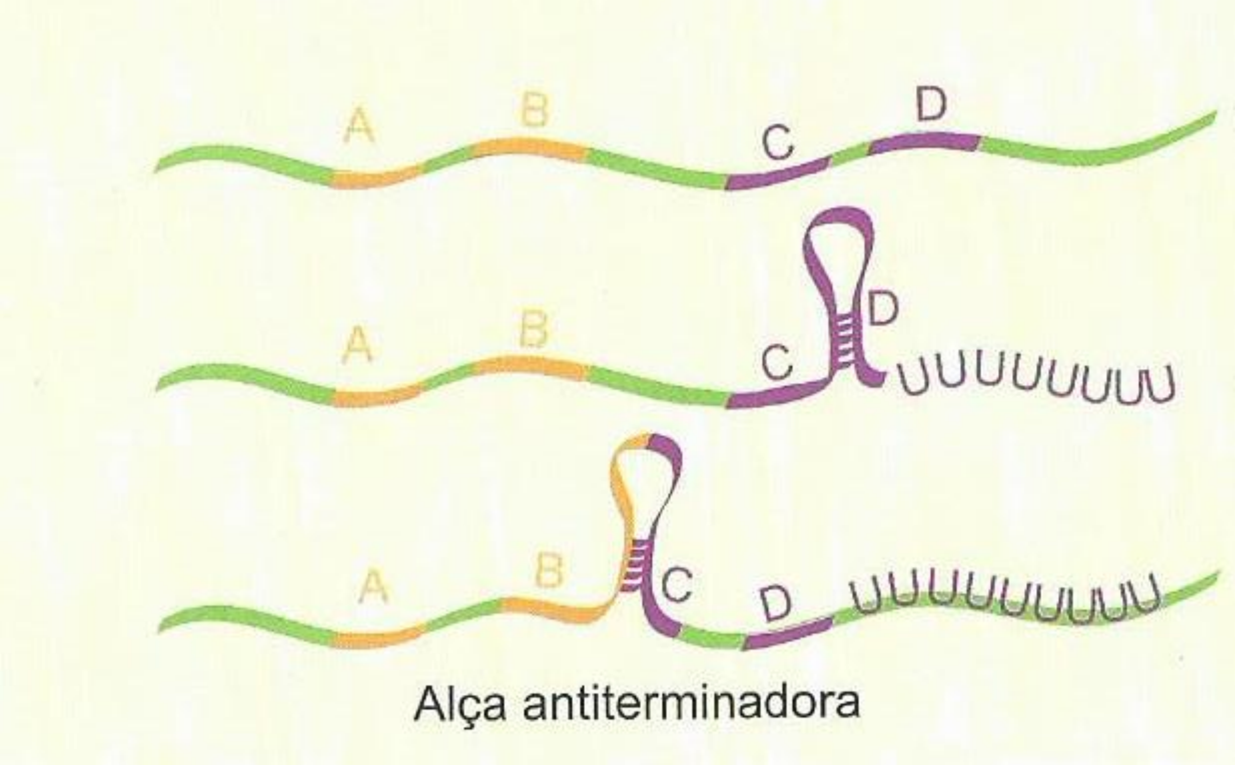


doi:10.1038/nrmicro2560

ANTITERMINAÇÃO

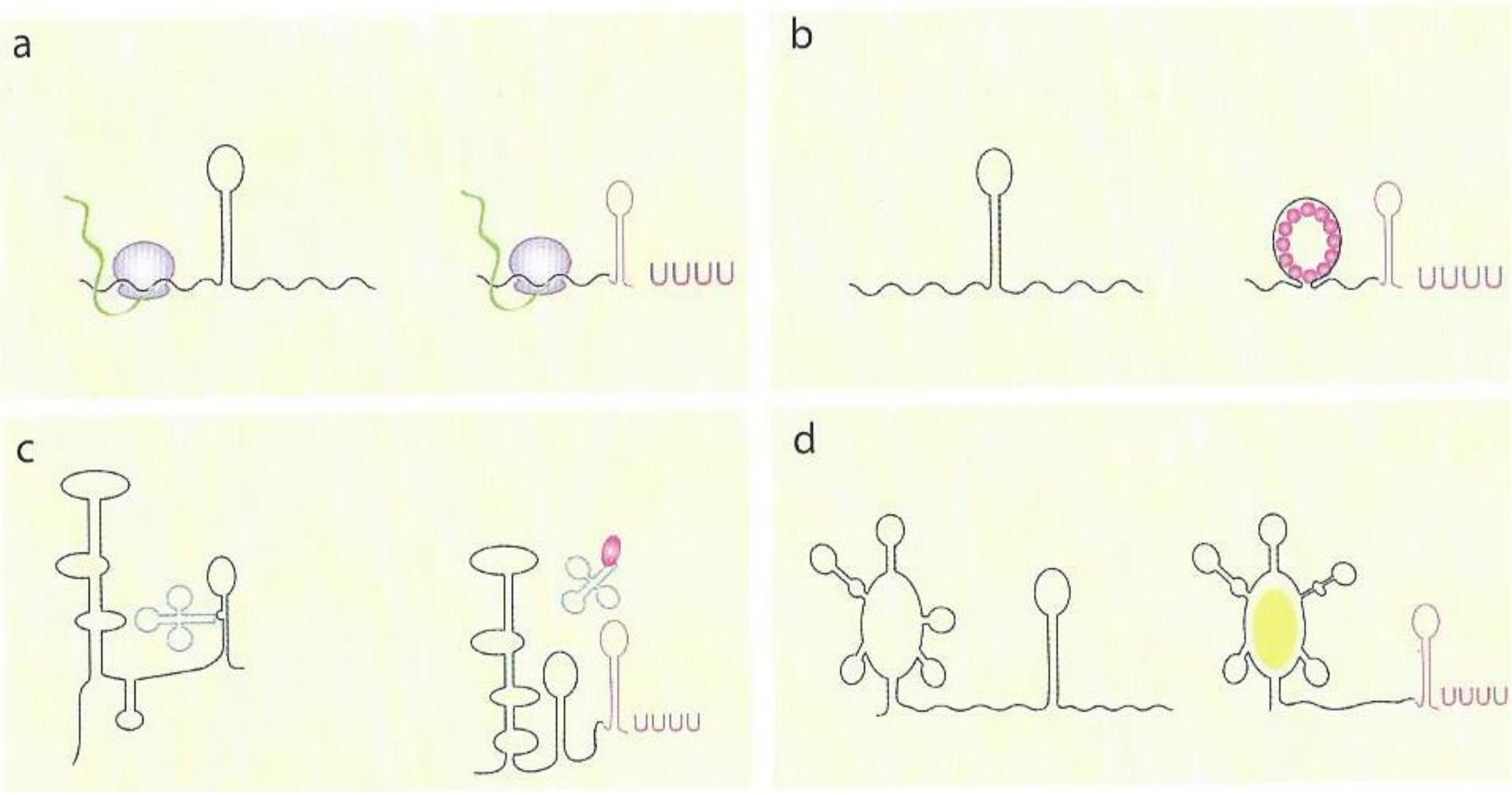
Sítio de paradas...pausa...mas não muito forte..

Maioria passiva em sequências intrinsecas...

