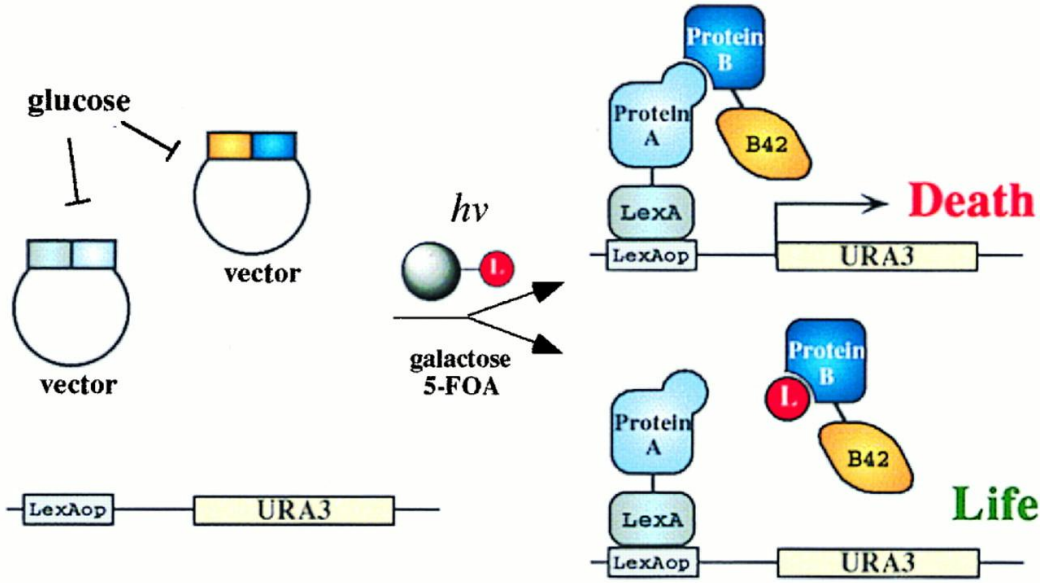
BMM5828/BTC5819 - Utilização de *Saccharomyces cerevisiae* como organismo modelo em biologia molecular

Interação de Pequenas Moléculas em Sistemas Biológicos



ICB, 17/11/2017

Objetivos

1. Definir Biologia Química e o seu propósito;

2. Conhecer as principais varreduras de compostos orgânicos realizadas em levedura;

3. Conhecer duas aplicações bem-sucedidas de Biologia Química em levedura: sensores de odores e produção de precursores de opióides;

Biologia Química

Síntese de pequenas moléculas orgânicas que são utilizadas como sonda biológicas

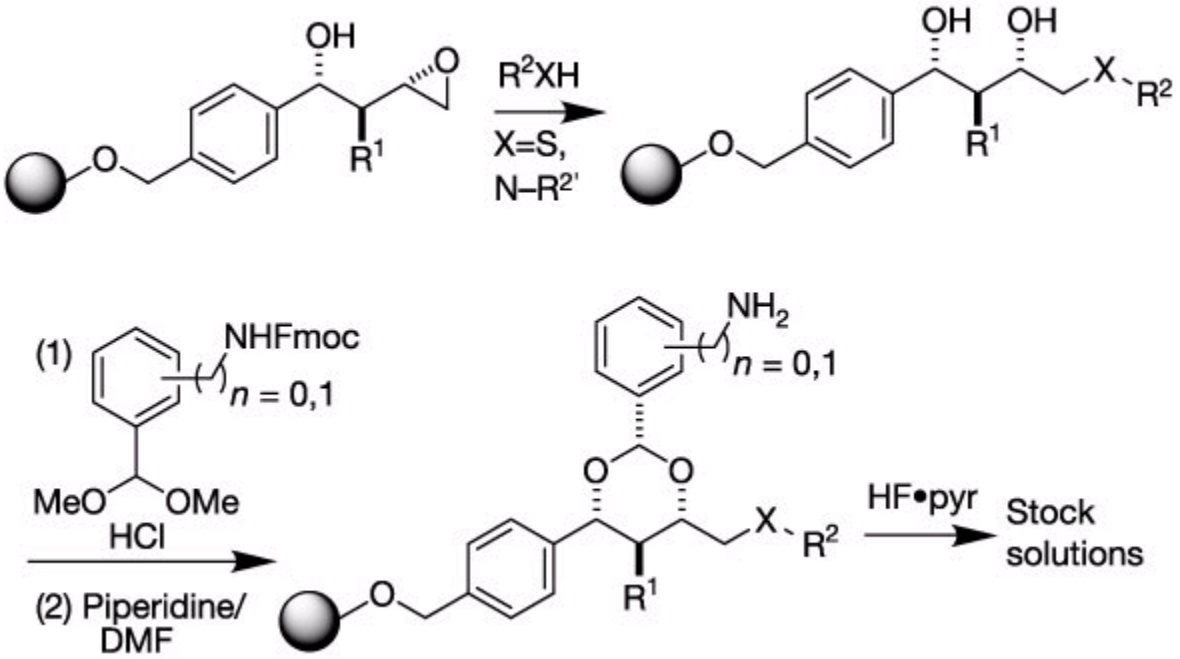
Interação de pequenas moléculas com sistemas biológicos

Varredura de bibliotecas de pequenas moléculas

Alteração de vias metabólicas para síntese de compostos orgânicos

Como obter as pequenas moléculas?

Síntese de compostos orgânicos orientada para a diversidade



http://www.broadinstitute.org/chembio/lab_schreiber/home.php

Nature **416**, 653-657 (11 April 2002) | doi:10.1038/416653a

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Publications citing ApexBio Products

Nature.
2017 Jan 19;541(7637):4...

Nature.
2016 Apr 21;532(7599):3...

Science.
2016 Aug 5;353(6299)59...

Nature Biotechnology.
2017 Jun;35(6):569-576

Cell.
2017 Aug 17. pii: S0092-...

Cell.
2017 Jul 13;170(2):312-

ZINC: a free database of commercially-available compounds for virtual screening

http://zinc.docking.org/

Como realizar a verredura dos compostos em *Saccharomces cerevisiae*?

Available online at www.sciencedirect.com

ScienceDirect

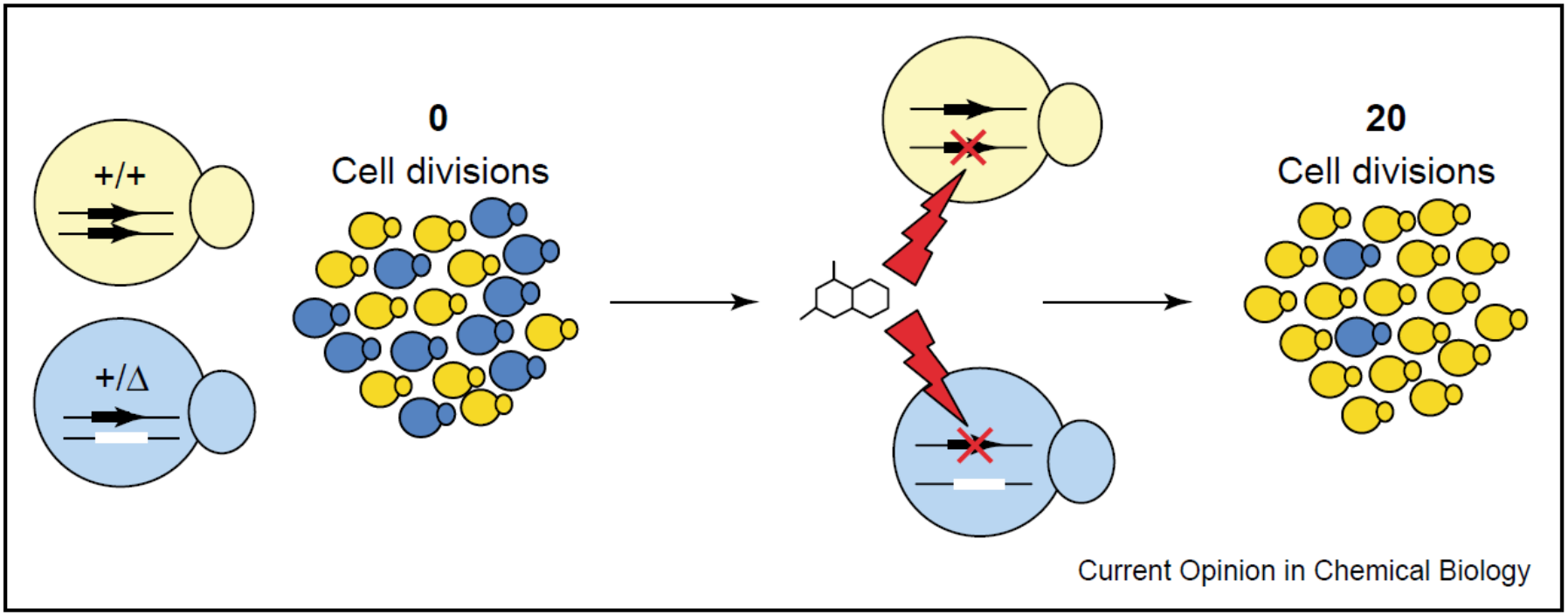
Current Opinion in Chemical Biology

Combining functional genomics and chemical biology to identify targets of bioactive compounds

Cheuk Hei Ho[1], Jeff Piotrowski[2], Scott J Dixon[3], Anastasia Baryshnikova[1], Michael Costanzo[1] and Charles Boone[1]

Current Opinion in Chemical Biology 2011, **15**:66–78

Haploinsuficiência fármaco-induzia



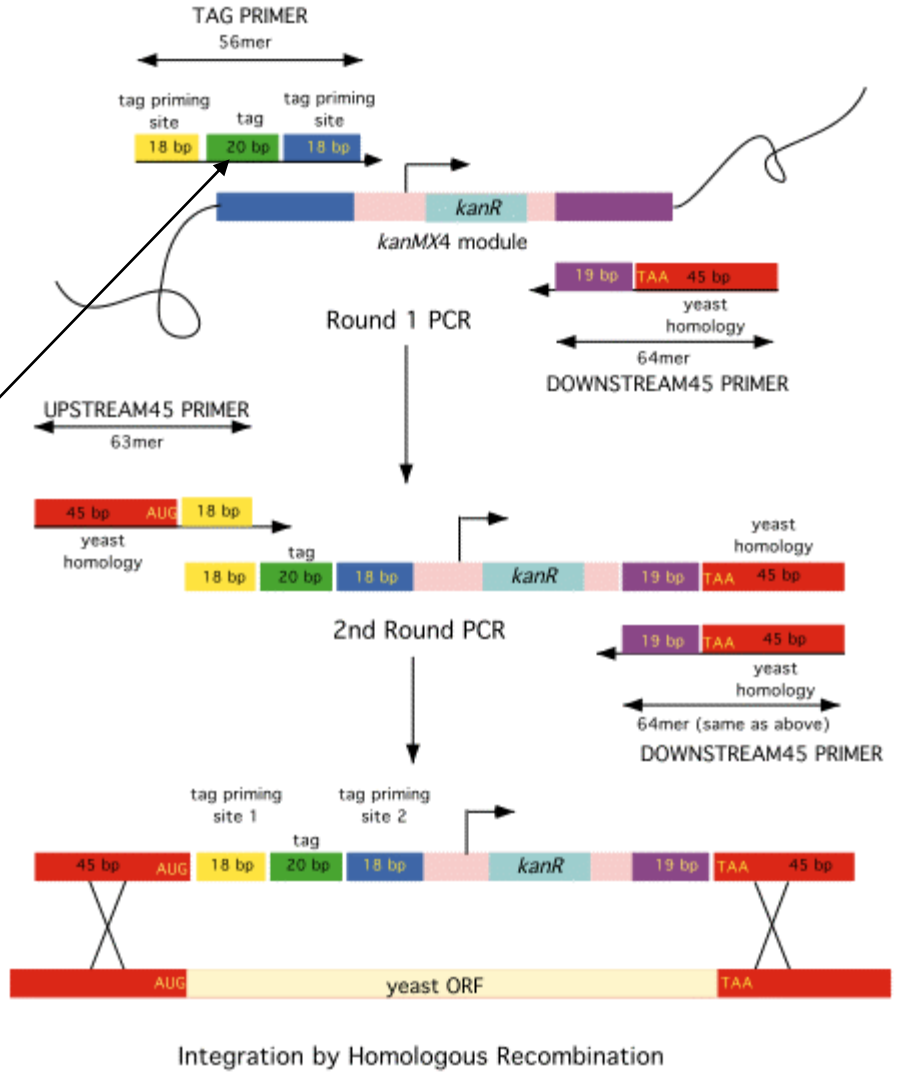
Armour and Lum (2005) *Curr Opin Chem Biol* 9: 20-24

