Genética de Fungos

Tipos de Reprodução

Os fungos são capazes de se propagar de diversas maneiras, através de núcleos haplóides, diplóides, poliplóides, aneuplóides, dicarions

- **VEGETATIVA - ASSEXUADA :**

não ocorre fusão de núcleos

- **SEXUAL:**

união núcleos – seguido de divisão meiótica

- **PARASEXUAL:**

ocorre união núcleos – divisão mitótica – haploidização por aneuploidia

Características do ciclo **Parassexual**

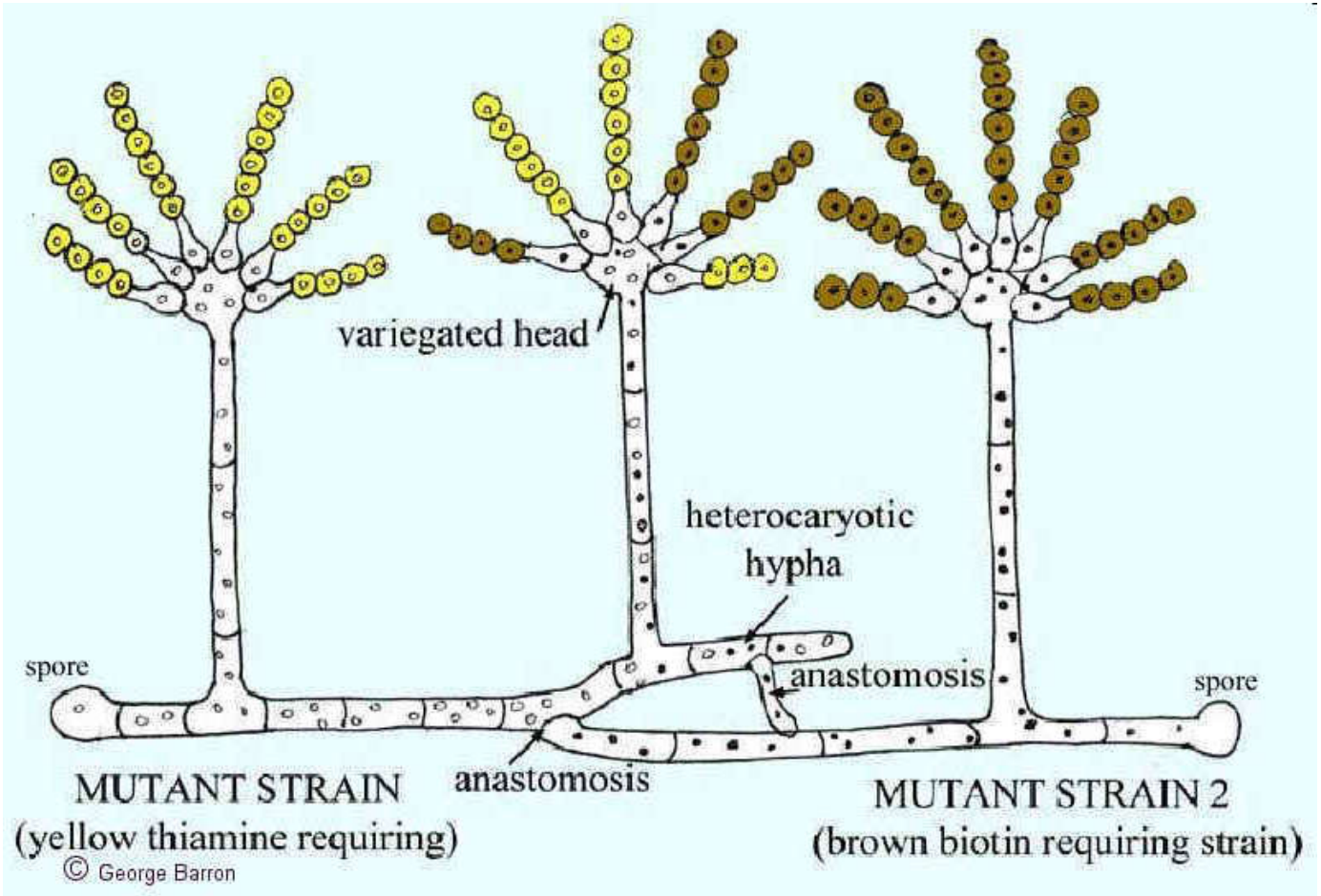
-ocorre recombinação na ausência de reprodução sexuada

-Fusão hifas compatíveis -> formação heterocarion que pode ser visualizado por setoração da colônia

-Cariogamia pode ocorrer e eventual recombinação mitótica.

-Haploidização por Aneuploidia (perda cromossômica)

Ciclo parassexual em *Aspergillus*



MycoAlbum CD - George Barron

Características da Reprodução Sexuada

Fusão de núcleos por:

1- Duas células morfologicamente idênticas mas de tipo sexual opostos (tipo **a** e tipo α)se unem (ex. *Saccharomyces cerevisiae*)

2- Fusão de células morfologicamente distintas como anterídios e ascogonios (com hifa tricógina, ex. *Laboulbenia formicarum*)

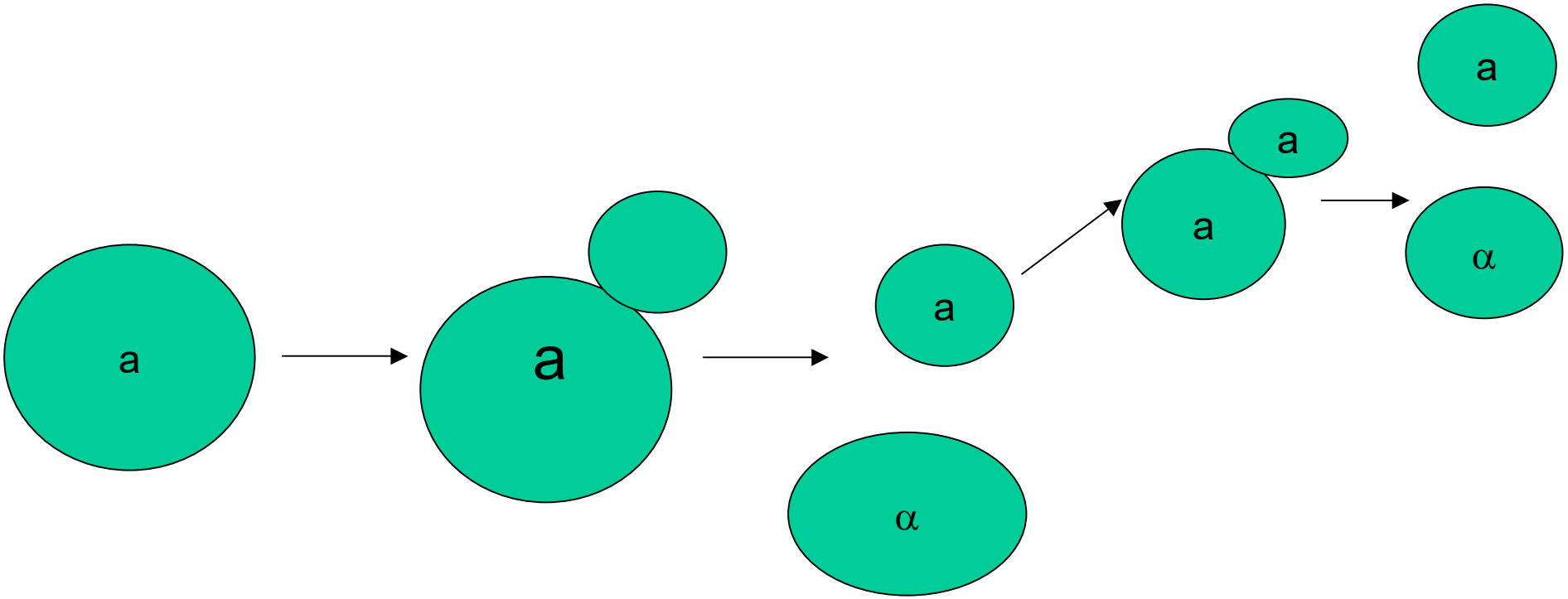
3- Por espermatização: transferência somente do núcleo do gameta masculino e recepção pela célula feminina. (ex. *Cronartium quercuum*)

4- Somatogamia: fusão de hifas somáticas indiferenciadas (ex. *Chytriomyces hyalinus*)