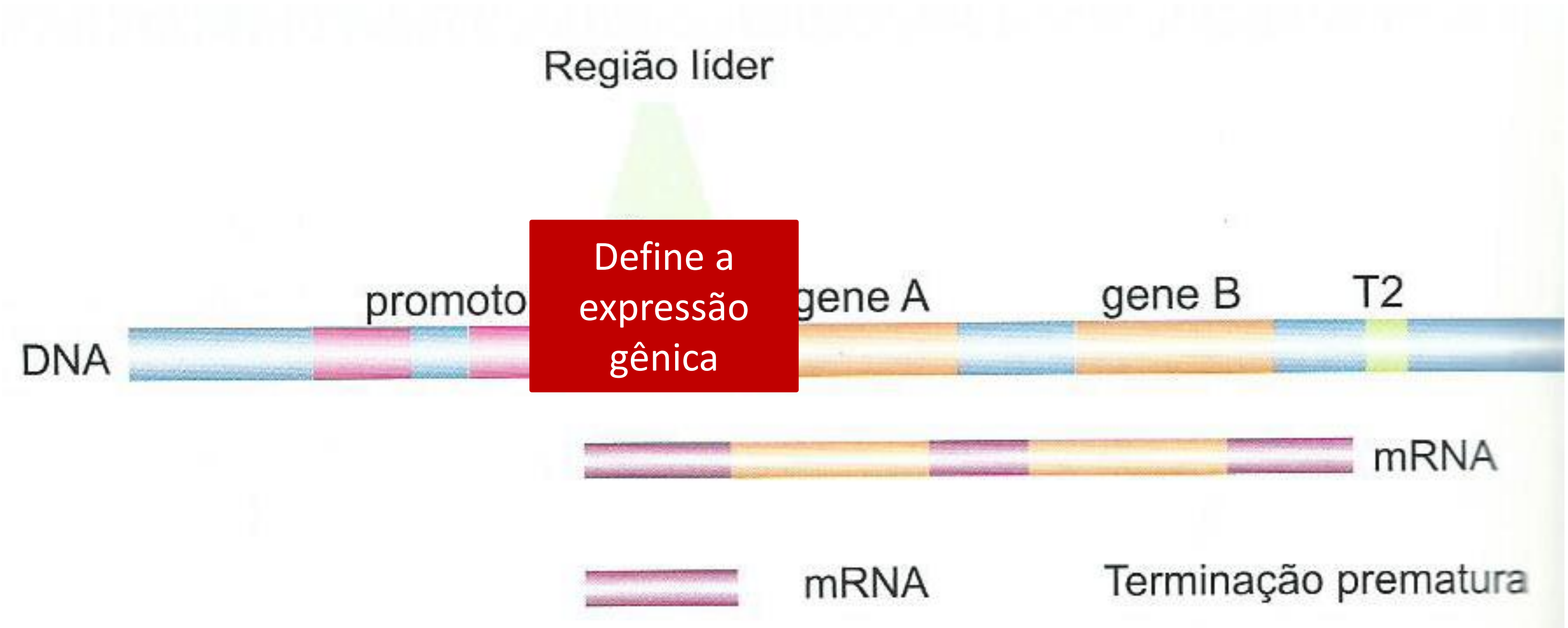


Alça antiterminadora

EVENTOS REGULADORES DA TERMINAÇÃO



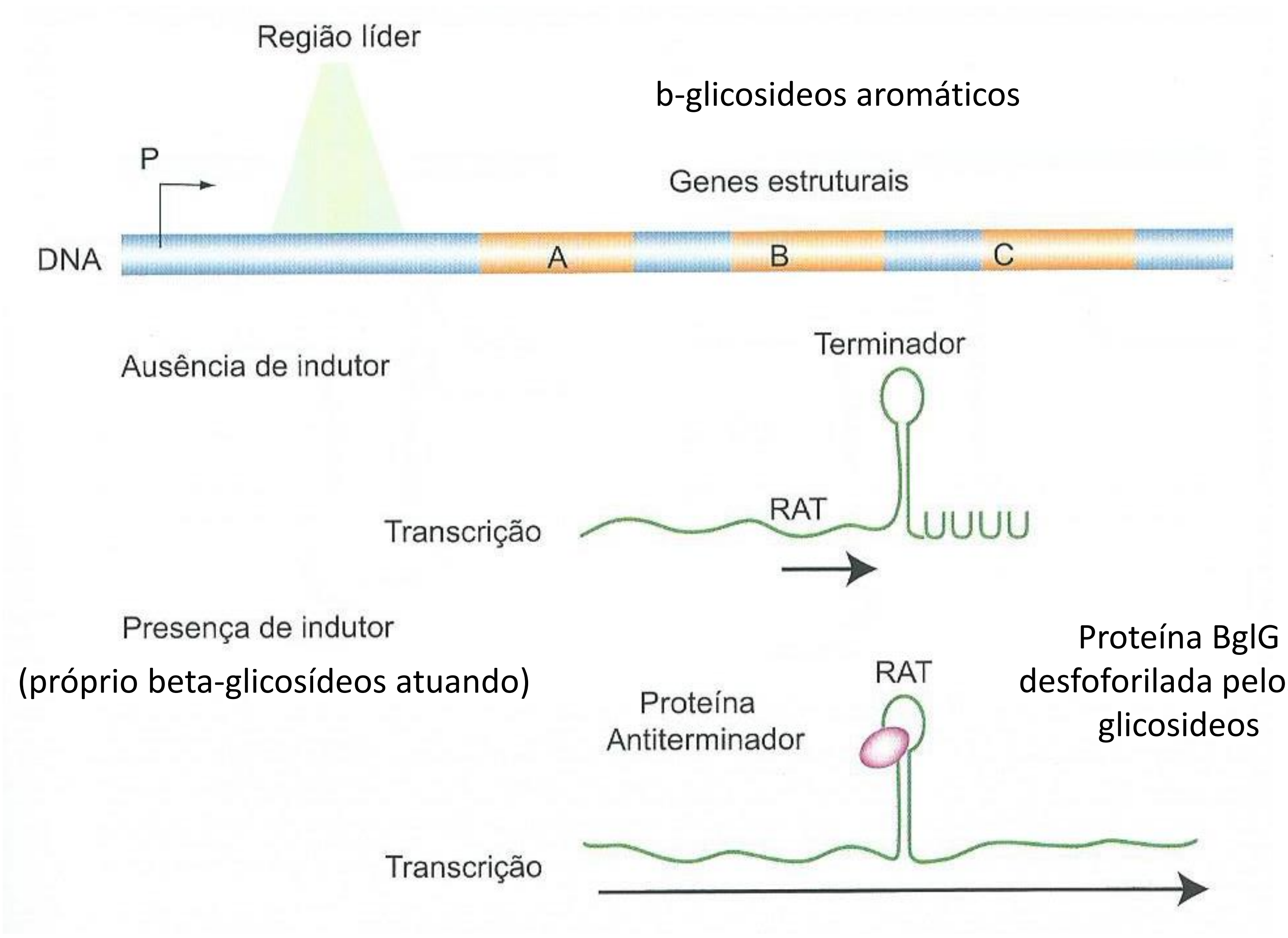
ESQUEMA DE ATENUAÇÃO..LOCALIZAÇÃO É TUDO..



Define a expressão gênica

Mesmos mecanismos citados anteriormente!

MODELO DE ANTITERMINAÇÃO

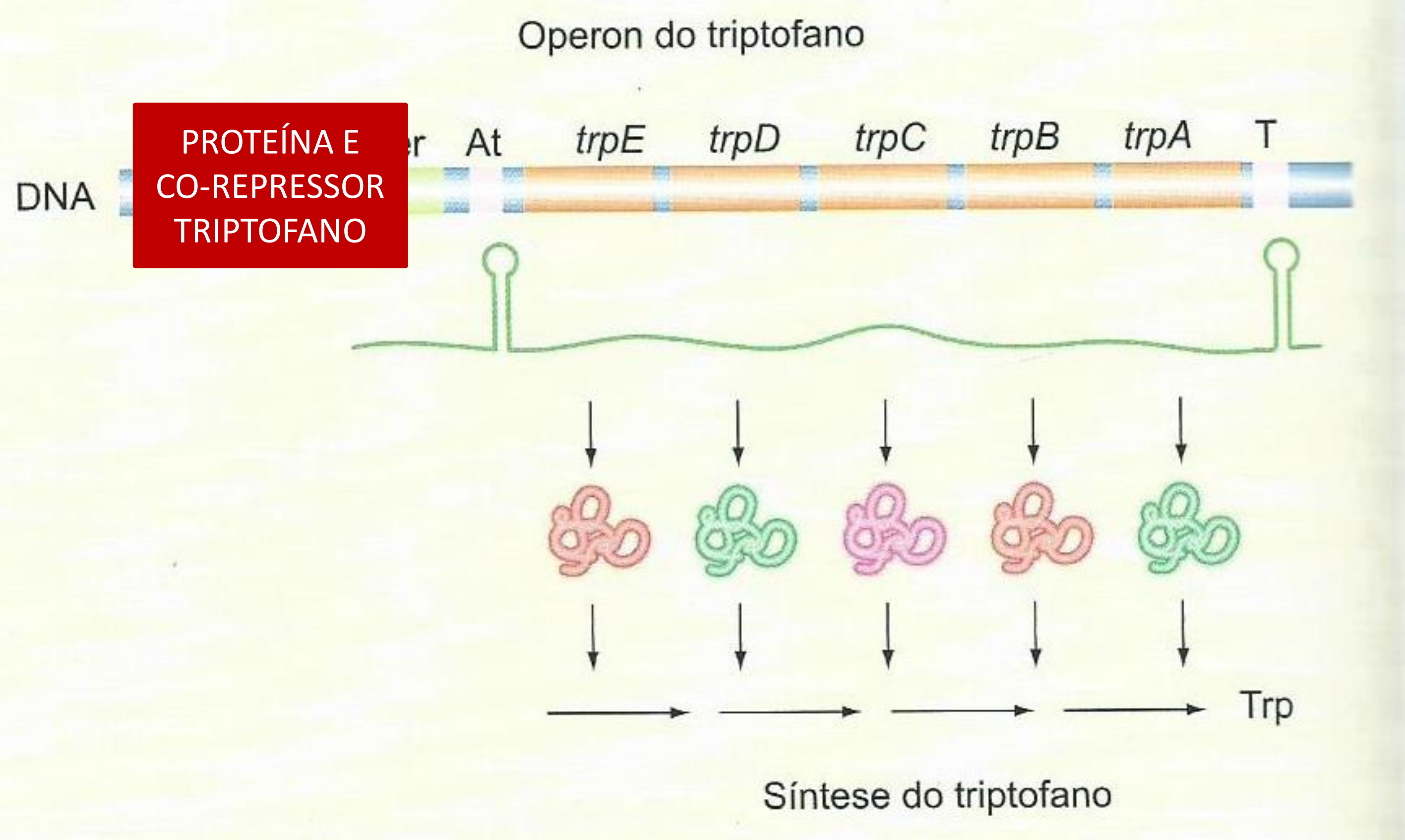


b-glicosideos aromáticos

(próprio beta-glicosídeos atuando)