A levedura como modelo de estudo da ação de pequenas moléculas

≅6000 coleção de diplóides heterozigotos

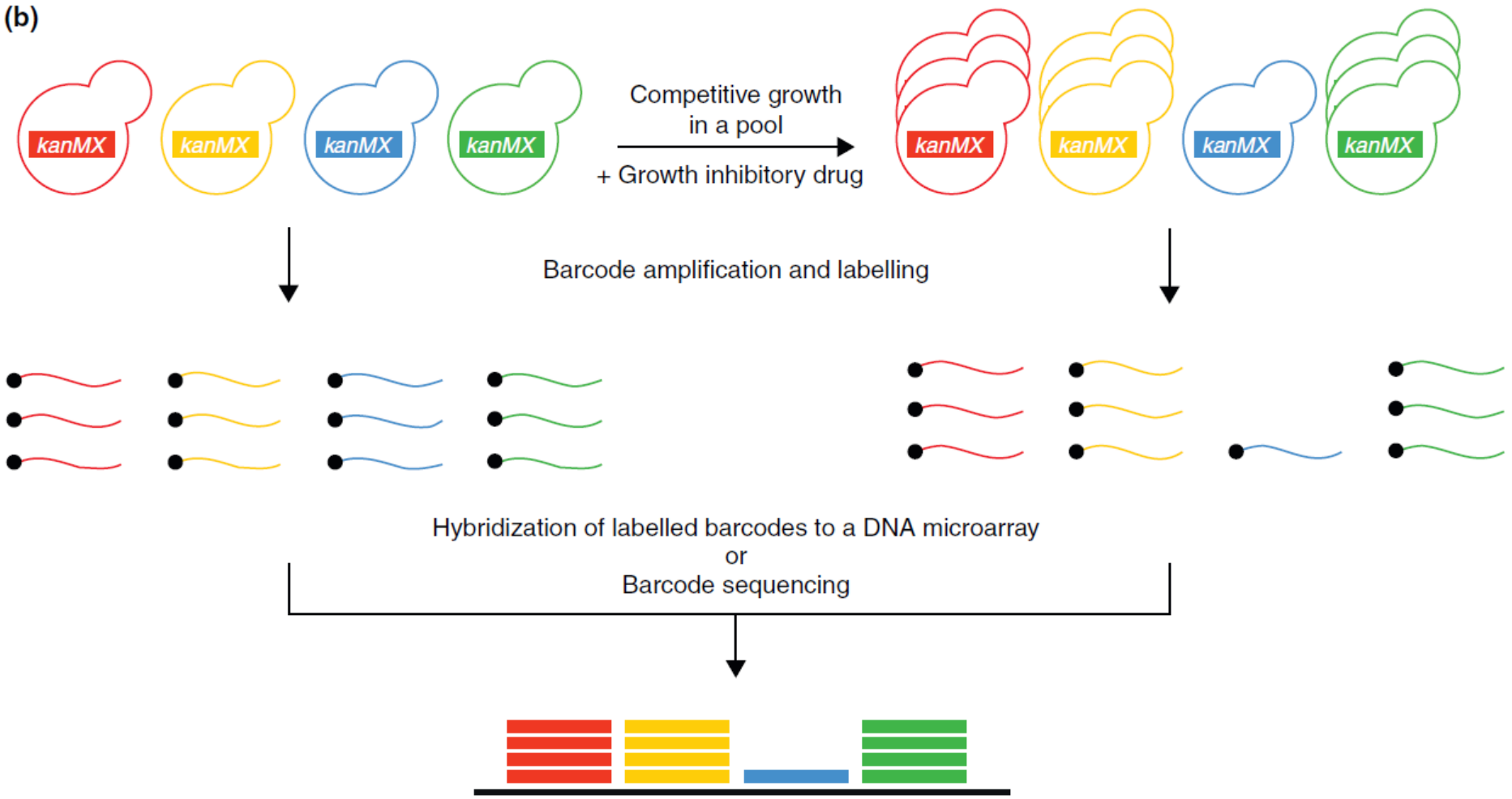
≅5000 haplóides viáveis

“código de barras” (20 pb)



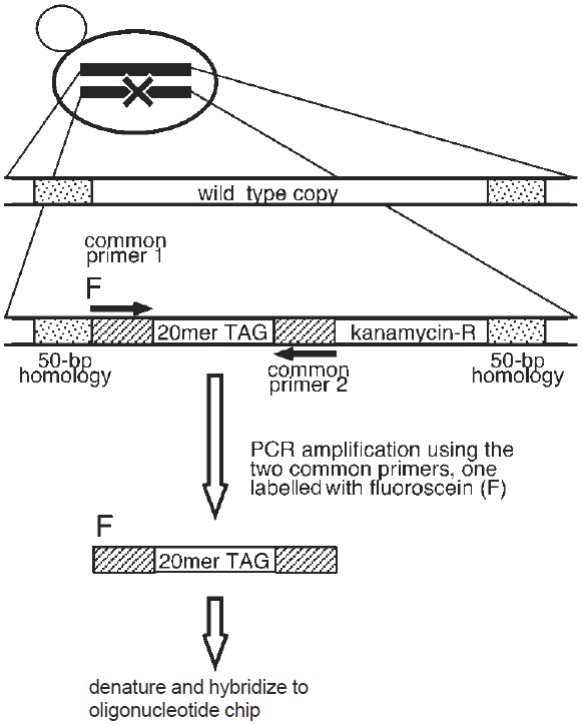
http://www-sequence.stanford.edu/group/yeast_deletion_project/first_round.html

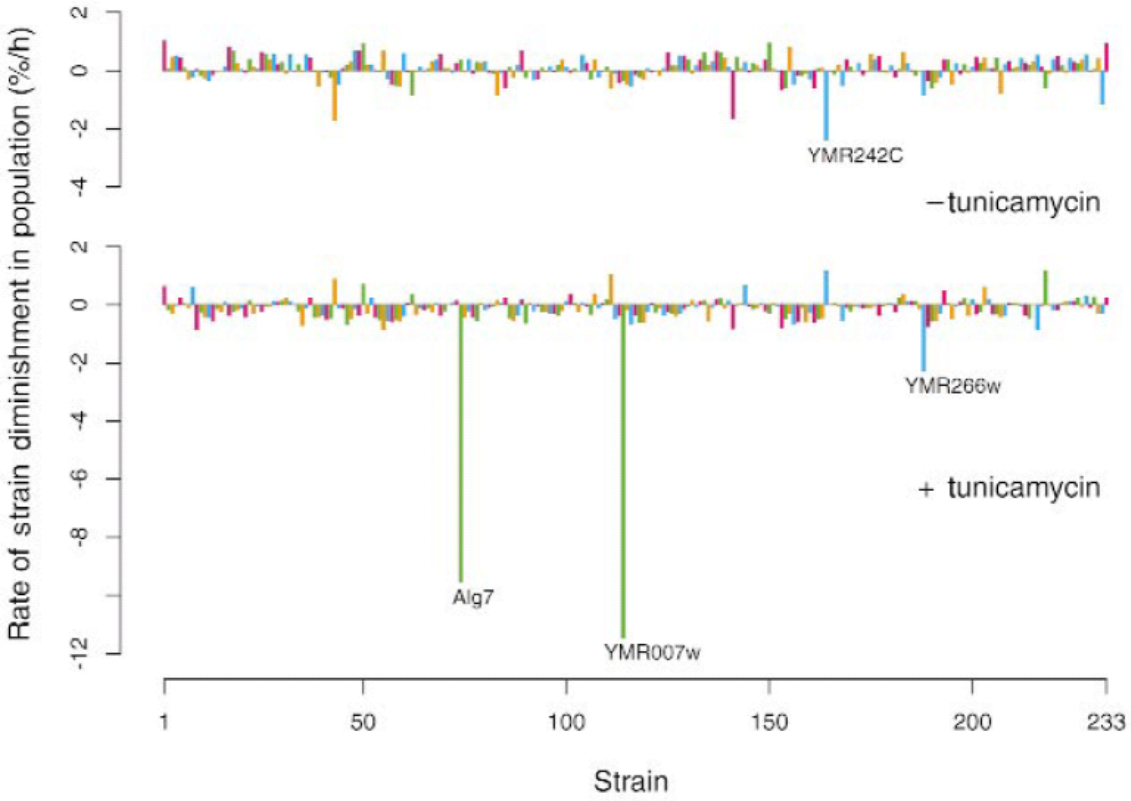
A levedura como modelo de estudo da ação de pequenas moléculas



