INTRODUÇÃO À GENÉTICA MOLECULAR

Maria Carolina Quecine
Departamento de Genética
mquecine@usp.br

SUMÁRIO

- LGN5809 – como será?
- Conteúdo programático
- Entendendo o que é genética molecular
- Aplicações da genética molecular
- Próxima aula

- Objetivo da disciplina – formação e não somente informação;
- Material disponível no e-Disciplinas;
- Participação ativa dos pós-graduandos;
- Relatórios semanais;
- Apresentação de seminários;
- Trabalho final.

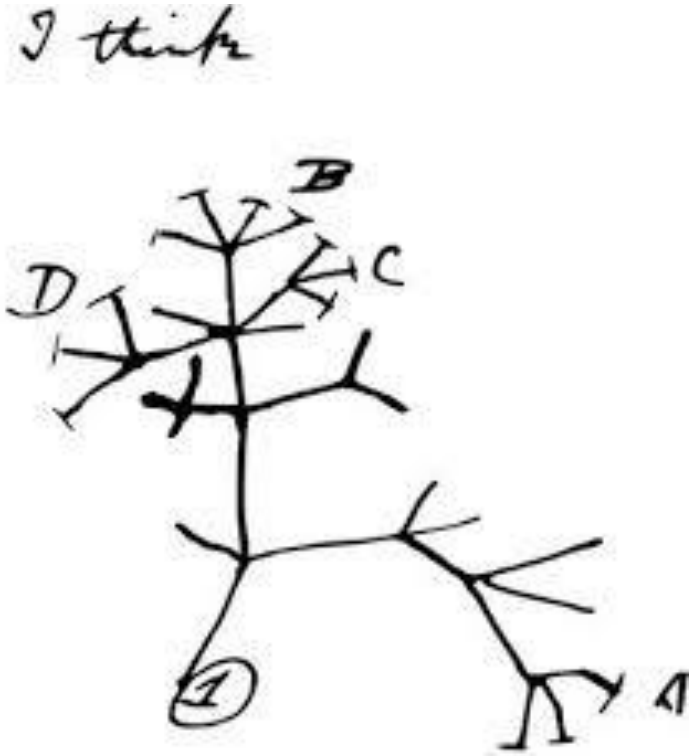
CONTEÚDO PROGRAMÁTICO

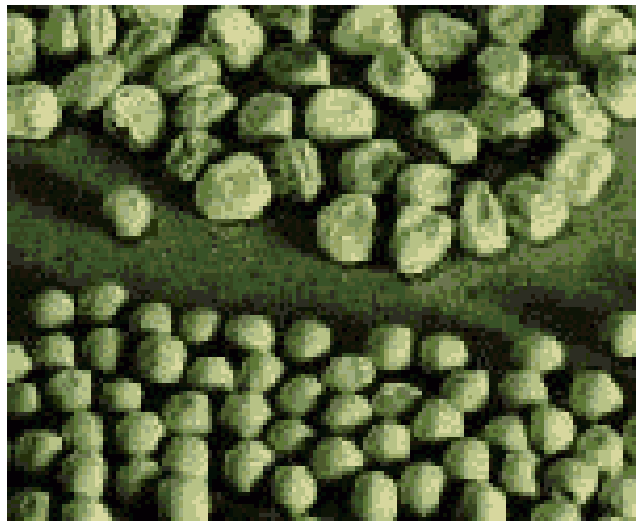
22 de Março – Introdução à Genética Molecular
29 de Abril – Moléculas Celulares: Um Enfoque Nas Proteínas
6 de Abril – Mecanismos Básicos Da Genética Molecular
12 de Abril – Arquitetura Genômica: Dos Genes Ao Genoma
19 de Abril– Sinalização Celular
26 de Abril – Controle Da Expressão Gênica Em Procariotos
3 de Maio - Controle Do Início Da Transcrição Em Eucariotos
10 de Maio – Epigenética de Plantas (Prof. Mateus Mondin)
17 de Maio – Controle Pós Transcricional Da Expressão Gênica
24 de Maio – Controle Pós Traducional Da Expressão Gênica
31 de Maio – Processamento e direcionamento de proteínas
7 de Junho –- Controle Genético Do Ciclo Celular
14 de Junho –RNA de Interferência
21 de Junho – CRISPR-Cas9 – Evolução ou engenharia genética?
28 de Junho– Biologia sintética
19 de Julho –- **Entrega final dos relatórios e trabalho**

O QUE É GENÉTICA MOLECULAR?

A **genética molecular** é a área da biologia que estuda a **estrutura e a função dos genes a nível molecular**. A genética molecular usa os métodos da genética e da biologia molecular para compreender padrões herdáveis nos seres vivos!

Adaptado:
https://pt.wikipedia.org/wiki/Gen%C3%A9tica_molecular

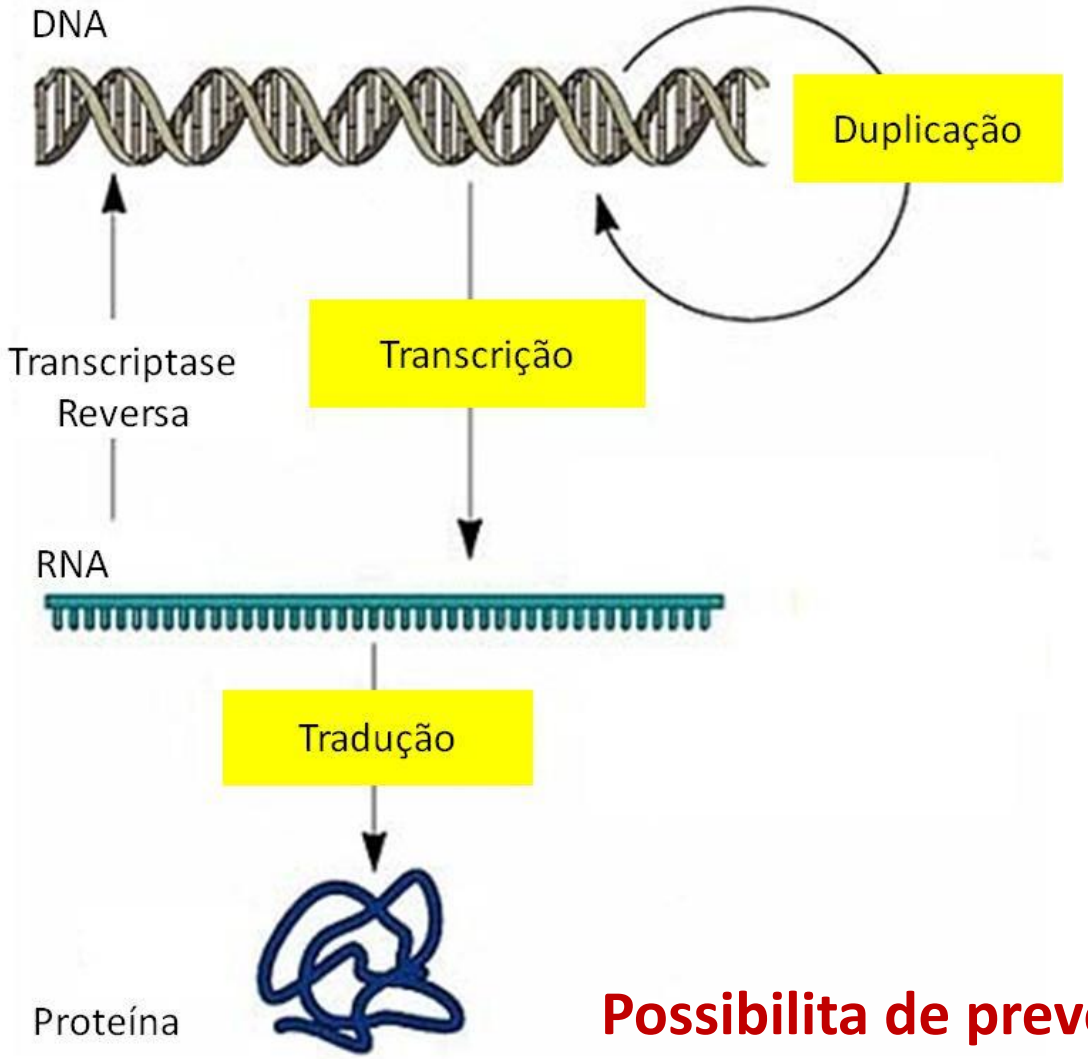




MENDEL: FATORES CONSTANTES QUE CONTROLAM CARACTERÍSTICAS FENOTÍPICAS

Mas porque a semente é lisa ou rugosa?

MAQUINÁRIA “CONSERVADA”



Possibilita de prever padrões de descendência!

MAS NÃO É TÃO SIMPLES...

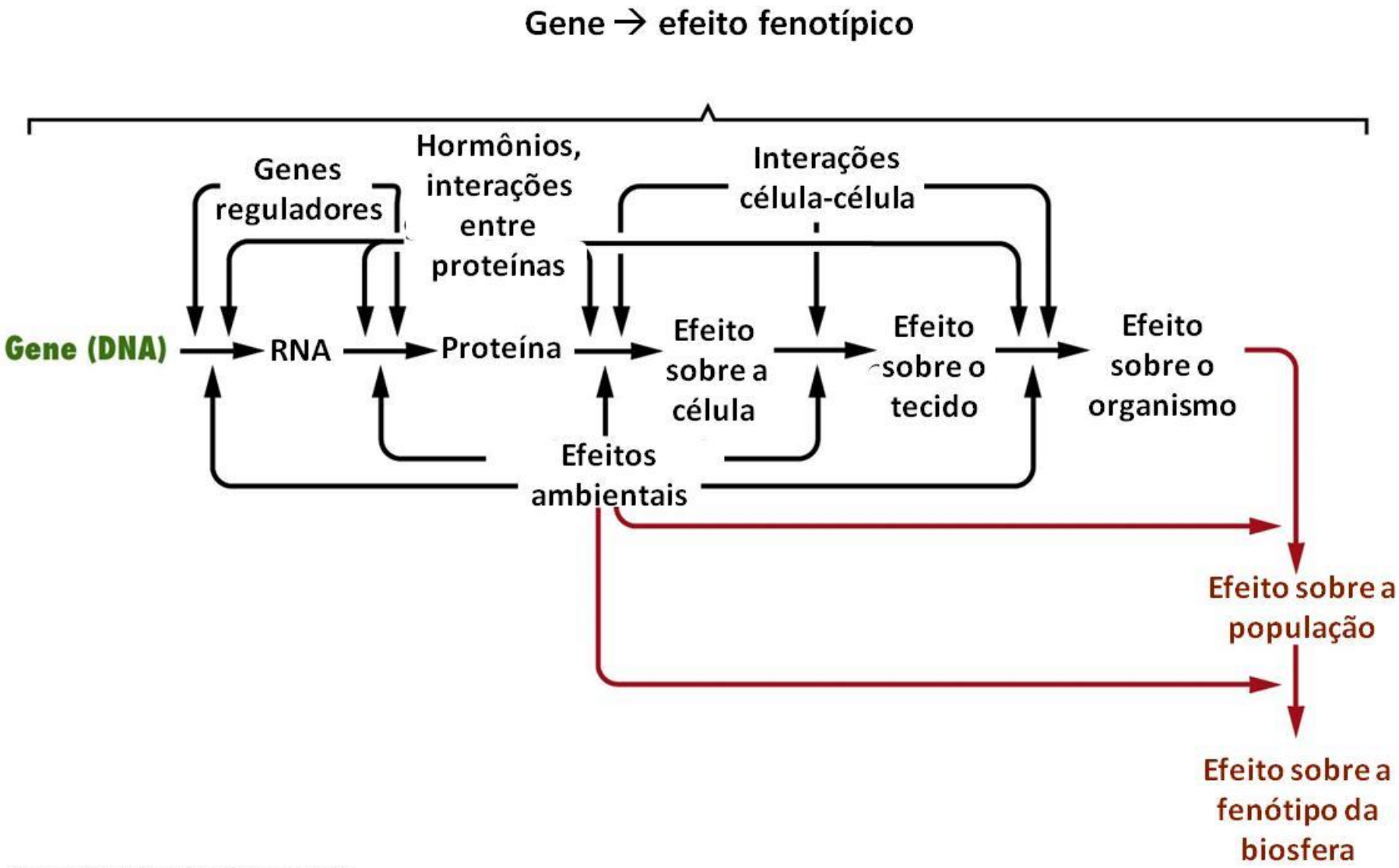
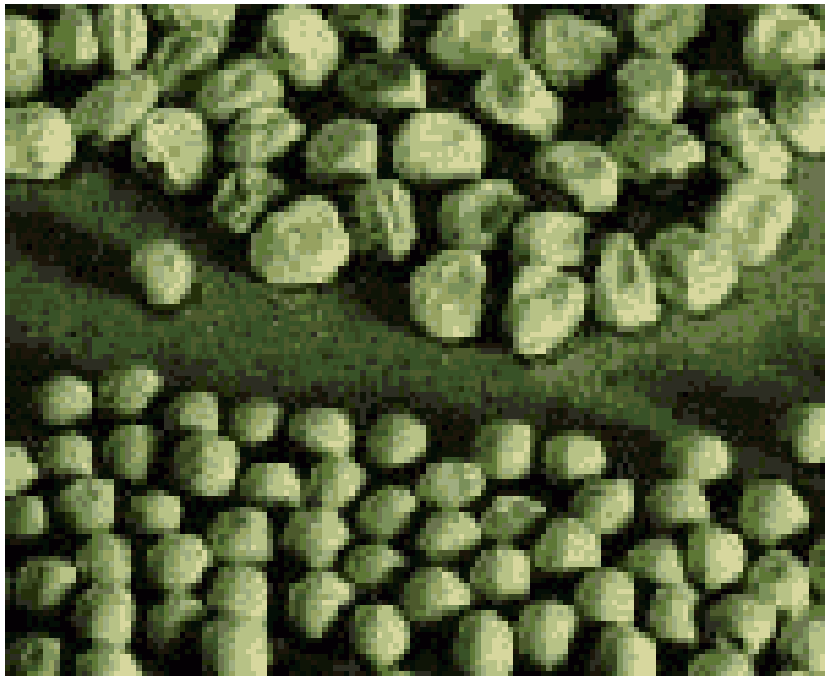


Figure 14-2 Principles of Genetics, 4/e

VOLTANDO À GENÉTICA MENDELIANA...



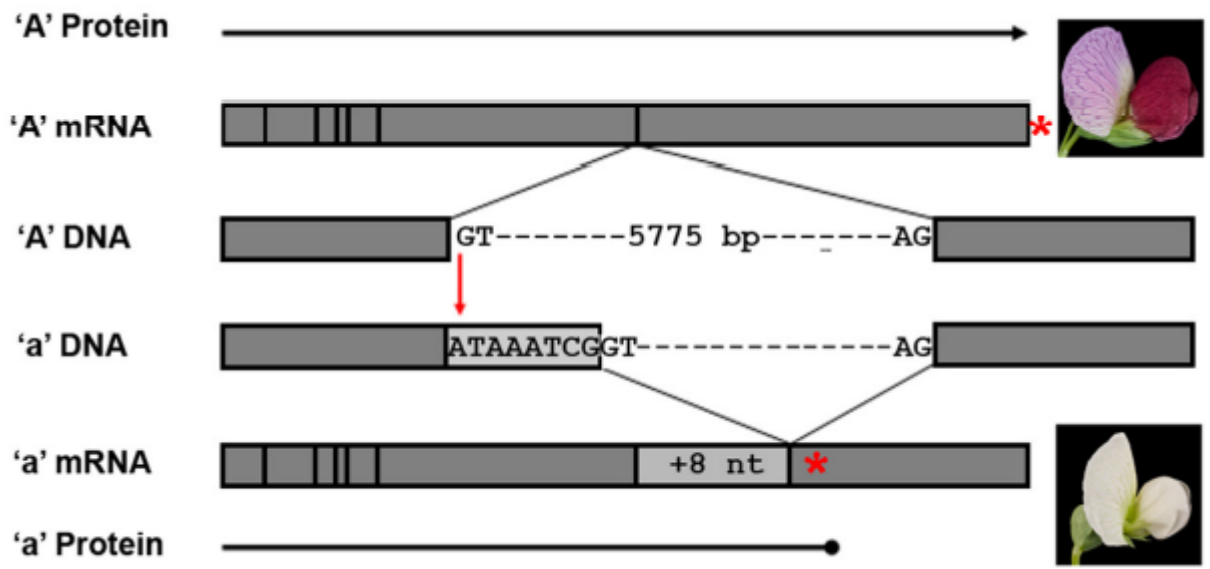
Qual a sequência do gene? Localização no genoma? Características do promotor? Expressão é constitutiva?