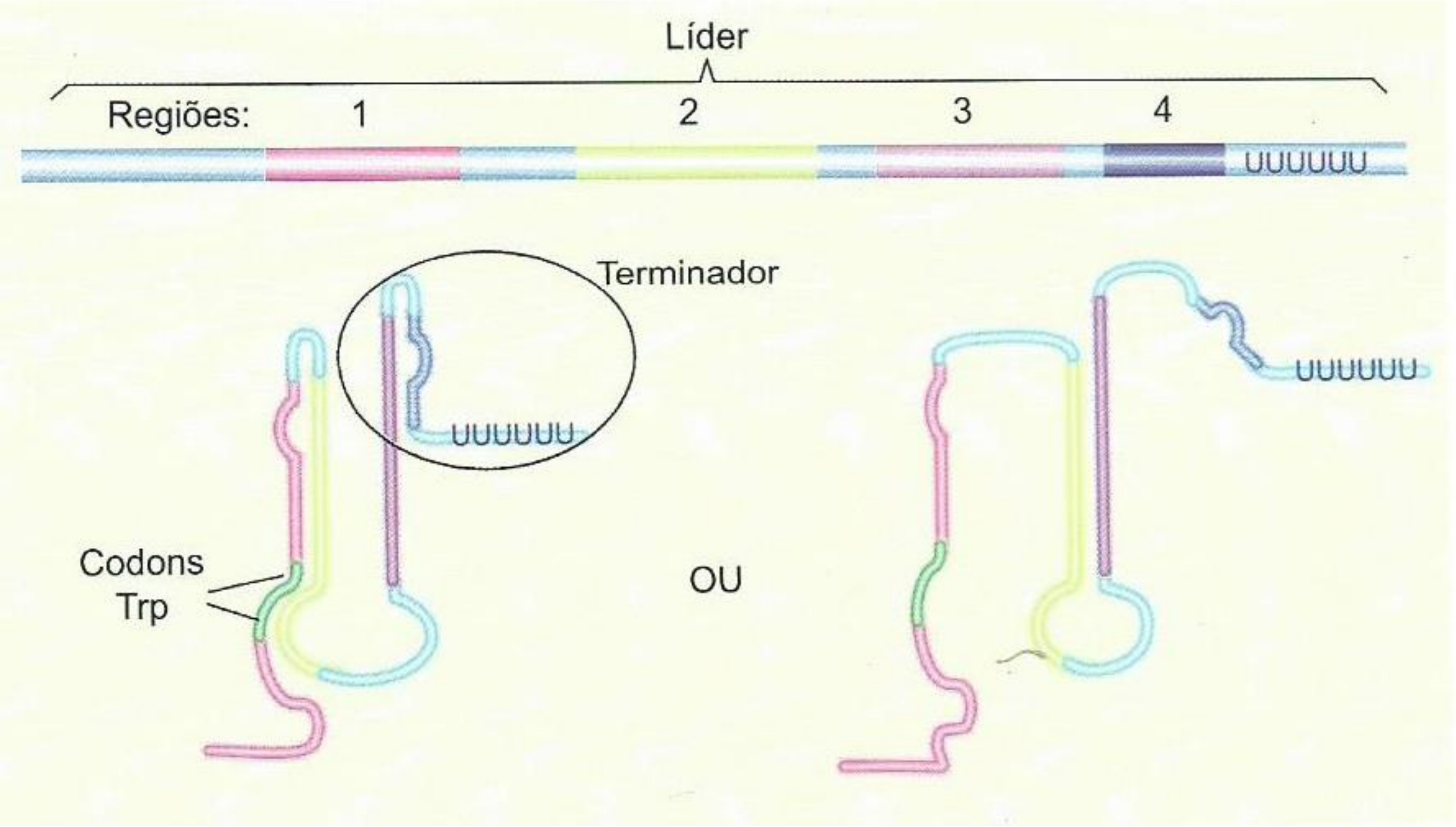
Proteína BglG desfoforilada pelos b-glicosideos