Current Opinion in Cell Biology

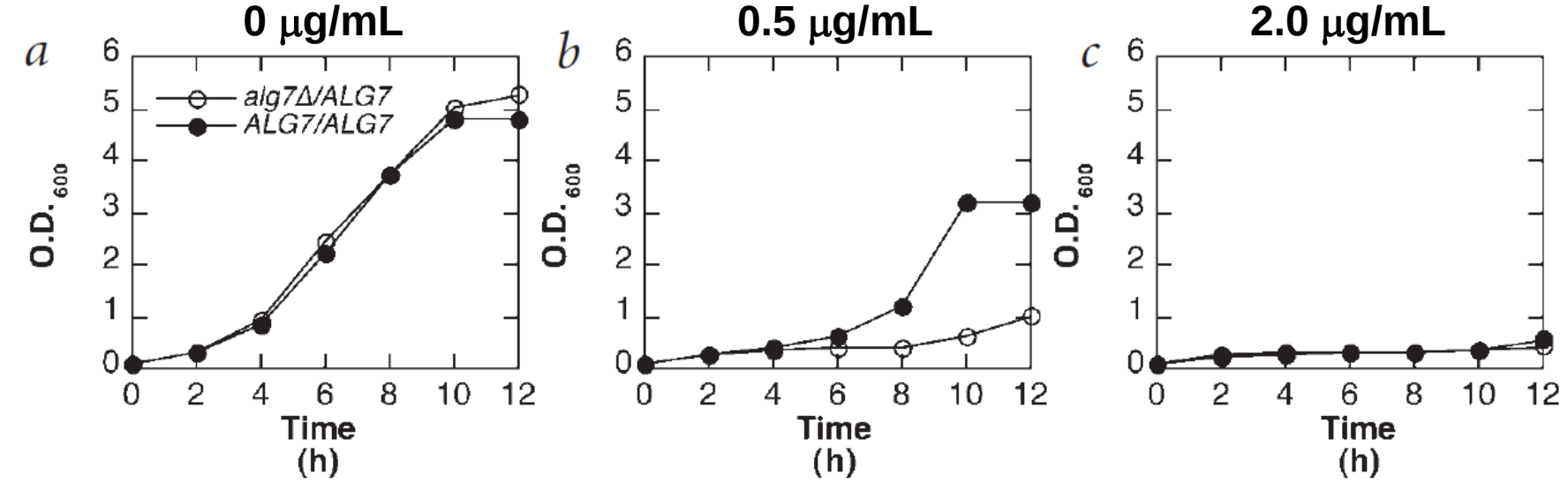
Haploinsuficiência fármaco-induzia



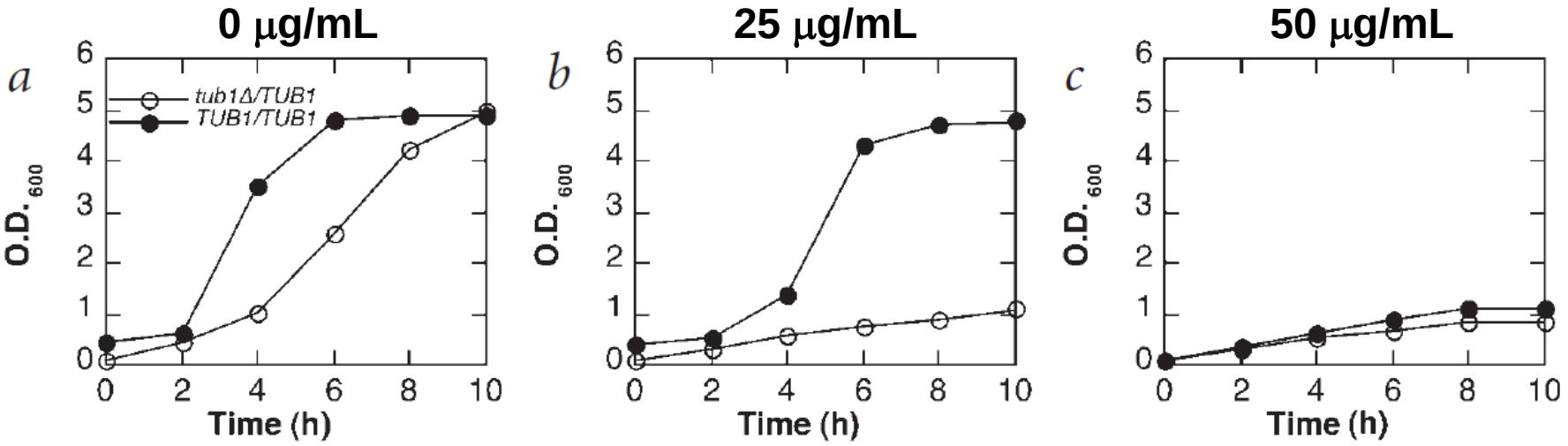


Giaever et al. (1999) *Nat Genet* 21: 278-283

Tunicamycin



Benomyl



Haploinsuficiência fármaco-induzia

Table 1 • Genes encoding known drug targets used in individual strain analysis

Gene	Protein; protein function	Inhibited by	Haplo-insufficiency*
HIS3	imidazoleglycerol-phosphate dehydratase; required for histidine biosynthesis	3-amino-triazole[21]	100 mM
ALG7	Asn-linked glycosyl transferase; required for Asn-linked glycosylation	tunicamycin[9]	0.6 μM
RNR2	small subunit of ribonucleotide reductase; required for nucleotide biosynthesis	hydroxyurea[22]	50 mM
TUB1	α-tubulin structural protein; essential component of mitotic apparatus	benomyl[7]	85 μM
TUB2	β-tubulin structural protein; essential component of mitotic apparatus	benomyl[7]	85 μM
ERG11	cytochrome P450 lanosterol 14a-demethylase; required for biosynthesis of ergosterol	fluconazole[23]	40 μM

*Haploinsufficiency: concentration of drug where $O.D._{600}$ wild type/$O.D._{600}$ heterozygote was greatest. Gene information obtained from the Saccharomyces Genome Database (http://genome-www.stanford.edu/Saccharomyces).

Giaever et al. (1999) *Nat Genet* 21: 278-283

Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action

Kristin Baetz*, Lianne McHardy[†], Ken Gable[‡], Tamsin Tarling[†], Delphine Rebérioux[†], Jenny Bryan[§¶], Raymond J. Andersen[‖], Teresa Dunn[‡], Phil Hieter*[†¶], and Michel Roberge[†]**

*Centre for Molecular Medicine and Therapeutics, Vancouver, BC, Canada V5Z 4H4; [¶]Biotechnology Laboratory and Departments of [†]Biochemistry and Molecular Biology, [§]Statistics, and [‖]Earth-Ocean Sciences and Chemistry, University of British Columbia, Vancouver, BC, Canada V6T 1Z3; and [‡]Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Edited by Francis S. Collins, National Institutes of Health, Bethesda, MD, and approved January 21, 2004 (received for review November 3, 2003)

Methods to systematically test drugs against all possible proteins in a cell are needed to identify the targets underlying their therapeutic action and unwanted effects. Here, we show that a genome-wide drug-induced haploinsufficiency screen by using yeast can reveal drug mode of action in yeast and can be used to predict drug mode of action in human cells. We demonstrate that dihydromotuporamine C, a compound in preclinical development that inhibits angiogenesis and metastasis by an unknown mechanism, targets sphingolipid metabolism. The systematic, unbiased and genome-wide nature of this technique makes it attractive as a general approach to identify cellular pathways affected by drugs.

alter the organization of filamentous actin at the cell margin (2). Identifying the mechanism of action of dhMotC is critical for evaluating its potential for cancer therapy but it is a difficult task, because dhMotC has no other known biological activities, it does not resemble other bioactive molecules, and its chemical structure revealed no clues to guide target identification.

By using a genome-wide haploinsufficiency screen, we show that dhMotC targets sphingolipid metabolism, resulting in decreased ceramide levels in yeast. Furthermore, exogenous ceramide partially rescues the effect of dhMotC on human cell survival, demonstrating that systematic genome-wide analysis of drug effects in yeast can be used to elucidate the mechanism of

Sensitivity of yeast to dhMotC.

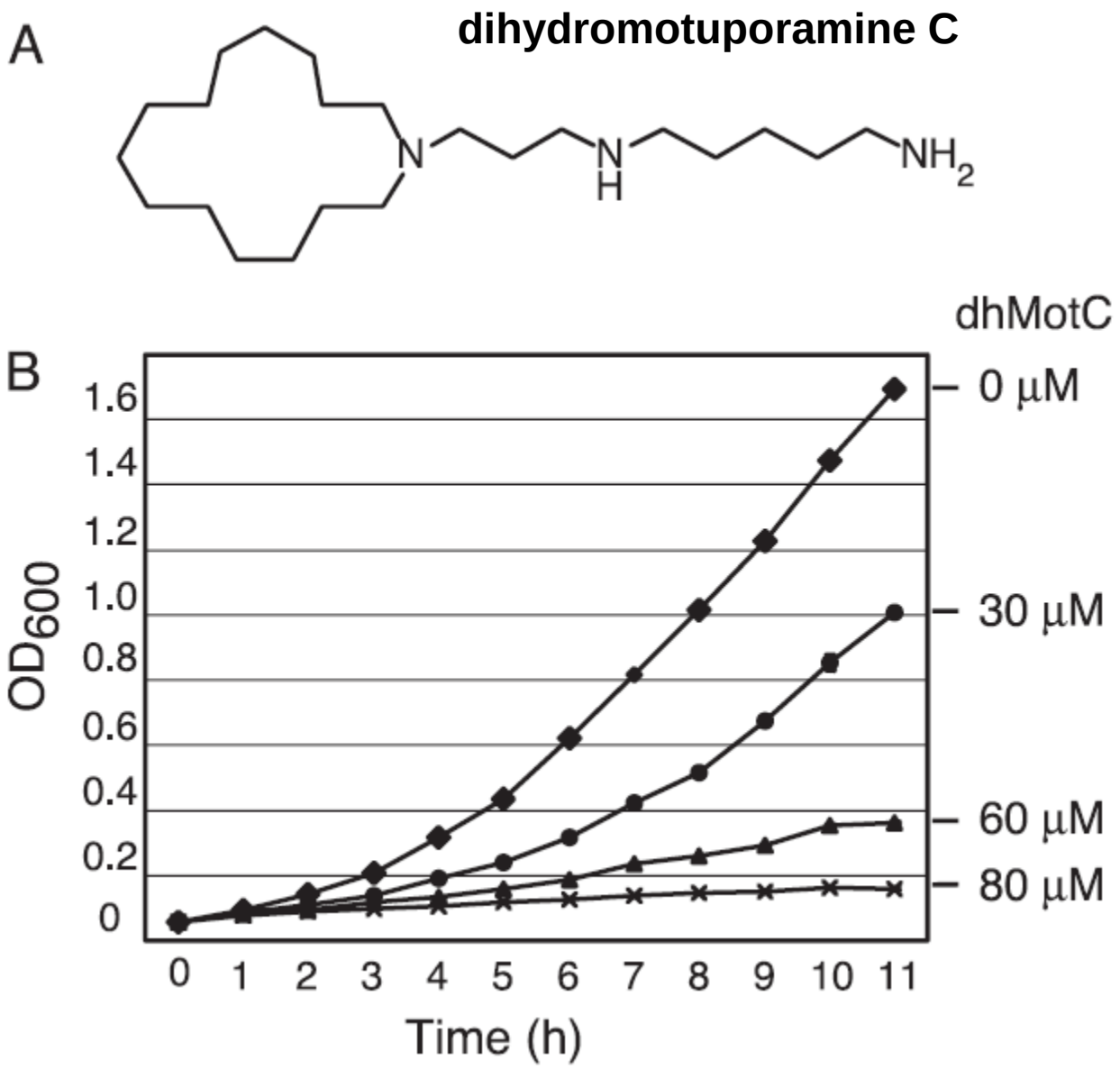


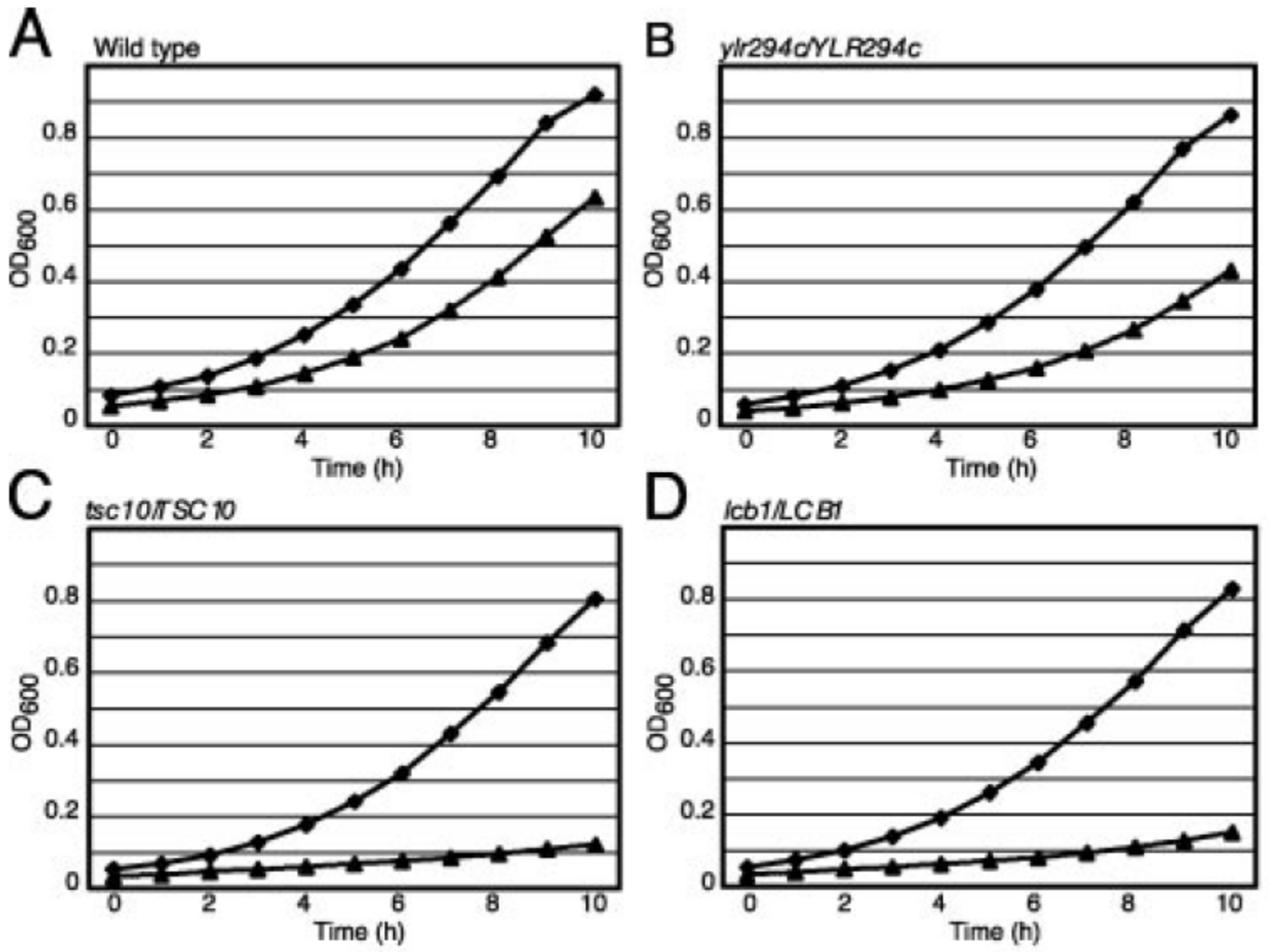
Table 1. Heterozygous deletion strains sensitive to dhMotC