OPEN ACCESS Freely available online

PLoS one

Identification of Mendel's White Flower Character

Roger P. Hellens[1], Carol Moreau[2], Kui Lin-Wang[1], Kathy E. Schwinn[3], Susan J. Thomson[4], Mark W. E. J. Fiers[4], Tonya J. Frew[4], Sarah R. Murray[4], Julie M. I. Hofer[2], Jeanne M. E. Jacobs[4], Kevin M. Davies[3], Andrew C. Allan[1], Abdelhafid Bendahmane[5], Clarice J. Coyne[6], Gail M. Timmerman-Vaughan[4], T. H. Noel Ellis[2]*



Mendel's Genes: Toward a Full Molecular Characterization

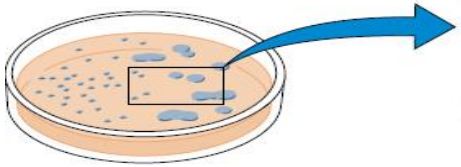
James B. Reid and John J. Ross[1]
School of Plant Science, University of Tasmania, Hobart, Tasmania 7001, Australia

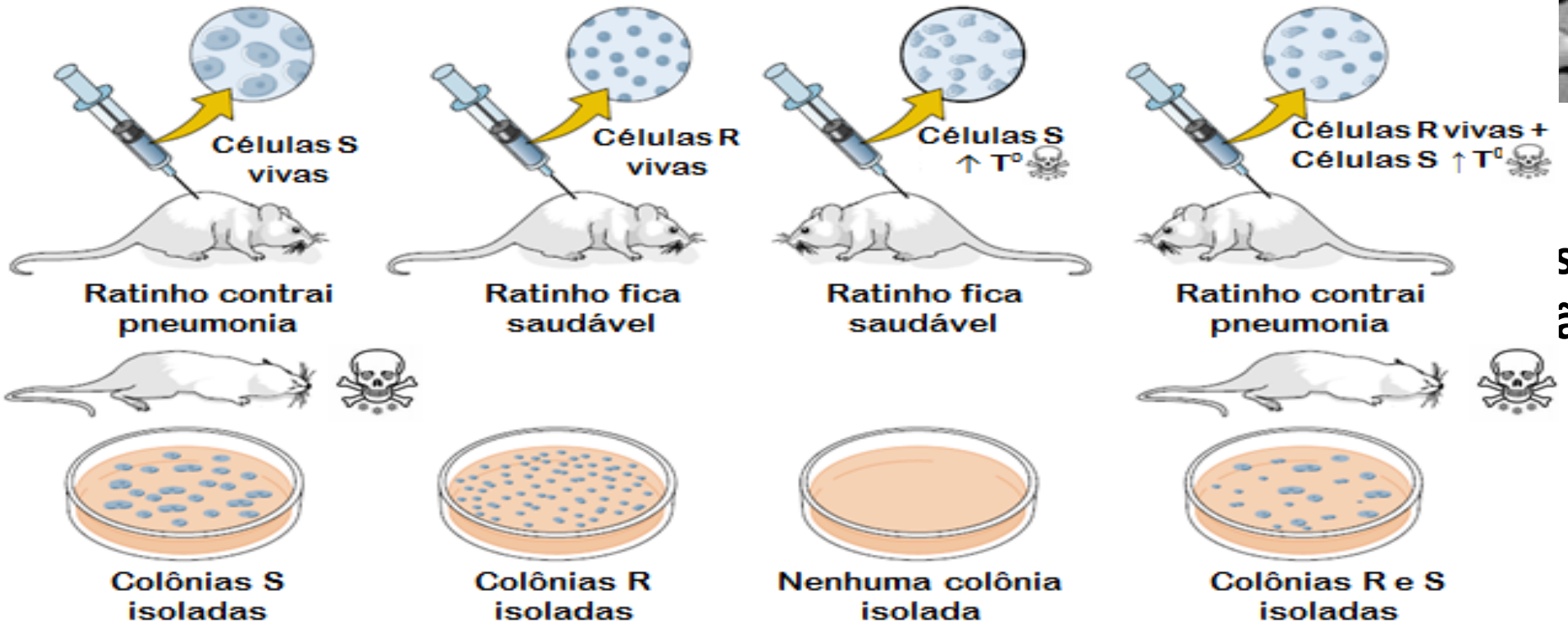
Table 1 Seven characters of *P. sativum* examined by Mendel and a summary of the genes, phenotypes, and presumed mutations involved

Trait	Dominant phenotype	Recessive phenotype	Symbol group	Linkage group	Cloned	Gene function	Molecular nature of mutation
Seed shape	Round	Wrinkled	*R*	V	Yes	Starch branching enzyme 1	0.8-kb insertion
Stem length	Tall	Dwarf	*LE*	III	Yes	GA 3-oxidase1	G-to-A substitution
Cotyledon color	Yellow	Green	*I*	I	Yes	Stay-green gene	6-bp insertion
Seed coat/flower color	Purple	White	*A*	II	Yes	bHLH transcription factor	G-to-A at splice site
Pod color	Green	Yellow	*GP*	V	No	Chloroplast structure in pod wall	Unknown
Pod form	Inflated	Constricted	*V?*	III	No	Sclerenchyma formation in pods	Unknown
Position of flowers	Axial	Terminal	*FA*	IV	No	Meristem function	Unknown

References are given in the text.

1928 - Frederick Griffith





s) foi
ão

QUAL É O PRINCÍPIO TRANSFORMANTE?

ELSEVIER

FEMS Microbiology Letters 149 (1997) 1–10

FEMS MICROBIOLOGY LETTERS

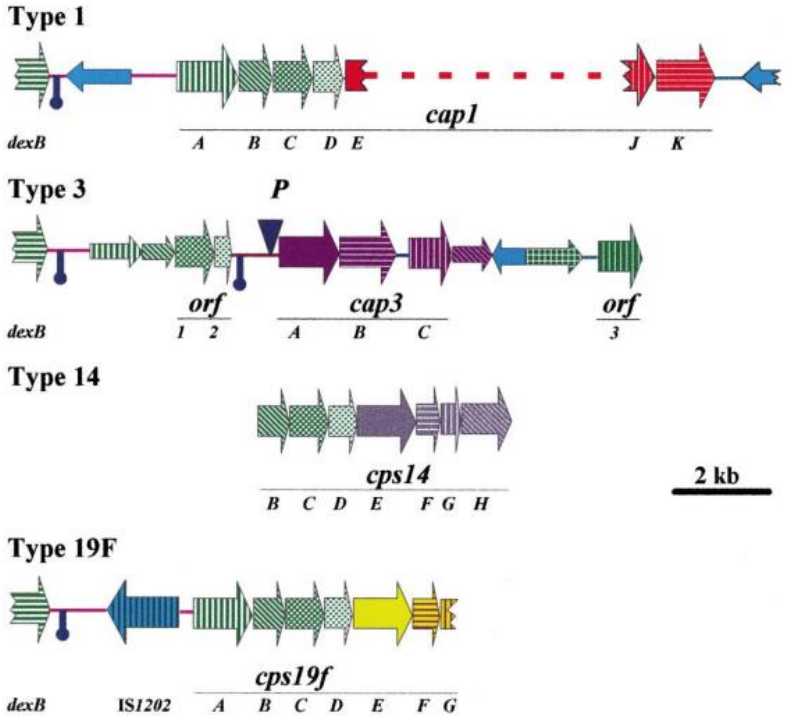
MiniReview

Molecular biology of the capsular genes of *Streptococcus pneumoniae*

Ernesto García *, Rubens López

Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Velázquez 144, 28006 Madrid, Spain

Received 20 November 1996; revised 13 January 1997; accepted 20 January 1997



scientific reports

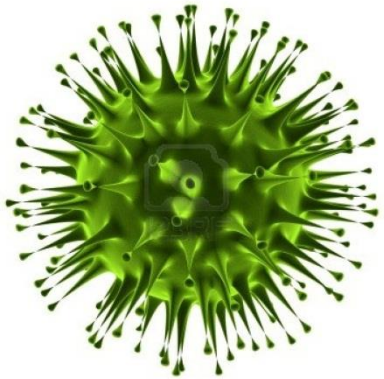
Check for updates

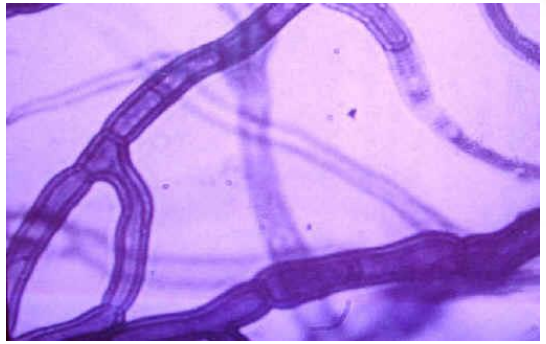
OPEN

Transformation of nonencapsulated *Streptococcus pneumoniae* during systemic infection

Jessica L. Bradshaw[1,2], Iftekhar M. Rafiqullah[1], D. Ashley Robinson[1] & Larry S. McDaniel[1]✉

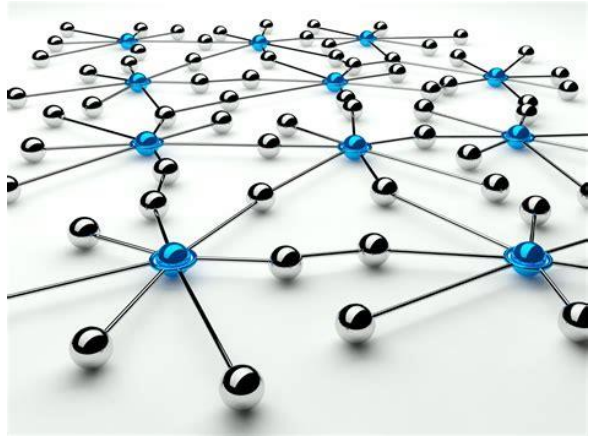
O ORGANISMO ESTUDADO FAZ TODA A DIFERENÇA!





VÁRIOS FATORES DEVEM SER CONSIDERADOS

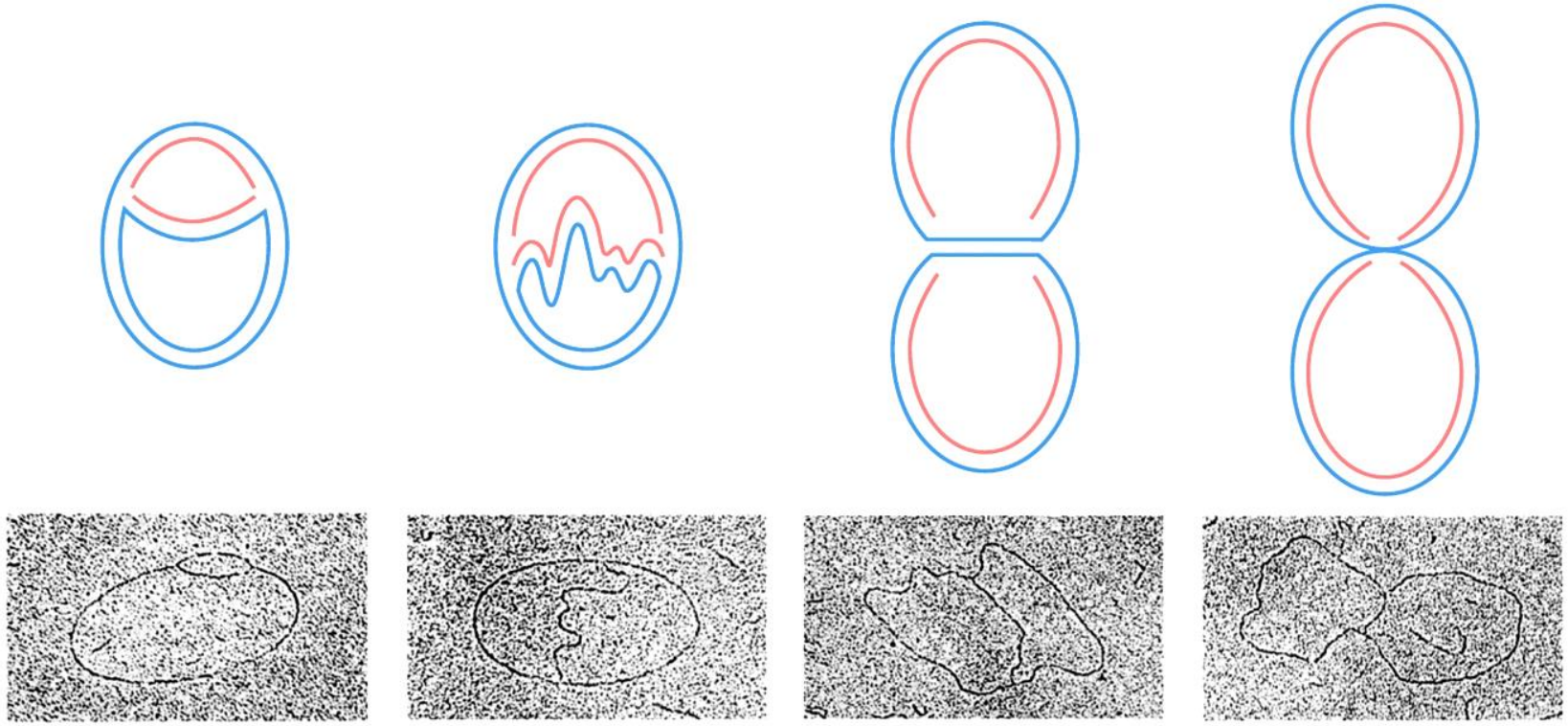
- Tipo celular
- Classificação taxonômica



- Padrão de herança genética
- Tamanho e arquitetura do genoma
- Tipo de reprodução

e outros...

PROCARIOTOS- REPLICAÇÃO É ÚNICA E BIDIRECIONAL



- A velocidade da forquillha de replicação de procarioto é cerca de 30.000 pb/min
- Poucos replicons...
- Fita líder com genes constitutivos

MECANISMOS CONSERVADOS… MAS NEM TANTO…

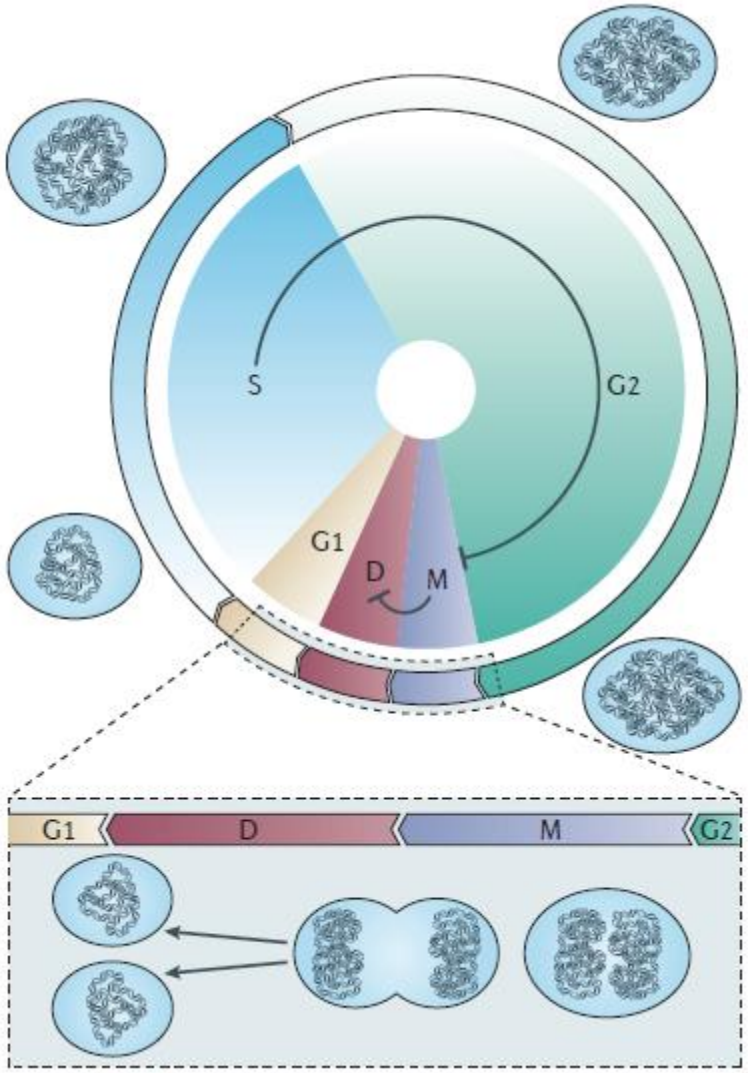
Table 1 | **Factors involved in bacterial replication initiation**

Factor	*Escherichia coli*	*Bacillus subtilis*	*Caulobacter crescentus*
Initiator	DnaA	DnaA	DnaA
Helicase	DnaB	DnaC	DnaB
Helicase loader	DnaC	DnaI, DnaB and DnaD	?
Regulatory factors	SeqA and Hda	YabA	CtrA
DNA architectural factors	HU, IHF and Fis	DnaB and DnaD	IHF

For historical reasons, the *B. subtilis* homologue of *E. coli* DnaB is named DnaC, while DnaC is termed DnaI. *B. subtilis* DnaB is unrelated to the *E. coli* replicative helicase. Fis, factor for inversion stimulation; IHF, integration host factor.