MODELO DE ATENUAÇÃO

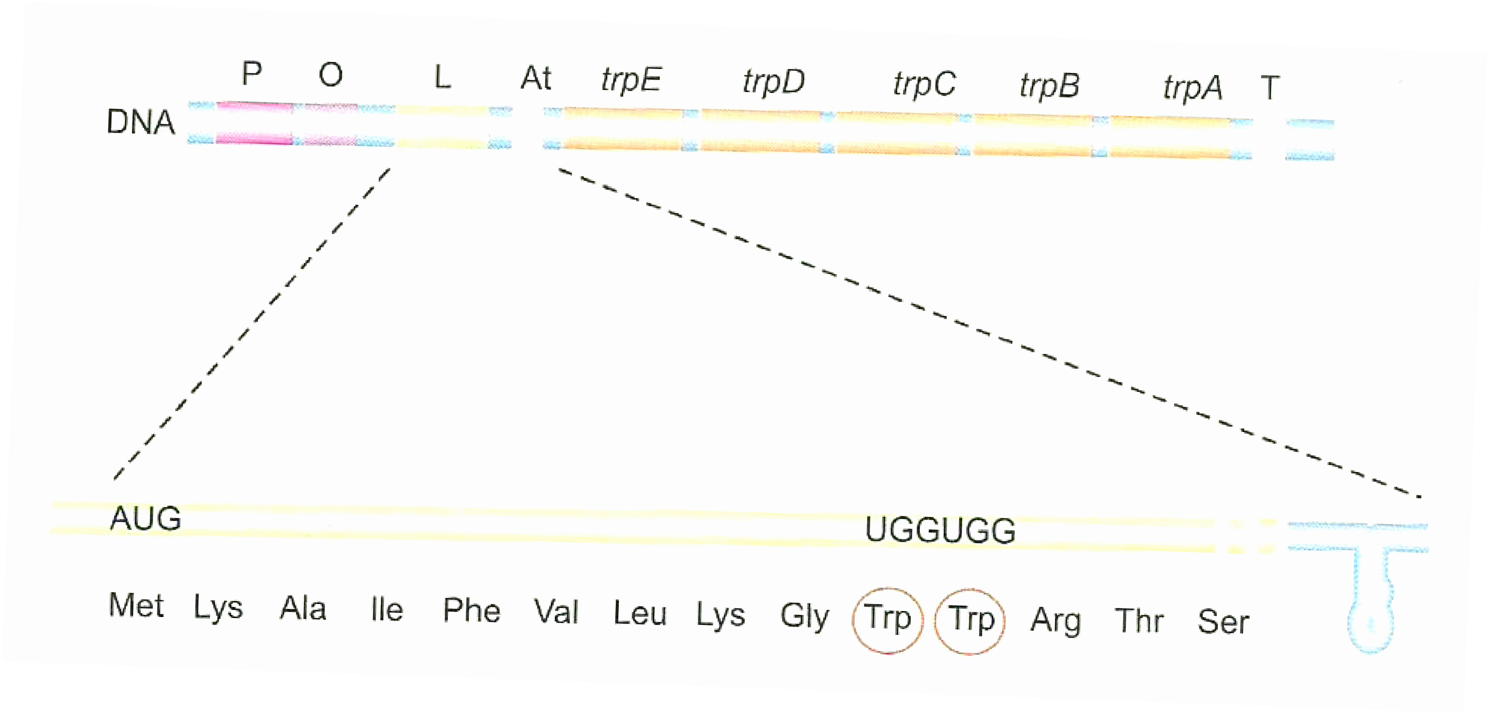


PROTEÍNA E
CO-REPRESSOR
TRIPTOFANO

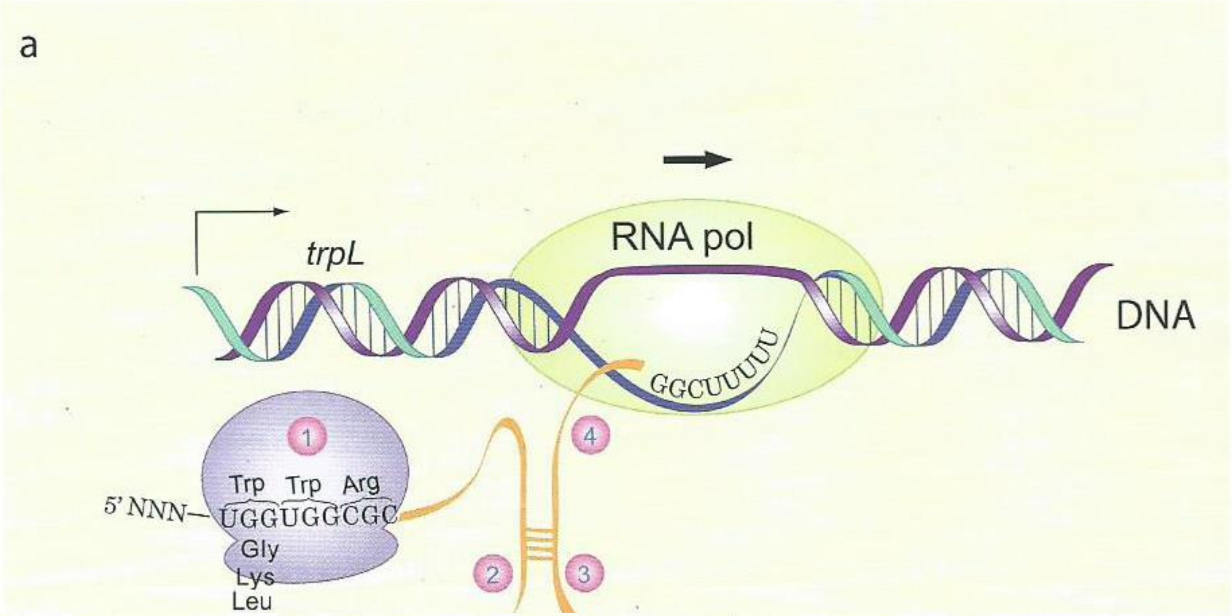
4 REGIÕES POSSÍVEIS…DUAS POSSIBILIDADE



LOGO NO INÍCIO DA REGIÃO LIDER…

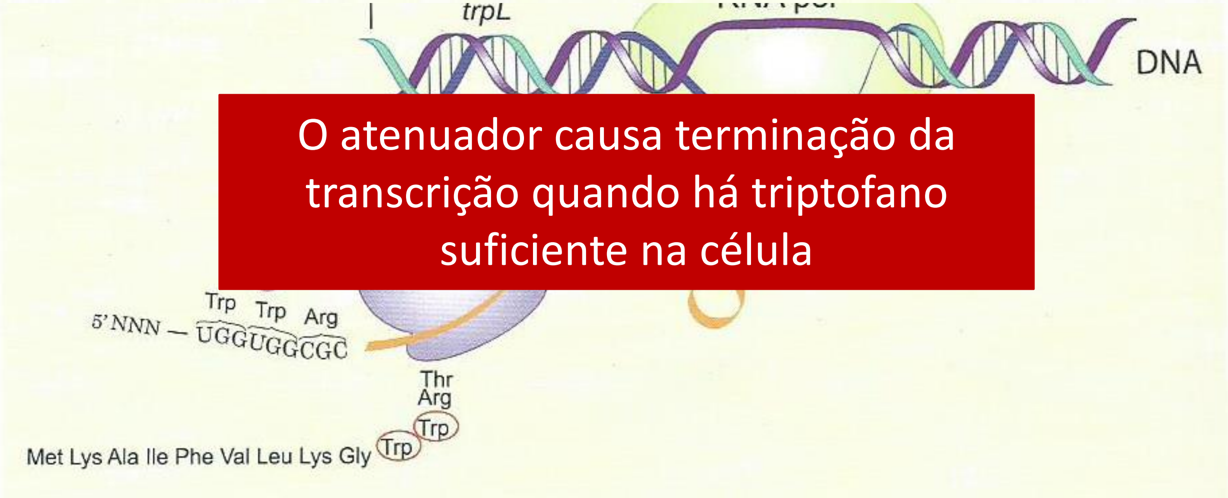


Perfeita sincrônia entre transcrição e tradução!



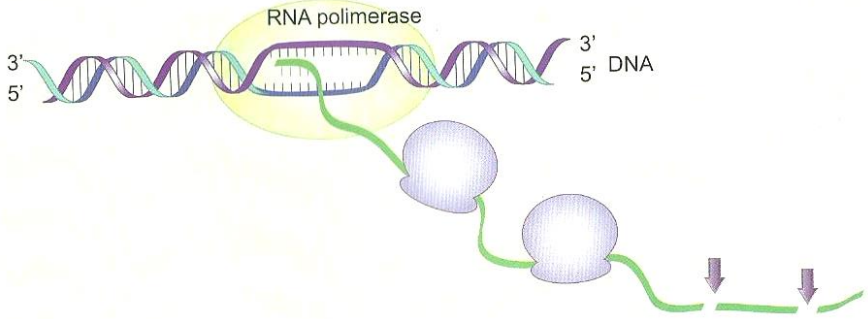
Met - Lys - His - Ile - Pro - Phe - Phe - Phe - Ala - Phe - Phe - Phe - Thr - Phe - Pro - Stop
5′ AUG AAA CAC AUA CCG UUU UUC UUC GCA UUC UUU UUU ACC UUC CCC UGA 3′

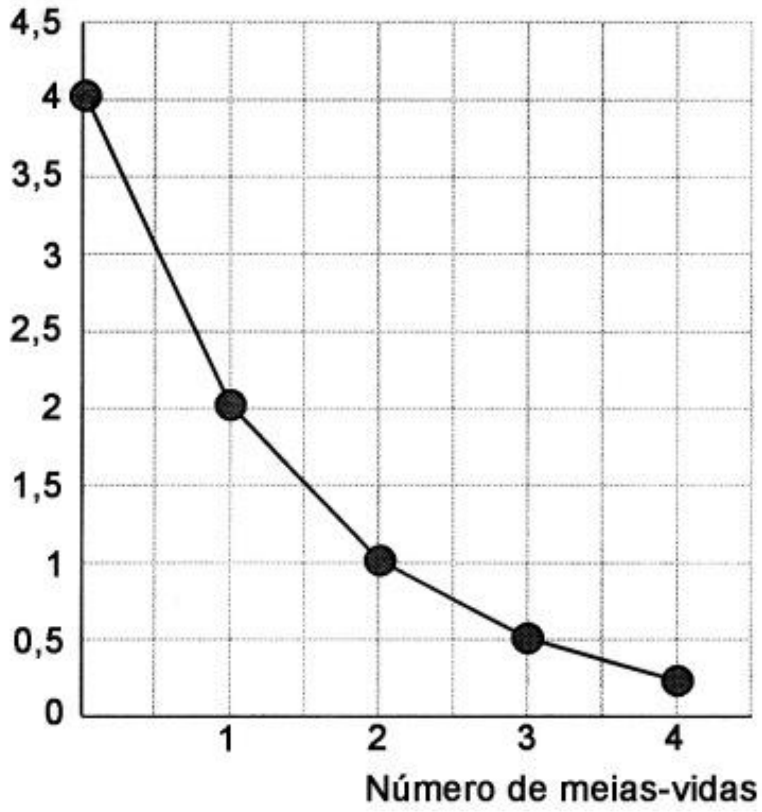
Met - Thr - Arg - Val - Gln - Phe - Lys - His - His - His - His - His - His - His - Pro - Asp -
5′ AUG ACA CGC GUU CAA UUU AAA CAC CAC CAU CAU CAC CAU CAU CCC GAC 3′



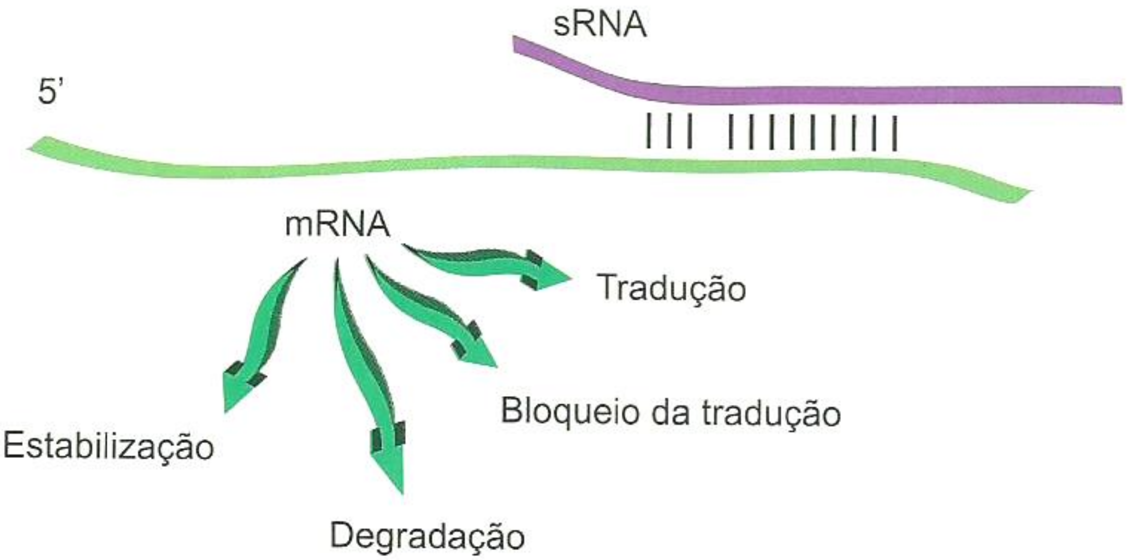
O atenuador causa terminação da transcrição quando há triptofano suficiente na célula