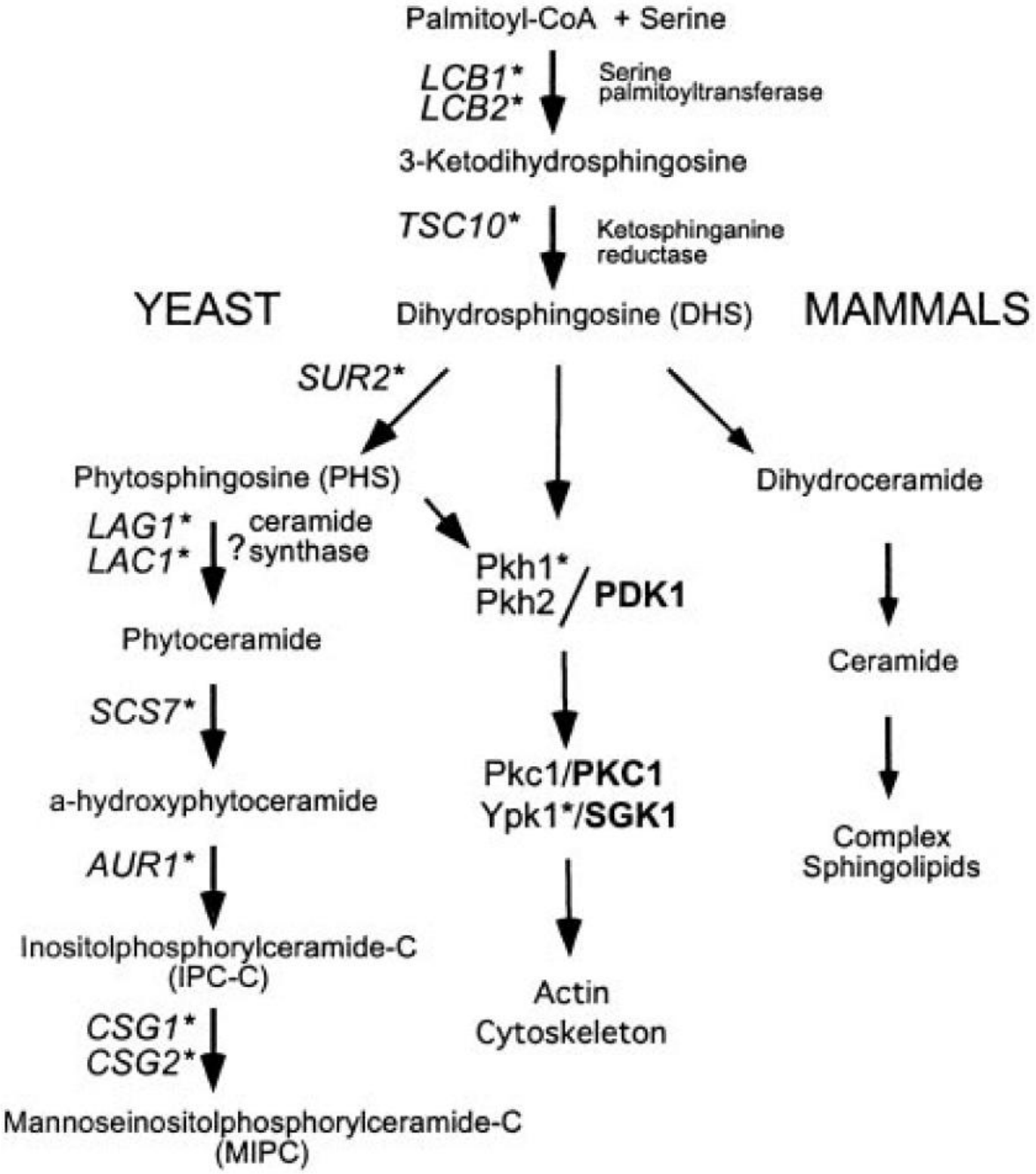
ORF	Name	Biological process†
YCL034W	*LSB5*	Actin filament organization
YNL314W	*DAL82*	Allantoin catabolism and transcription initiation from polymerase II promoter
YML099C	*ARG81*	Arginine metabolism
YBR078W	*ECM33*	Cell wall organization and biogenesis
*YNL267W**	*PIK1**	Cytokinesis, post-Golgi transport, and signal transduction
YLR286C	*CTS1*	Cytokinesis, completion of separation
YDL192W	*ARF1*	ER-to-Golgi transport and intra-Golgi transport
YBR290W	*BSD2*	Heavy metal ion transport and protein vacuolar targeting
YLR025W	*SNF7*	Late endosome to vacuole transport
YHR147C	*MRPL6*	Protein biosynthesis
*YOL040C**	*RPS15**	Protein biosynthesis
YAL005C	*SSA1*	Protein folding and protein-nucleus import, translocation
YIL047C	*SYG1*	Signal transduction
*YBR265W**	*TSC10**	Sphingolipid biosynthesis
*YMR296C**	*LCB1**	Sphingolipid biosynthesis
*YJR007W**	*SUI2**	Translation initiation
*YML092C**	*PRE8**	Ubiquitin-dependent protein catabolism
YER140W	*YER140W*	Unknown
YER188W	*YER188W*	Unknown
YGR205W	*YGR205W*	Unknown
YLR294C	*YLR294C*	Unknown

*Essential genes.

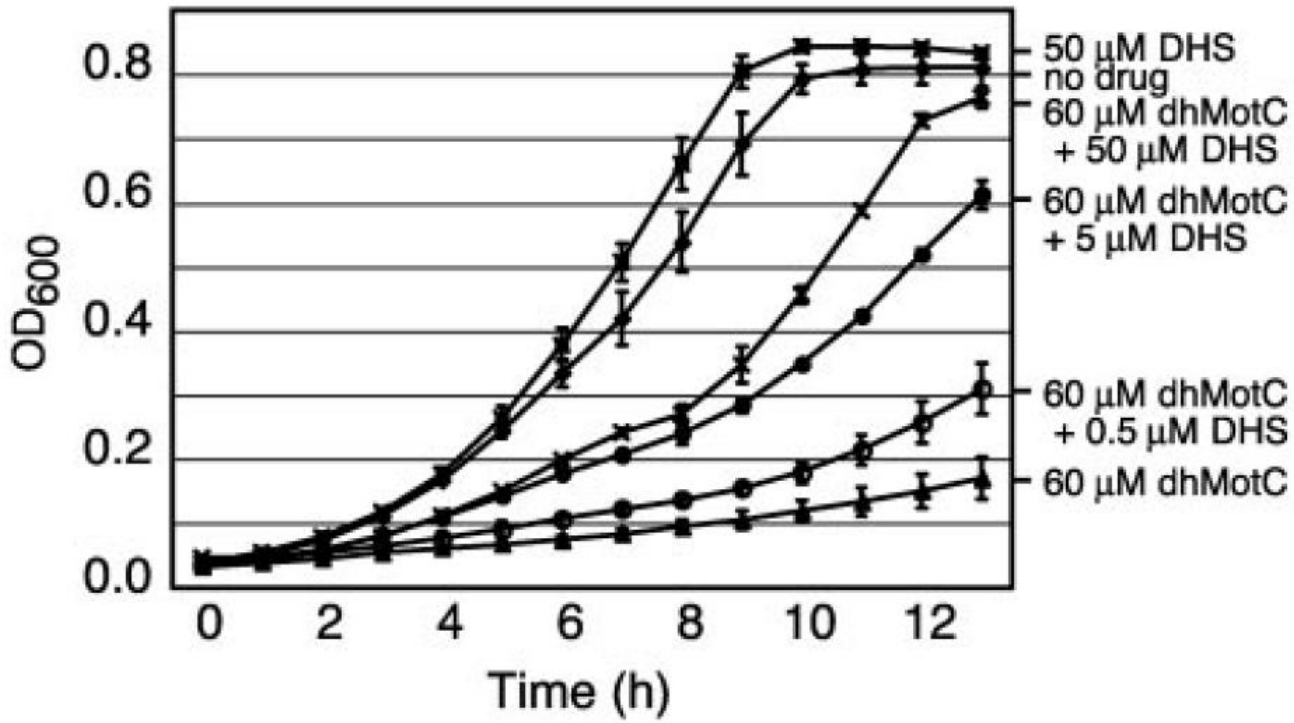
†Biological process according to their *Saccharomyces* Genome Database report.

lcb1/LCB1 and tsc10/TSC10 strains are supersensitive to dhMotC

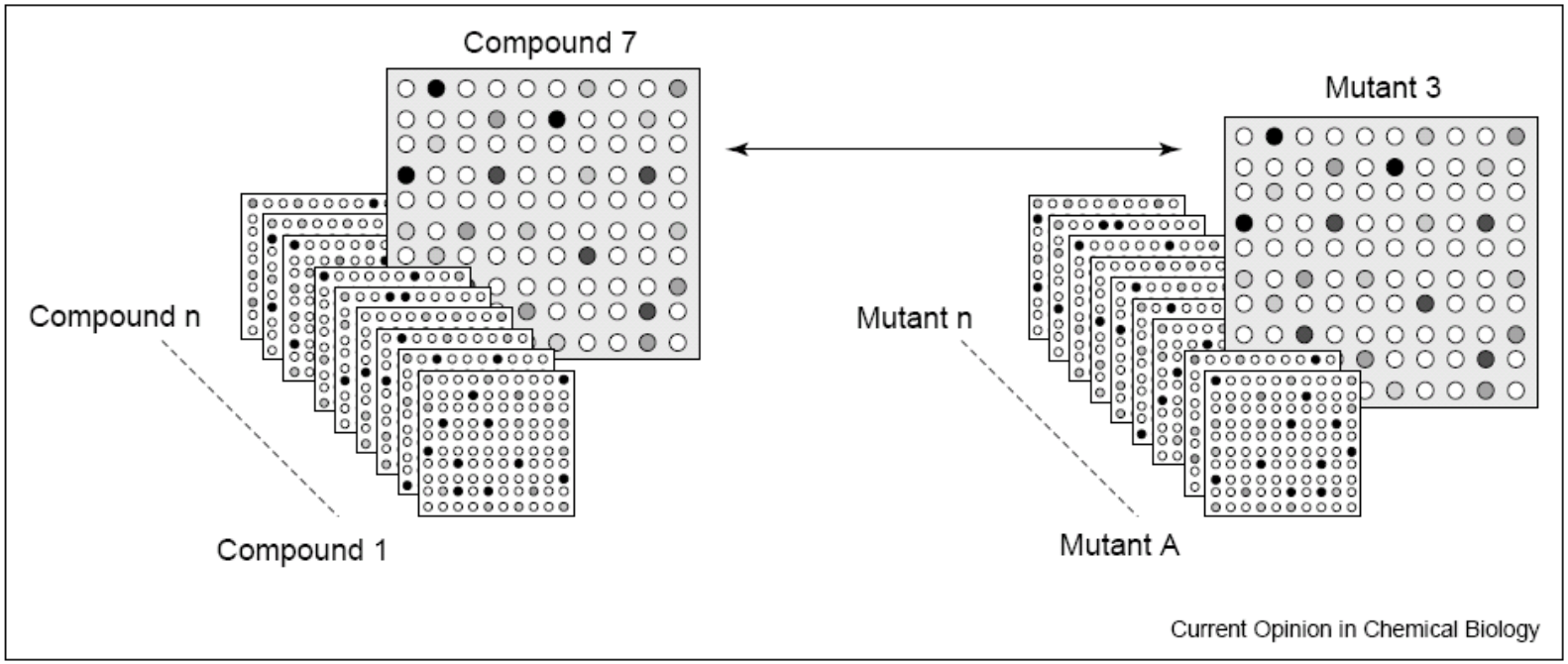



Palmitoyl-CoA + Serine
LCB1*
LCB2*
Serine palmitoyltransferase
3-Ketodihydrosphingosine
TSC10*
Ketosphinganine reductase
YEAST
Dihydrosphingosine (DHS)
MAMMALS
SUR2*
Phytosphingosine (PHS)
Dihydroceramide
LAG1*
LAC1*
?
ceramide synthase
Pkh1*
Pkh2
PDK1
Phytoceramide
Ceramide
SCS7*
a-hydroxyphytoceramide
Pkc1/PKC1
Ypk1*/SGK1
Complex Sphingolipids
AUR1*
Inositolphosphorylceramide-C (IPC-C)
Actin Cytoskeleton
CSG1*
CSG2*
Mannoseinositolphosphorylceramide-C (MIPC)