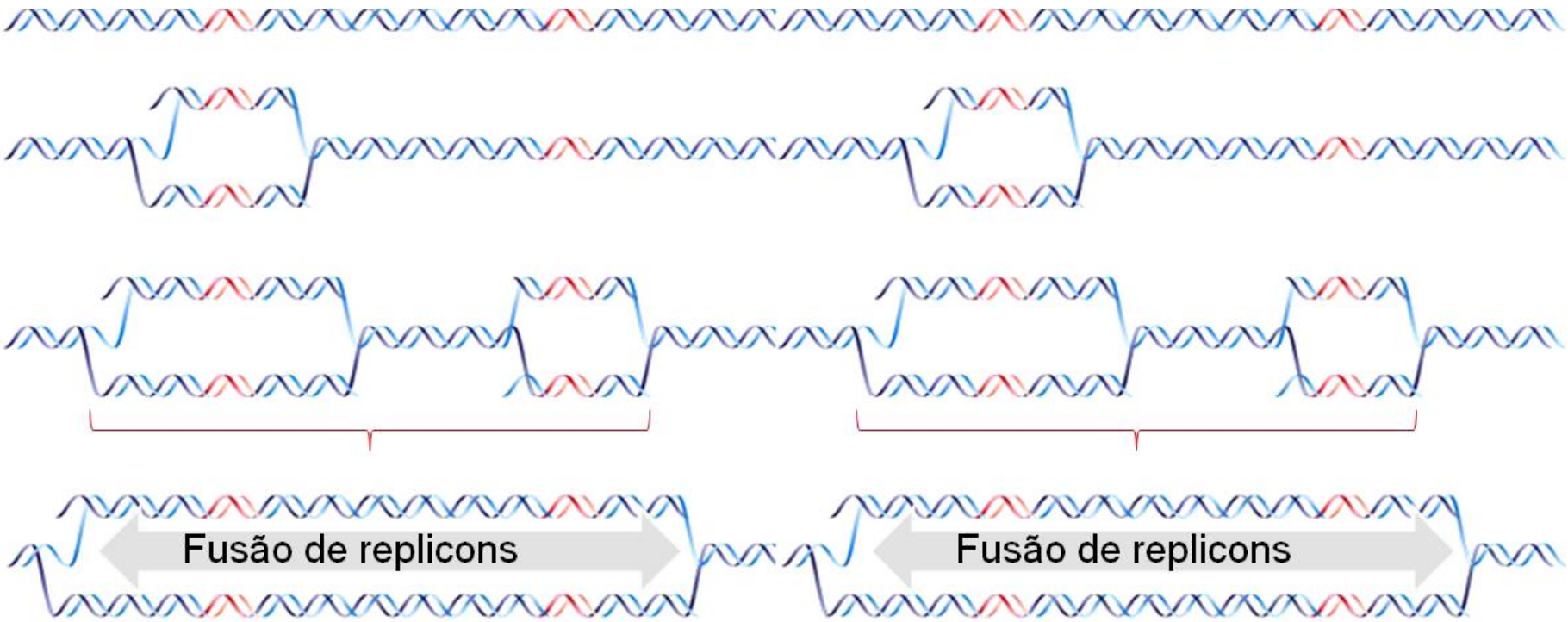
dnaA – mais conservada

Outros genes dependente da espécie …



Arquéia ciclo celular com eventos parecido com de eucariotos

EUCARIOTOS...VÁRIAS FORQUILHAS DE REPLICAÇÃO



- A velocidade da forquillha de replicação de eucarioto é cerca de 3.000 pb/min;

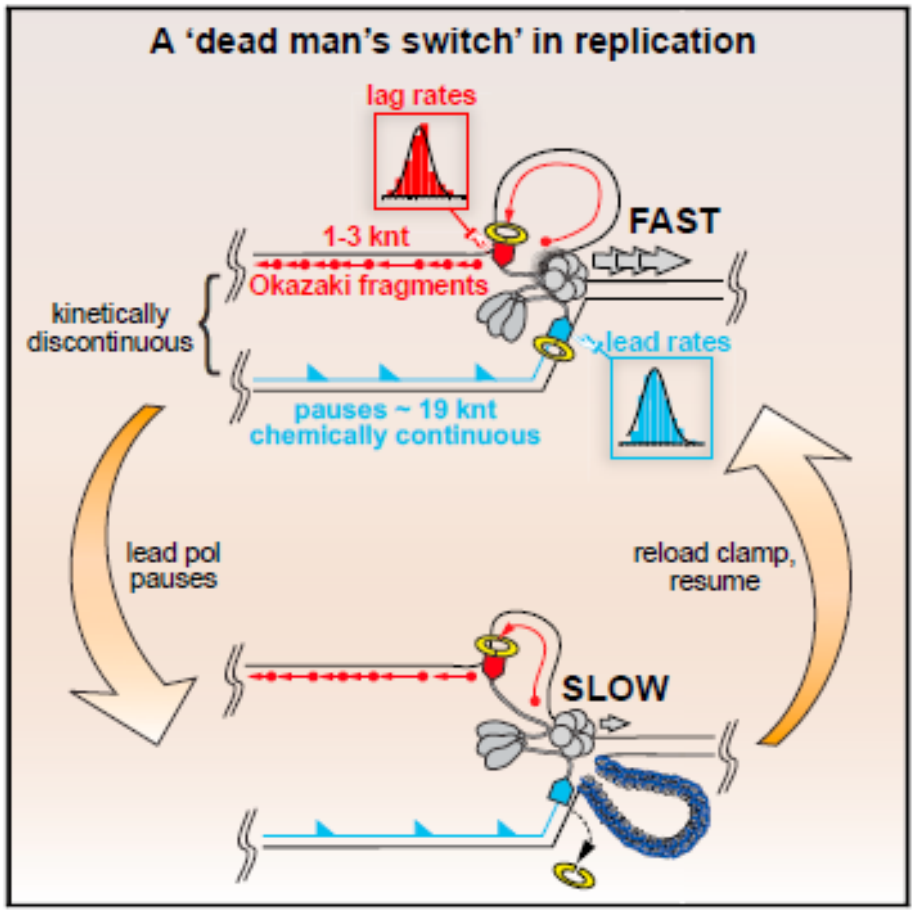
- Os replicons de eucariotos têm cerca de 40-100 kb e são iniciados em tempos diferentes. (não sabemos todos os fatores que determinam qual origem e em que momento ela fica ativa - O *timing* da replicação pode, por ex. ser determinado pela atividade do gene: genes mais transcritos são replicados primeiro).

Article

Cell

Independent and Stochastic Action of DNA Polymerases in the Replisome

Graphical Abstract



Authors

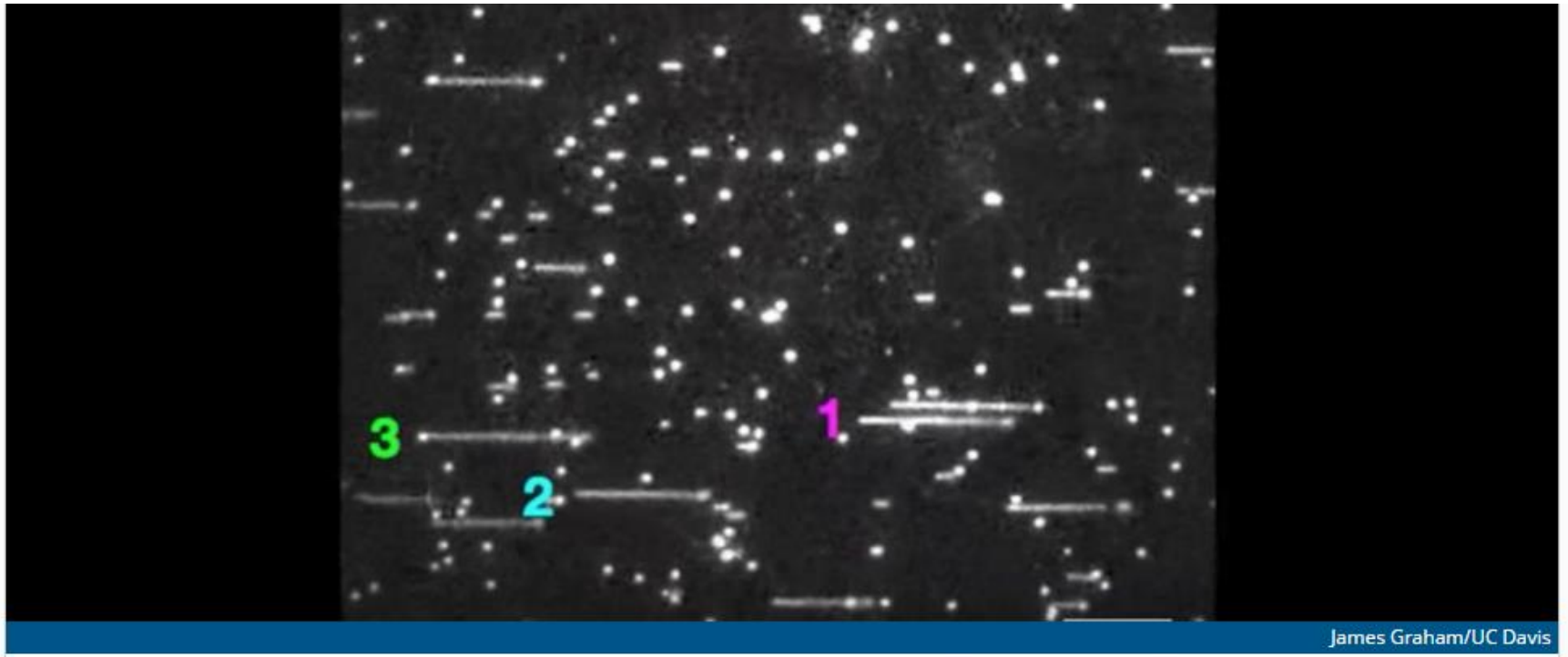
James E. Graham, Kenneth J. Marians, Stephen C. Kowalczykowski

Correspondence

kmarians@sloankettering.edu (K.J.M.), sckowalczykowski@ucdavis.edu (S.C.K.)

In Brief

Polymerases within the replisome operate independently and discontinuously, and they are not coordinated.



DNA Replication Has Been Filmed For The First Time, And It's Not What We Expected

"It undermines a great deal of what's in the textbooks."

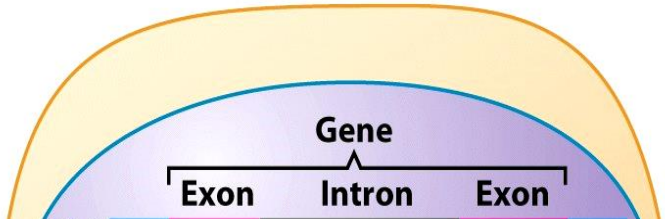
BEC CREW 19 JUN 2017

http://www.sciencealert.com/dna-replication-has-been-filmed-for-the-first-time-and-it-s-stranger-than-we-thought

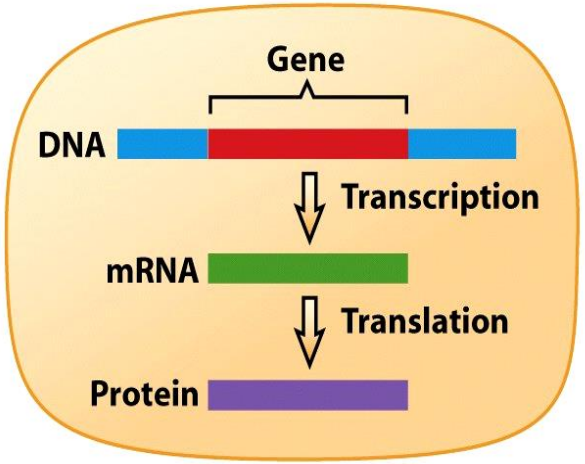
TRANSCRIÇÃO – as diferenças parecem aumentar!!!

✓Nos **eucariotos** a transcrição ocorre no núcleo, enquanto a tradução ocorre no citoplasma.

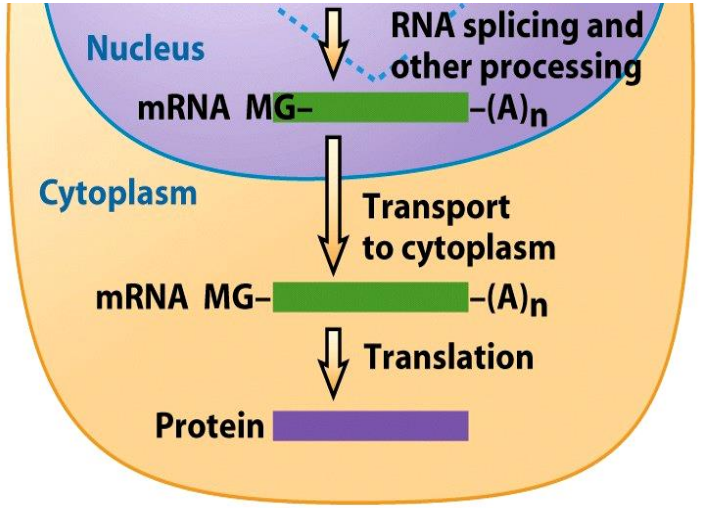
✓Já nos **procariotos** tal separação celular não existe, sendo os dois processos acoplados.



Vital importância no controle de expressão!!!



(a) **Prokaryotes.**



(b) **Eukaryotes.**

GENE TÍPICO DE PROCARIOTOS

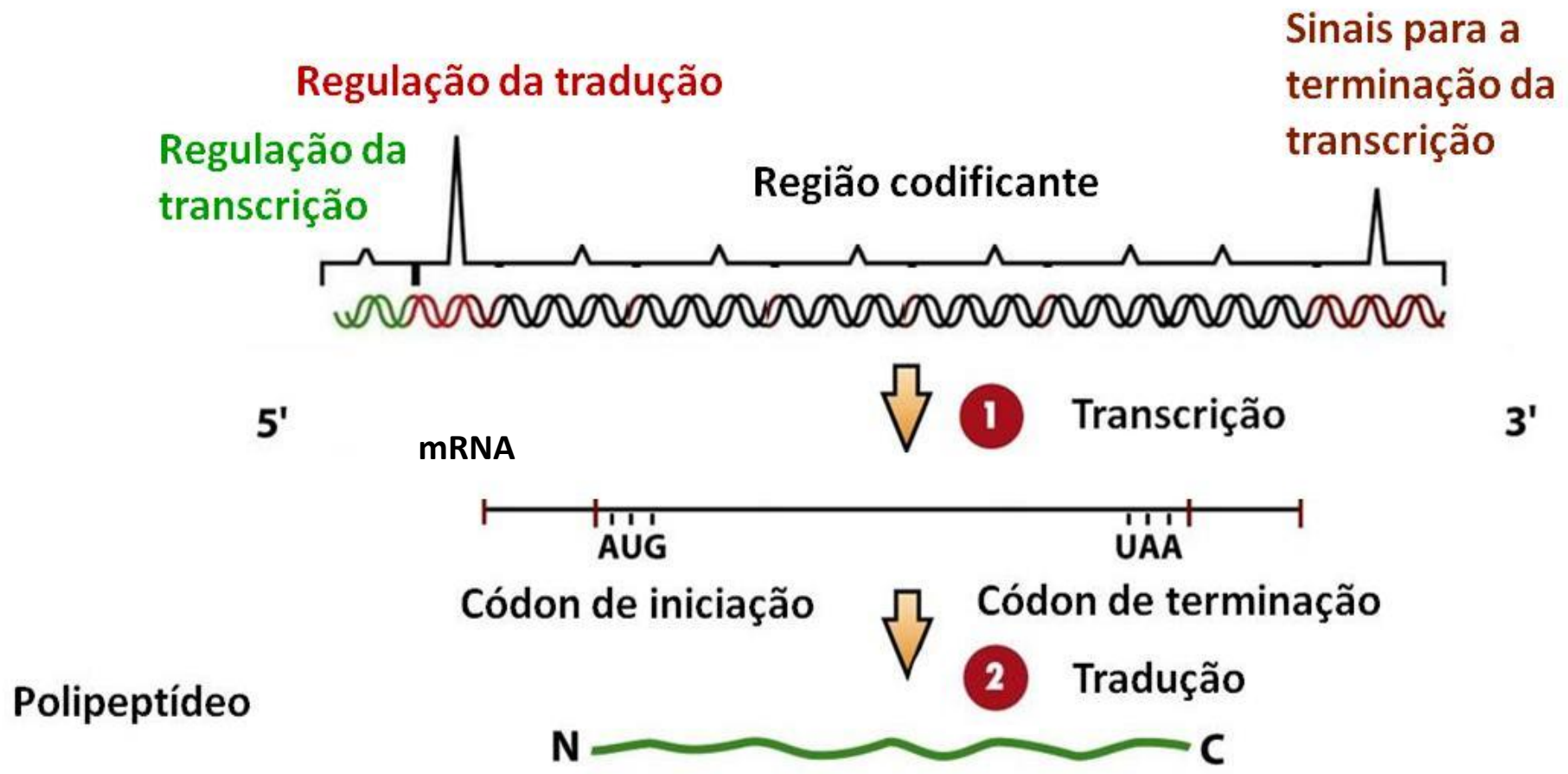
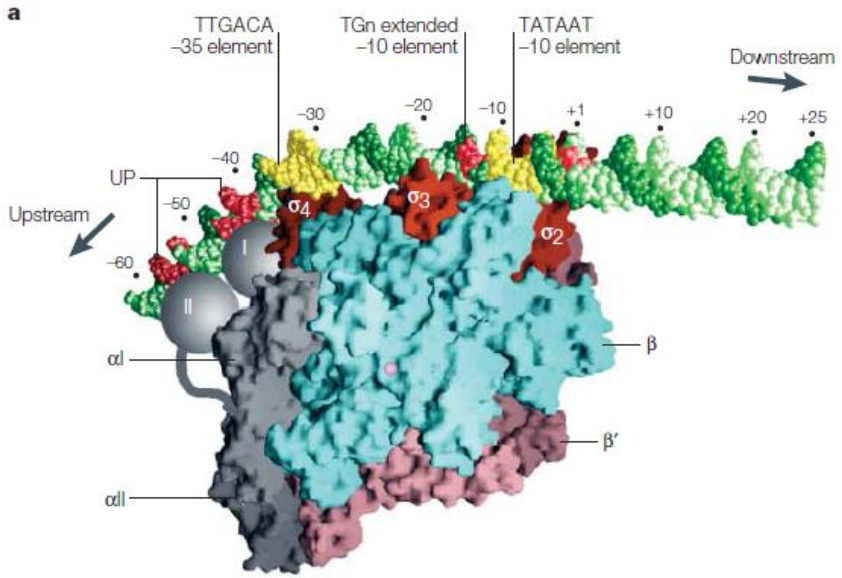
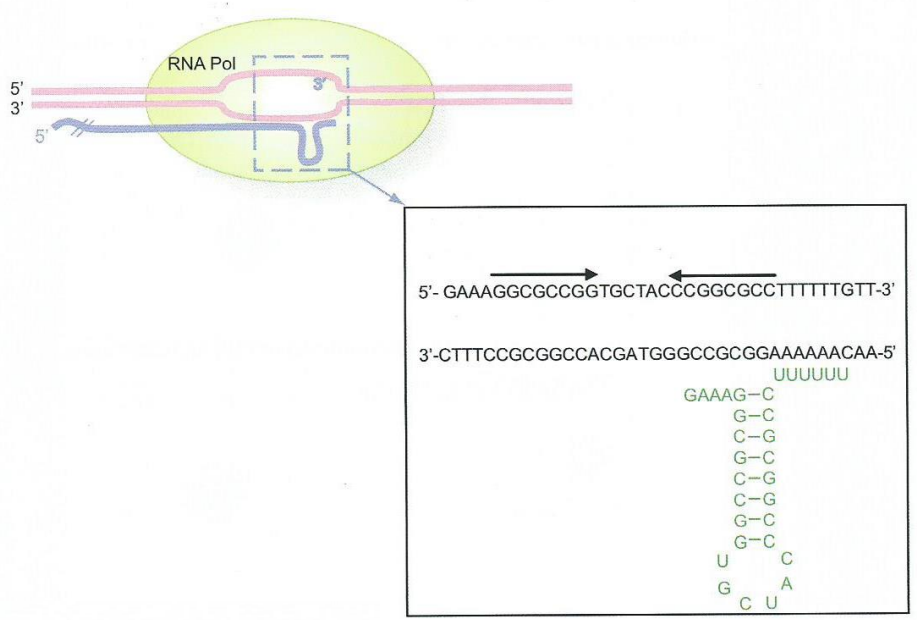
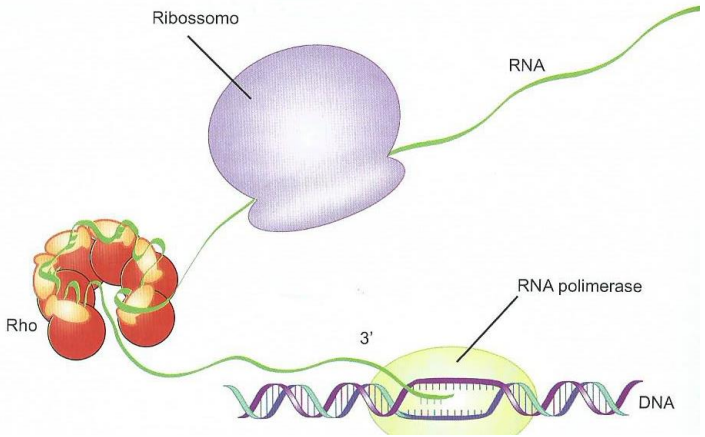