CONTROLE PÓS TRANSCRICIONAL



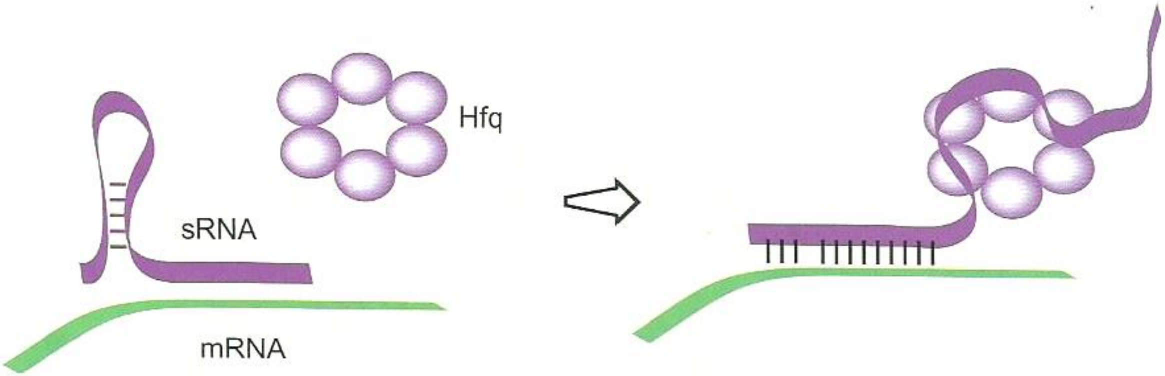


sRNA E SUAS DIVERSAS FUNÇÕES

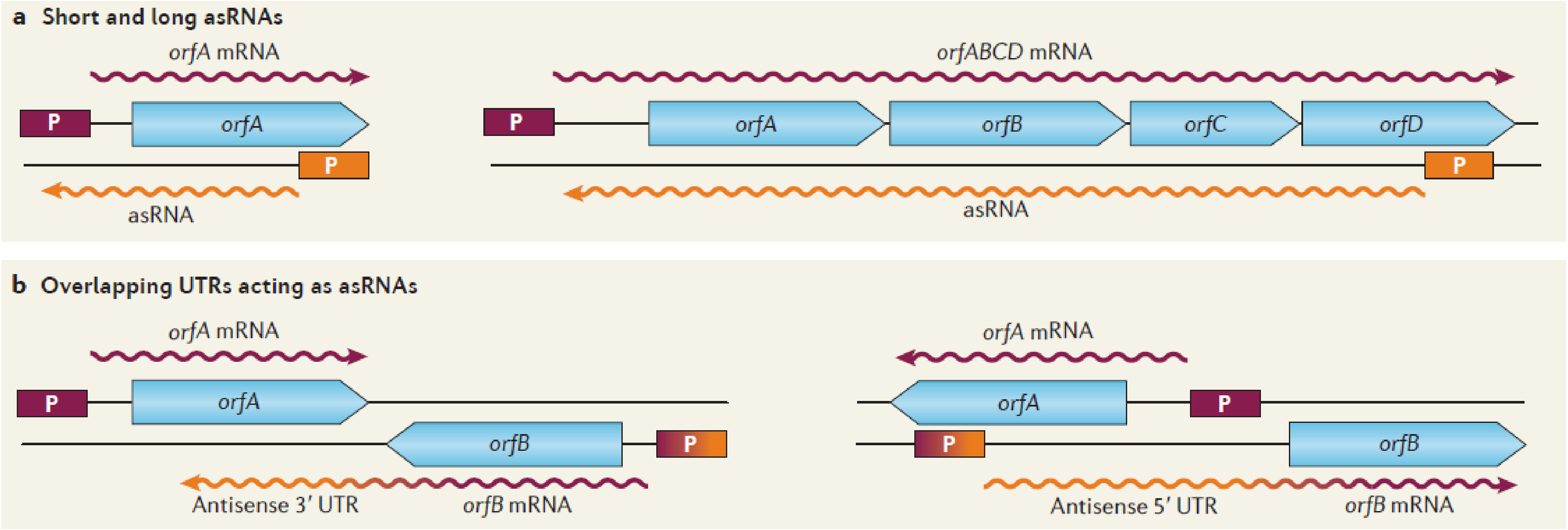


Geralmente presentes em regiões intergênicas – controle em trans

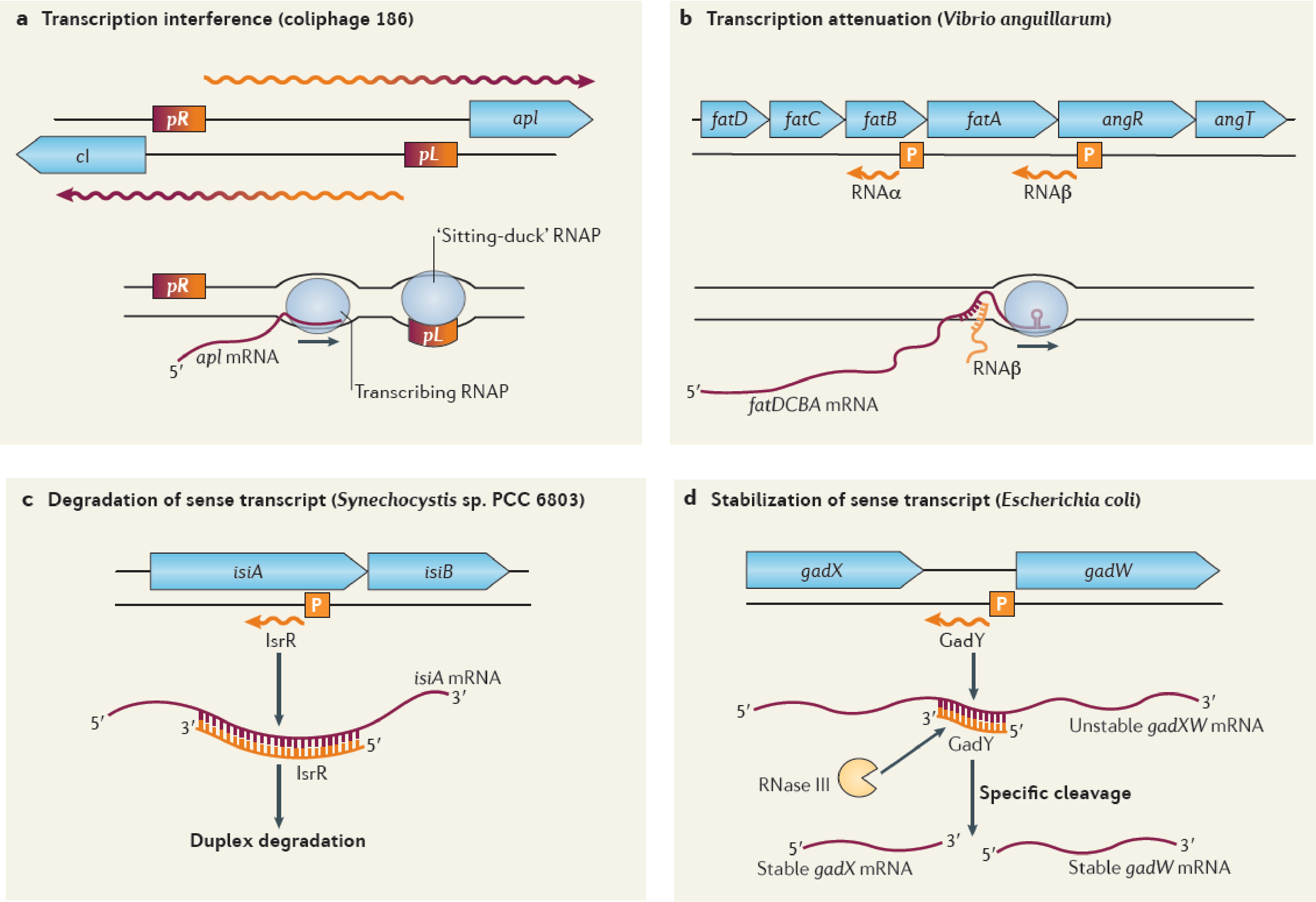
Estabilização da interação



VÁRIOS TIPOS DE RNA ANTISENSO



Diferentes modelos para atividade RNA antisense

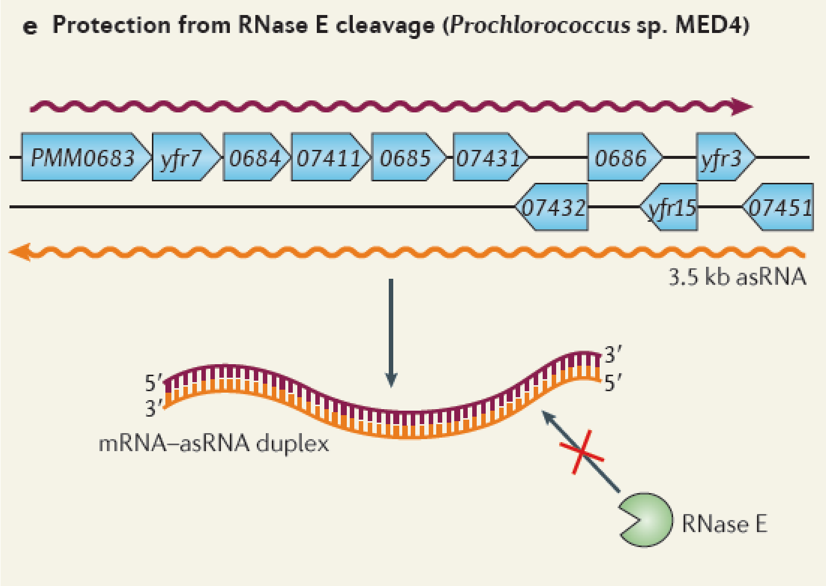


a Transcription interference (coliphage 186)