DHS suppresses growth inhibition by dhMotC

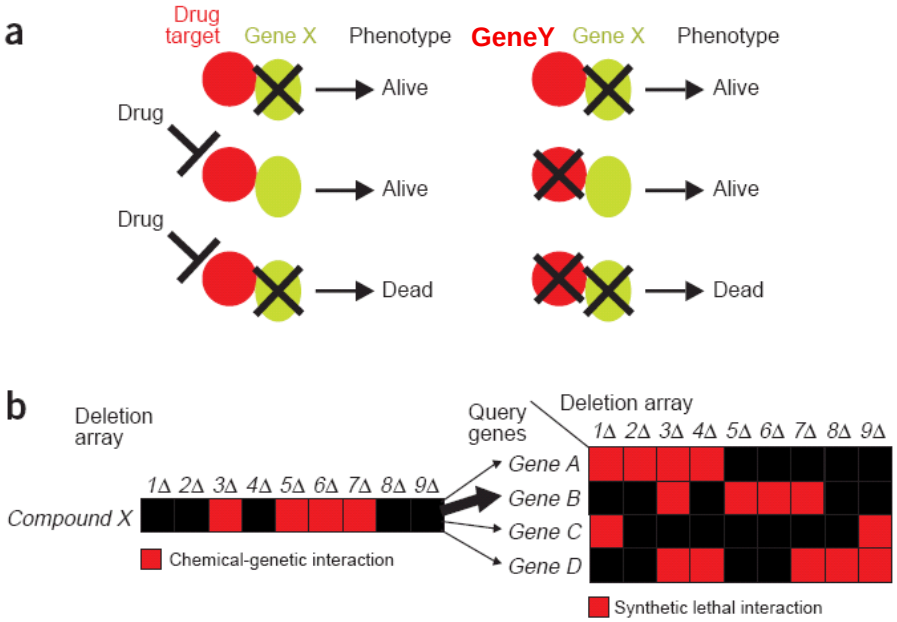


Pareamento de padrões



Armour and Lum (2005) *Curr Opin Chem Biol* 9: 20-24

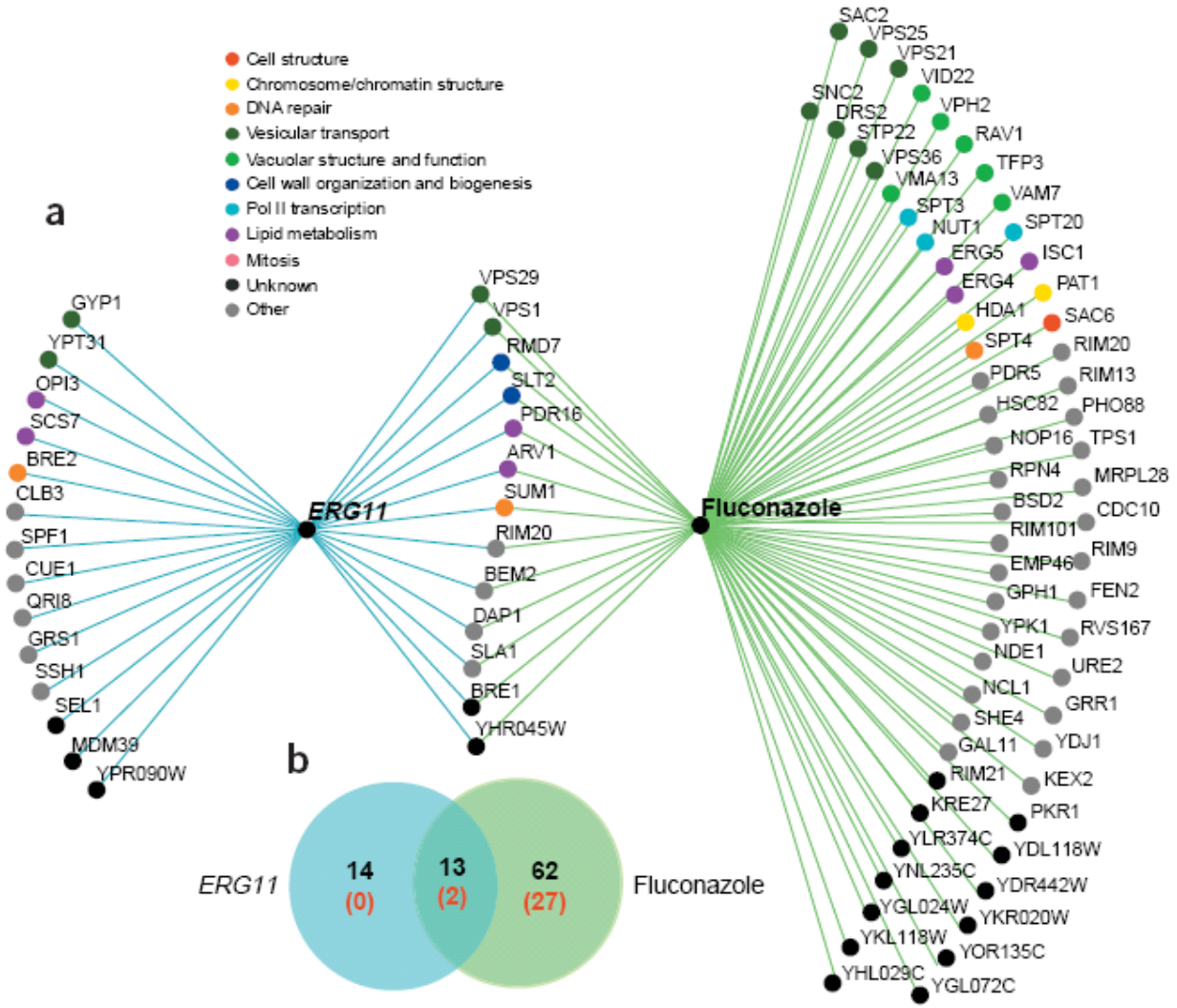
Chemical-genetic interactions can be modelled by synthetic genetic interactions



Parsons et al. (2004) *Nat Biotechnol* 22: 62-69

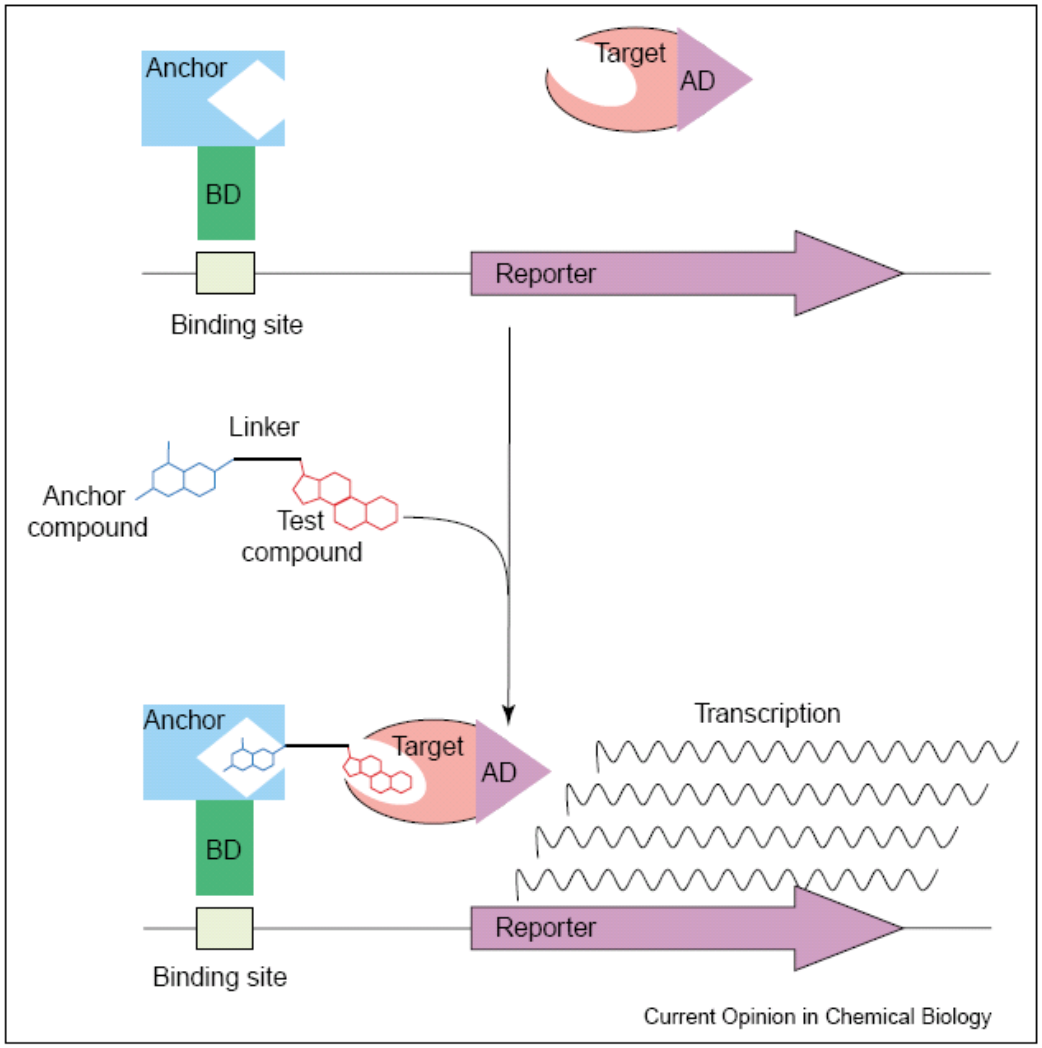
http://thecellmap.org/

Overlap between the chemical-genetic profile of fluconazole and the genetic interaction profile of ERG11