Figure 14-1b Principles of Genetics, 4/e

CONTROLE DA TRANSCRIÇÃO - PROCARIOTO







GENE TÍPICO DE EUCARIOTOS

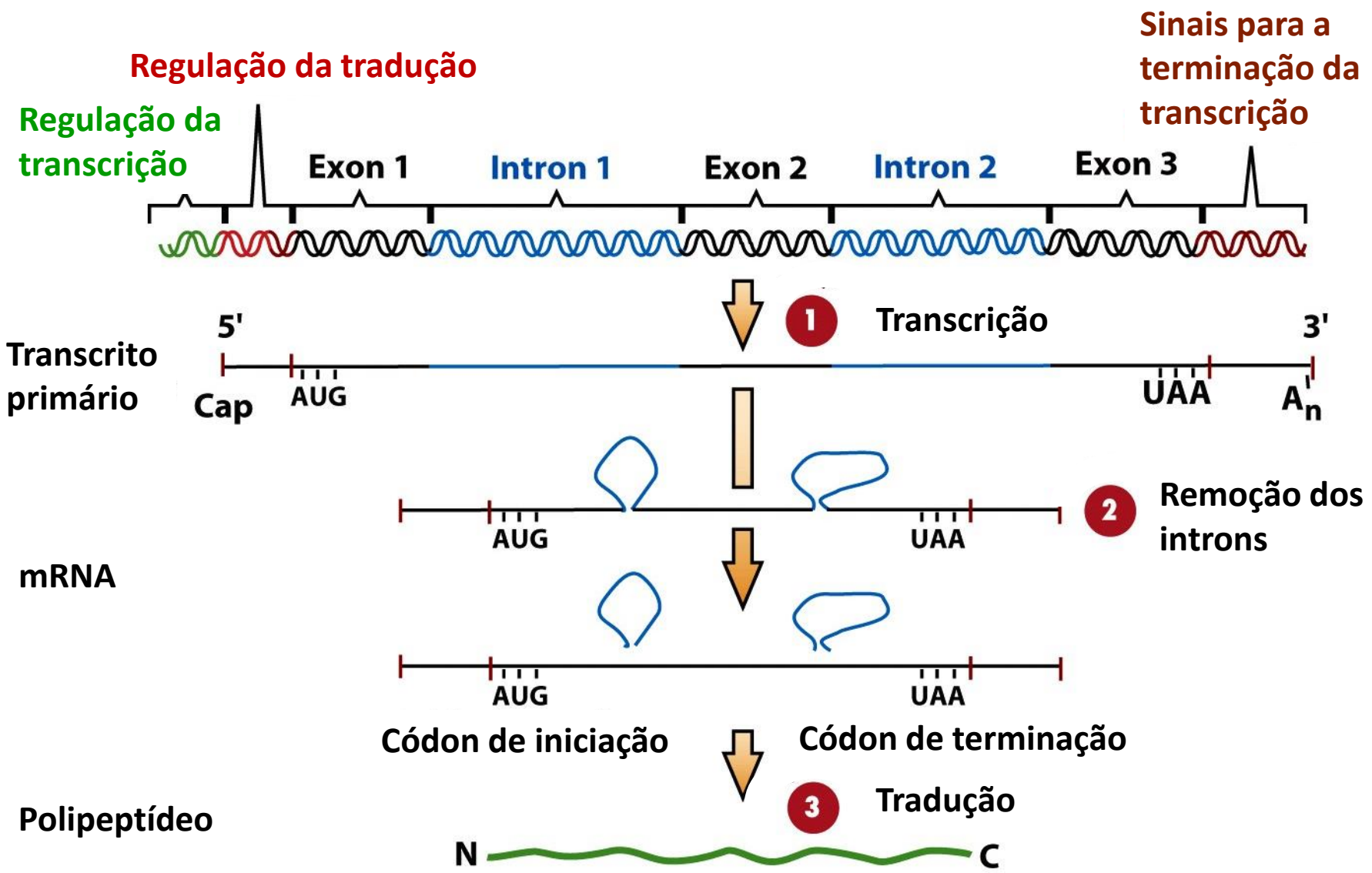
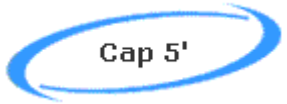


Figure 14-1b Principles of Genetics, 4/e

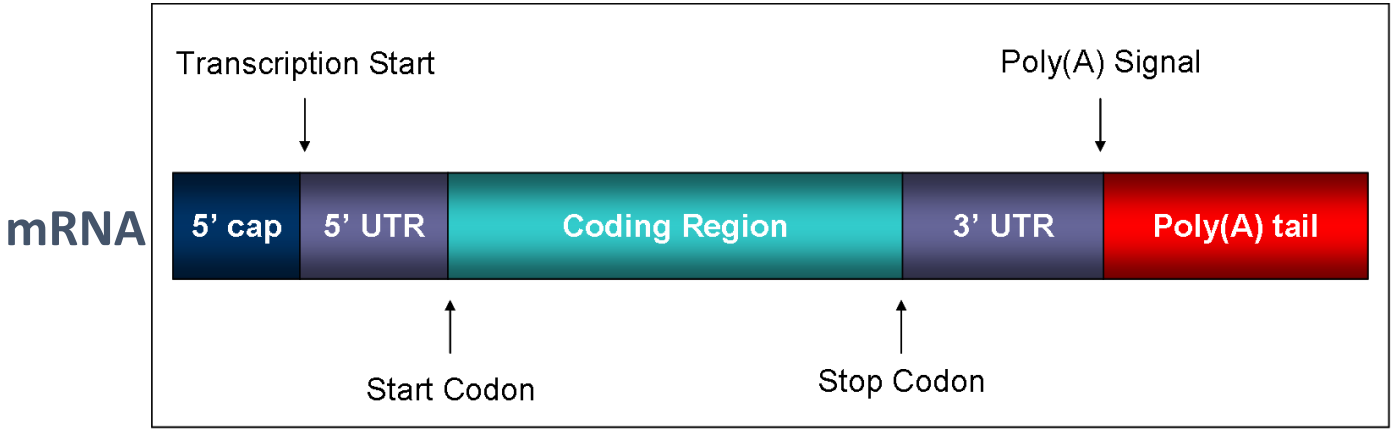
PROCESSAMENTO DO RNA (TRANSCRITO) PRIMÁRIO EM EUCARIOTOS

- As modificações que podem ocorrer nos transcritos nucleares são basicamente de três tipos:

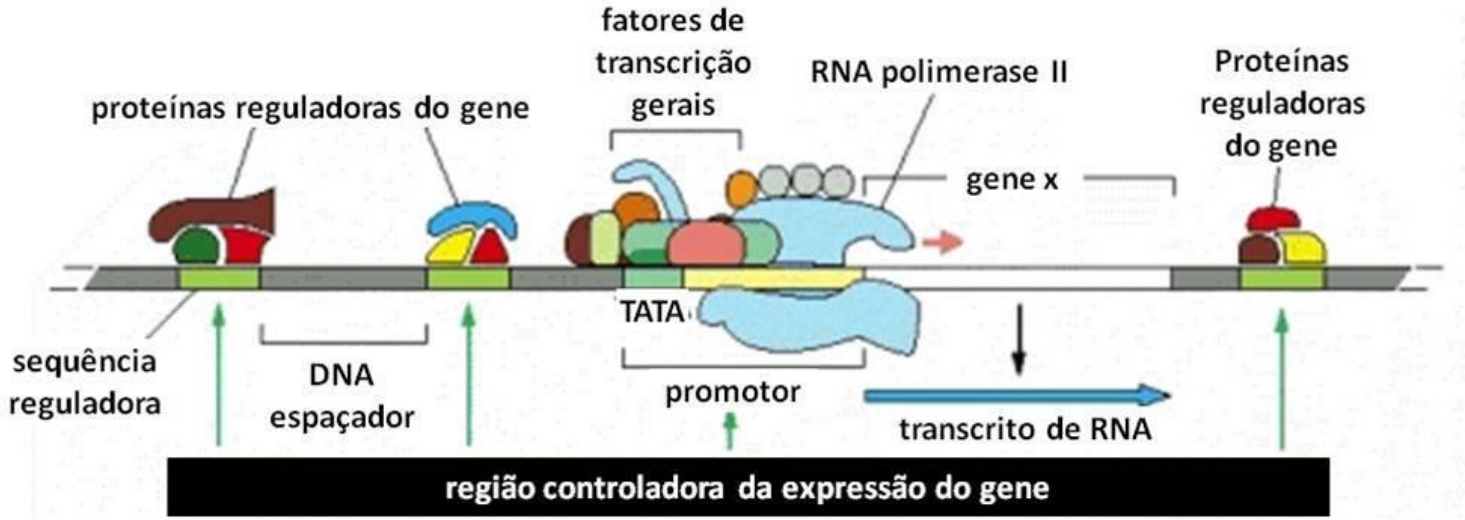
  - Capeamento ("capping") do terminal 5';
  - Poliadenilação do terminal 3';
  - Montagem de segmentos codificadores ("*splicing*").

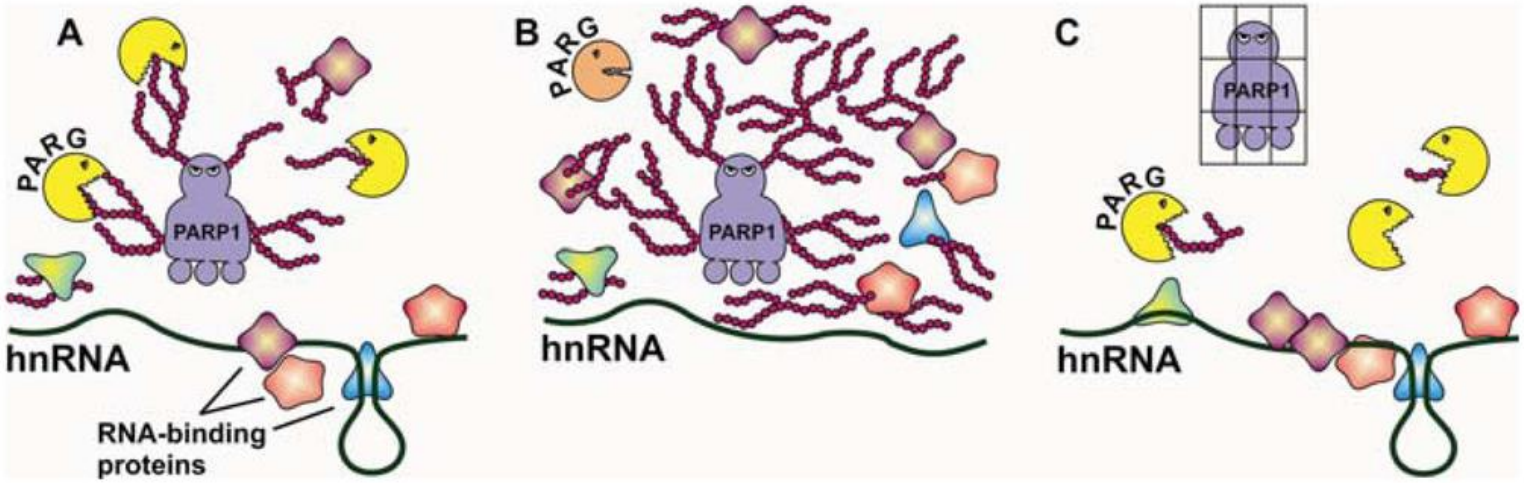


- Este conjunto de modificações no transcrito nuclear originará o mRNA, pronto para migrar para o citoplasma.



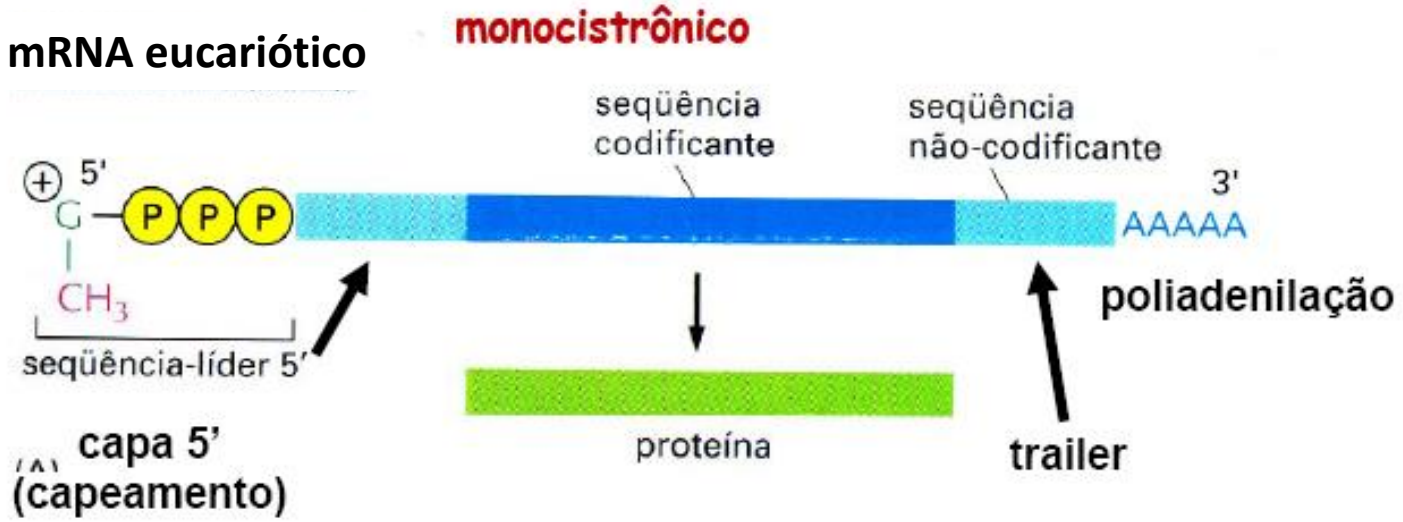
CONTROLE DA TRANSCRIÇÃO - EUCARIOTOS





CÍSTRONS: PROCARIOTOS X EUCARIOTOS

Cadê o promotor do meu gene?



Pesquisadores tem integrado genética e variações transcricionais para conduzir o estudo de associação de transcriptoma amplo (transcription-wide association study - TWAS) correlacionando expressão de genes e características complexas.

ARTICLES

nature genetics

Integrative approaches for large-scale transcriptome-wide association studies

Alexander Gusev[1–3], Arthur Ko[4,5], Huwenbo Shi[6], Gaurav Bhatia[1–3], Wonil Chung[1], Brenda W J H Penninx[7], Rick Jansen[7], Eco J C de Geus[8], Dorret I Boomsma[8], Fred A Wright[9], Patrick F Sullivan[10–12], Elina Nikkola[4], Marcus Alvarez[4], Mete Civelek[13], Aldons J Lusis[4,13], Terho Lehtimäki[14], Emma Raitoharju[14], Mika Kähönen[15], Ilkka Seppälä[14], Olli T Raitakari[16,17], Johanna Kuusisto[18], Markku Laakso[18], Alkes L Price[1–3], Päivi Pajukanta[4,5] & Bogdan Pasaniuc[4,6,19]

PERSPECTIVE
https://doi.org/10.1038/s41588-019-0385-z
nature genetics

Opportunities and challenges for transcriptome-wide association studies

Michael Wainberg[1], Nasa Sinnott-Armstrong[2], Nicholas Mancuso[3], Alvaro N. Barbeira[4], David A. Knowles[5,6], David Golan[2], Raili Ermel[7], Arno Ruusalepp[7,8], Thomas Quertermous[9], Ke Hao[10], Johan L. M. Björkegren[8,10,11,12]*, Hae Kyung Im[4]*, Bogdan Pasaniuc[3,13,14]*, Manuel A. Rivas[15]* and Anshul Kundaje[1,2]*

Transcriptome-wide association studies (TWAS) integrate genome-wide association studies (GWAS) and gene expression datasets to identify gene–trait associations. In this Perspective, we explore properties of TWAS as a potential approach to prioritize causal genes at GWAS loci, by using simulations and case studies of literature-curated candidate causal genes for schizophrenia, low-density-lipoprotein cholesterol and Crohn's disease. We explore risk loci where TWAS accurately prioritizes the likely causal gene as well as loci where TWAS prioritizes multiple genes, some likely to be non-causal, owing to sharing of expression quantitative trait loci (eQTL). TWAS is especially prone to spurious prioritization with expression data from non-trait-related tissues or cell types, owing to substantial cross-cell-type variation in expression levels and eQTL strengths. Nonetheless, TWAS prioritizes candidate causal genes more accurately than simple baselines. We suggest best practices for causal-gene prioritization with TWAS and discuss future opportunities for improvement. Our results showcase the strengths and limitations of using eQTL datasets to determine causal genes at GWAS loci.