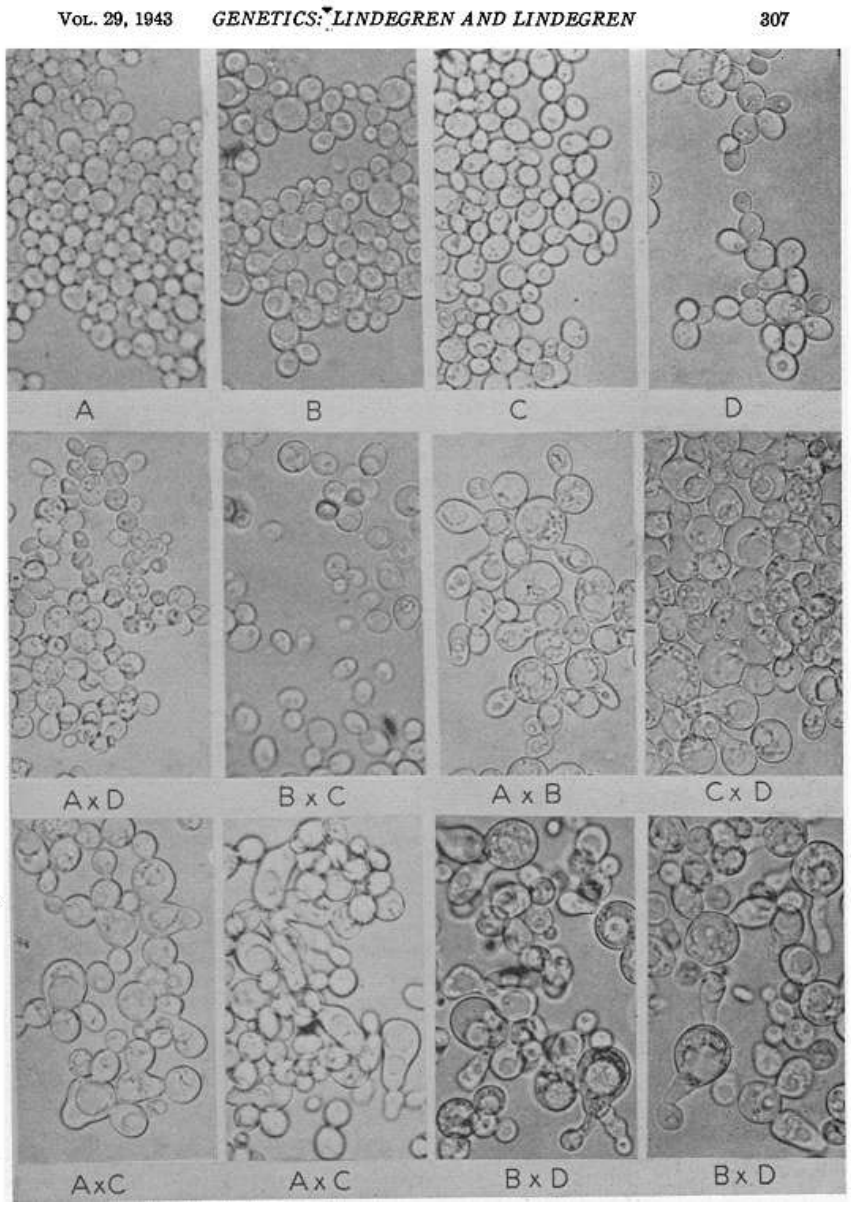
Promiscuidades de *S. cerevisiae* –

o gene homotálico HO e a mudança de sexo

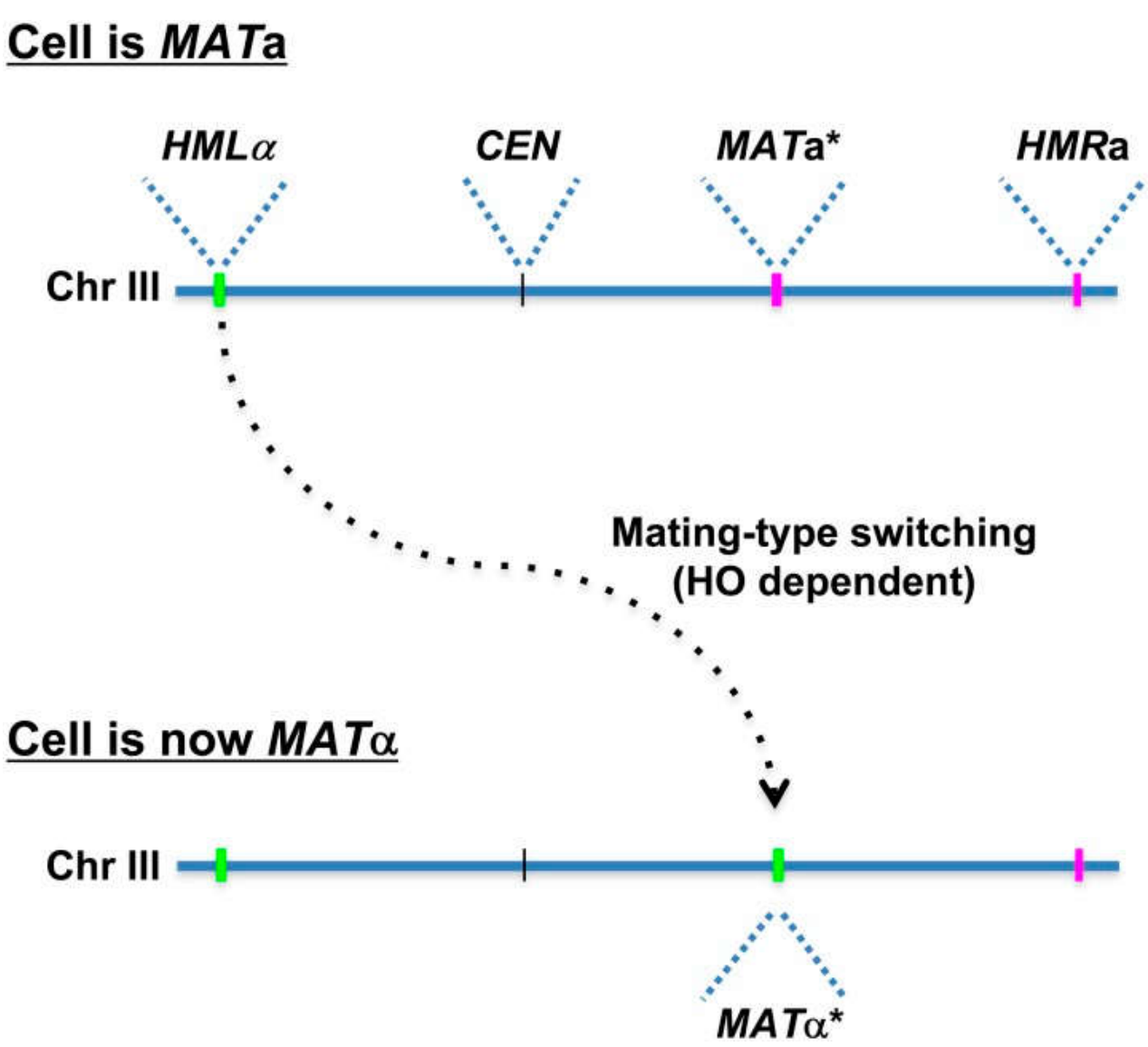
Divisão celular assimétrica por brotamento
"*budding yeast*"



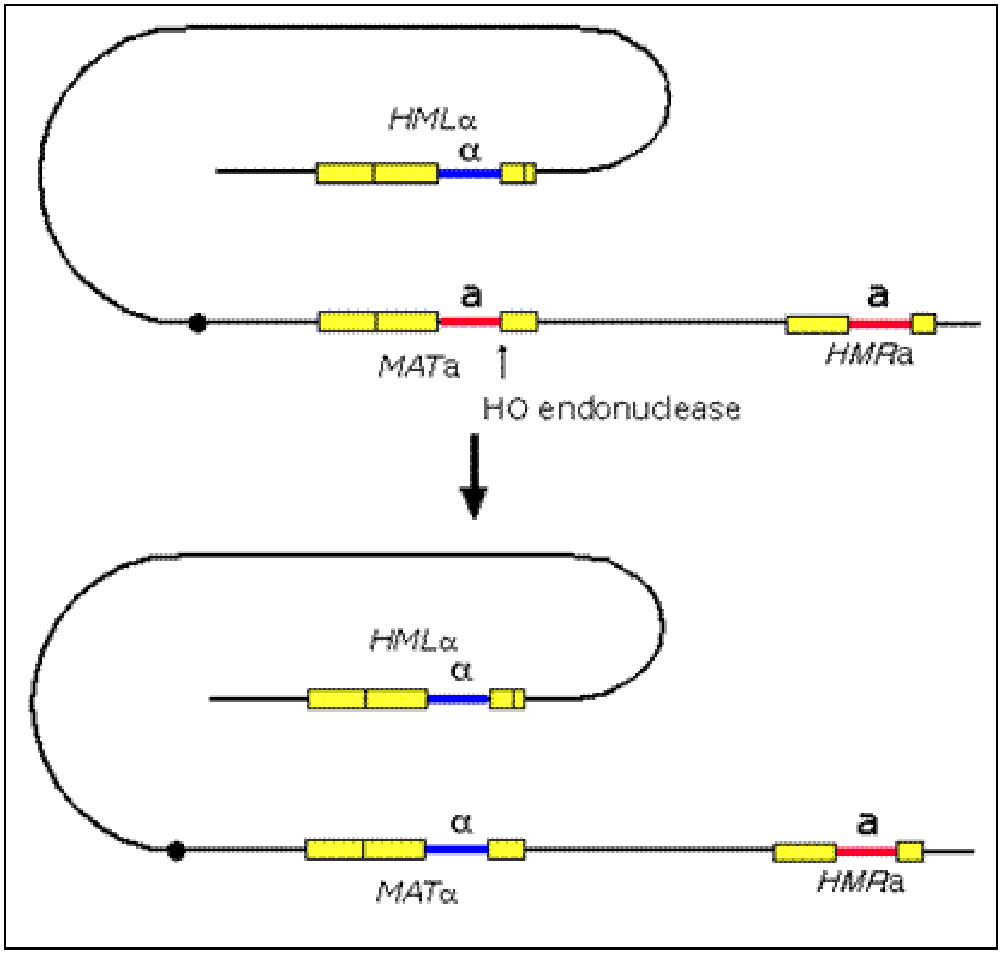
Exemplos de cruzamentos:



Proc Natl Acad Sci U S A. **29**: 306-308 (1943)



Genetics, Vol. 197, 33–48 May 2014


HMLα
α
a
a
MATa
HMRa
HO endonuclease
HMLα
α
α
a
MATα
HMRa

Reprodução sexuada em **Basidiomicetos** :

- dentro do basídio ocorre cariogamia e meiose
-fase dicariótica é originada a partir da fusão de micélios compatíveis

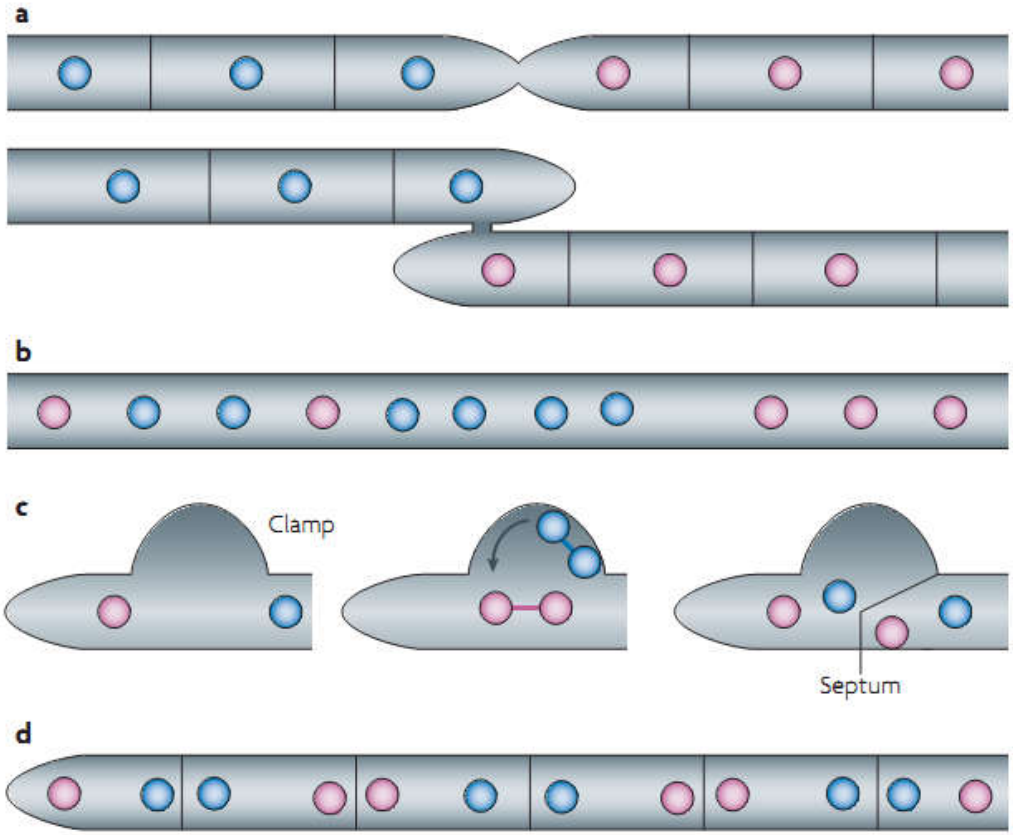


Figure 3 | **Overview of dikaryon formation. a** | Hyphae of many types of filamentous

Nature Reviews Microbiology 7, 875-886

Reprodução sexuada em Basidiomicetos :

-Pelo menos 4 loci gênicos estão envolvidos na compatibilidade sexual : Aα Aβ Bα e Bβ
–> as hifas devem diferir em Aα ,e/ou Aβ, Bα e/ou Bβ

Table 16-1 Six possible dikaryon genotypes in *Schizophyllum commune*.

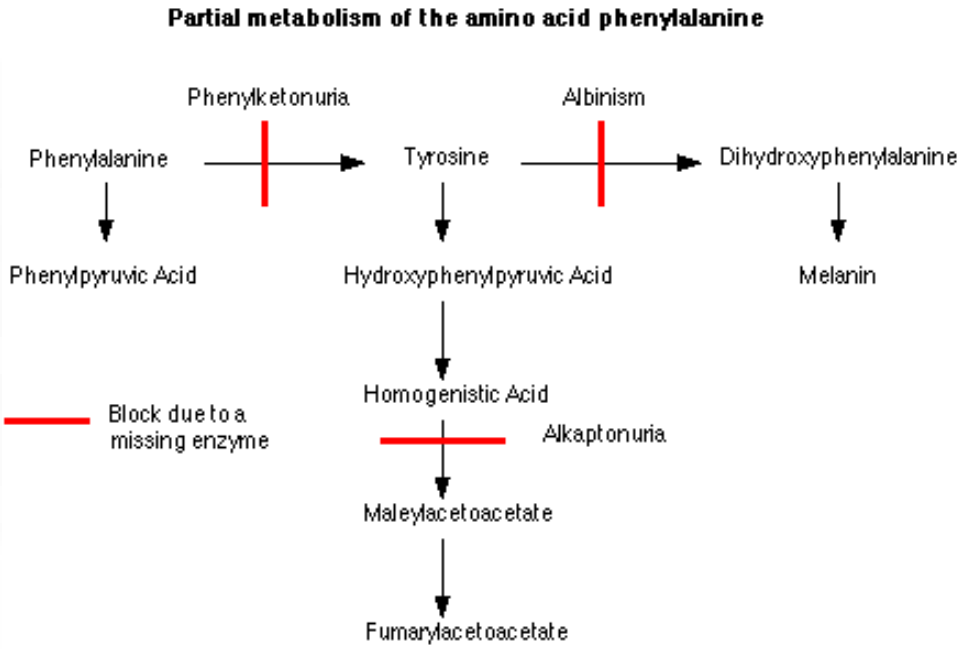
Individual homokaryon genotypes	Type of reaction
1. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2$	Incompatible
2. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_2\ A\beta_1\ B\alpha_2\ B\beta_2$	Compatible
3. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_1\ A\beta_2\ B\alpha_1\ B\beta_1$	Compatible
4. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_2\ A\beta_2\ B\alpha_2\ B\beta_1$	Compatible
5. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_2\ A\beta_1\ B\alpha_1\ B\beta_2$	Partially compatible
6. $A\alpha_1\ A\beta_1\ B\alpha_1\ B\beta_2 + A\alpha_1\ A\beta_1\ B\alpha_2\ B\beta_2$	Partially compatible
combined genotype of dikaryon	

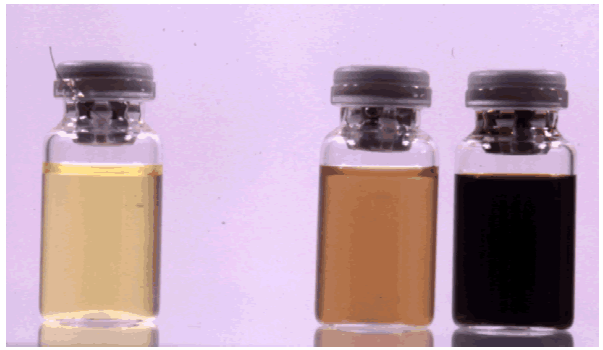
Note: The different alleles are designated by the subscripts 1 and 2. The genotypes of the homokaryon mates are shown at either side of the plus sign. The type of reaction exhibited by the resulting dikaryon genotype is shown in the right column. Modified from Novotny *et al.*, 1991.