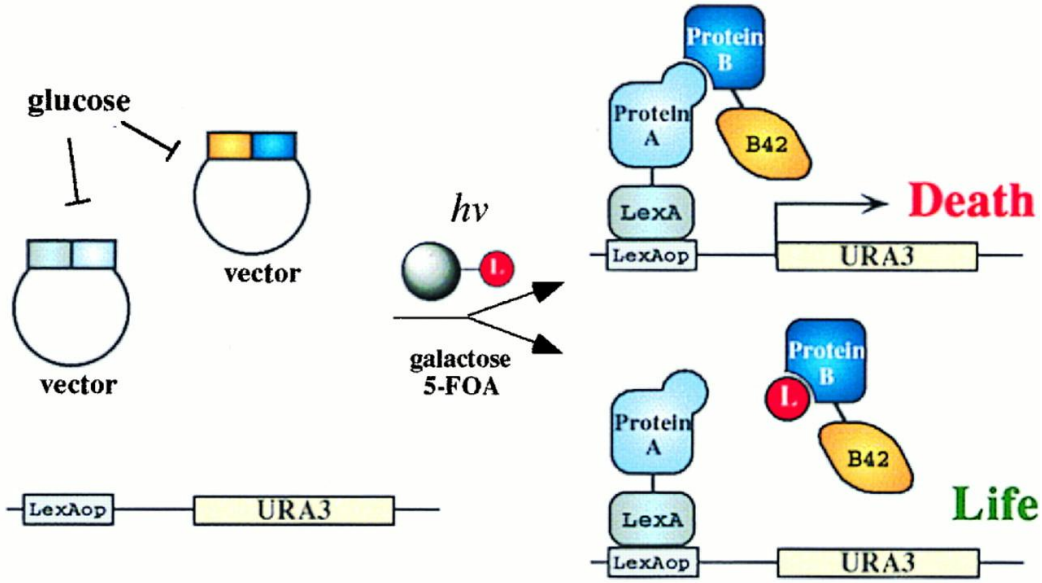
Parsons et al. (2004) *Nat Biotechnol* 22: 6-69

Triplo-híbrido com pequenas moléculas



Armour and Lum (2005) *Curr Opin Chem Biol* 9: 20-24

Duplo-híbrido reverso com pequenas moléculas

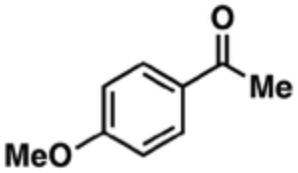


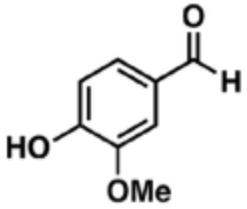
Huang and Schreiber (1997) *PNAS* 94: 13396-13401

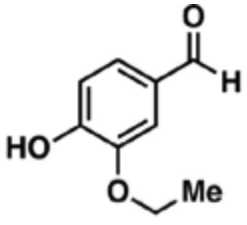
É possível construir uma levedura com olfato?

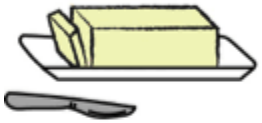
Os odores são pequenas moléculas com estrutura química conhecida

Vanilla

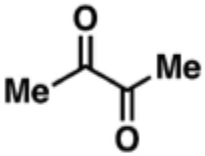


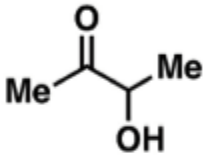




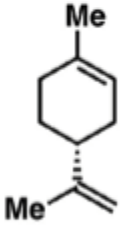


Butter

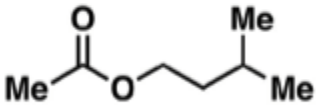




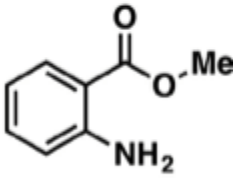
Orange



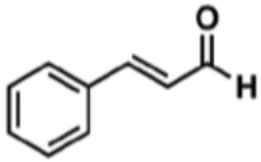
Banana



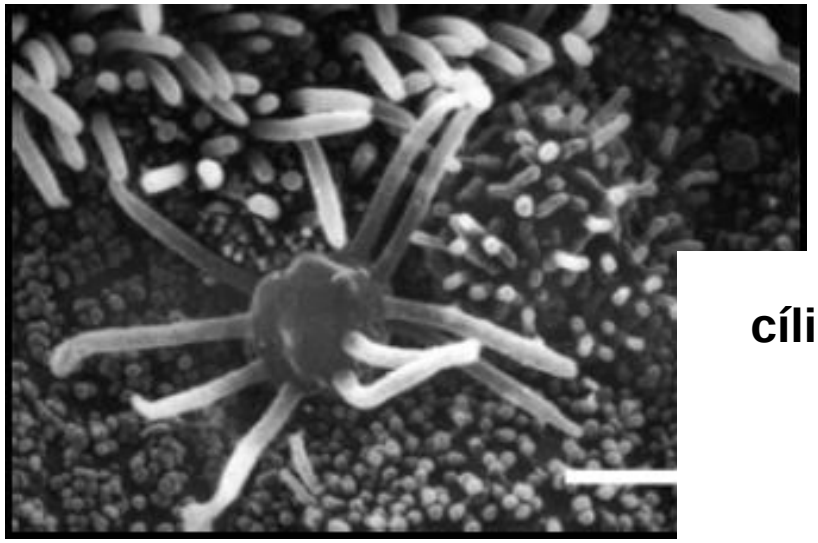
Grape

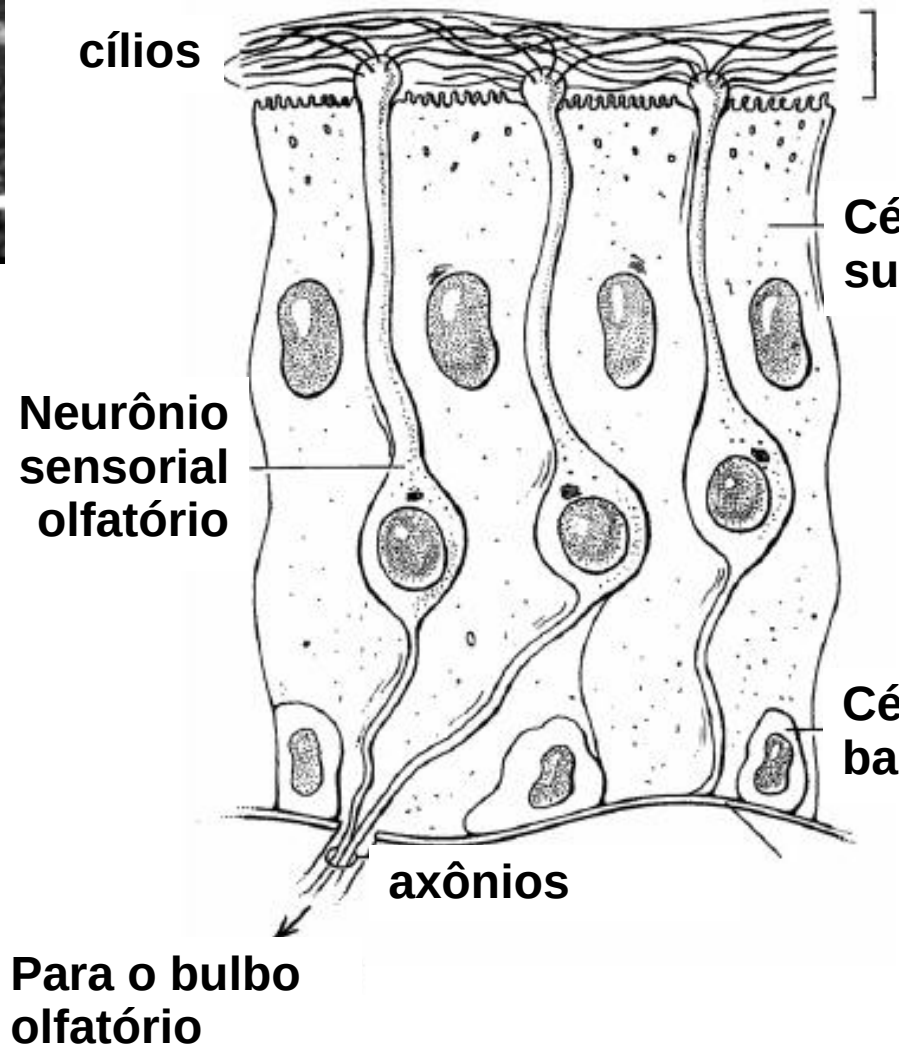


Cinnamon



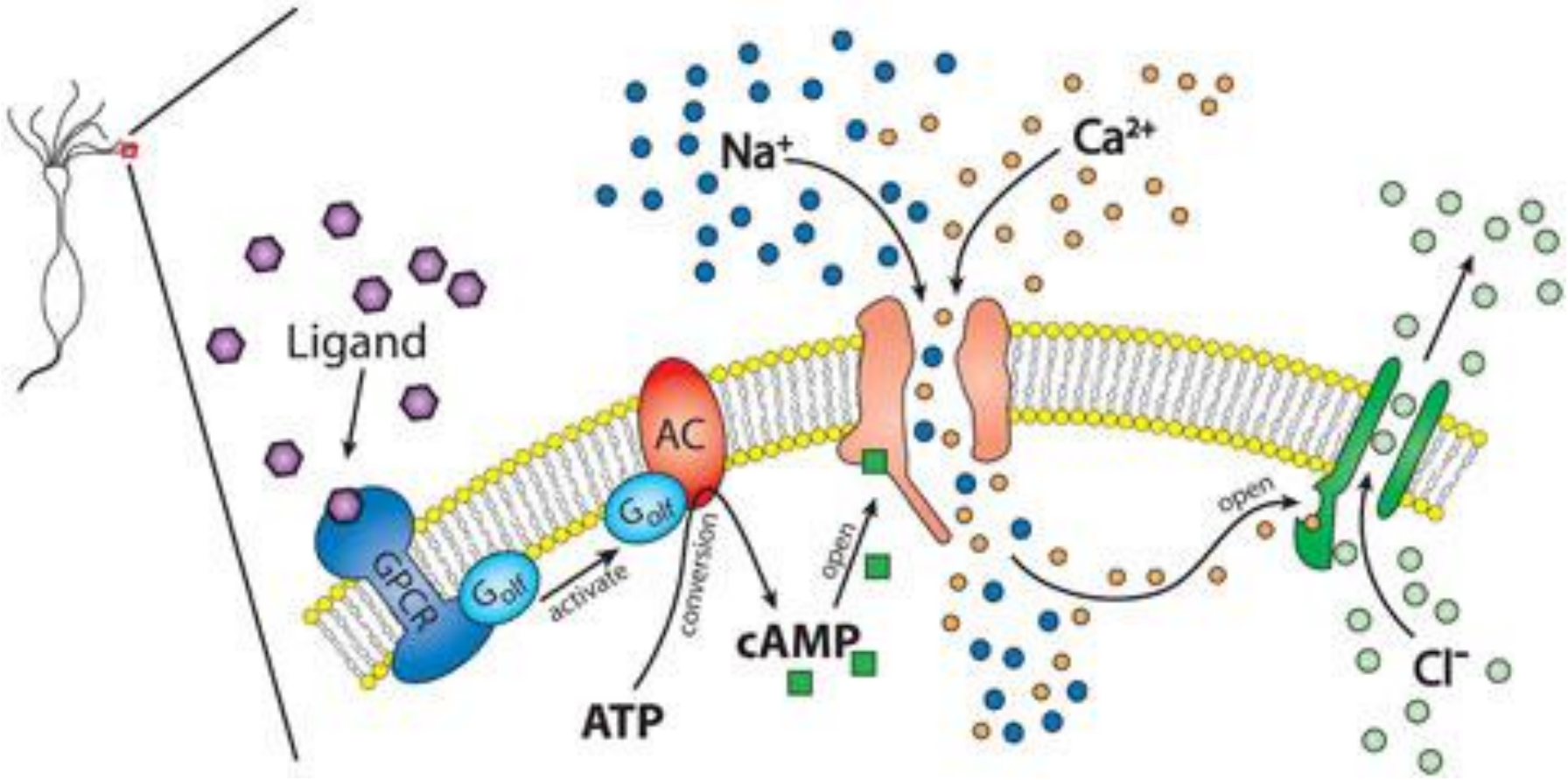
Quais são as células que sentem os odores no nariz?