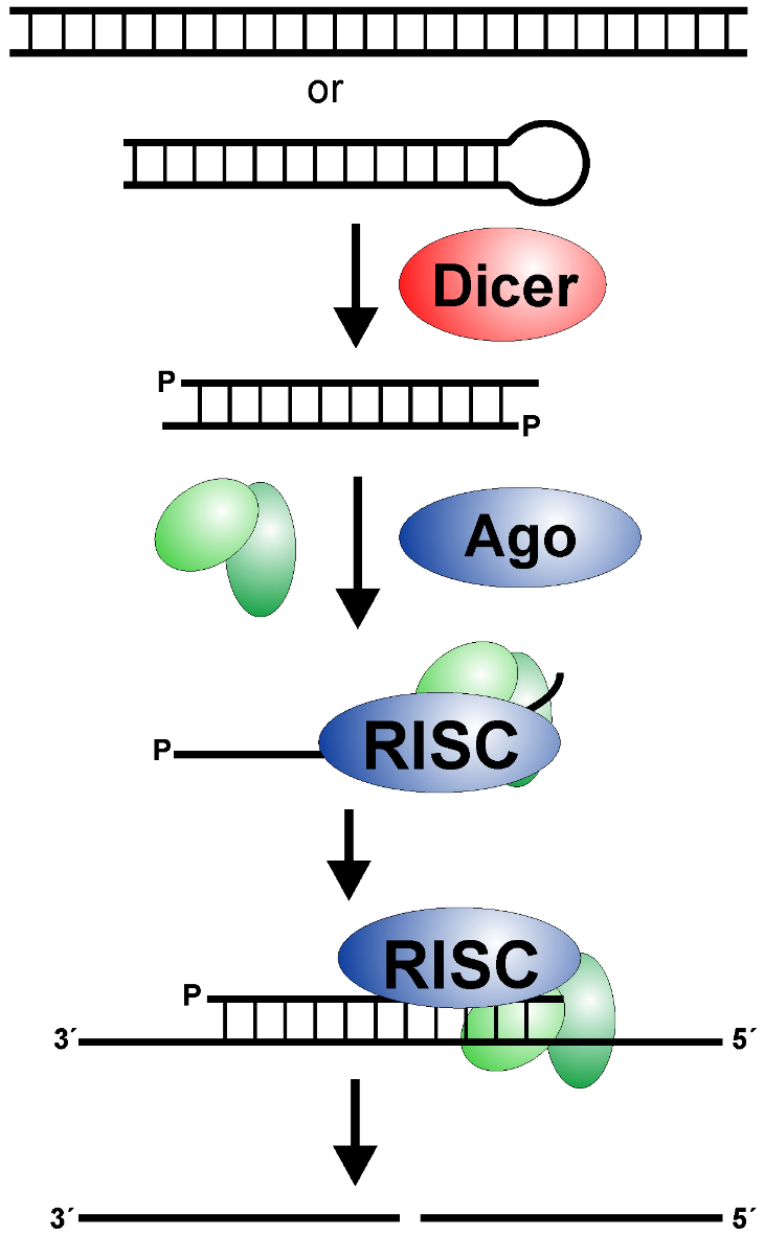
Published online 16 October 2022

Nucleic Acids Research, 2023, Vol. 51, Database issue ***D1179–D1187***
https://doi.org/10.1093/nar/gkac821

TWAS Atlas: a curated knowledgebase of transcriptome-wide association studies

Mingming Lu[1,2,3,†], Yadong Zhang[1,2,†], Fengchun Yang[4,†], Jialin Mai[1,2,3,†], Qianwen Gao[1,2,3], Xiaowei Xu[4], Hongyu Kang[4], Li Hou[4], Yunfei Shang[1,2,3], Qiheng Qain[1,2,3], Jie Liu[5], Meiye Jiang[1,2,3], Hao Zhang[1,2,3], Congfan Bu[1,2], Jinyue Wang[6], Zhewen Zhang[1,2], Zaichao Zhang[7], Jingyao Zeng[1,2,*], Jiao Li[4,*] and Jingfa Xiao[1,2,3,*]

O QUE É ESSA IMAGEM?



https://www.gene-quantification.de/rnai.html

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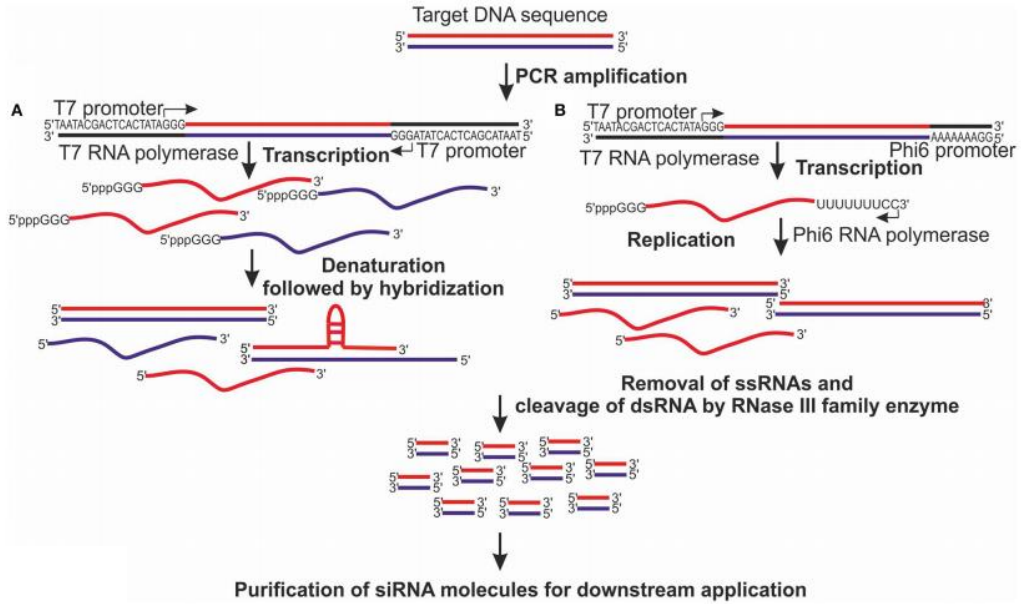
frontiers in Microbiology

MINI REVIEW
published: 11 September 2018
doi: 10.3389/fmicb.2018.02151

RNA Interference as a Prospective Tool for the Control of Human Viral Infections

Alesia Levanova and Minna M. Poranen*

Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland



International Journal of *Molecular Sciences*

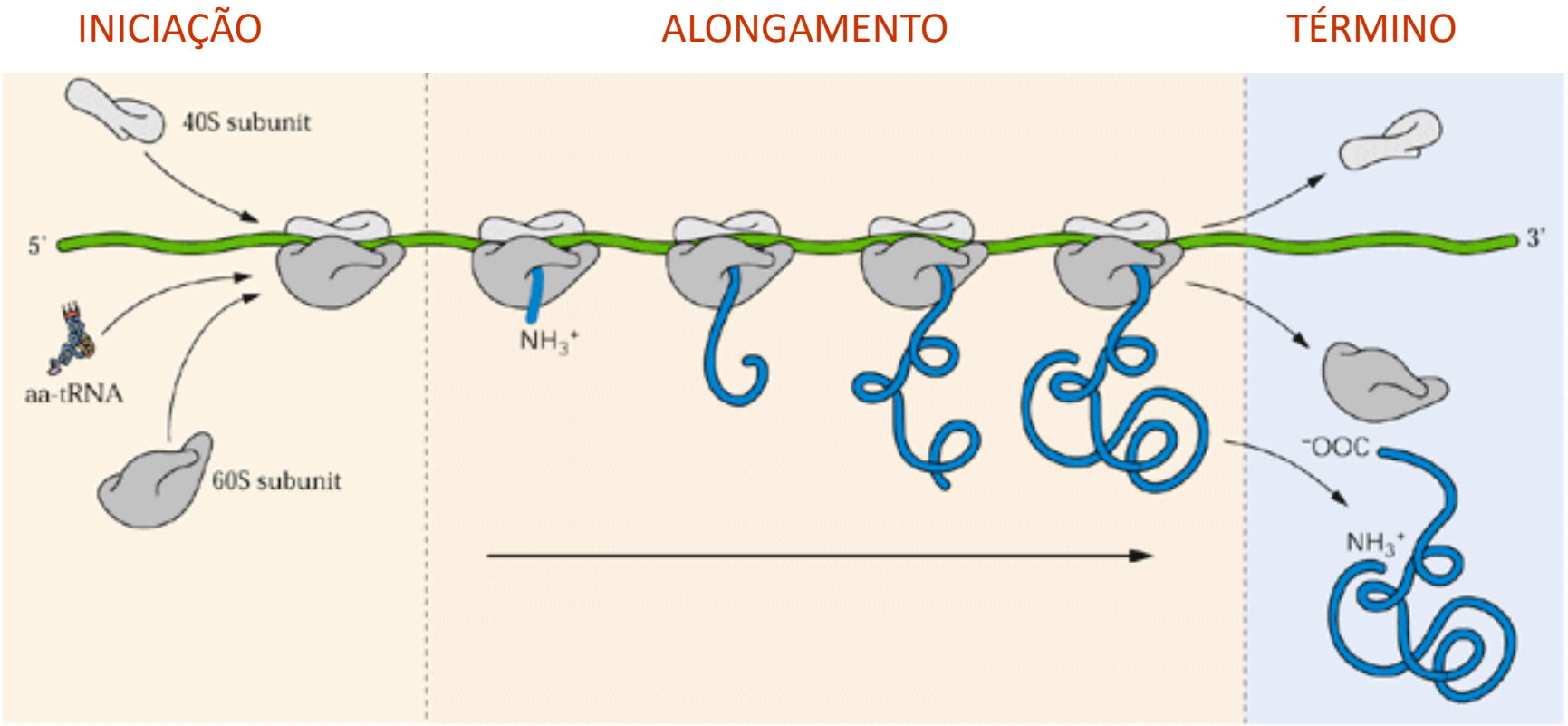
MDPI

Review

RNA Interference: Promising Approach to Combat Plant Viruses

Sehrish Akbar [1,†], Yao Wei [1,†] and Mu-Qing Zhang [1,2,*]

TRADUÇÃO



DOBRAMENTO E PROCESSAMENTO DE PROTEÍNAS DE ACORDO COM O TIPO CELULAR

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 7-24 Essential Cell Biology 3/e (© Garland Science 2010)

5′ 3′

Qual hospedeiro utilizar??

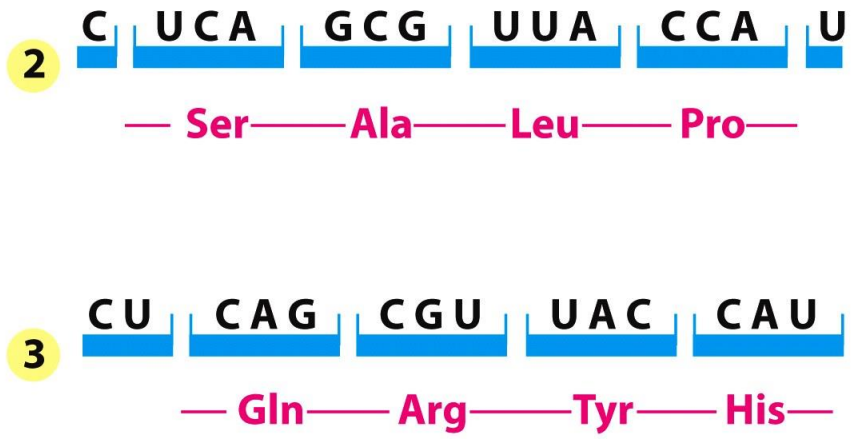


Figure 7-25 Essential Cell Biology 3/e (© Garland Science 2010)

Os codóns usados variam drasticamente!

Molecular Cell
Review

Codon Bias as a Means to Fine-Tune Gene Expression

Tessa E.F. Quax,[1,2,4] Nico J. Claassens,[1,4] Dieter Söll,[3] and John van der Oost[1,*]
[1]Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, the Netherlands
[2]Institut für Biologie II, Albert Ludwig Universität Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany
[3]Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06520-8114, USA
[4]Co-first author
*Correspondence: john.vanderoost@wur.nl
http://dx.doi.org/10.1016/j.molcel.2015.05.035

Table 2. Coding Sequence Features Relevant for Synthetic Gene Design

Global Host and Condition-Related Codon Usage
codon usage frequency of highly expressed genes (CAI)
tRNA gene copy numbers (tAI)
balance tRNA supply/mRNA codon demand (nTE)[a]
tRNA expression levels[a]
charged tRNA levels[a]
tRNA modification levels[a]
synonymous codon co-occurrence bias[a]
non-synonymous codon pair bias[a]

SINAIS PARA O INÍCIO DA TRADUÇÃO

PROCARIOTO

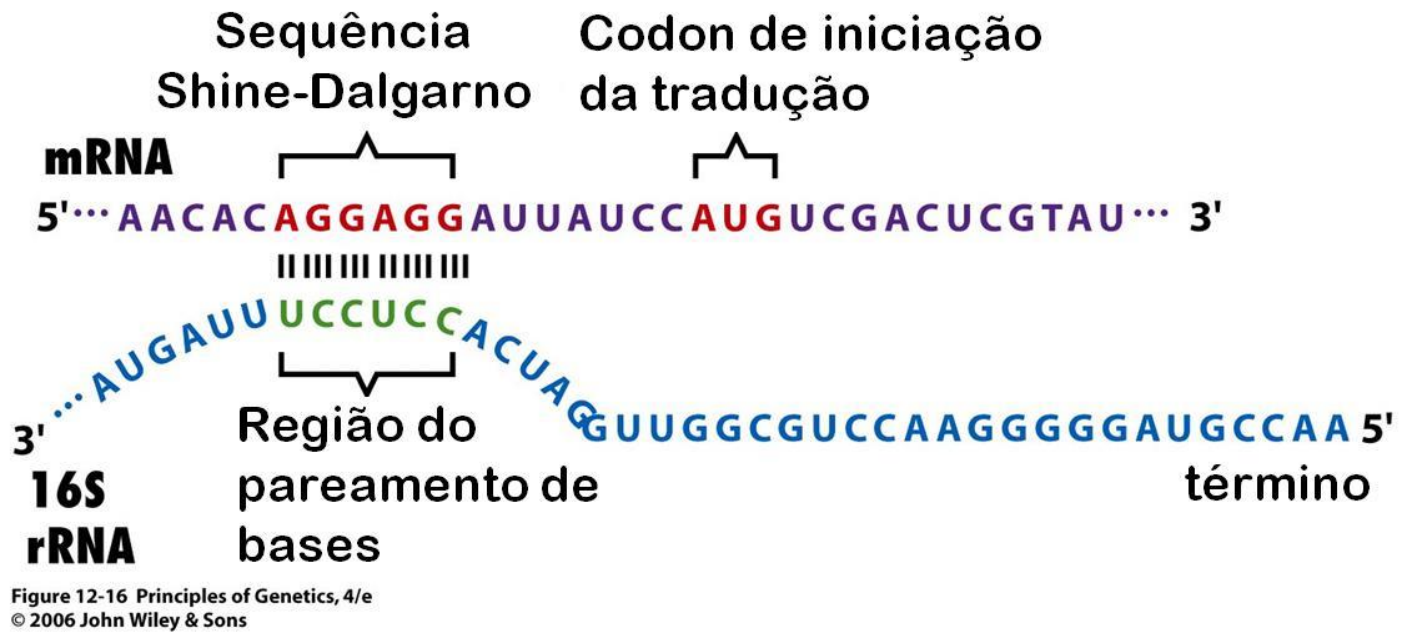


Figure 12-16 Principles of Genetics, 4/e
© 2006 John Wiley & Sons

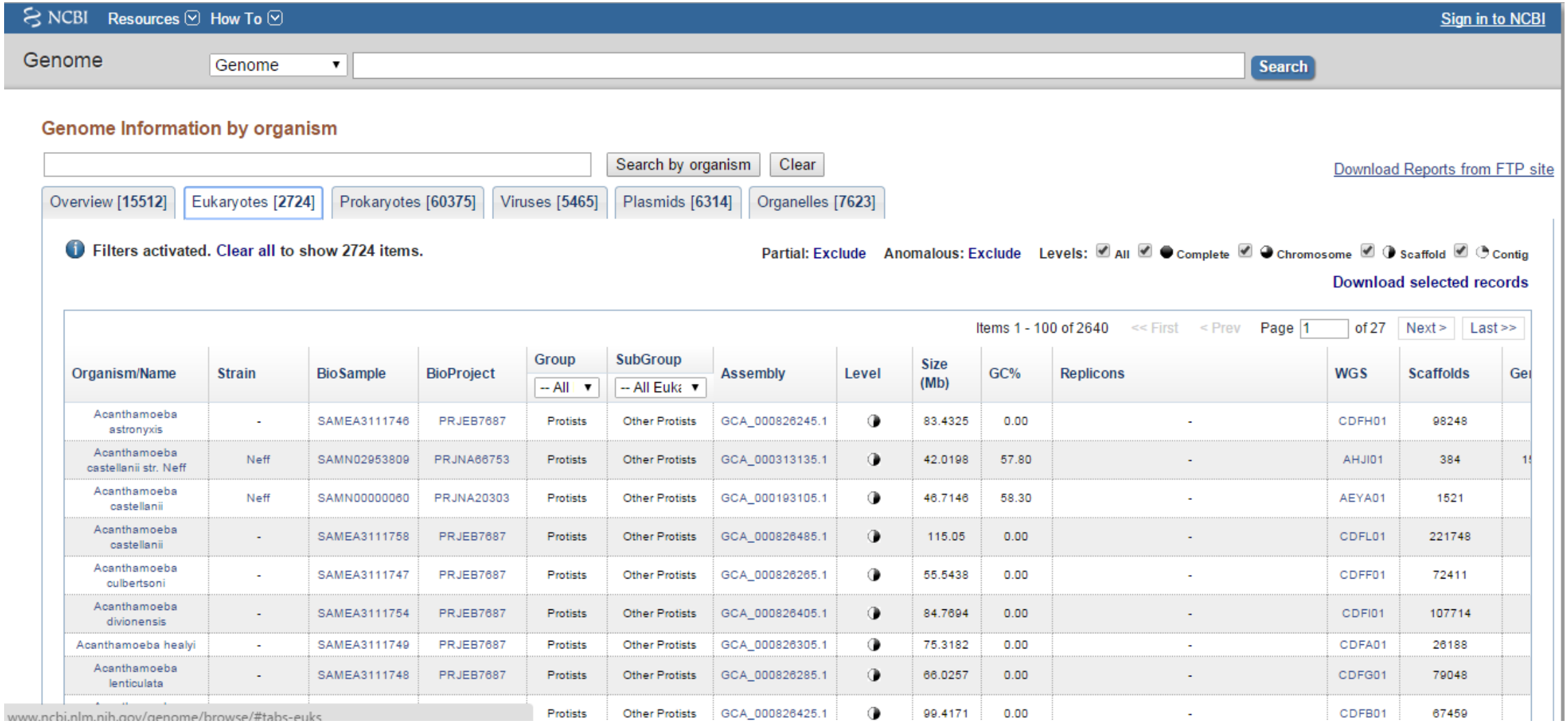
EUCARIOTO

(Regras de Kozak)