Alexopouolous – Introductory Mycology

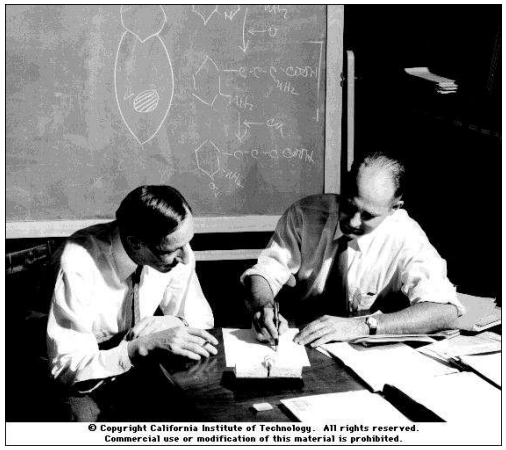
FUNGOS COMO MODELO GENÉTICO

Archibald E. Garrod: escreveu em 1909 sobre erros inatos do metabolismo –





Beadle – Tatum e o nascimento da Genética-Bioquímica

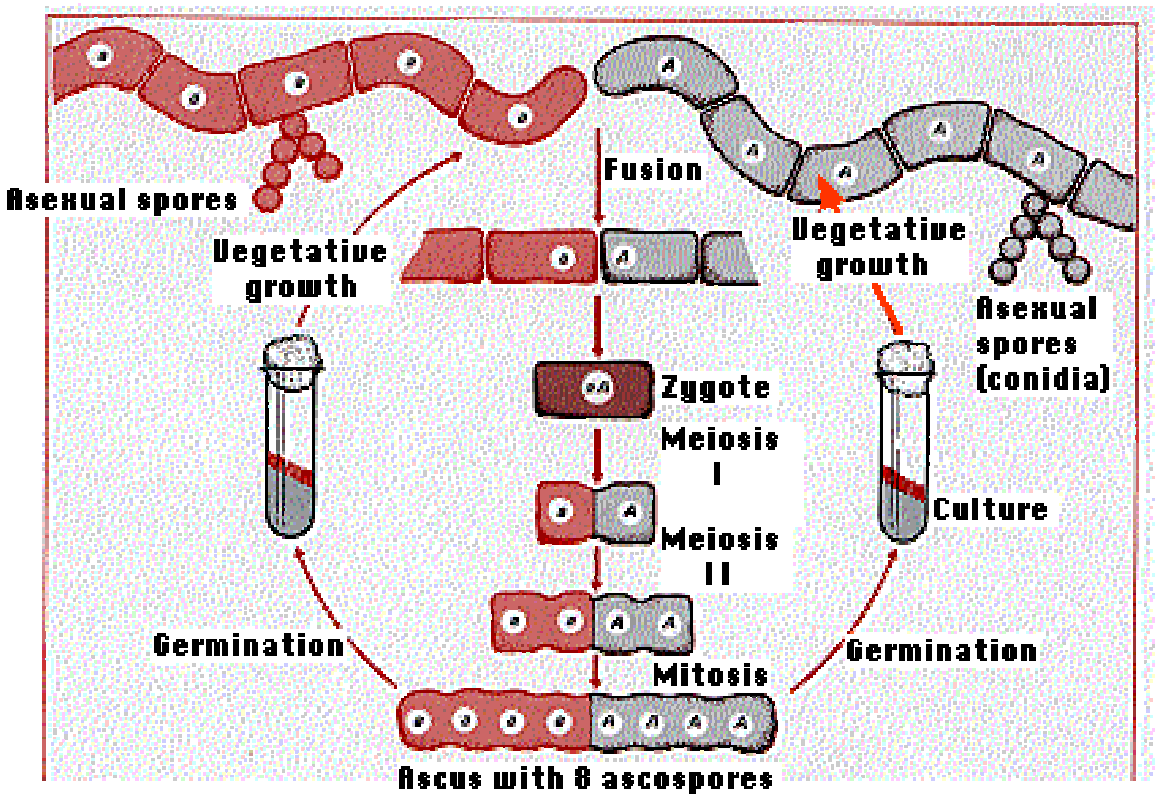


Proposta de 1 ENZIMA = 1 GENE - 1941 em *Neurospora crassa*.

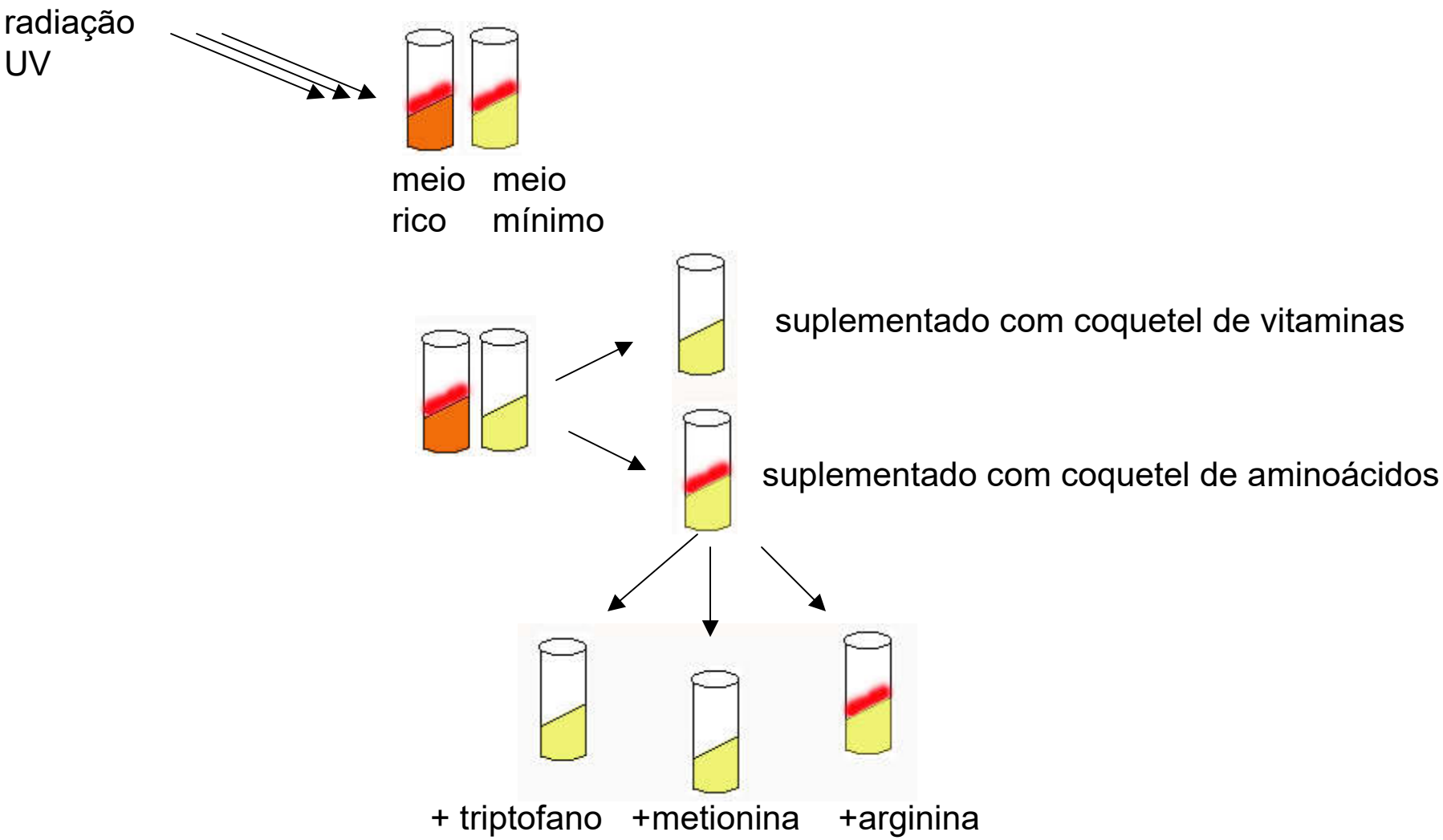
Início dos trabalhos com fungos como organismos modelos

↓

Biotecnologia


Asexual spores
Vegetative growth
Fusion
Vegetative growth
Asexual spores (conidia)
Zygote
Meiosis I
Meiosis II
Culture
Germination
Germination
Mitosis
Ascus with 8 ascospores

Experimentos de Beadle e Tatum com *Neurospora crassa*



Ao finalizar esse experimento Beadle e Tatum isolaram centenas de mutantes que verificaram pertencer a três classes de mutantes com deficiência para a síntese de arginina.

Como é possível distinguir classes diferentes de mutantes? Isto é, como saber se dois mutantes estão afetando o mesmo gene ou genes diferentes de uma mesma via metabólica?

Teste de Complementação Genético

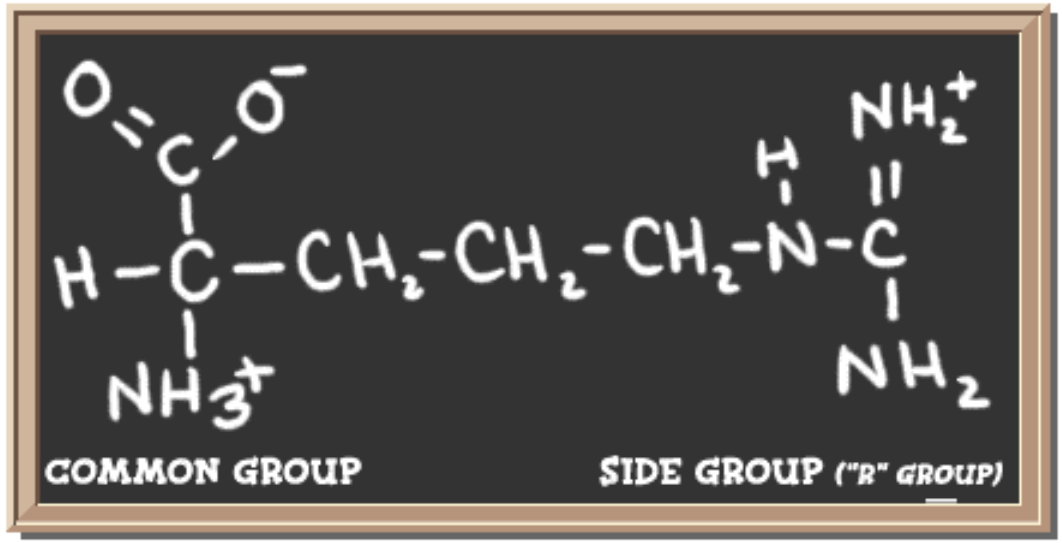
Conclusão:
Mutante 1 e Mutante 2 são de um mesmo grupo de complementação
Mutante 3 e Mutante 4 são de outro grupo de complementação

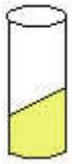
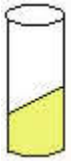
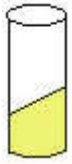
Sabia-se que mutantes para síntese de Arginina acumulavam :

1 – Ornitina, Citrulina ou nenhum deles

2– A suplementação dos mutantes com esses compostos resultou em diferentes respostas de crescimento

Arginina



Mutantes	+ Arginina	+ Citrulina	+ Ornitina
A			
B			
C			

Nature Reviews | Genetics

Precursor $\xrightarrow{\text{Enzima A}}$ **Ornitina** $\xrightarrow{\text{Enzima B}}$ **Citrulina** $\xrightarrow{\text{Enzima C}}$ **Arginina**

Exercício : Um mutante da enzima A (*a*) foi cruzado com um mutante da enzima B (*b*), gerando um diplóide duplo heterozigoto com fenótipo arg+ . Na sequência induziu-se a esporulação do diplóide , qual é o fenótipo esperado dos esporos resultantes dessa meiose?

Mut 1 (aB) arg- x Mut 2 (Ab) arg-

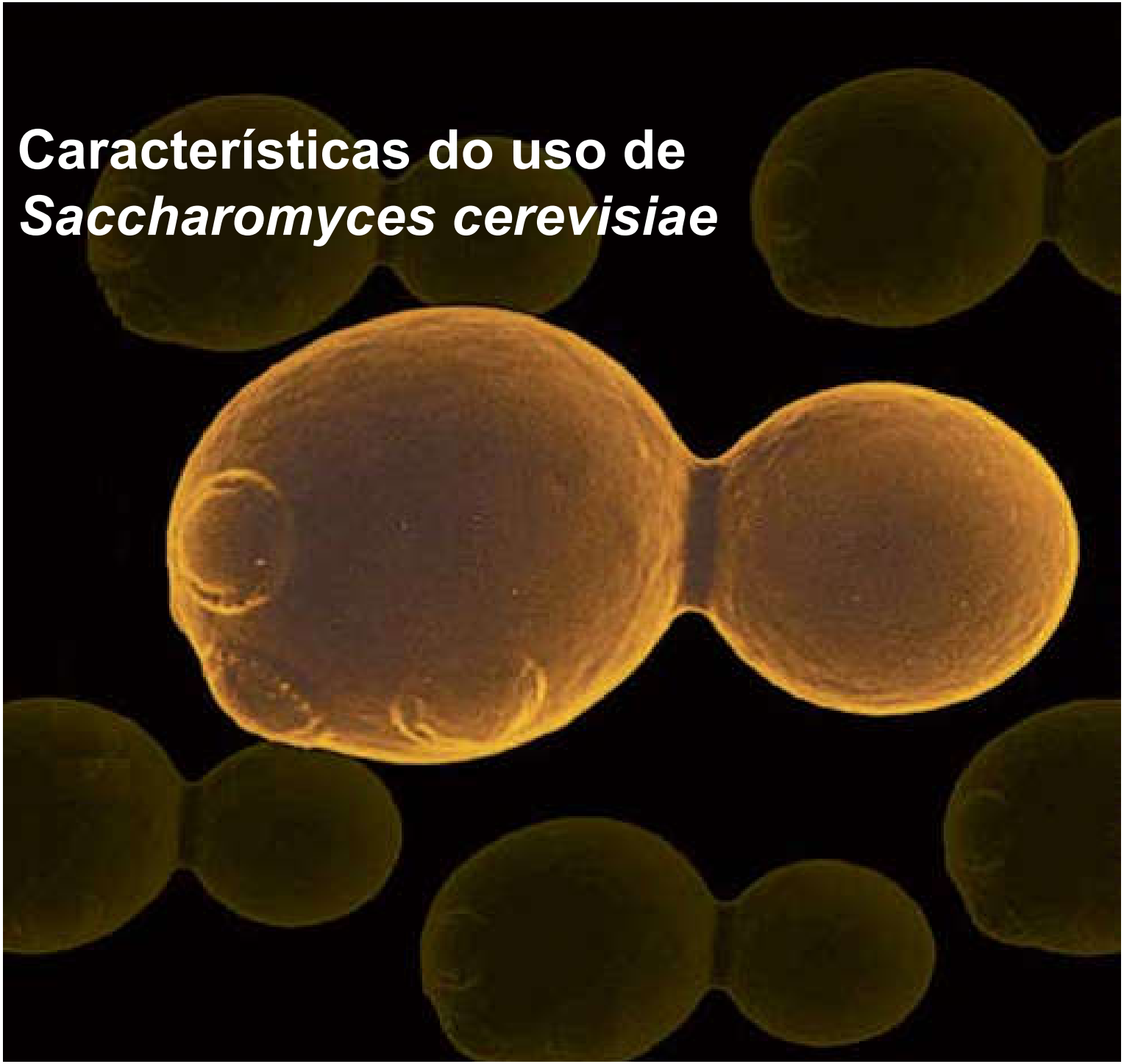
↓

Diplóide AaBb arg+

↓ R!