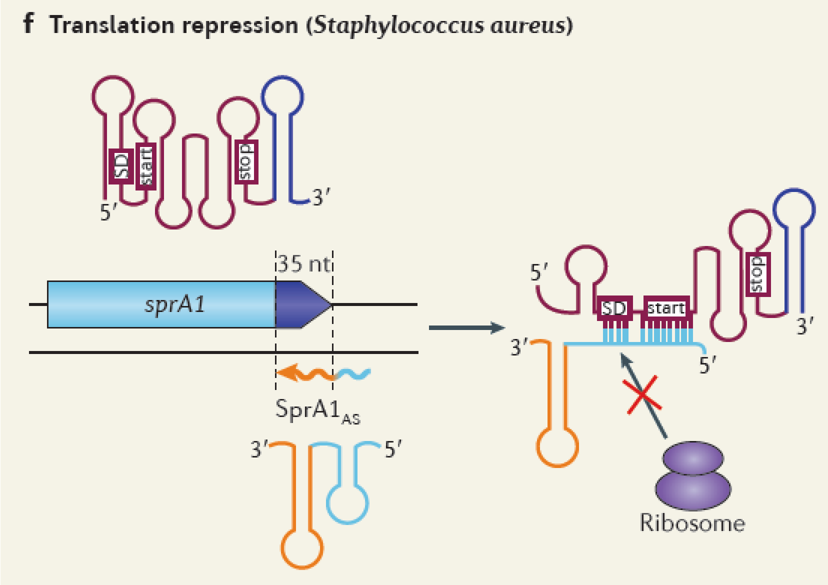
b Transcription attenuation (*Vibrio anguillarum*)

c Degradation of sense transcript (*Synechocystis* sp. PCC 6803)

d Stabilization of sense transcript (*Escherichia coli*)