5´ - GCC (A ou G) CC **AUG**G - 3`

SINAL PARA O INÍCIO DA TRADUÇÃO EM EUCARIOTOS

UMA QUANTIDADE ENORME DE GENOMAS E DADOS RELACIONADOS…



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Partial: Exclude Anomalous: Exclude Levels: All Complete Chromosome Scaffold Contig

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Organism/Name	Strain	BioSample	BioProject	Group -- All	SubGroup -- All Euka	Assembly	Level	Size (Mb)	GC%	Replicons	WGS	Scaffolds	Ge
Acanthamoeba astronyxis	-	SAMEA3111746	PRJEB7687	Protists	Other Protists	GCA_000826245.1		83.4325	0.00	-	CDFH01	98248	
Acanthamoeba castellanii str. Neff	Neff	SAMN02953809	PRJNA66753	Protists	Other Protists	GCA_000313135.1		42.0198	57.80	-	AHJI01	384	1
Acanthamoeba castellanii	Neff	SAMN00000060	PRJNA20303	Protists	Other Protists	GCA_000193105.1		46.7146	58.30	-	AEYA01	1521	
Acanthamoeba castellanii	-	SAMEA3111758	PRJEB7687	Protists	Other Protists	GCA_000826485.1		115.05	0.00	-	CDFL01	221748	
Acanthamoeba culbertsoni	-	SAMEA3111747	PRJEB7687	Protists	Other Protists	GCA_000826265.1		55.5438	0.00	-	CDFF01	72411	
Acanthamoeba divionensis	-	SAMEA3111754	PRJEB7687	Protists	Other Protists	GCA_000826405.1		84.7694	0.00	-	CDFI01	107714	
Acanthamoeba healyi	-	SAMEA3111749	PRJEB7687	Protists	Other Protists	GCA_000826305.1		75.3182	0.00	-	CDFA01	26188	
Acanthamoeba lenticulata	-	SAMEA3111748	PRJEB7687	Protists	Other Protists	GCA_000826285.1		66.0257	0.00	-	CDFG01	79048	
				Protists	Other Protists	GCA_000826425.1		99.4171	0.00	-	CDFB01	67459	

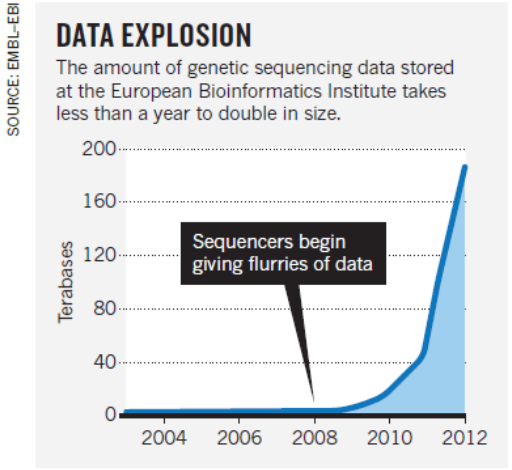
www.ncbi.nlm.nih.gov/genome/browse/#tabs-euks

http://www.ncbi.nlm.nih.gov/genome/browse/

TECHNOLOGY FEATURE

THE BIG CHALLENGES OF BIG DATA

As they grapple with increasingly large data sets, biologists and computer scientists uncork new bottlenecks.



NÃO É MAIS UM PROBLEMA...???

International Journal of Information Management 48 (2019) 63–71

Contents lists available at ScienceDirect

International Journal of Information Management

journal homepage: www.elsevier.com/locate/ijinfomgt

Artificial intelligence for decision making in the era of Big Data – evolution, challenges and research agenda

Yanqing Duan[a,*], John S. Edwards[b], Yogesh K Dwivedi[c]

[a] University of Bedfordshire, United Kingdom
[b] Aston University, United Kingdom
[c] School of Management, Swansea University, United Kingdom

Current Opinion in Biotechnology

Volume 76, August 2022, 102714

The future of Artificial Intelligence for the BioTech Big Data landscape

Fausto Artico [1] ✉, Arthur L Edge III [2], Kyle Langham [3]

Andreas Sundquist says amounts of data are now larger than the tools used to analyse them.

"If I could, I would routinely look at all sequenced cancer genomes. With the current infrastructure, that's impossible."

The 100,000 Genomes Project

The project will sequence 100,000 genomes from around 70,000 people. Participants are NHS patients with a rare disease, plus their families, and patients with cancer.

The aim is to create a new genomic medicine service for the NHS – transforming the way people are cared for. Patients may be offered a diagnosis where there wasn't one before. In time, there is the potential of new and more effective treatments.

The project will also enable new medical research. Combining genomic sequence data with medical records is a ground-breaking resource. Researchers will study how best to use genomics in healthcare and how best to interpret the data to help patients. The causes, diagnosis and treatment of disease will also be investigated. We also aim to kick-start a UK genomics industry. This is currently the largest national sequencing project of its kind in the world.

Anunciado em 2012...

https://www.genomicsengland.co.uk/the-100000-genomes-project/

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100,000 Genomes Project hits halfway milestone

Category: News report 21 February 2018 In collaboration with the Press Association

50.000 já sequenciados em Março de 2018 e em Dezembro de 2018 anunciada a conclusão do projeto!!!

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6 March 2018

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SCIENCEINSIDER | EUROPE

Sequencing projects will screen 200,000 newborns for disease genes

Projects in the U.K. and New York City face questions of cost and ethics

12 DEC 2022 · 7:01 PM · BY JOCELYN KAISER

https://www.science.org/content/article/sequencing-projects-will-screen-200-000-newborns-disease-genes

Ético?

Epigenome: The symphony in your cells

A slew of papers reveals the chemical tweaks to DNA in a wealth of different cells — as explained with the help of a small orchestra.

Kerri Smith

18 February 2015

http://www.nature.com/news/epigenome-the-symphony-in-your-cells-1.16955

PLOS | GENETICS

RESEARCH ARTICLE

The Epigenomic Landscape of Prokaryotes

Matthew J. Blow[1,2]*, Tyson A. Clark[3], Chris G. Daum[1,2], Adam M. Deutschbauer[4], Alexey Fomenkov[5], Roxanne Fries[1,2], Jeff Froula[1,2], Dongwan D. Kang[1,2], Rex R. Malmstrom[1,2], Richard D. Morgan[5], Janos Posfai[5], Kanwar Singh[1,2], Axel Visel[1,2], Kelly Wetmore[4], Zhiying Zhao[1,2], Edward M. Rubin[1,2], Jonas Korlach[3], Len A. Pennacchio[1,2], Richard J. Roberts[5]*

1 Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, California, United States of America, **2** U.S. Department of Energy Joint Genome Institute, Walnut Creek, California, United States of America, **3** Pacific Biosciences, Menlo Park, California, United States of America, **4** Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, United States of America, **5** New England Biolabs, Ipswich, Massachusetts, United States of America

* MJBlow@lbl.gov (MJB); roberts@neb.com (RJR)

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Uso do SMRT para detectar padrões de metilação no genoma de 230 procariotos.

ARTICLE

https://doi.org/10.1038/s41467-018-08103-y OPEN

Metaepigenomic analysis reveals the unexplored diversity of DNA methylation in an environmental prokaryotic community

Satoshi Hiraoka[1,2], Yusuke Okazaki[3], Mizue Anda[4], Atsushi Toyoda[5], Shin-ichi Nakano[3] & Wataru Iwasaki[1,4,6]

ANNUAL REVIEWS

Annual Review of Microbiology

Beyond Restriction Modification: Epigenomic Roles of DNA Methylation in Prokaryotes

Brian P. Anton and Richard J. Roberts

New England Biolabs, Ipswich, Massachusetts 01938, USA; email: anton@neb.com, roberts@neb.com

ESTAMOS ABRINDO A CAIXA DE PANDORA...

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III

By OSWALD T. AVERY, M.D., COLIN M. MacLEOD, M.D., and MACLYN McCARTY,* M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

Plate 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microörganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

TABLE IV

Titration of Transforming Activity of Preparation 44

Transforming principle Preparation 44*		Quadruplicate tests							
		1		2		3		4	
Dilution	Amount added	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form
	μg.								
10^{-2}	1.0	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-2.5}$	0.3	+	SIII	+	SIII	+	SIII	+	SIII
10^{-3}	0.1	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-3.5}$	0.03	+	SIII	+	SIII	+	SIII	+	SIII
10^{-4}	0.01	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-4.5}$	0.003	−	R only	+	SIII	−	R only	+	SIII
10^{-5}	0.001	−	R "	−	R only	−	R "	−	R only
Control	None	−	R "	−	R "	−	R "	−	R "

* Solution from which dilutions were made contained 0.5 mg. per cc. of purified material. 0.2 cc. of each dilution added to quadruplicate tubes containing 2.0 cc. of standard serum broth. 0.05 cc. of a 10^{-4} dilution of a blood broth culture of R36A is added to each tube.

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without

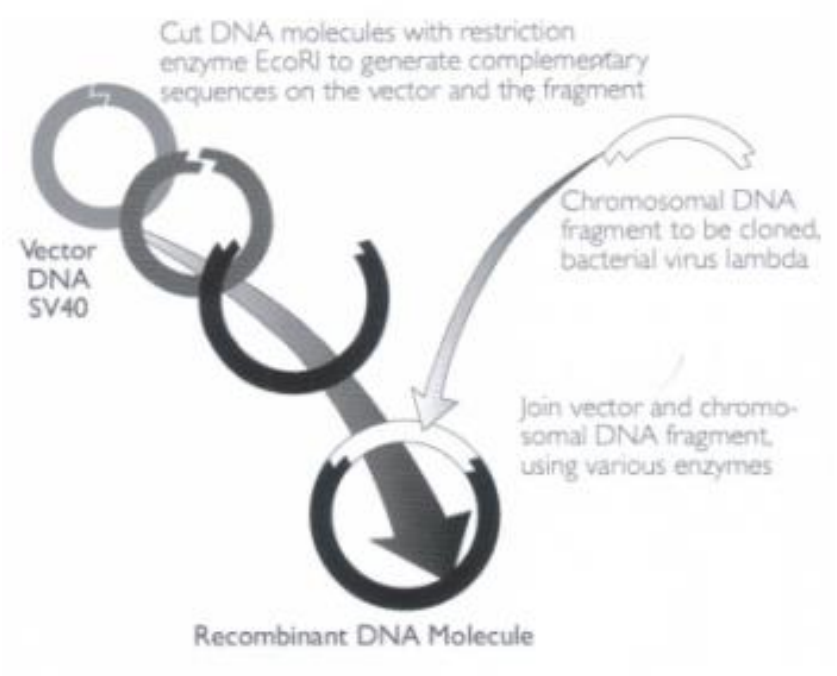
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Our model suggests possible explanations for a number of other phenomena. For example, spontaneous mutation may be due to a base occasionally occurring in one of its less likely tautomeric forms. Again, the pairing between homologous chromosomes at meiosis may depend on pairing between specific bases. We shall discuss these ideas in detail elsewhere.

For the moment, the general scheme we have proposed for the reproduction of deoxyribonucleic acid must be regarded as speculative. Even if it is correct, it is clear from what we have said that much remains to be discovered before the picture of genetic duplication can be described in detail. What are the polynucleotide precursors? What makes the pair of chains unwind and separate? What is the precise role of the protein? Is the chromosome one long pair of deoxyribonucleic acid chains, or does it consist of patches of the acid joined together by protein?

Despite these uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems—the molecular basis of the template needed for genetic replication. The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.

1972 - Paul Berg realizou a primeira experiência bem sucedida onde foram ligadas duas cadeias genéticas diferentes: ele ligou uma cadeia de DNA do fago λ junto ao operon da galactose de *Escherichia coli*, inserindo-os no DNA do vírus SV40.



Jackson, D.A., Symons, R.H. & Berg, P. Biochemical method for inserting new genetic information into DNA of simian virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. Proc Nat Acad Sci USA **69**, 2904-2909 (October 1972)

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3240–3244, November 1973

Construction of Biologically Functional Bacterial Plasmids *In Vitro*

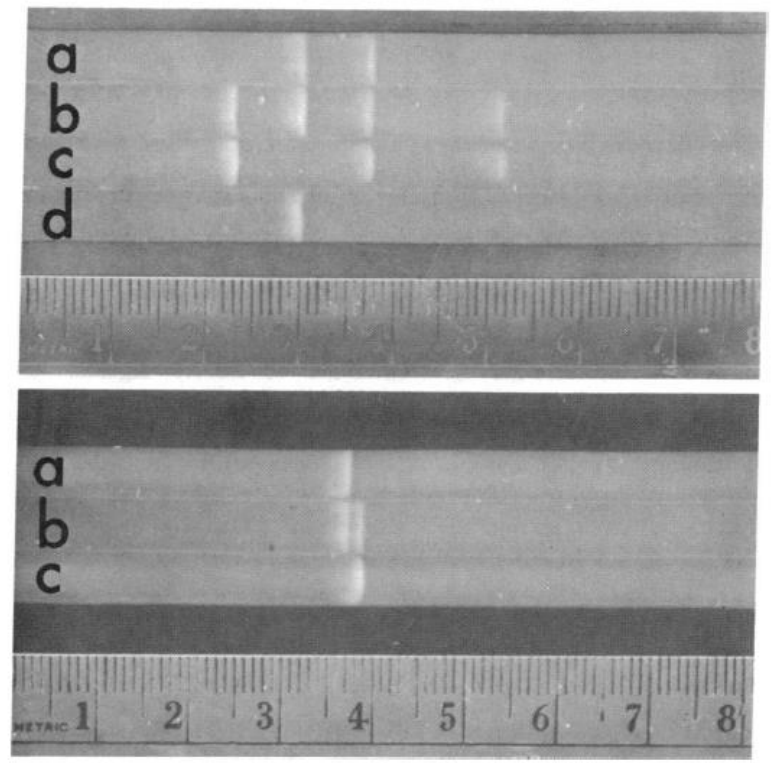
(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

Communicated by Norman Davidson, July 18, 1973

ABSTRACT **The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated frag-**



If the K-T boundary isotopic spike is indeed the result of impact-related acid rain, the oceanic strontium isotope record may reveal other large impacts. The seawater strontium curve of Burke *et al.* (*9*), which spans the past 500 million years, shows at least two other prominent high spikes in the $^{87}Sr/^{86}Sr$ ratio, one in the mid-Cretaceous, at ~100 million years, and the other in the Pennsylvanian, at ~290 million years. The first appears to precede by a few million years the mass extinction event at the Cenomanian-Turonian boundary. There is also a large increase in $^{87}Sr/^{86}Sr$ across the Permian-Triassic boundary (*9*), the time of the most extreme mass extinction in the Phanerozoic record (*17*). However, the increase appears to be rather gradual, extending over 20 million to 25 million years, and is thus quite different in character from the K-T spike. Nevertheless, data are sparse for this interval, and more work will be required to determine the exact nature of the increase.

The occurrence of a spike toward higher values in the seawater $^{87}Sr/^{86}Sr$ record at the K-T boundary is tantalizing evidence for

18. I thank many colleagues at Scripps for comments on the ideas expressed in this report, in particular G. Arrhenius, S. Galer, J. Gieskes, M. Kastner, D. Lal, G. Lugmair, and H.-G. Stosch. Comments from two anonymous reviewers also improved the original manuscript. I thank P. Hey for preparation of the manuscript. This work was supported in part by grants from the National Science Foundation and the National Aeronautics and Space Administration.

28 September 1987; accepted 7 December 1987

Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase

RANDALL K. SAIKI, DAVID H. GELFAND, SUSANNE STOFFEL, STEPHEN J. SCHARF, RUSSELL HIGUCHI, GLENN T. HORN, KARY B. MULLIS,* HENRY A. ERLICH

A thermostable DNA polymerase was used in an in vitro DNA amplification procedure, the polymerase chain reaction. The enzyme, isolated from *Thermus aquaticus*, greatly simplifies the procedure and, by enabling the amplification reaction to be performed at higher temperatures, significantly improves the specificity, yield, sensitivity, and length of products that can be amplified. Single-copy genomic sequences were amplified by a factor of more than 10 million with very high specificity, and DNA segments up to 2000 base pairs were readily amplified. In addition, the method was used to amplify and detect a target DNA molecule present only once in a sample of 10^5 cells.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase." Science 239 (1988): 487-491.