aB arg-
Ab arg-
AB arg+
ab arg-

Características do uso de *Saccharomyces cerevisiae*



Book cover:
From a to α
Hiten Madhani

Saccharomyces cerevisiae:

Ascomiceto → ascos de paredes finas

Na natureza →locais com pouca disponibilidade de água e alta oferta de açúcar : exsudato de plantas

Uso pelo homem:

→ preparação de alimentos e bebidas desde o início da civilização

→ Modelo de estudo da célula eucariótica

→ Modelo de estudo:

Estrutura celular muito semelhante aos animais

Crescimento rapido

Facilidade de cultivo e seleção em meios diferenciados

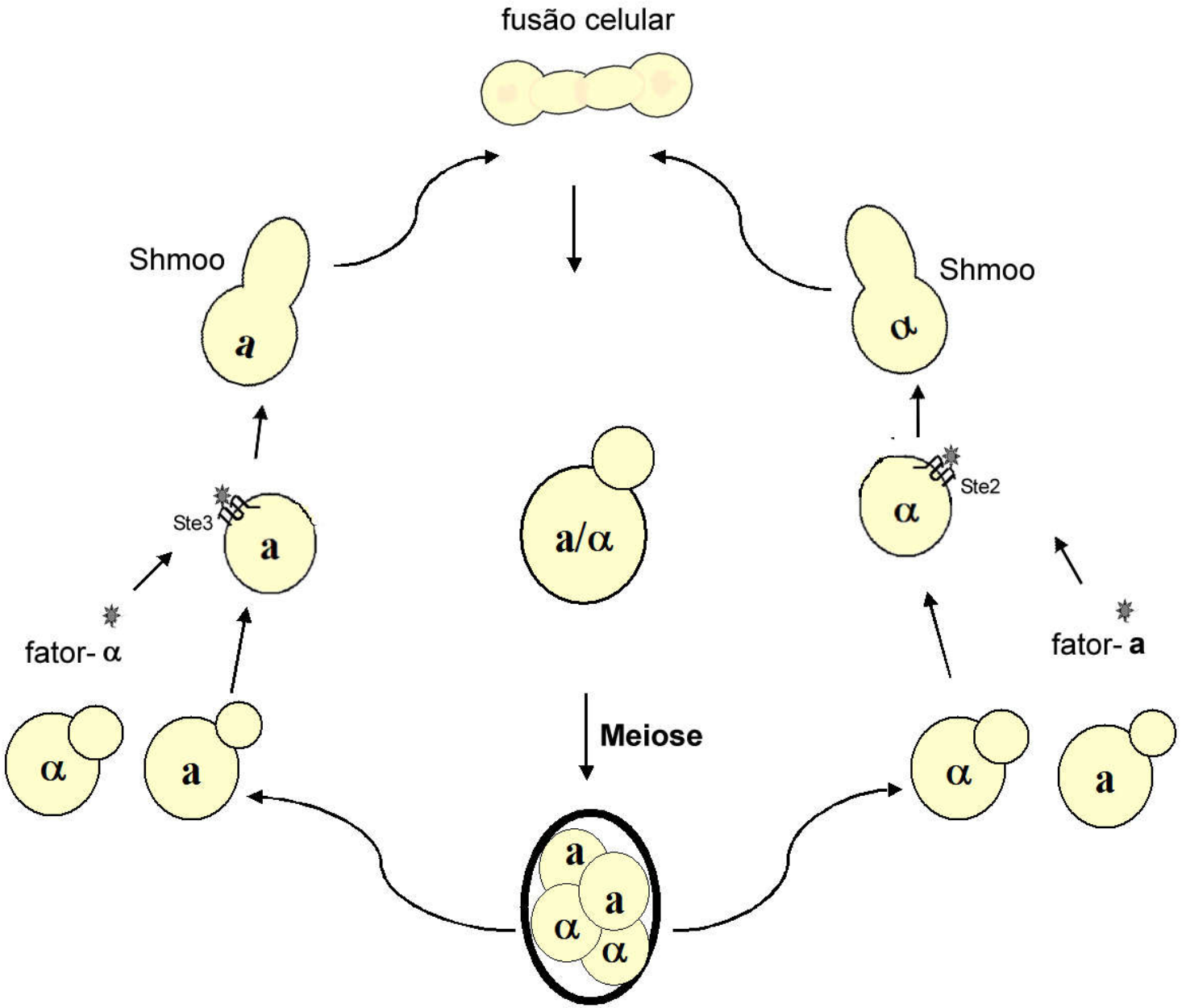
Manipulação genética bem determinada e sistema verstátil de transformação gênica

Ciclo de vida com estado haplóide e diplóide estáveis.

Yeast and Nobel Laureates

1907- Eduard Buchner	Cell Free Fermentation	(Chemistry)
.		
.		
.		
2001- Leland H. Hartwell	Key regulators of cell cycle	(Medicine)
2004 – Avram Hershko	Ubiquitin-mediated protein degradation	(Chemistry)
2006 – Rober Kornberg	Eukaryotic transcription	(Chemistry)
2009 – Elizabeth H. Blackburn	Telomeres and telomerases	(Medicine)
2013 – Randy Schekman	Secretory Pathway	(Medicine)
2016 – Yoshinori Ohsumi	Mechanisms for Autophagy	(Medicine)

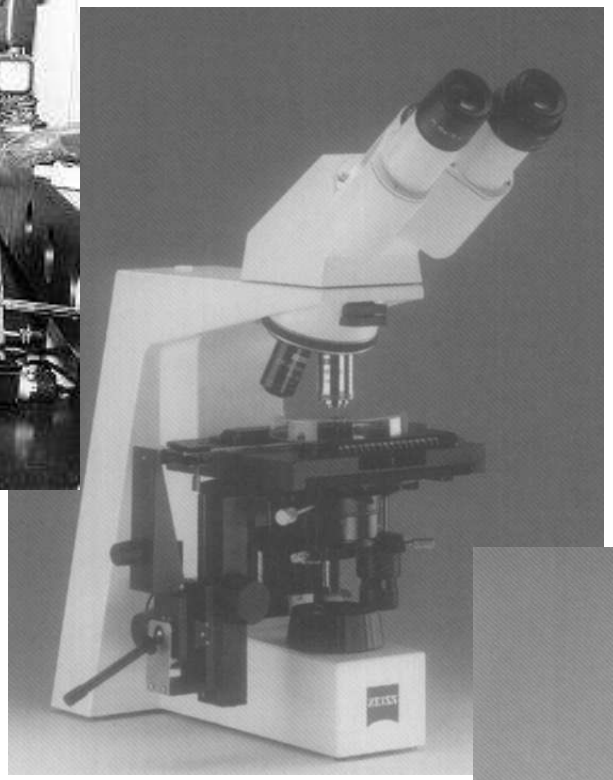
Saccharomyces cerevisiae

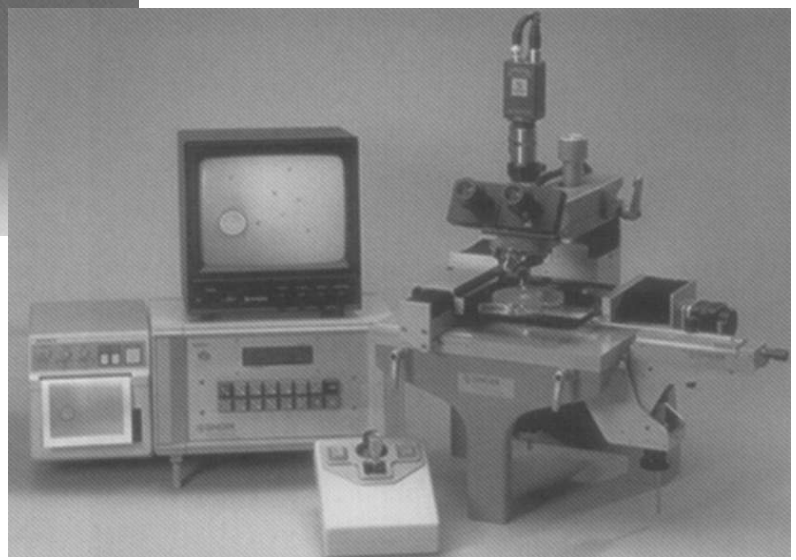


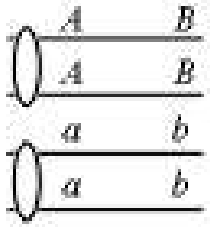
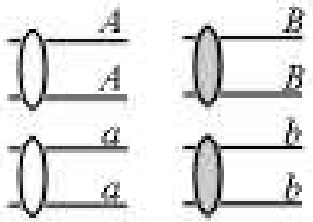
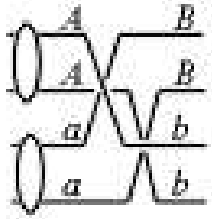
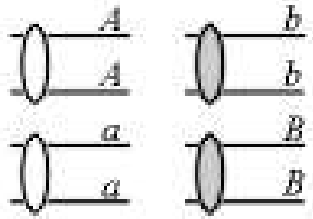
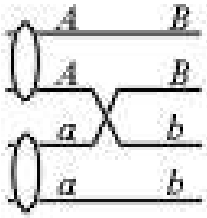
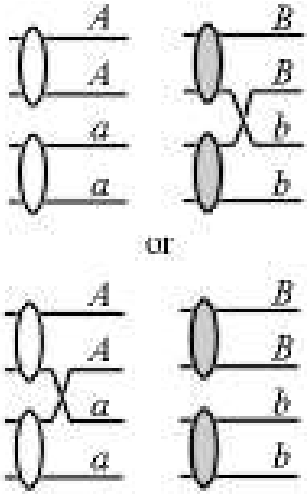
Ira Herskowitz

Micromanipulador de tetrades

Amar Klar





Tetrad type	Genes on homologous chromosomes	Genes on nonhomologous chromosomes
Parental ditype (PD) *A B* *A B* *a b* *a b*	No crossover 	No crossover 
Non-parental ditype (NPD) *A b* *A b* *a B* *a B*	Double crossover 	No crossover 
Tetratype (T) *A B* *A b* *a B* *a b*	Single crossover 	Single crossovers 