e **Protection from RNase E cleavage (*Prochlorococcus* sp. MED4)**



f **Translation repression (*Staphylococcus aureus*)**

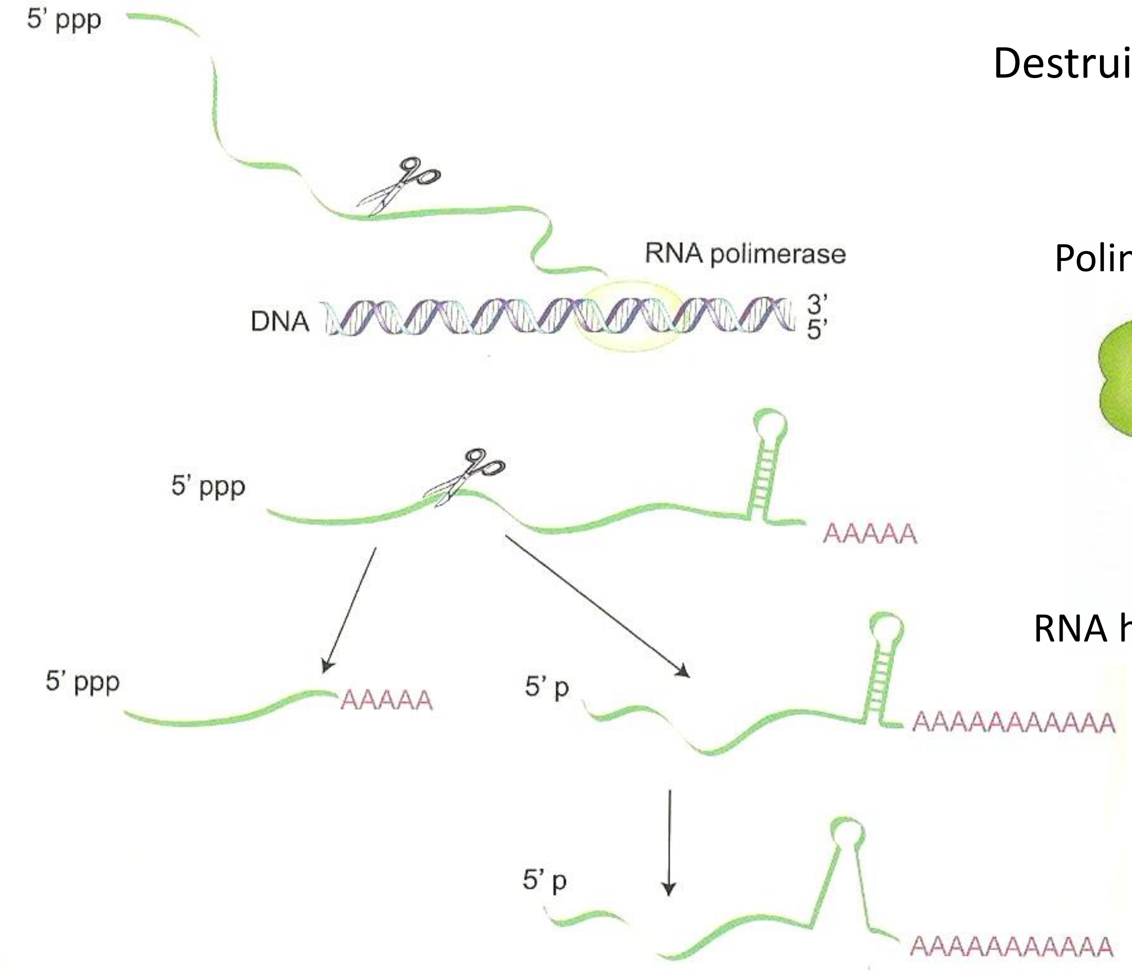
doi: 10.1038/nrmicro2934

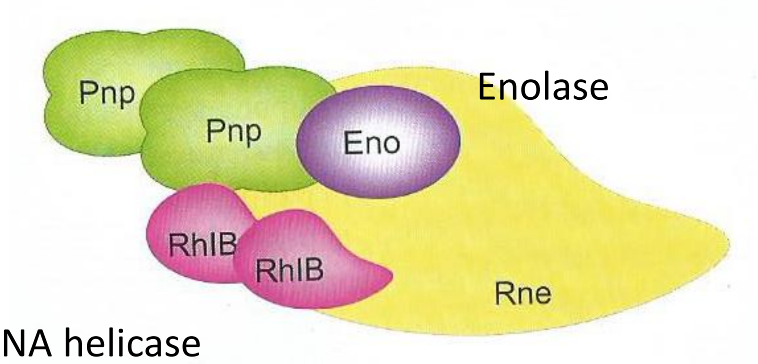
RNAase

Controle da expressão

Reciclagem de ribonucleotídeos

Destruição de RNA aberrantes





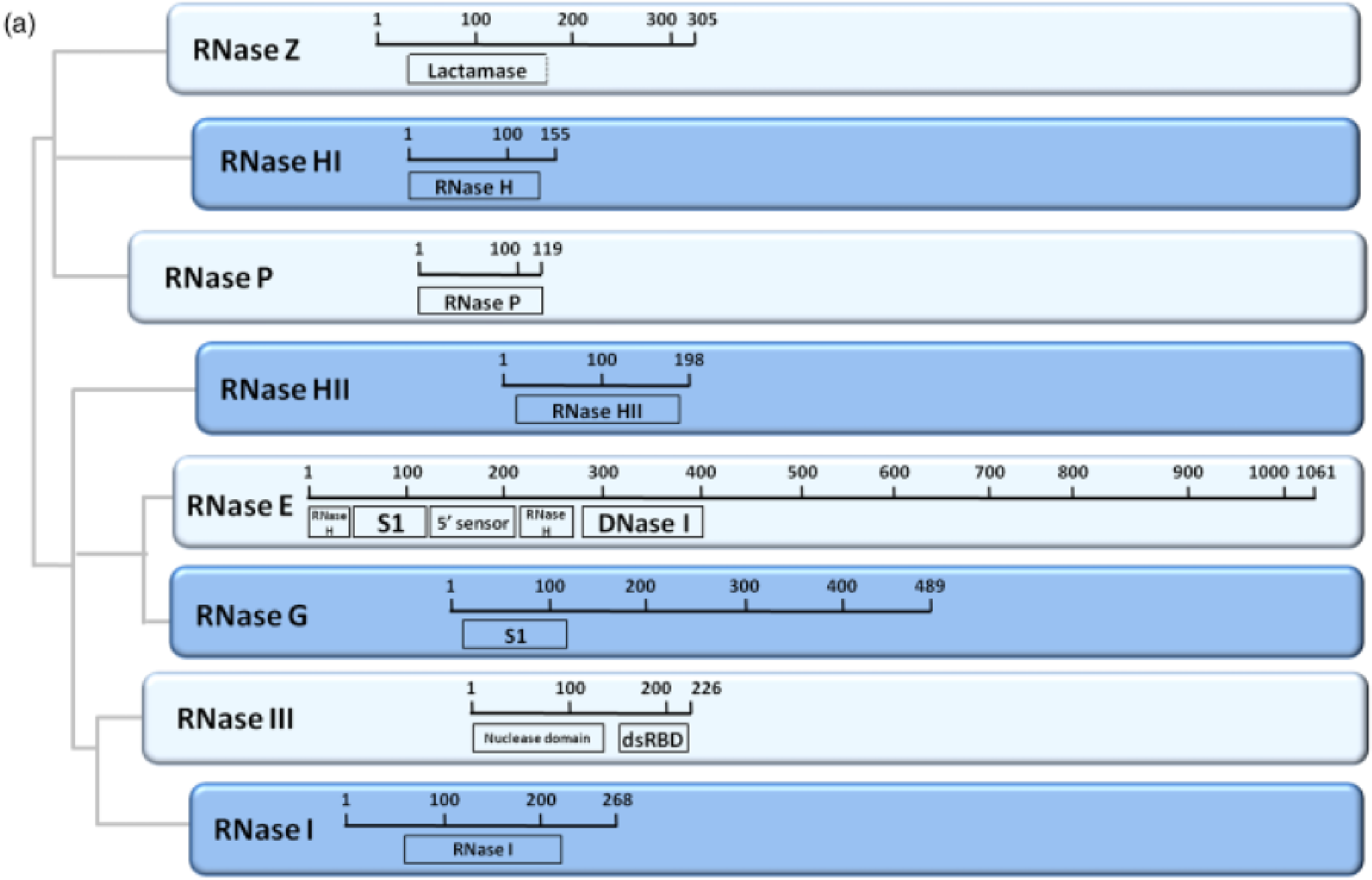
Polinucleotídeo fosforilase

Enolase

RNA helicase

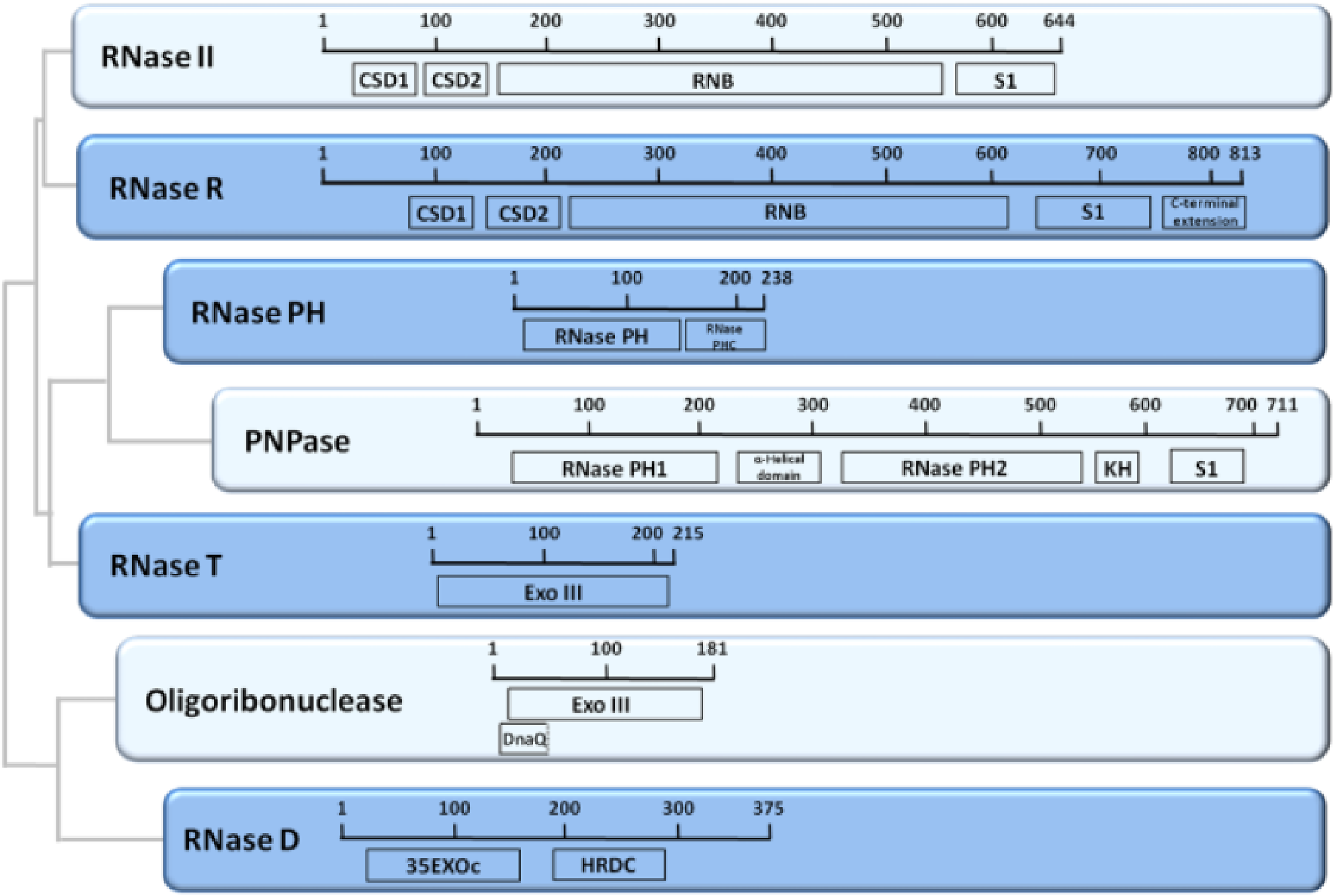
RNAasee

EndoRNAase



FEMS Microbiol Rev 34 (2010) 883–923

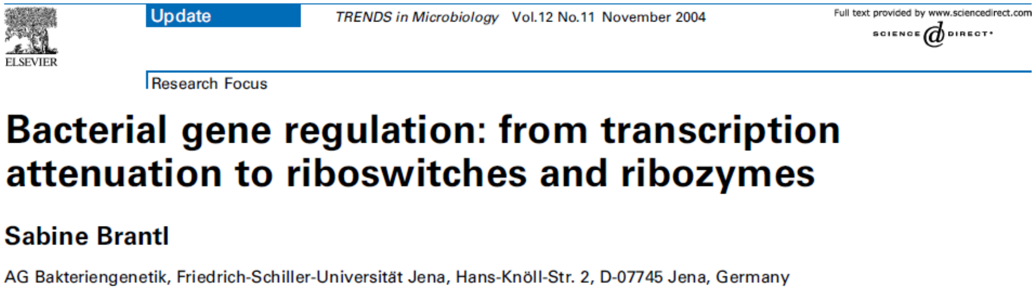
EnxoRNAase



FEMS Microbiol Rev 34 (2010) 883–923

RIBOSWITCHES

Riboswitchs são segmentos regulatórios do mRNA onde se ligam pequenas moléculas resultando na mudança da tradução. Assim, o mRNA que contem riboswitch está diretamente envolvido na regulaçao de sua própria atividade muitas vezes em resposta da concentração de sua propria molécula produzida.