BIOLOGIA SINTÉTICA

ARTICLE

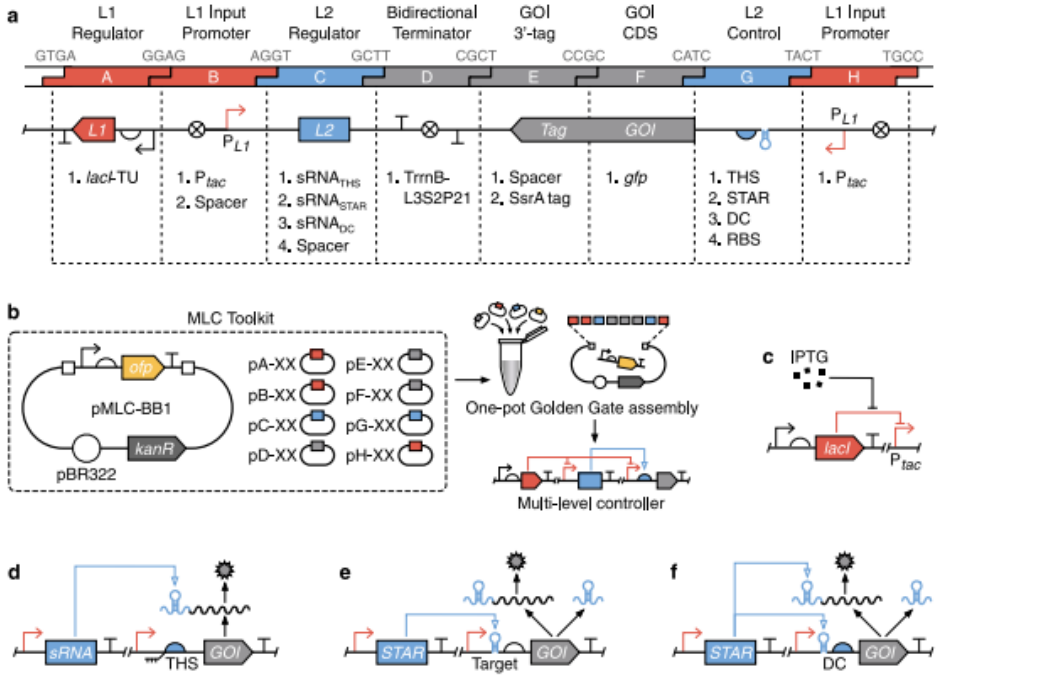
https://doi.org/10.1038/s41467-021-21995-7 OPEN

Check for updates

Harnessing the central dogma for stringent multi-level control of gene expression

F. Veronica Greco [1], Amir Pandi [2], Tobias J. Erb [2,3], Claire S. Grierson [1,4] & Thomas E. Gorochowski [1,4]✉

Strictly controlled inducible gene expression is crucial when engineering biological systems where even tiny amounts of a protein have a large impact on function or host cell viability. In these cases, leaky protein production must be avoided, but without affecting the achievable range of expression. Here, we demonstrate how the central dogma offers a simple solution to this challenge. By simultaneously regulating transcription and translation, we show how basal expression of an inducible system can be reduced, with little impact on the maximum expression rate. Using this approach, we create several stringent expression systems displaying >1000-fold change in their output after induction and show how multi-level regulation can suppress transcriptional noise and create digital-like switches between 'on' and 'off' states. These tools will aid those working with toxic genes or requiring precise regulation and propagation of cellular signals, plus illustrate the value of more diverse regulatory designs for synthetic biology.



CONSTRUINDO NOVAS ROTAS ...

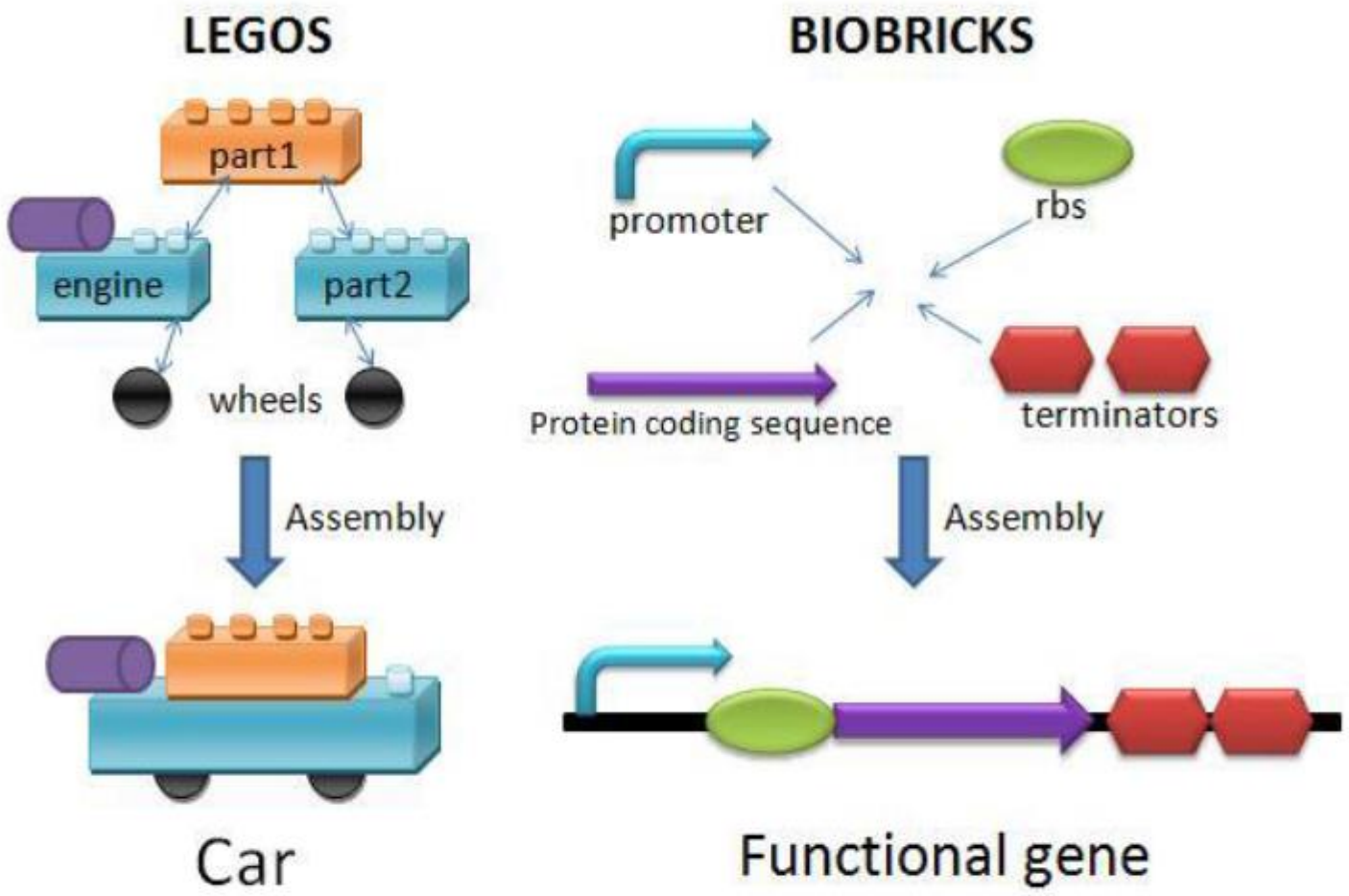


Figure1: Comparison between the concept of Legos and Biobricks

http://www.biochem.hku.hk/synbio/?page_id=148

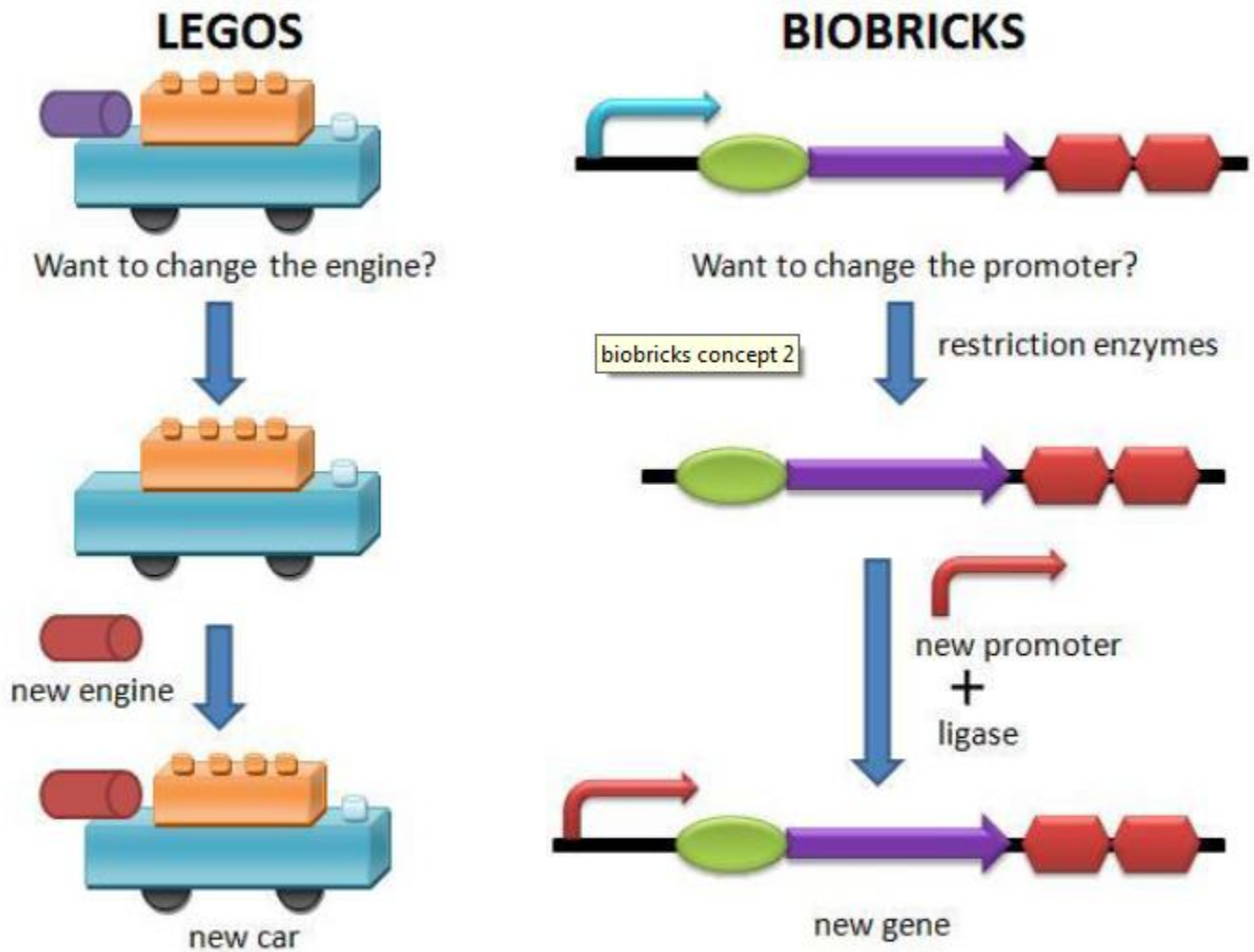


Figure2: The concept of interchangeable parts in biobricks

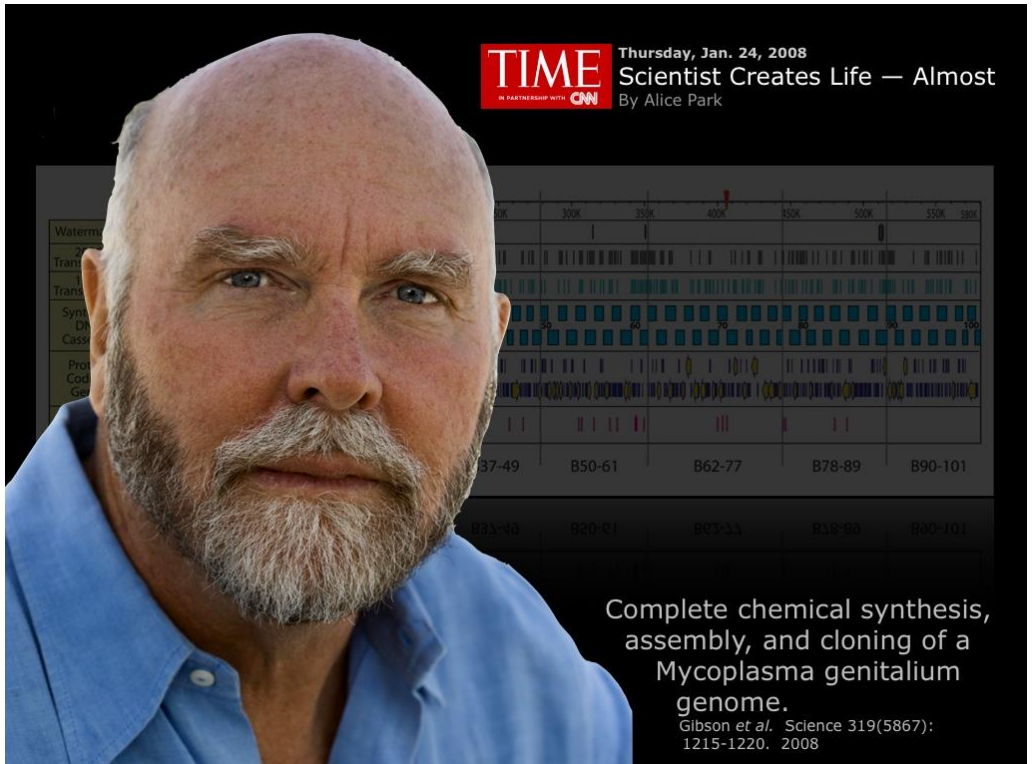
http://www.biochem.hku.hk/synbio/?page_id=148

GENOMA MÍNIMO

- *Mycoplasma genitalium* (580.070 pb) – 477 genes:

  - ~120 genes não são necessários para o crescimento em laboratório
  - ~350 genes são necessários para o crescimento em laboratório

***M .genitalium* JCVI-1.0**



http://www.sciencemag.org/content/319/5867/1215.abstract

PRIMEIRO ORGANISMO SINTÉTICO

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome
Daniel G. Gibson *et al.*
Science **329**, 52 (2010);
DOI: 10.1126/science.1190719

RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

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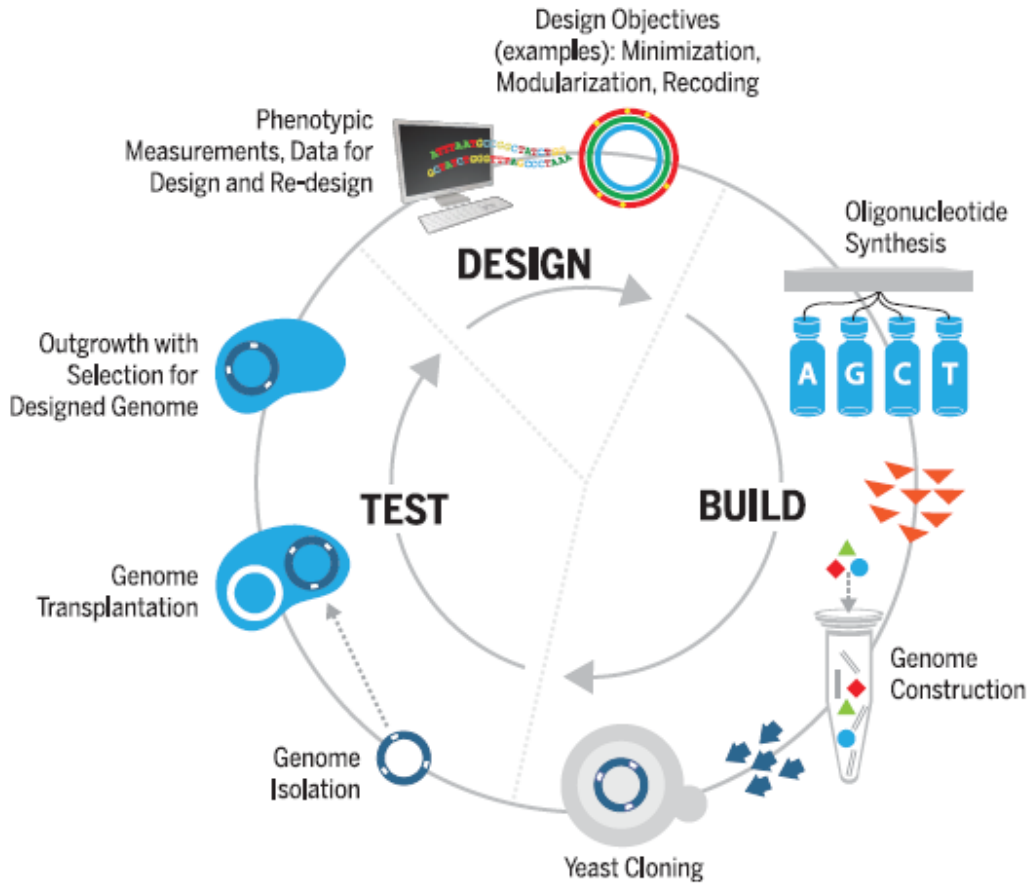
RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,[1*†] Ray-Yuan Chuang,[1†‡] Vladimir N. Noskov,[1] Nacyra Assad-Garcia,[1] Thomas J. Deerinck,[2] Mark H. Ellisman,[2] John Gill,[3] Krishna Kannan,[3] Bogumil J. Karas,[1] Li Ma,[1] James F. Pelletier,[4§] Zhi-Qing Qi,[3] R. Alexander Richter,[1] Elizabeth A. Strychalski,[4] Lijie Sun,[1||] Yo Suzuki,[1] Billyana Tsvetanova,[3] Kim S. Wise,[1] Hamilton O. Smith,[1,3] John I. Glass,[1] Chuck Merryman,[1] Daniel G. Gibson,[1,3] J. Craig Venter[1,3*]

We used whole-genome design and complete chemical synthesis to minimize the 1079–kilobase pair synthetic genome of *Mycoplasma mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology combined with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kilobase pairs, 473 genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design.



nature chemical biology

ARTICLE

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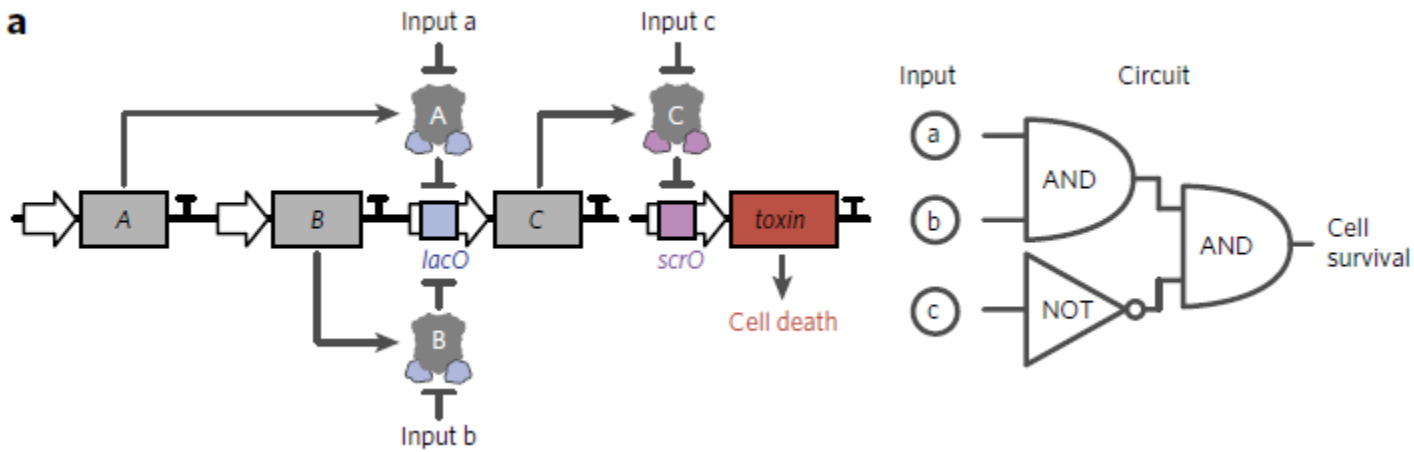
‘Deadman’ and ‘Passcode’ microbial kill switches for bacterial containment

Clement T Y Chan[1–3,7], Jeong Wook Lee[1–3,7], D Ewen Cameron[1–3,7], Caleb J Bashor[1–3] & James J Collins[1–6*]

Biocontainment systems that couple environmental sensing with circuit-based control of cell viability could be used to prevent escape of genetically modified microbes into the environment. Here we present two engineered safeguard systems known as the ‘Deadman’ and ‘Passcode’ kill switches. The Deadman kill switch uses unbalanced reciprocal transcriptional repression to couple a specific input signal with cell survival. The Passcode kill switch uses a similar two-layered transcription design and incorporates hybrid LacI-GalR family transcription factors to provide diverse and complex environmental inputs to control circuit function. These synthetic gene circuits efficiently kill *Escherichia coli* and can be readily reprogrammed to change their environmental inputs, regulatory architecture and killing mechanism.