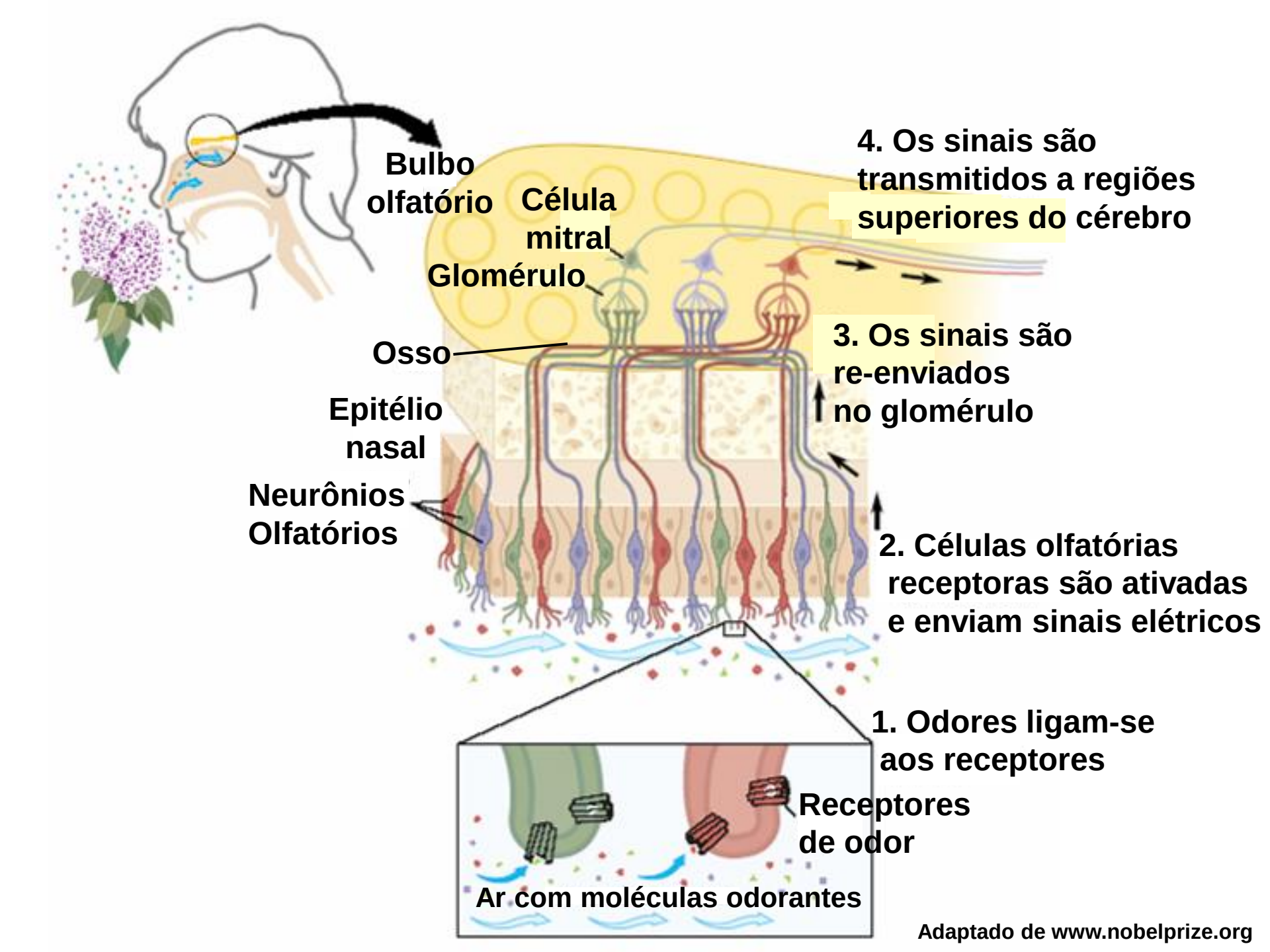

cílios
muco
Células de suporte
Neurônio sensorial olfatório
Célula-tronco basal
axônios
Para o bulbo olfatório

Como os cílios neurônios sensoriais olfatórios “sentem” os odores?

O mecanismo do receptor olfatório

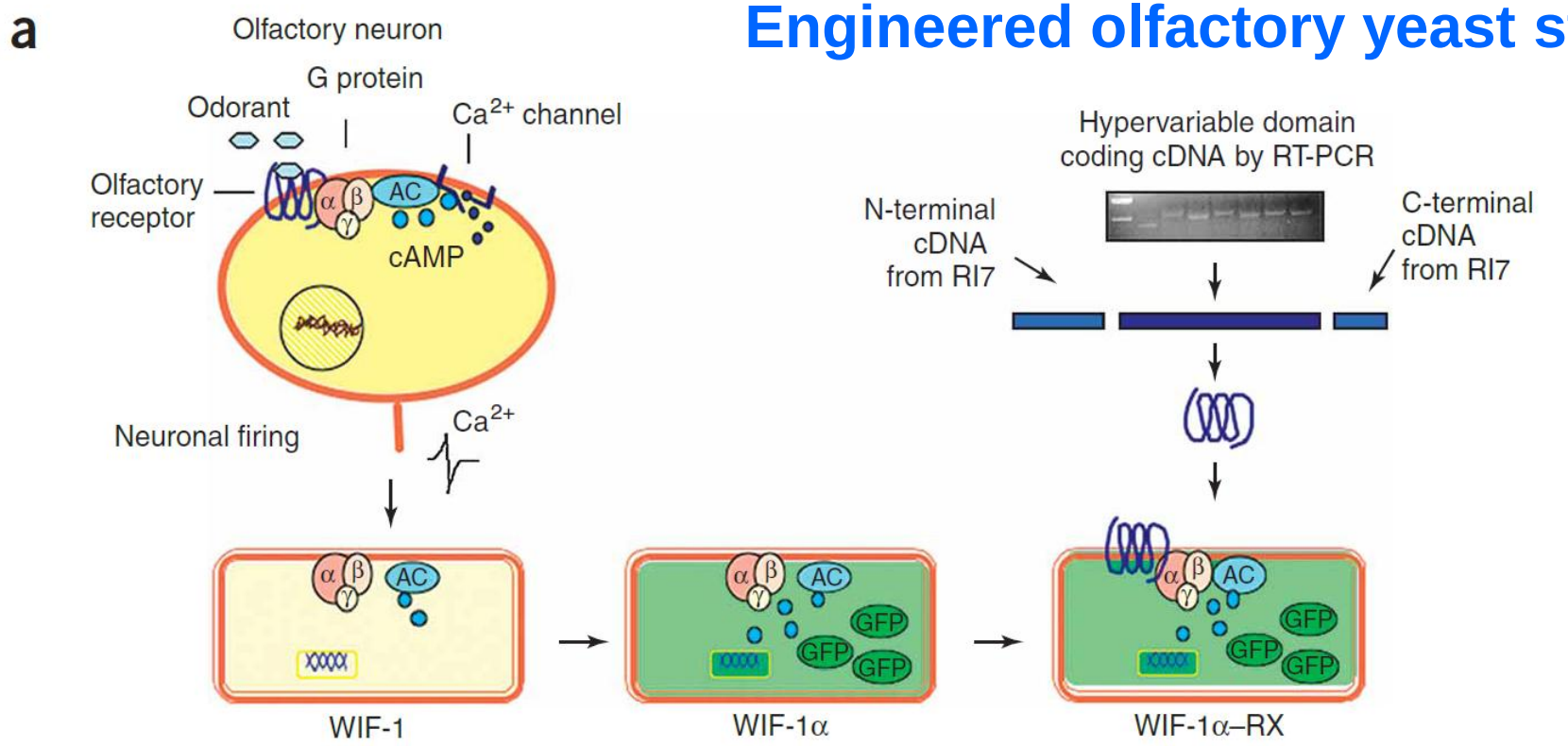


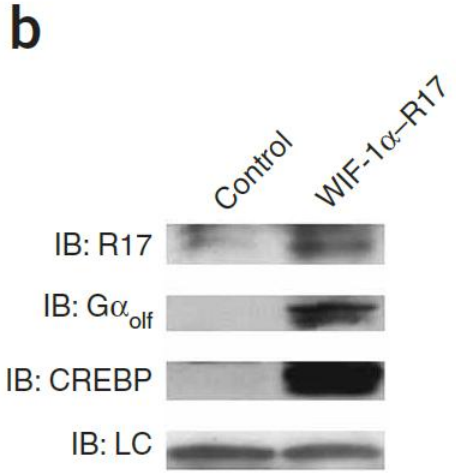
Como a resposta aos odores é enviada ao cérebro?