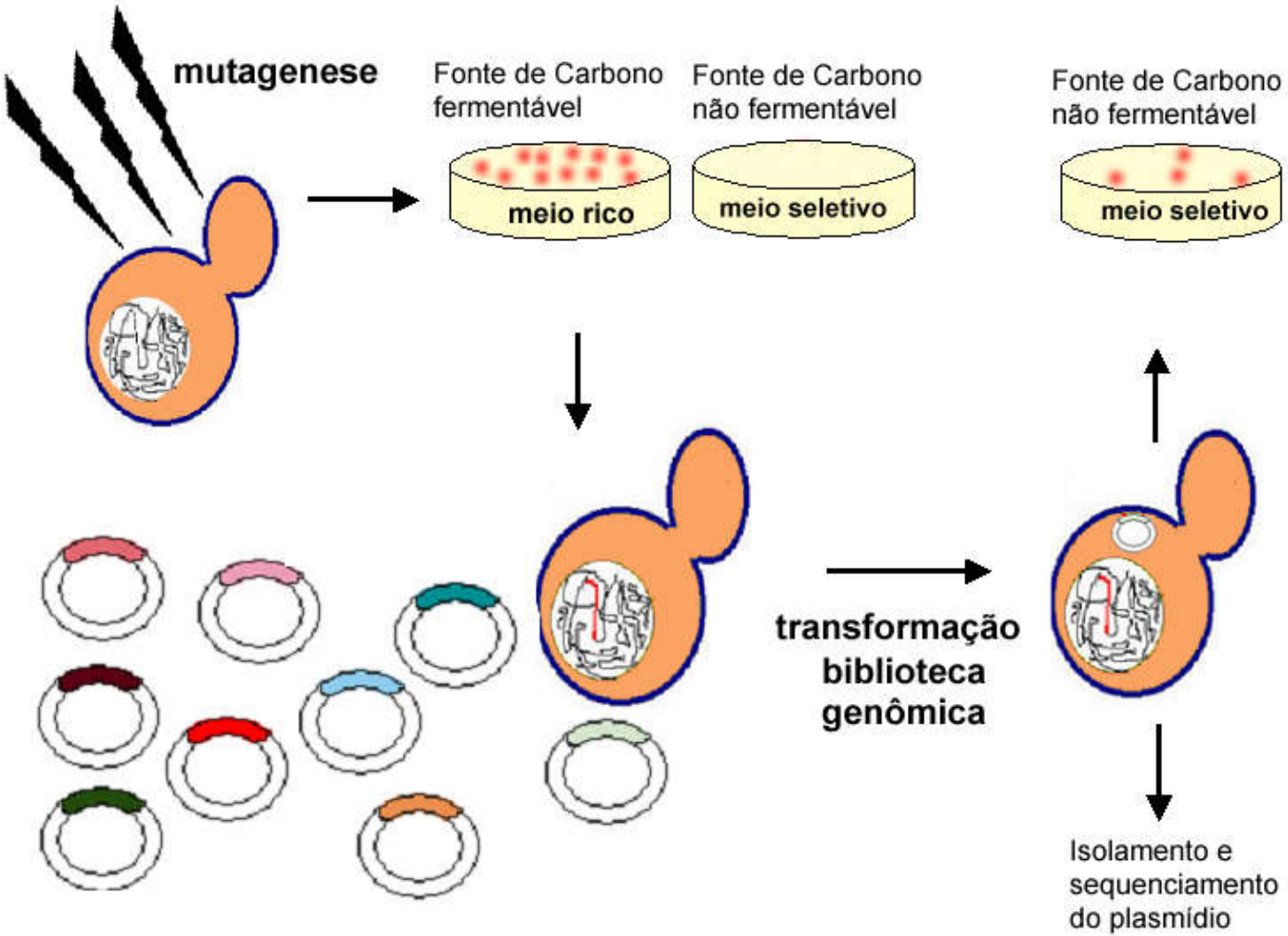
$$cM = \frac{100}{2}\left[\frac{T + 6NPD}{PD + NPD + T}\right]$$

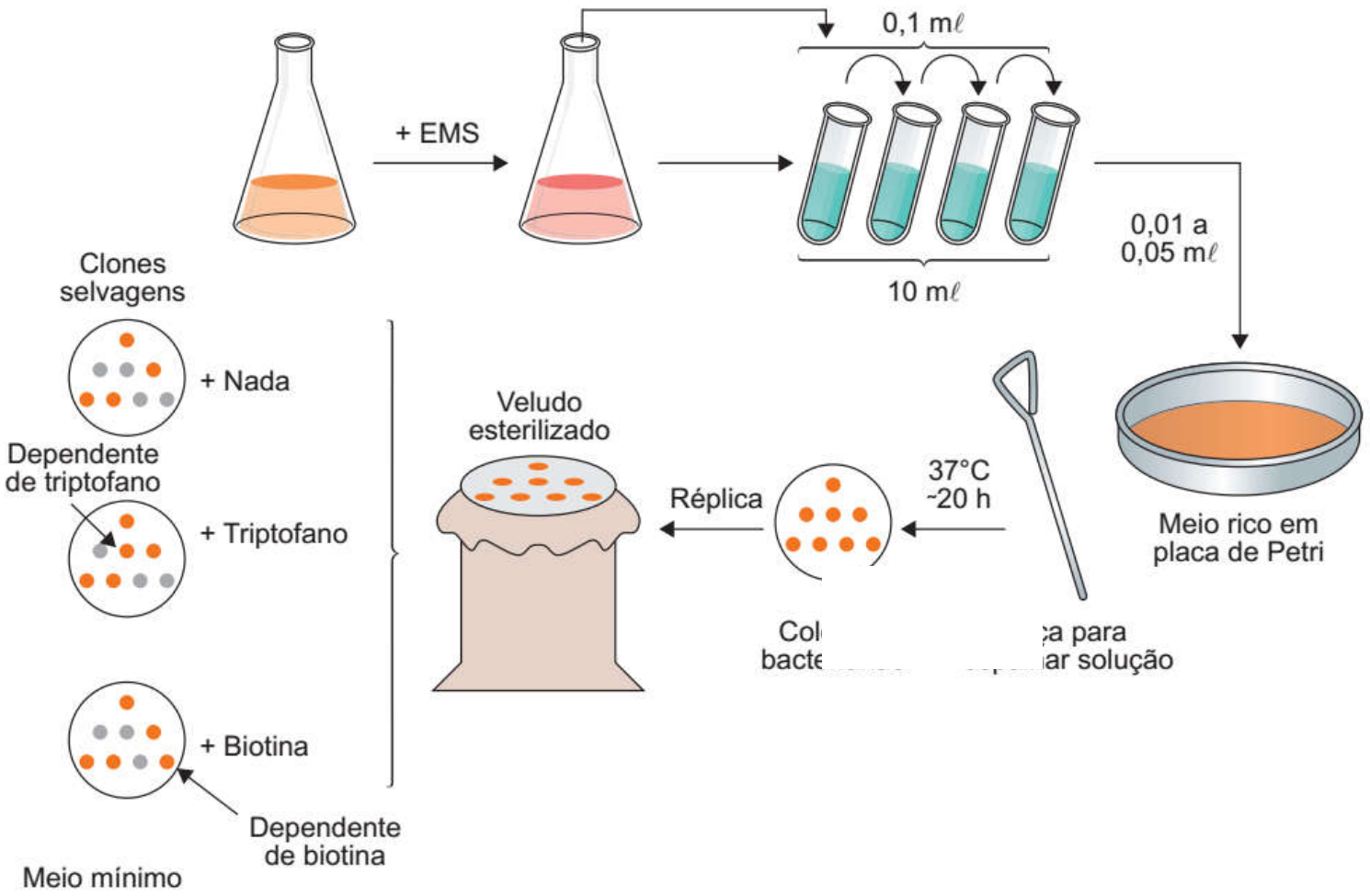
A reprodução sexuada em *S. cerevisiae* foi utilizada por muito tempo com fonte de definição para mapeamento cromossômico

Table 6.1. Genetic nomenclature, using *ARG2* as an example

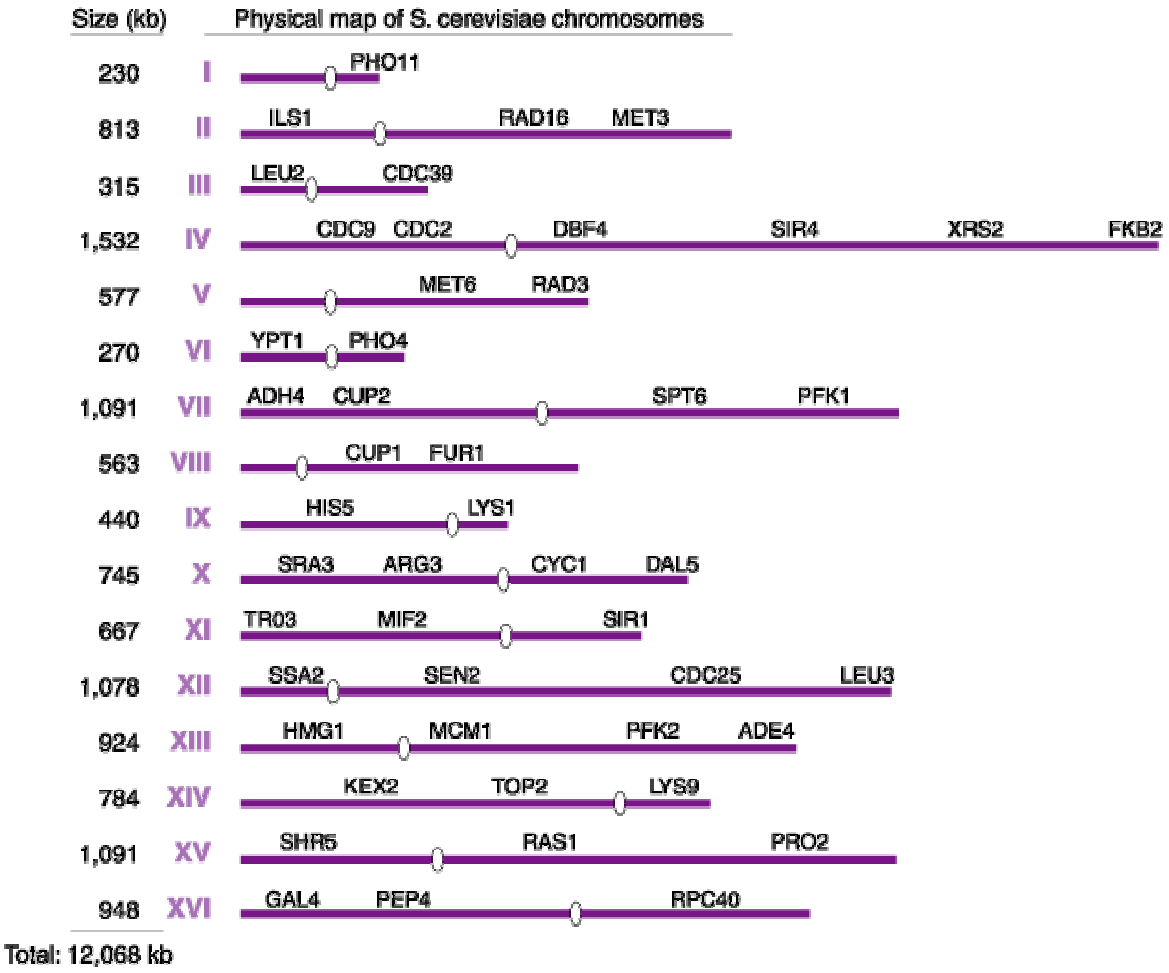
Gene symbol	Definition
ARG+	All wild-type alleles controlling arginine requirement
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
arg2-	Any arg2 allele confering an arginine requirement
ARG2+	The wild-type allele
arg2-9	A specific allele or mutation
Arg+	A strain not requiring arginine
Arg-	A strain requiring arginine
Arg2p	The protein encoded by ARG2
Arg2 protein	The protein encoded by ARG2
ARG2 mRNA	The mRNA transcribed from ARG2
arg2-D1	A specific complete or partial deletion of ARG2
ARG2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *ARG2* remains functional and dominant
arg2::*LEU2*	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *arg2* is or became nonfunctional
arg2-10::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and the specified arg2-10 allele which is nonfunctional
cyc1-arg2	A fusion between the *CYC1* and *ARG2* genes, where both are nonfunctional
PCYC1-ARG2	A fusion between the *CYC1* promoter and *ARG2*, where the *ARG2* gene is functional

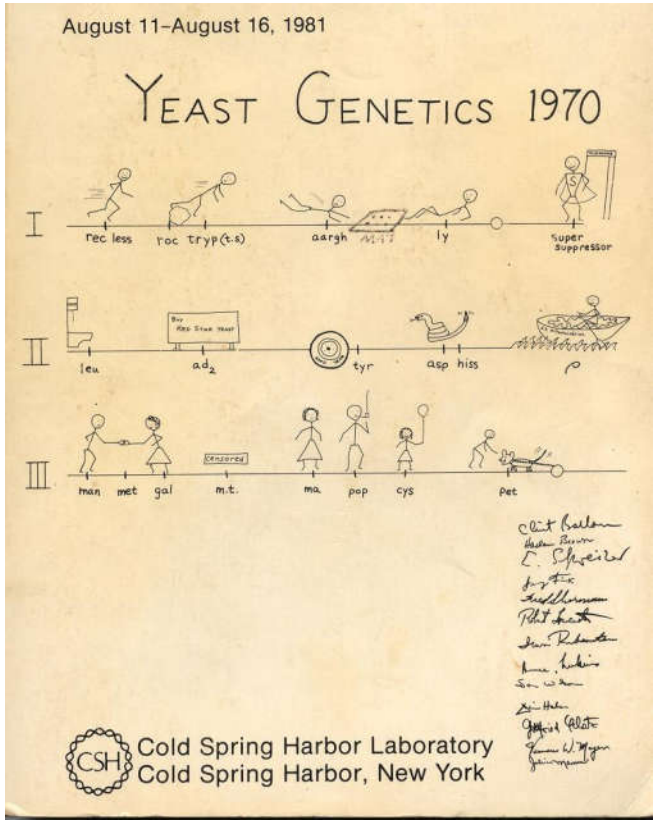
A manipulação genética é favorável e de fácil execução, como no isolamento de mutantes com deficiência respiratória




+ EMS
0,1 mℓ
10 mℓ
0,01 a
0,05 mℓ
Meio rico em
placa de Petri
37°C
~20 h
Réplica
Veludo
esterilizado
Clones
selvagens
+ Nada
Dependente
de triptofano
+ Triptofano
+ Biotina
Dependente
de biotina
Meio mínimo

1º Eucarioto a ter seu genoma sequenciado (1996) – base de todos estudos “ômicos”