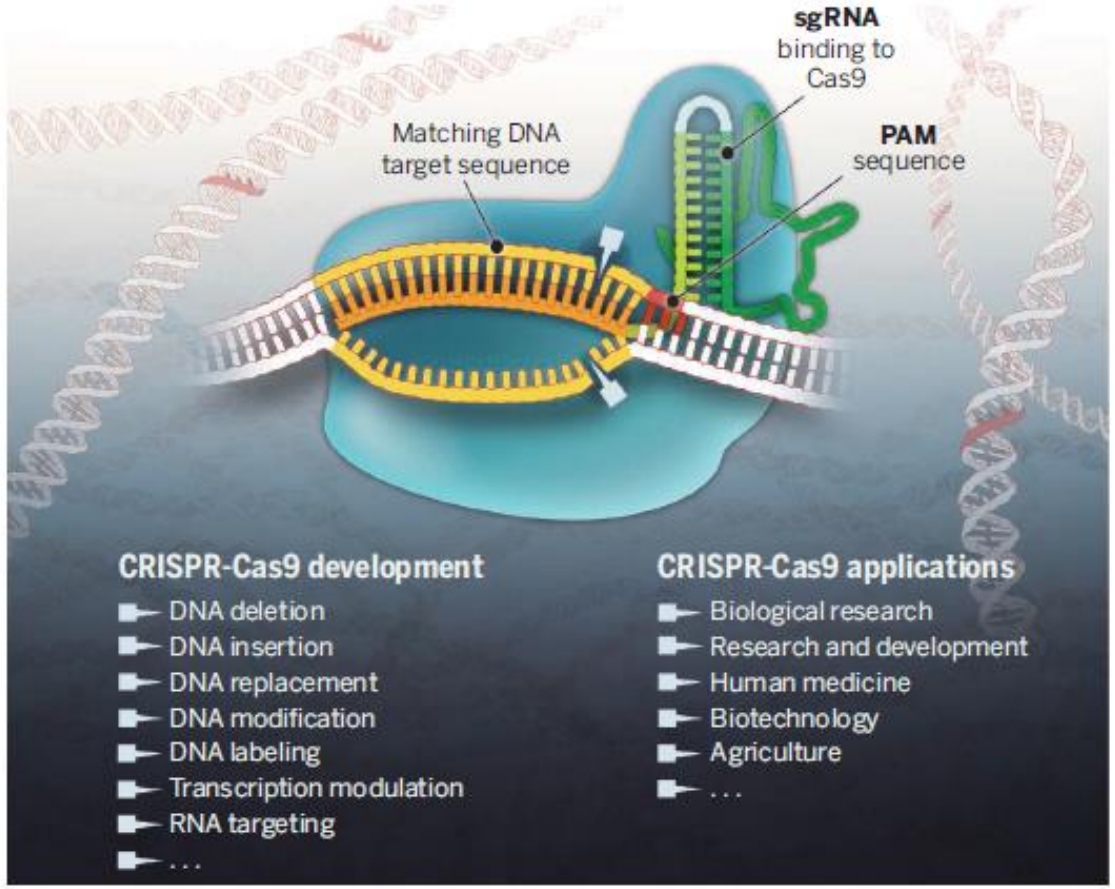
REVIEW

GENOME EDITING

The new frontier of genome engineering with CRISPR-Cas9

Jennifer A. Doudna[1,2,3]* and Emmanuelle Charpentier[4,5,6]*

The advent of facile genome engineering using the bacterial RNA-guided CRISPR-Cas9 system in animals and plants is transforming biology. We review the history of CRISPR (clustered regularly interspaced palindromic repeat) biology from its initial discovery through the elucidation of the CRISPR-Cas9 enzyme mechanism, which has set the stage for remarkable developments using this technology to modify, regulate, or mark genomic loci in a wide variety of cells and organisms from all three domains of life. These results highlight a new era in which genomic manipulation is no longer a bottleneck to experiments, paving the way toward fundamental discoveries in biology, with applications in all branches of biotechnology, as well as strategies for human therapeutics.



The Cas9 enzyme (blue) generates breaks in double-stranded DNA by using its two catalytic centers (blades) to cleave each strand of a DNA target site (gold) next to a PAM sequence (red) and matching the 20-nucleotide sequence (orange) of the single guide RNA (sgRNA). The sgRNA includes a dual-RNA sequence derived from CRISPR RNA (light green) and a separate transcript (tracrRNA, dark green) that binds and stabilizes the Cas9 protein. Cas9-sgRNA–mediated DNA cleavage produces a blunt double-stranded break that triggers repair enzymes to disrupt or replace DNA sequences at or near the cleavage site. Catalytically inactive forms of Cas9 can also be used for programmable regulation of transcription and visualization of genomic loci.

RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection

Wenhui Hu[a,1,2], Rafal Kaminski[a,1], Fan Yang[a], Yonggang Zhang[a], Laura Cosentino[a], Fang Li[a], Biao Luo[b], David Alvarez-Carbonell[c], Yoelvis Garcia-Mesa[c], Jonathan Karn[c], Xianming Mo[d], and Kamel Khalili[a,2]

[a]Department of Neuroscience, Center for Neurovirology and The Comprehensive NeuroAIDS Center, Temple University School of Medicine, Philadelphia, PA 19140; [b]Cancer Genome Institute, Fox Chase Cancer Center, Temple University School of Medicine, Philadelphia, PA 19111; [c]Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106; and [d]Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China

Edited by Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, Bethesda, MD, and approved June 19, 2014 (received for review March 19, 2014)

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Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

Genome editing has potential for the targeted correction of germline mutations. Here we describe the correction of the heterozygous *MYBPC3* mutation in human preimplantation embryos with precise CRISPR–Cas9-based targeting accuracy and high homology-directed repair efficiency by activating an endogenous, germline-specific DNA repair response. Induced double-strand breaks (DSBs) at the mutant paternal allele were predominantly repaired using the homologous wild-type maternal gene instead of a synthetic DNA template. By modulating the cell cycle stage at which the DSB was induced, we were able to avoid mosaicism in cleaving embryos and achieve a high yield of homozygous embryos carrying the wild-type *MYBPC3* gene without evidence of off-target mutations. The efficiency, accuracy and safety of the approach presented suggest that it has potential to be used for the correction of heritable mutations in human embryos by complementing preimplantation genetic diagnosis. However, much remains to be considered before clinical applications, including the reproducibility of the technique with other heterozygous mutations.

LETTERS

nature biotechnology

High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells

Yanfang Fu[1–4], Jennifer A Foden[1–3], Cyd Khayter[1–3], Morgan L Maeder[1–3,5], Deepak Reyon[1–4], J Keith Joung[1–5] & Jeffry D Sander[1–4]

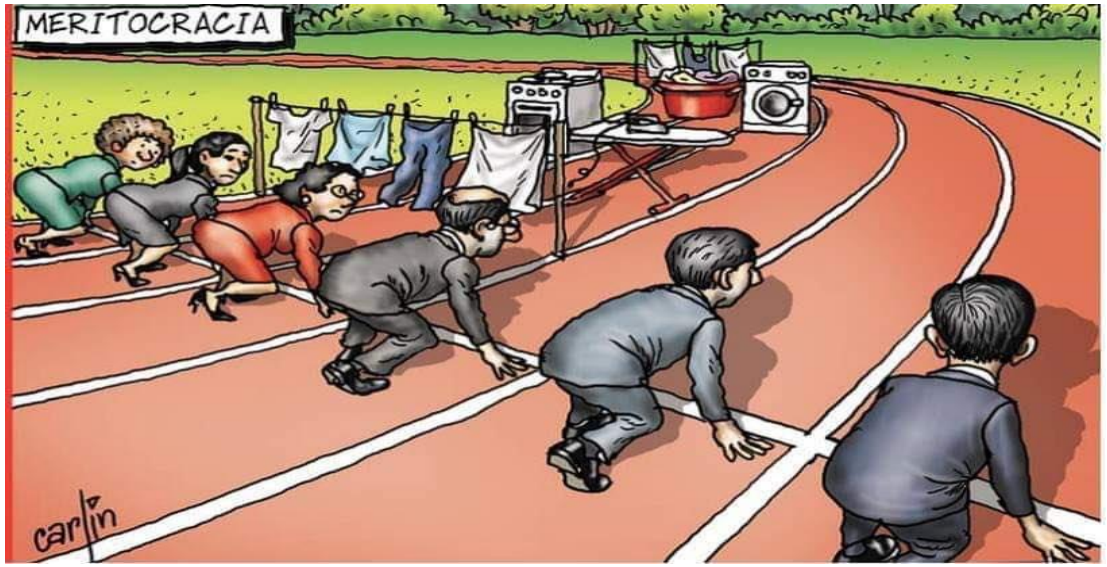
Clustered, regularly interspaced, short palindromic repeat (CRISPR) RNA-guided nucleases (RGNs) have rapidly emerged as a facile and efficient platform for genome editing. Here, we use a human cell–based reporter assay to characterize off-target cleavage of CRISPR-associated (Cas)9-based RGNs. We find that single and double mismatches are tolerated to varying degrees depending on their position along the guide RNA (gRNA)-DNA interface. We also readily detected off-target alterations induced by four out of six RGNs targeted to endogenous loci in human cells by examination of partially mismatched sites. The off-target sites we identified harbored up to five mismatches and many were mutagenized with frequencies comparable to (or higher than) those observed at the intended on-target site. Our work demonstrates that RGNs can be highly active even with imperfectly matched RNA-DNA interfaces in human cells, a finding that might confound their use in research and therapeutic applications.

To begin to define the determinants of the specificity of RGNs in human cells, we carried out a large-scale test in which we assessed the effects of systematically mismatching various positions within multiple gRNA–target DNA interfaces. To do this, we used a quantitative human cell–based EGFP disruption assay, previously described by our laboratory[15], that enables rapid quantification of targeted nuclease activities (**Fig. 1a**). In this assay, the activities of nucleases targeted to a single integrated *EGFP* reporter gene can be quantified by assessing loss of fluorescence signal in human U2OS.EGFP cells caused by inactivating frameshift insertion/deletion (indel) mutations introduced by error prone nonhomologous end-joining (NHEJ) repair of nuclease-induced double-stranded breaks (**Fig. 1a** and Online Methods). We used three ~100-nt single gRNAs (sgRNAs) targeted to different sequences within *EGFP* (**Supplementary Fig. 2**); each of these sgRNAs efficiently directed Cas9-mediated disruption of EGFP expression (**Supplementary Results**).

In initial experiments, we tested the effects of single-nucleotide mismatches at 19 of 20 nucleotides in the complementary targeting

Jennifer Doudna e Emmanuelle Charpentier, vencedoras do Prêmio Nobel de Química 2020 (Foto: Alexander Heinl/picture alliance via Getty Images)

GANHADORAS DO PRÊMIO NOBEL DE QUIMICA EM 2020



Biologia sintética associada à técnica de CRISPR-Cas9

ARTICLE

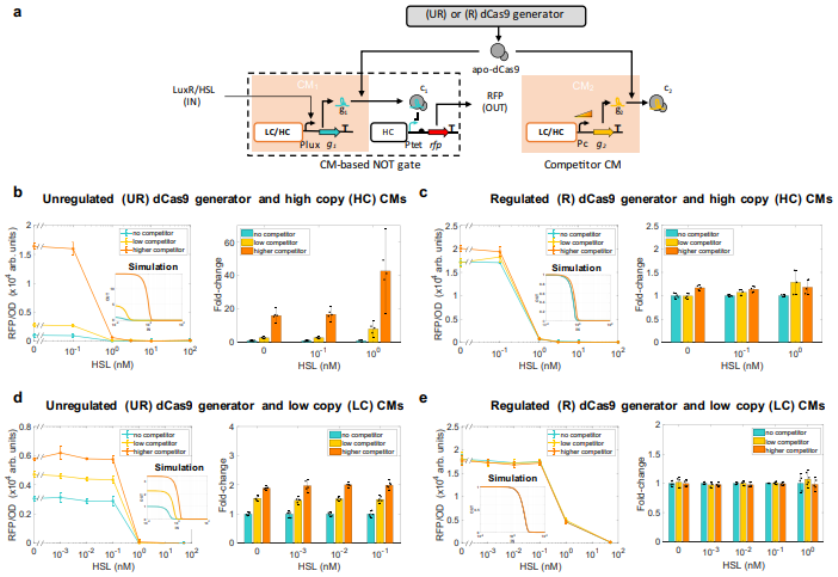
https://doi.org/10.1038/s41467-021-21772-6 OPEN

Check for updates

dCas9 regulator to neutralize competition in CRISPRi circuits

Hsin-Ho Huang [1,5], Massimo Bellato [2,5], Yili Qian [1], Pablo Cárdenas [3], Lorenzo Pasotti[2], Paolo Magni[2] & Domitilla Del Vecchio [1,4]✉

CRISPRi-mediated gene regulation allows simultaneous control of many genes. However, highly specific sgRNA-promoter binding is, alone, insufficient to achieve independent transcriptional regulation of multiple targets. Indeed, due to competition for dCas9, the repression ability of one sgRNA changes significantly when another sgRNA becomes expressed. To solve this problem and decouple sgRNA-mediated regulatory paths, we create a dCas9 concentration regulator that implements negative feedback on dCas9 level. This allows any sgRNA to maintain an approximately constant dose-response curve, independent of other sgRNAs. We demonstrate the regulator performance on both single-stage and layered CRISPRi-based genetic circuits, zeroing competition effects of up to 15-fold changes in circuit I/O response encountered without the dCas9 regulator. The dCas9 regulator decouples sgRNA-mediated regulatory paths, enabling concurrent and independent regulation of multiple genes. This allows predictable composition of CRISPRi-based genetic modules, which is essential in the design of larger scale synthetic genetic circuits.



LGN5809 E A GENÉTICA MOLECULAR...

PRÓXIMA AULA – 29 de Março

Aula “Moléculas celulares: um enfoque nas proteínas“ – Formação dos grupos e apresentação temas

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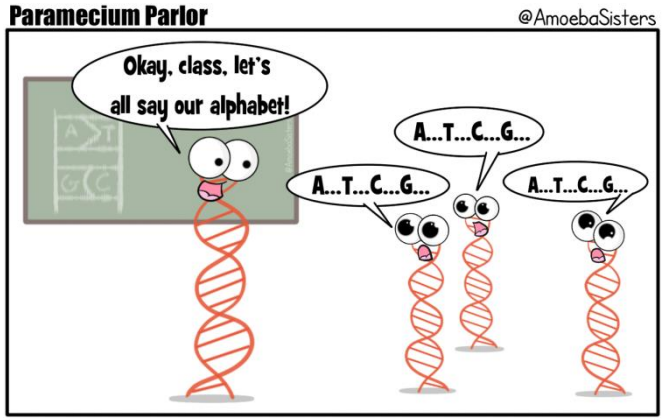

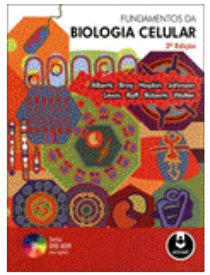

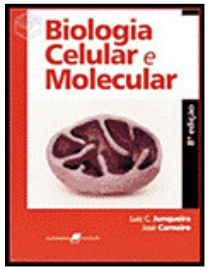

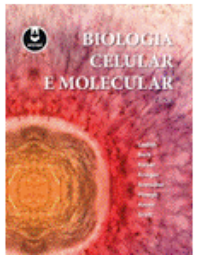

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