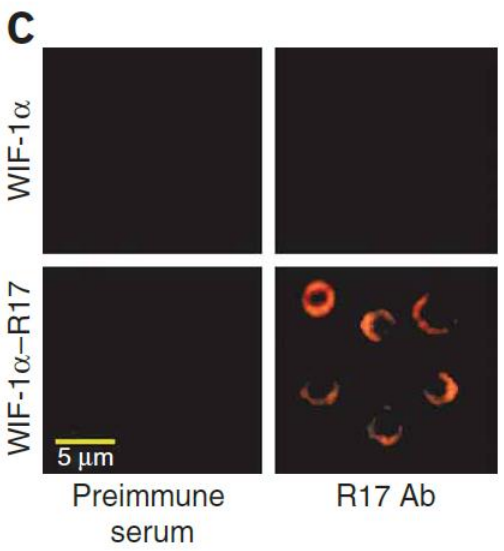


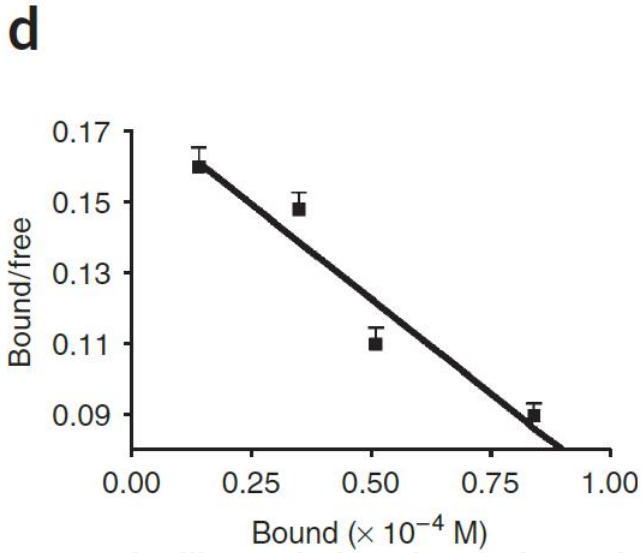
Adaptado de www.nobelprize.org

Engineered olfactory yeast strain



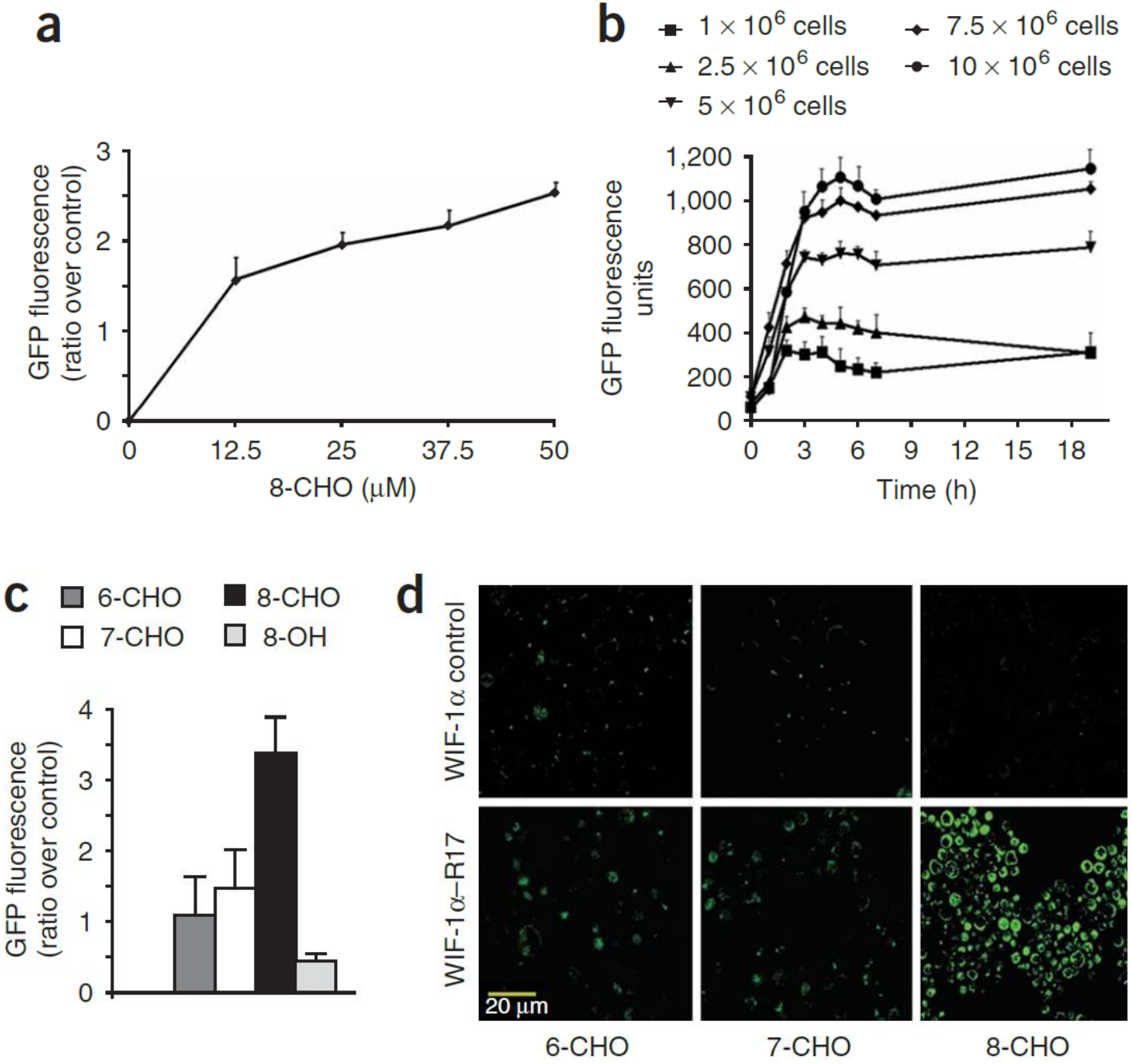






Rhadika et al., (2007) *Nat Chem Biol*,3: 325-330

Indução, sensibilidade e especificidade do sensor

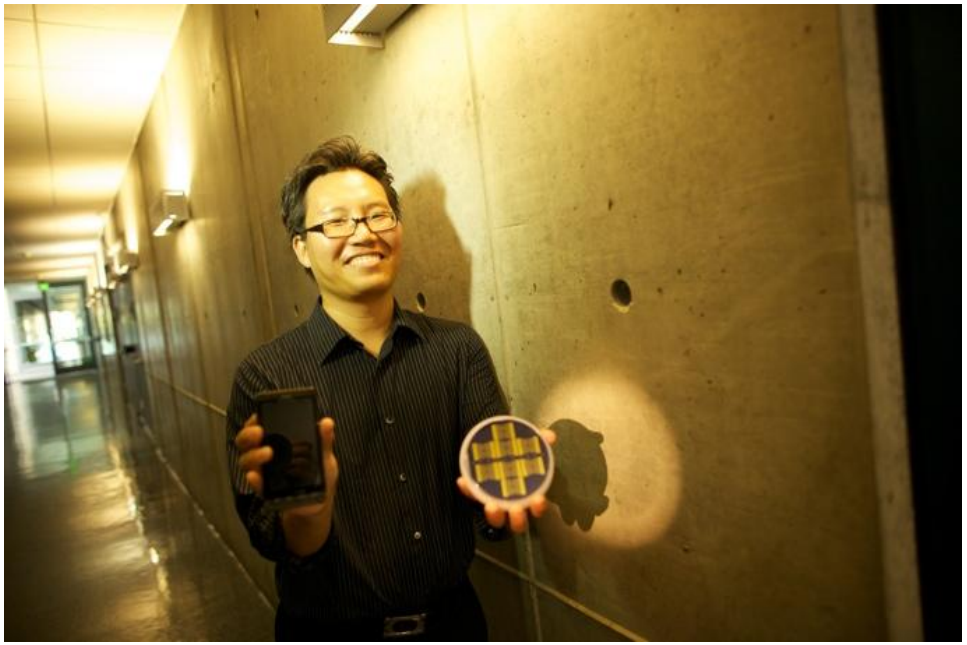


TEMPLE UNIVERSITY

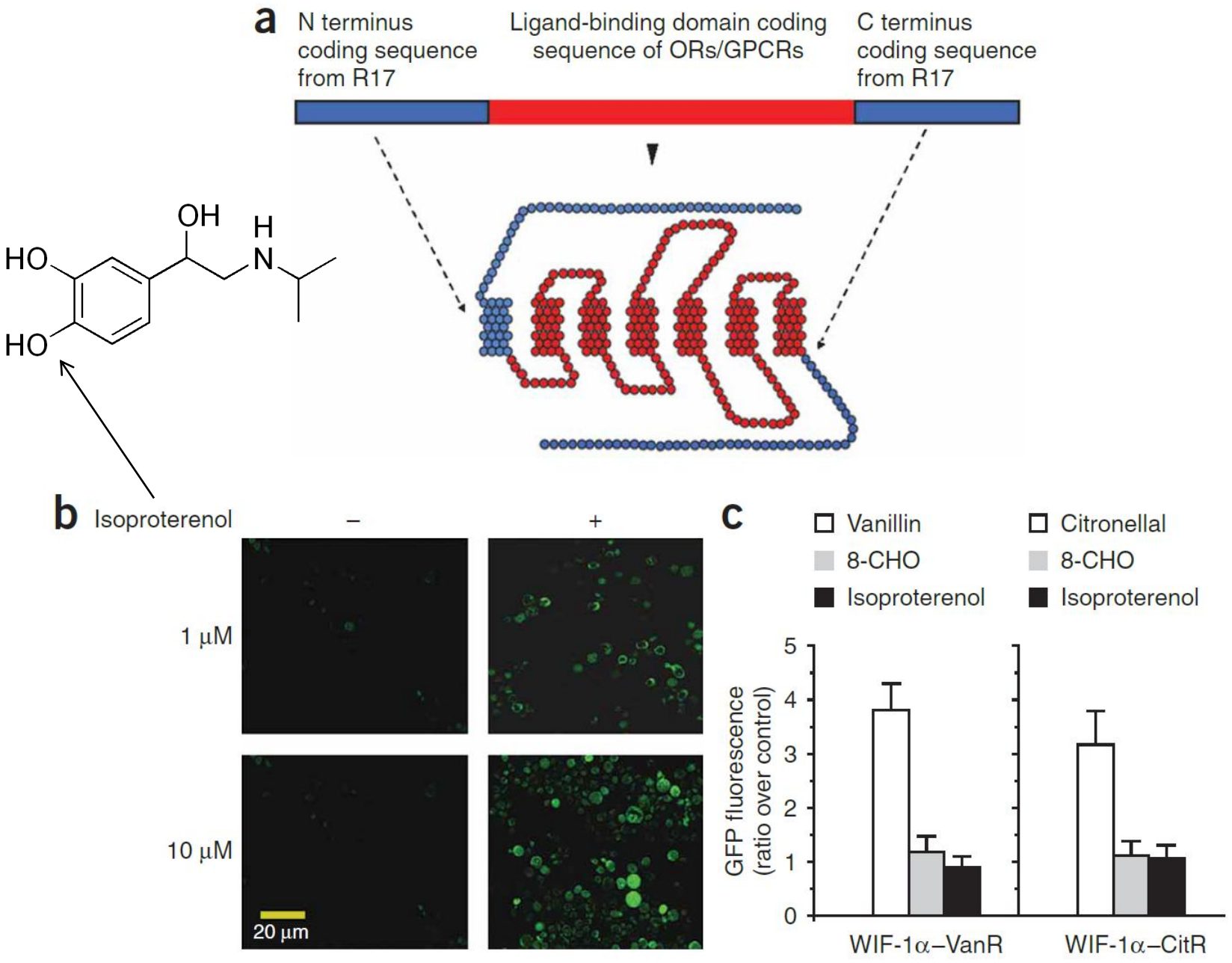
Yeast nose

UC RIVERSIDE

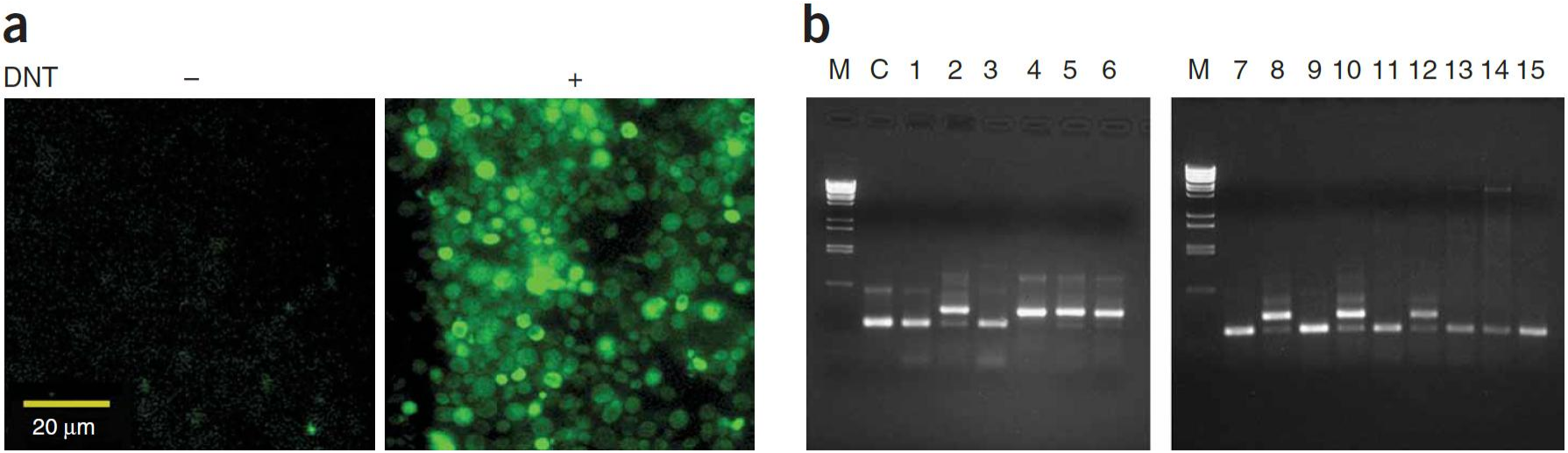
Electronic nose



Expandindo a diversidade sensorial do sistema



Clonagem do sensor de 2,4-dinitrotolueno (DNT)



c

TMII

MLIIIAIRNHPTLHKPMYSGNYFMVNLALAEVCYTSNIVPKML

TMIII

LIIIADQKTISVVGCLAQFYFFGSLAATECLLLAVMSYDRYLA

TMIV

ICQPLRYPILMTGSLCFRLAIGSWFCCFFLTAITMVLLCRQNF

TMV

CGPNEIDHFFCDFAPLIHLSCHLSCMDT

Rhadika et al., (2007) *Nat Chem Biol*,3: 325-330

Resumo

1. Biologia Química utiliza pequenas moléculas para sondar os sistemas biológicos;

2. A síntese de pequenas moléculas é orientada para a diversidade, a fim de se obter a biblioteca para varredura;

3. Haploinsuficiência fármaco-induzida, Y2H reverso e Y3H são estratégias possíveis de Biologia Química em levedura;

4. Aplicações da Biologia Química em levedura incluem desenvolvimento de sensores de pequenas moléculas;