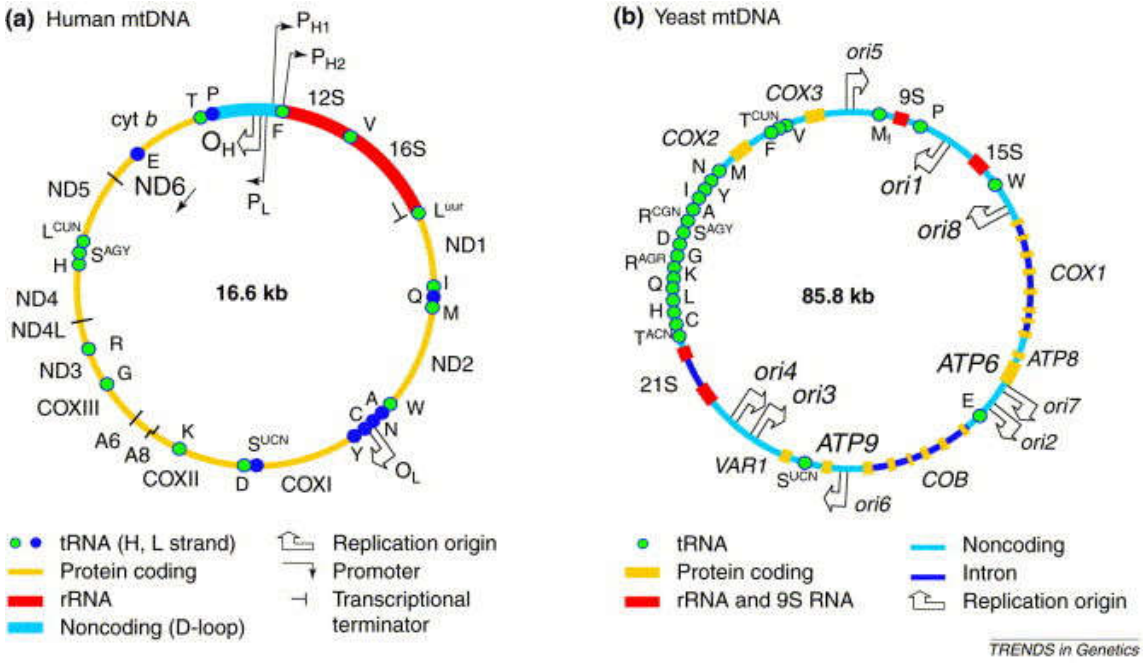


Hemiascomycetous yeast genomes are intron-poor.

	S. cerevisiae	*S. servazzii*	*S. kluyveri*	*K. marxianus*	*C. tropicalis*	*D. hansenii*	*P. angusta*	*Y. lipolytica*
Number of detected introns	260	23	36	19	12	14	30	19
Total number of genes	5261	1410	1406	1301	1130	1119	2320	1078
Number of genes with introns	252	22	36	17	11	12	27	19
Proportion of genes with introns [a]	4.8	1.6	2.6	1.3	1.0	1.1	1.2	1.7

[a] Expressed in percentages

Bon *et al.*, 2003



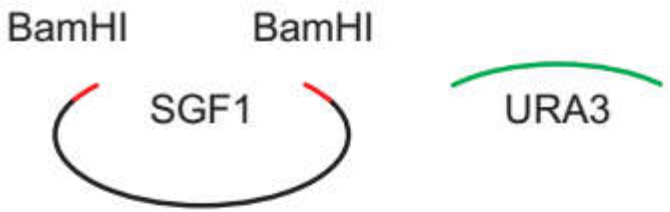
Animais e fungos apresentam variações no código genético mitocondrial.

First letter	Second letter				Third letter
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*Stop*	(*Stop*) Trp	A
	Leu	Ser	*Stop*	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile (Met)	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	(Ile) Met	Thr	Lys	(Arg) *Stop*	A
	Met	Thr	Lys	(Arg) *Stop*	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

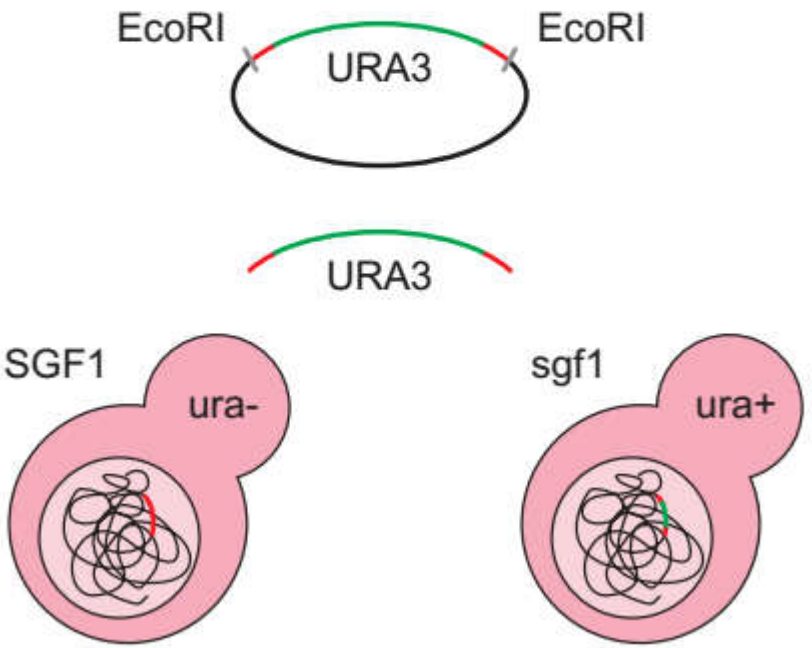
Disponibilidade de mutantes nulos para cada uma das 6000 ORFs (desde 1999)

Genética clássica	Genética reversa
Fenótipo mutante ↓	Sequência DNA ↓
Alelo mutante	Alelo mutante
Sequência DNA ↓	Fenótipo mutante ↓

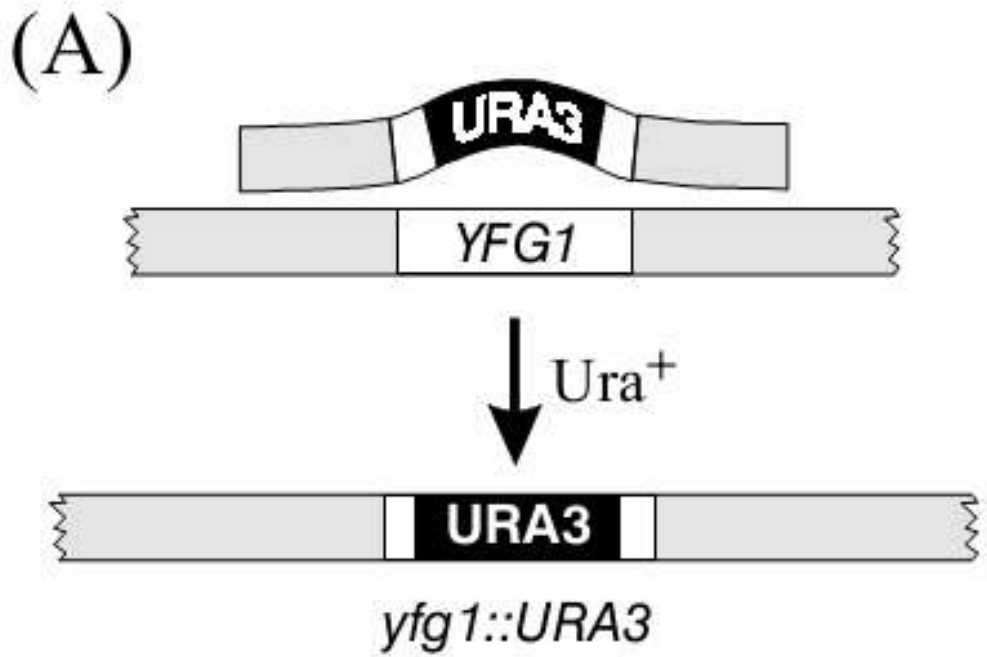
Esquema para inativação
Do gene SGF1



Emprego da Genética Reversa



Necessidade de
pareamento homólogo
entre segmentos de DNA
para recombinação
ocorrer



Modified from: F. Sherman, Yeast genetics. •
The Encyclopedia of Molecular Biology and Molecular Medicine, •
pp. 302-325, Vol. 6. Edited by R. A. Meyers, VCH Publisher, Weinheim, Germany, 1997.•

Uso de plasmídios tal qual em *Escherichia coli*

Tipos de Plasmídios:
Multicópias
Integrativos
Centroméricos

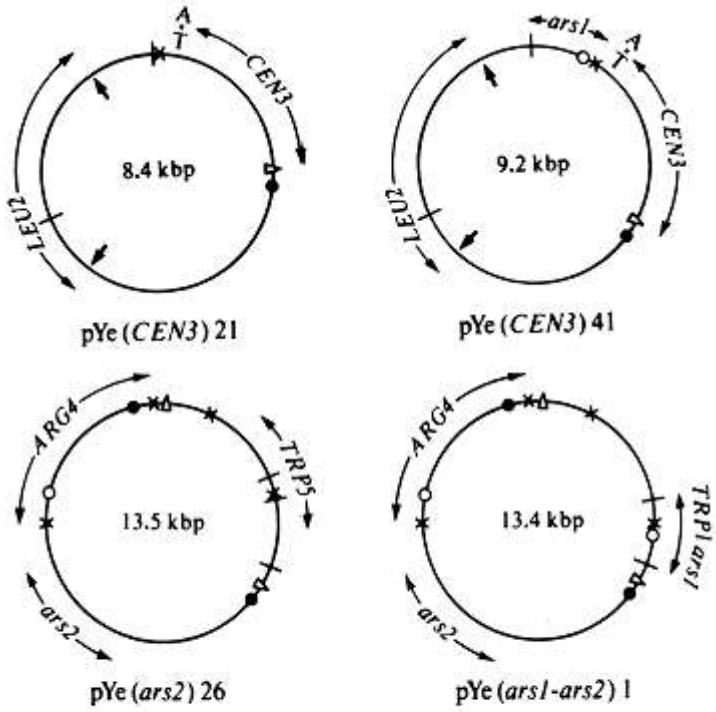


Fig. 2 Physical maps of various plasmid DNAs. The maps show the location of *Eco*RI (–|–), *Hin*dIII (–×–), *Bam*HI (–△–), *Pst*I (–↑–), *Sal*I (–●–) and *Bgl*II (–○–) sites in the DNAs and indicate approximate locations of pertinent replicators and genes. The construction and use of these plasmids is described in the text. Their sizes are indicated in kilobase pairs (kbp).

Nature. Vol 287, No. 5782, pp. 504-509, October 9 1980

Características dos plasmídios de levedura

Table 5. Components of common yeast plasmid vectors

	YIp	YEp	YRp	YCp
Plasmid				
E. coli genes or segments				
ori, *bla*; *tet*	+	+	+	+
Yeast genes or segments				
URA3; *HIS3*; *LEU2*; *TRP1*; *LYS2*; *etc.*	+	+	+	+
leu2-d	0	+	+	0
2 μm; 2 μm-*ori REP3*;	0	+	0	0
ARS1; *ARS2*; *ARS3*; *etc.*	0	0	+	+
CEN3; *CEN4*; *CEN11*; *etc.*	0	0	0	+
Host (yeast) markers				
ura3-52; *his3-Δ1*; *leu2-Δ1*; *trp1-Δ1*; *lys2-201*; *etc.*	+	+	+	+
Stability	++	+	±	+

Modified from: F. Sherman, Yeast genetics. •
The Encyclopedia of Molecular Biology and Molecular Medicine, •
pp. 302-325, Vol. 6. Edited by R. A. Meyers, VCH Publisher, Weinheim, Germany, 1997.•

Estudo de Mutantes:

→Mutantes nulos (genética reversa)

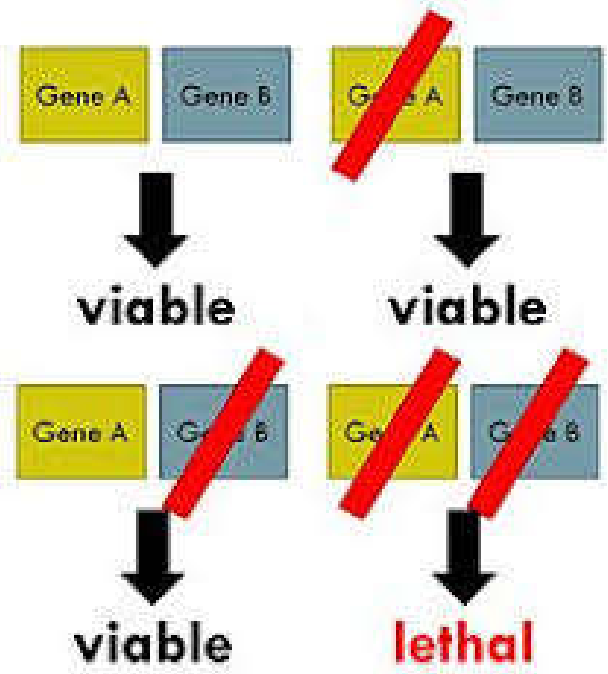
→Mutantes condicionais (sensível a temperatura)

→Mutações dominantes (como determinar se um mutante hisX é dominante?)

→Mutações Supressoras (por epistasia – por excesso – por desvio de via)

Mutações Letais (como definir? – sintéticos letais?)