ELSEVIER

Update

TRENDS in Microbiology Vol.12 No.11 November 2004

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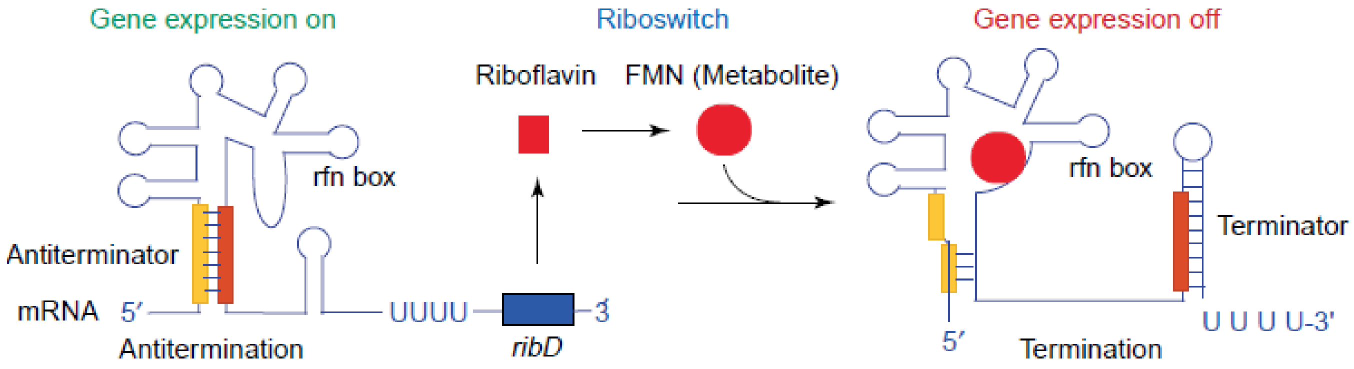
Research Focus

Bacterial gene regulation: from transcription attenuation to riboswitches and ribozymes

Sabine Brantl

AG Bakteriengenetik, Friedrich-Schiller-Universität Jena, Hans-Knöll-Str. 2, D-07745 Jena, Germany



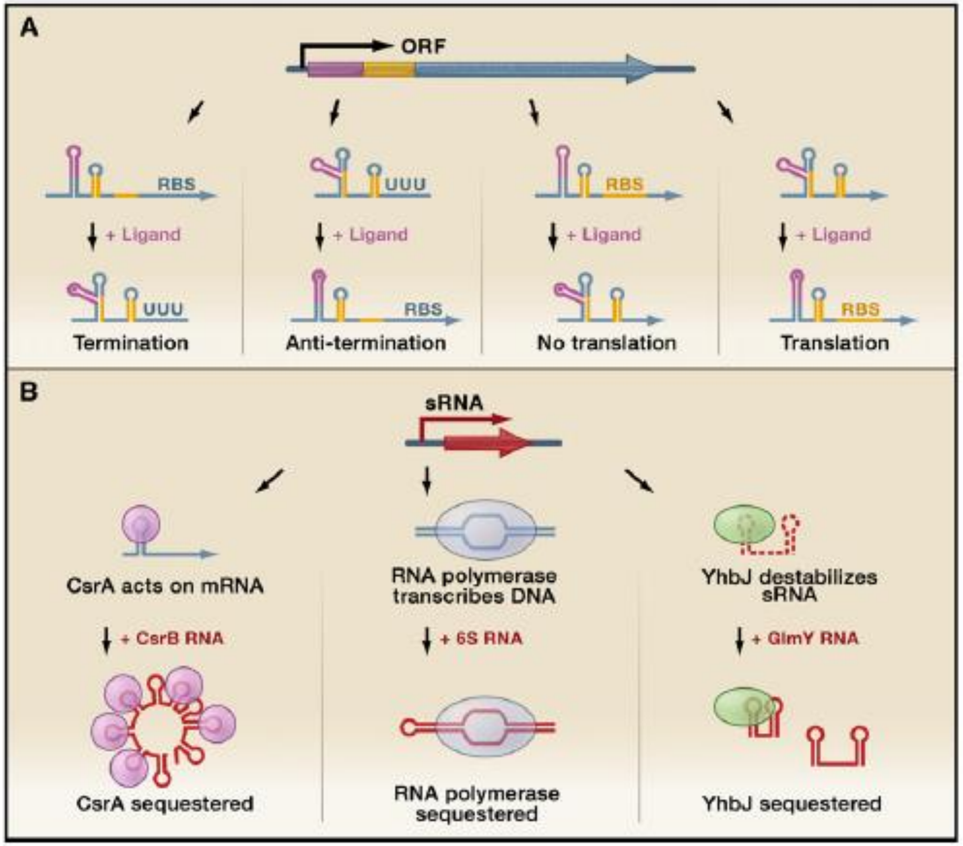
Leading Edge
Review

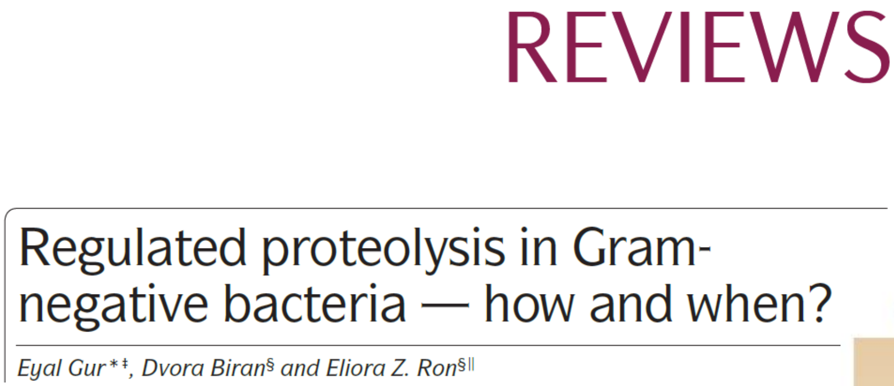
Cell

Regulatory RNAs in Bacteria

Lauren S. Waters[1] and Gisela Storz[1,*]
[1]Cell Biology and Metabolism Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development,
Bethesda, MD 20892, USA
*Correspondence: storz@helix.nih.gov
DOI 10.1016/j.cell.2009.01.043

Bacteria possess numerous and diverse means of gene regulation using RNA molecules, including mRNA leaders that affect expression in *cis*, small RNAs that bind to proteins or base pair with target RNAs, and CRISPR RNAs that inhibit the uptake of foreign DNA. Although examples of RNA regulators have been known for decades in bacteria, we are only now coming to a full appreciation of their importance and prevalence. Here, we review the known mechanisms and roles of regulatory RNAs, highlight emerging themes, and discuss remaining questions.

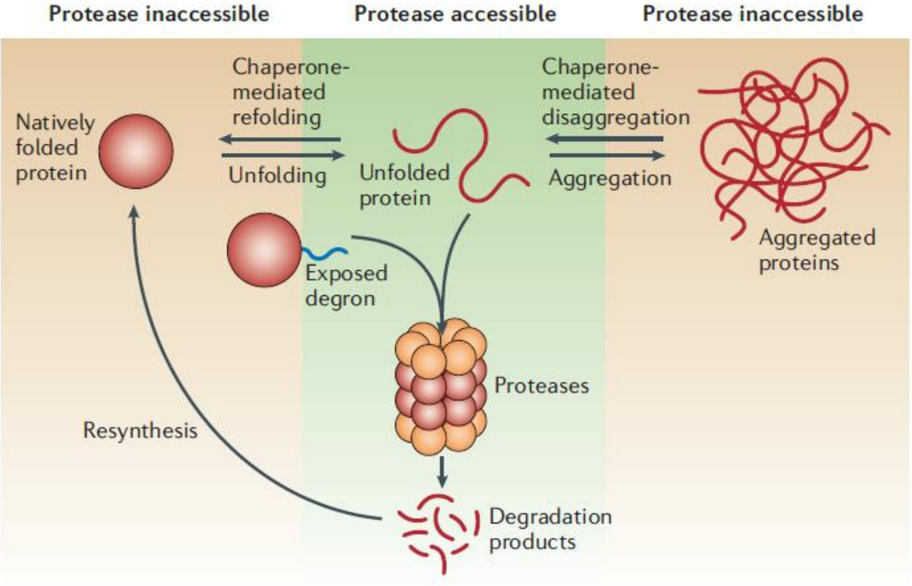


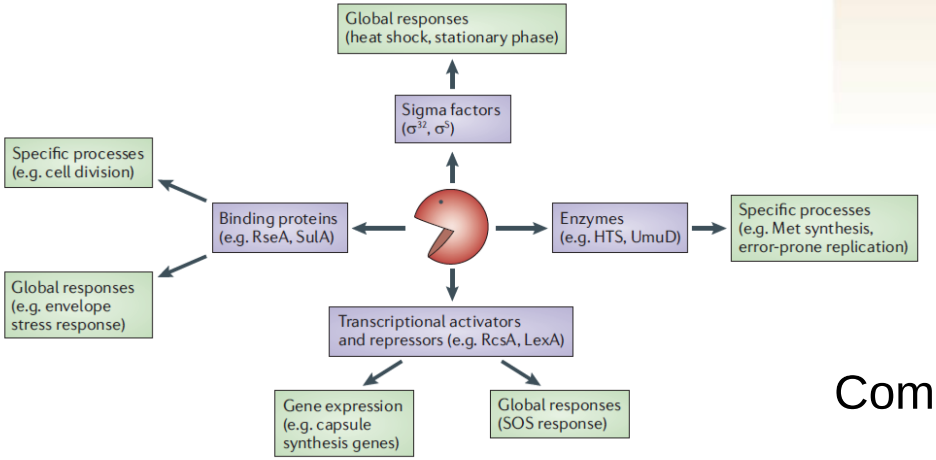


REVIEWS

Regulated proteolysis in Gram-negative bacteria — how and when?

Eyal Gur[*,‡], *Dvora Biran*[§] *and Eliora Z. Ron*[§,||]





Complementa o controle transcricional!!!

PRÓXIMA AULA

Controle Do Início Da Transcrição Em Eucariotos

Métodos de transformação de procariontes e eucariontes: conceitos, organismos modelos e desafios