Letalidade sintética



Tipos de supressão genética

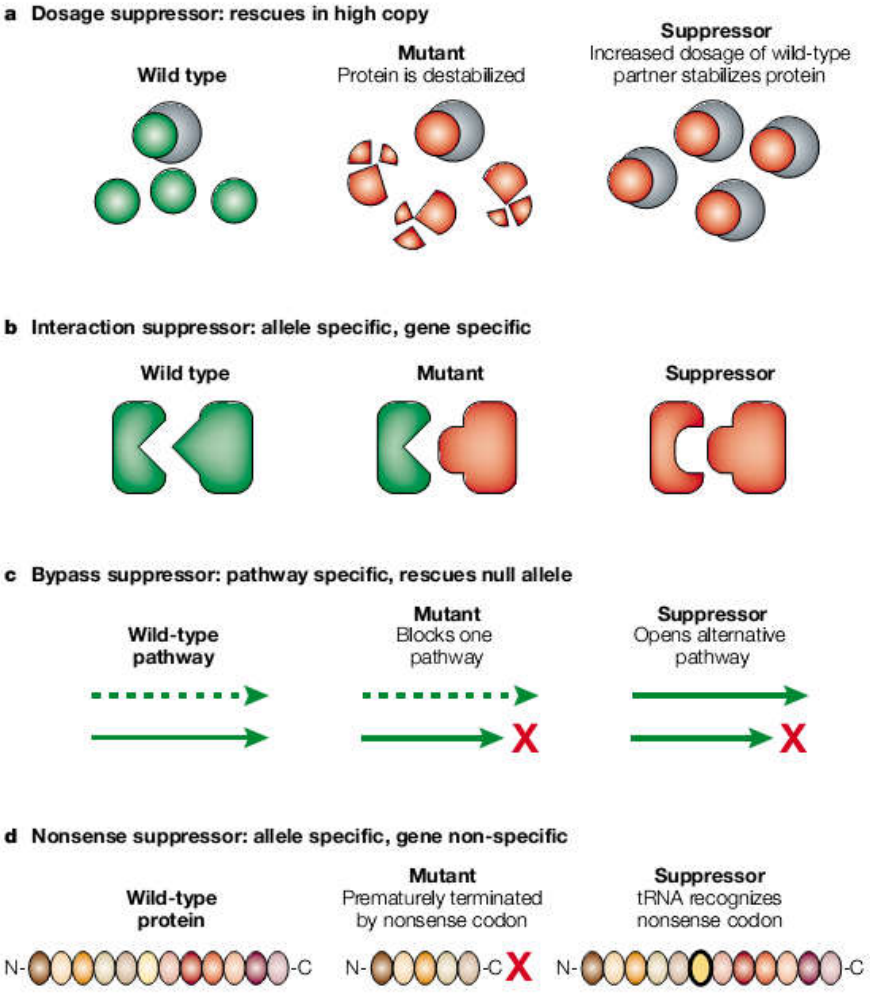
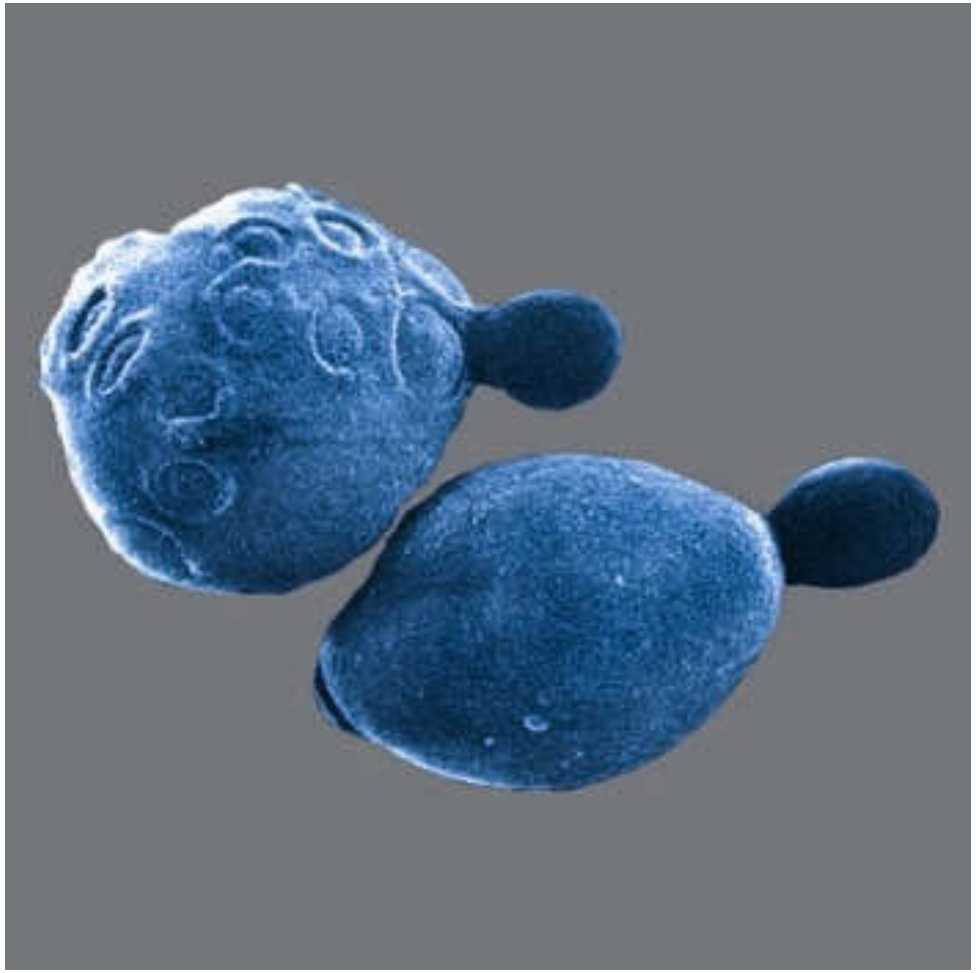
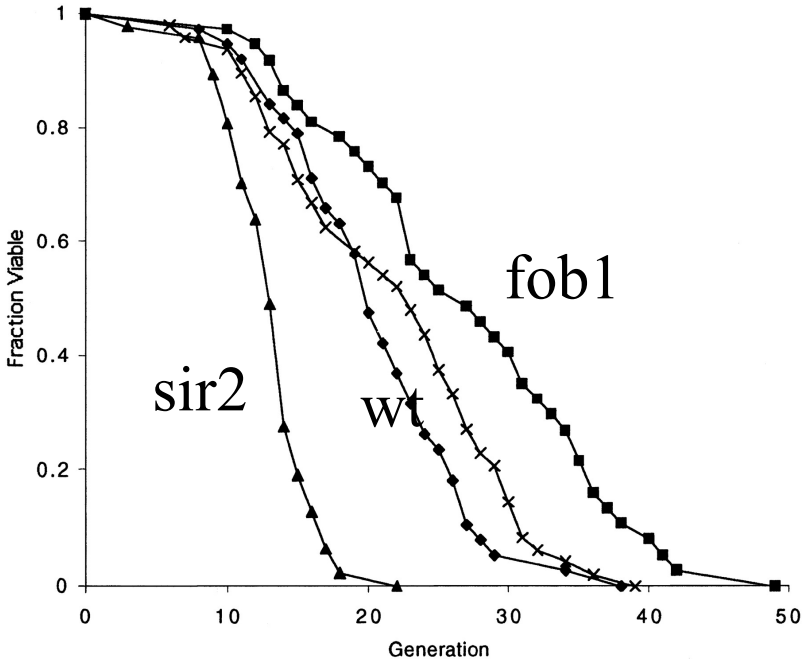


Figure 3 | **Suppressor mechanisms.** Depending on the allele and gene specificity associated with suppressors, mechanisms can be inferred, as shown. **a** | Dosage suppressors encode proteins that stabilize the mutant product when they are expressed at high levels. **b** | An interaction suppressor restores the interaction between the mutant product and its partner(s). **c** | A bypass suppressor activates an alternative pathway to the wild-type pathway. **d** | A nonsense suppressor encodes a tRNA molecule that recognizes a premature termination codon and inserts an amino acid at that position.

Algumas aplicações

Estudo do envelhecimento





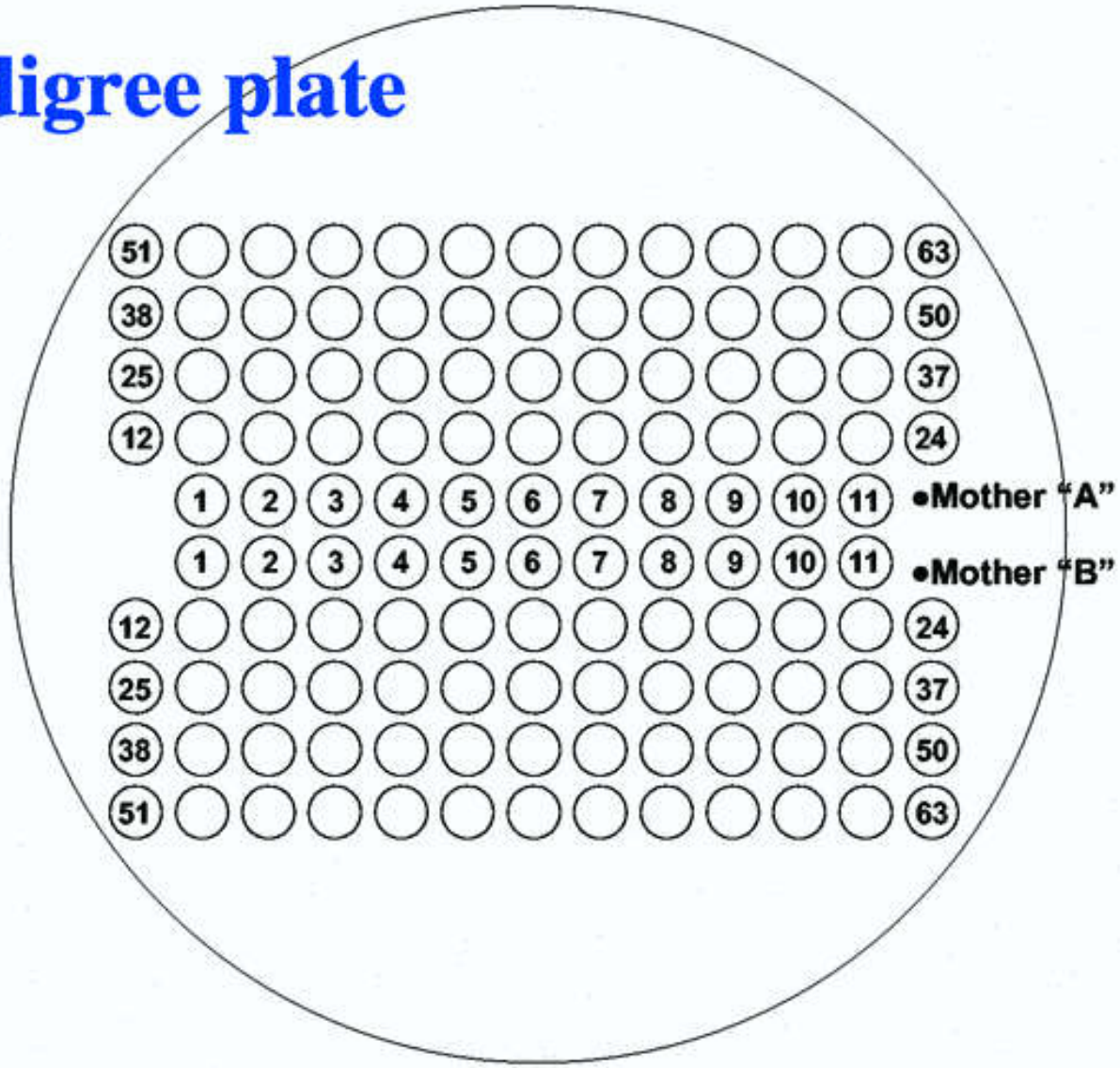
Perda de heterozigosidade e câncer

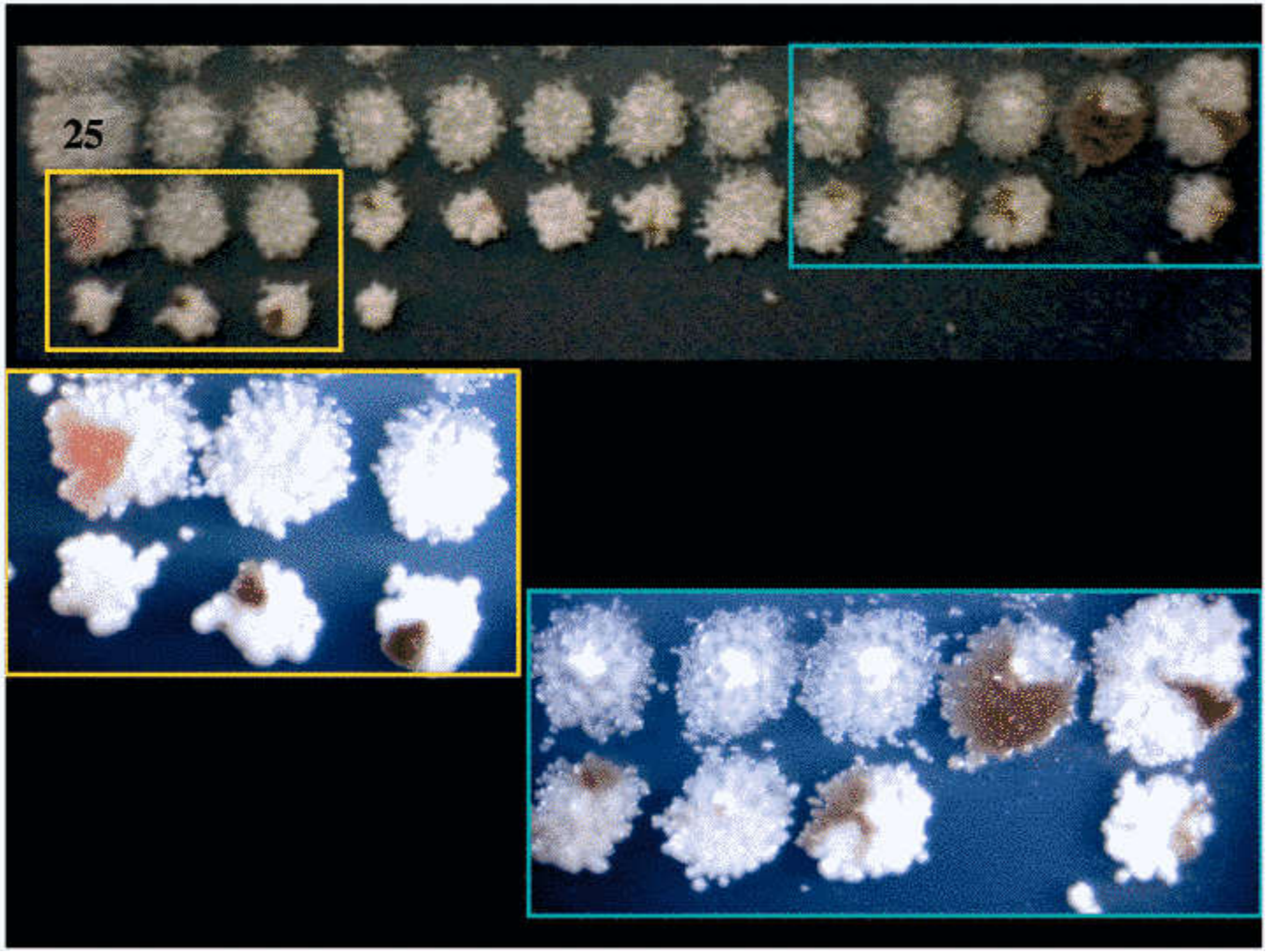
Color Assays for LOH

MET15/met15 ---> met15/met15

ADE2/ade2 ---> ade2/ade2

Pedigree plate




25

Biotecnológicas

Produção de glicoproteínas humanizadas em levedura

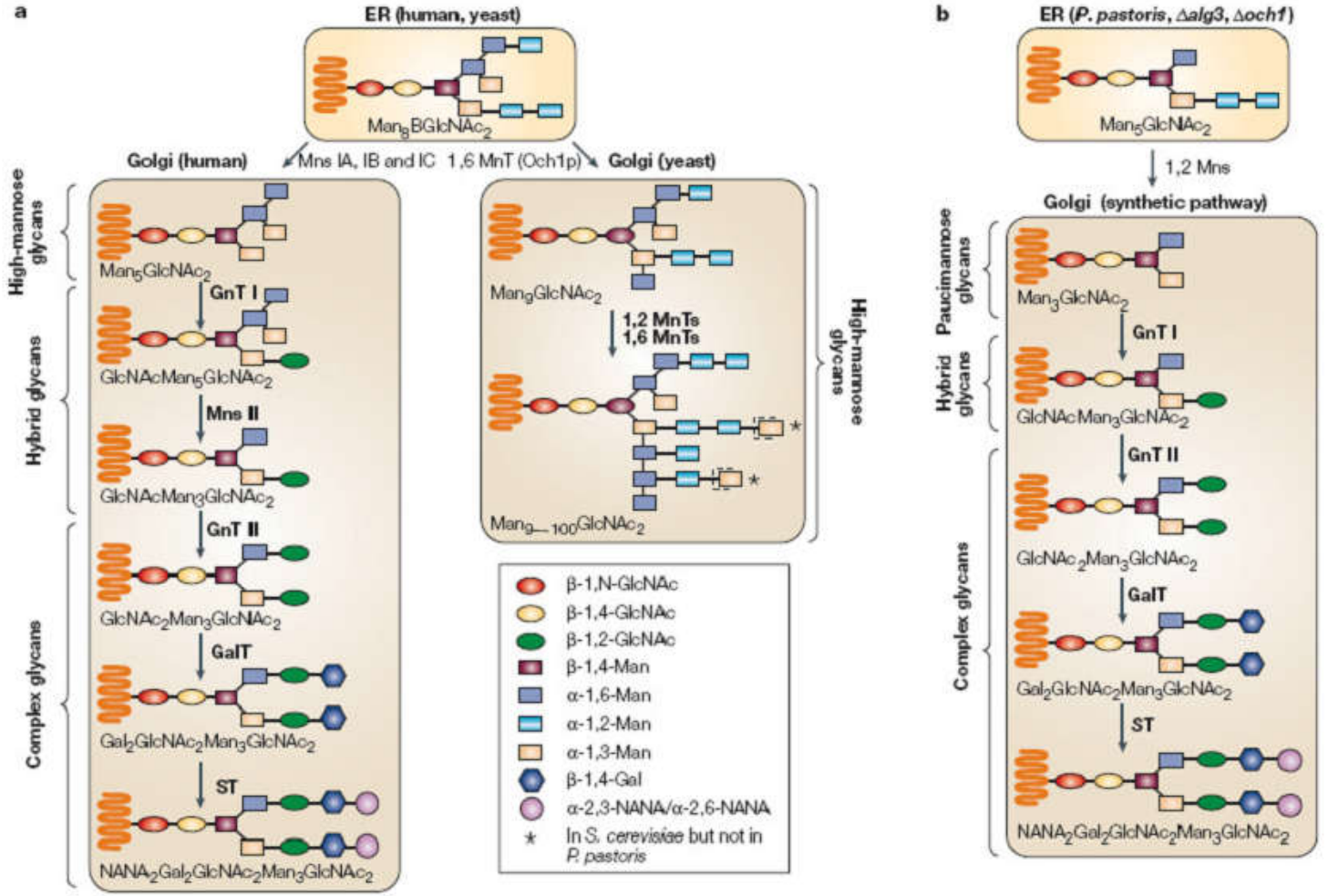
THE HUMANIZATION OF *N*-GLYCOSYLATION PATHWAYS IN YEAST

Stefan Wildt and Tillman U. Gerngross†*

Glycosylation engineering in yeast: the advent of fully humanized yeast
Stephen R Hamilton[1] and Tillman U Gerngross[1,2]

Production of humanized glycoproteins in bacteria and yeasts
Yasunori Chiba[1,2] and Yoshifumi Jigami[1]

Principais vias de N-glicosilação em humanos e levedura



Wildt and Gerngross (2005) *Nat Rev Microbiol* 3: 119-128

Table 1 Therapeutic proteins produced in the yeasts *S. cerevisiae* and *P. pastoris*

Products on the market

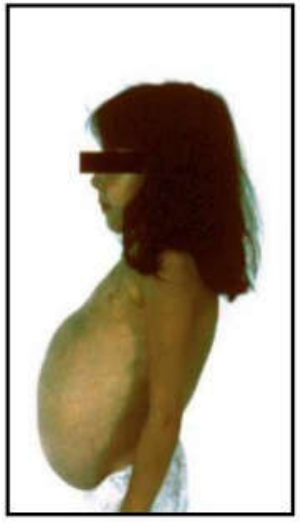
Commercial name	Recombinant protein	Company	Expression system
Actrapid	Insulin	NovoNordisk	*S. cerevisiae*
Ambirix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Comvax	Hepatitis B surface antigen	Merck	*S. cerevisiae*
Elitex	Urate oxidase	Sanofi-Synthelabo	*S. cerevisiae*
Glucagen	Glucagon	Novo Nordisk	*S. cerevisiae*
HBVAXPRO	Hepatitis B surface antigen	Aventis Pharma	*S. cerevisiae*
Hexavac	Hepatitis B surface antigen	Aventis Pasteur	*S. cerevisiae*
Infanrix-Penta	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Leukine	Granulocyte-macrophage colony stimulating factor	Berlex	*S. cerevisiae*
Novolog	Insulin	Novo Nordisk	*S. cerevisiae*
Pediarix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*
Procomvax	Hepatitis B surface antigen	Aventis Pasteur	*S. cerevisiae*
Refuldan	Hirudin/lepirudin	Hoechst	*S. cerevisiae*
Regranex rh	Platelet-derived growth factor	Ortho-McNeil Phama (US), Janssen-Cilag (EU)	*S. cerevisiae*
Revasc	Hirudin/desirudin	Aventis	*S. cerevisiae*
Twinrix	Hepatitis B surface antigen	GlaxoSmithKline	*S. cerevisiae*

Wildt and Gerngross (2005) *Nat Rev Microbiol* 3: 119-128

Doença de Gaucher - afeta lisossomo tratamento com Glucocerobridase → ainda não produzida em levedura!

Cerezyme→ cada dose USD 432,97
No Brasil 726 pacientes – USD 153.000.000

Response to Enzyme Therapy



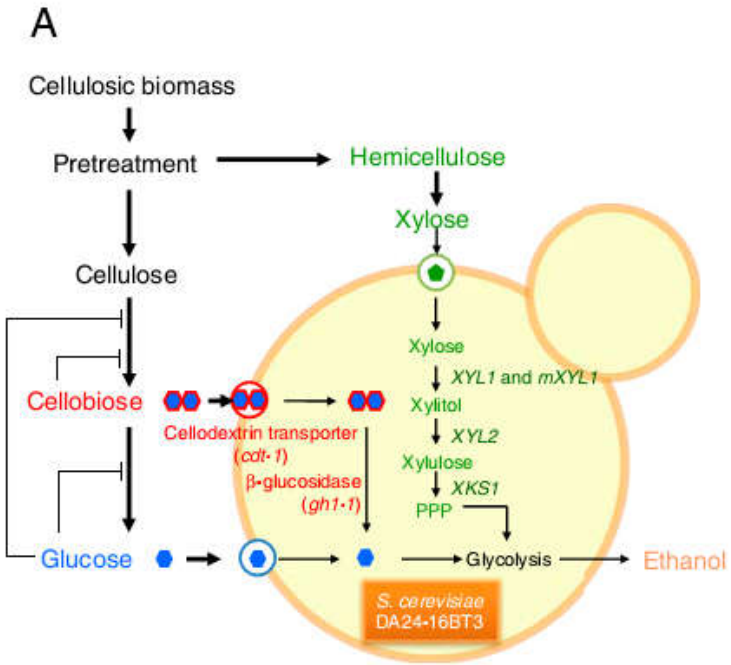
Pretreatment
Age 8 Years, 8 Months

Post-treatment
Age 10 Years, 10 Months

Na produção de etanol

Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation

Suk-Jin Ha[a,b,1], Jonathan M. Galazka[c,1], Soo Rin Kim[a,b], Jin-Ho Choi[a,b], Xiaomin Yang[d], Jin-Ho Seo[e], N. Louise Glass[f], Jamie H. D. Cate[c,g,2], and Yong-Su Jin[a,b,2]



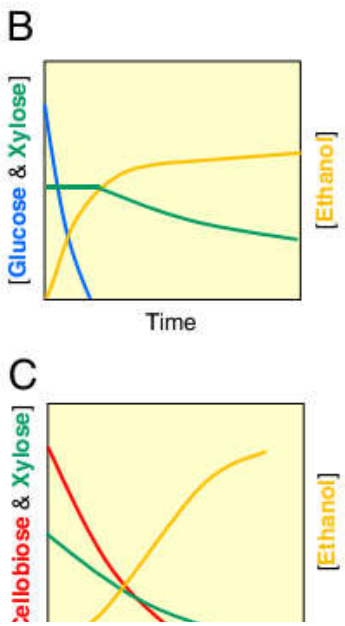


Fig. 1. Strategy for simultaneous cofermentation of cellobiose and xylose without glucose repression. (*A*) A strain improvement strategy to engineer yeast capable of fermenting two nonmetabolizable sugars (cellobiose and xylose). The cellodextrin assimilation pathway consists of a cellodextrin transporter (*cdt-1*) and an intracellular β-glucosidase (*gh1-1*) from the filamentous fungus *N. crassa*. The modified xylose metabolic pathway utilizes xylose reductase isoenzymes (wild-type XR and a mutant XR^{R276H}), xylitol dehydrogenase (*XYL2*), and xylulokinase (*XKS1*) from the xylose-fermenting yeast *P. stipitis*. (*B*) Schematic fermentation profile of a sugar mixture containing glucose and xylose by the engineered *S. cerevisiae*. Glucose fermentation represses xylose fermentation completely so that xylose fermentation begins only after glucose depletion (analogous fermentation result shown in Fig. 5*A*). (*C*) Schematic fermentation profile of a sugar mixture containing cellobiose and xylose by the engineered *S. cerevisiae*. Cellobiose [illegible]neously utilized, as neither consumption of the other (analogous fermentation result shown in Fig. 5*B*).

An Introduction to the Genetics and Molecular Biology of the Yeast *Saccharomyces cerevisiae*

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• 1998 •

Barros, M. H. . Genética dos Fungos. In: Flavio Alterthum. (Org.). Microbiologia. 6 ed. São Paulo: Atheneu, 2015, v. 1, p. 559-566.