

Synthetic Biology in Drug Discovery and Combating Drug Resistance

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The impact of Synthetic Biology on the discovery and development of new drugs

Synthetic Biology enables...

- understanding the molecular basis of disease
- the Design of Synthetic Biology-derived Biopharmaceuticals for
 - Treatment: Designer phages as adjuvant for antibiotics
 - Prevention: Synthesis of safe vaccines and Elimination of the pathogen population
 - Compliance: Synthesis of smart drug depots
- Small Molecule Drug Discovery
 - for combating drug resistance in tuberculosis

REVIEWS

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The impact of synthetic biology on drug discovery

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Understanding Diseases using Synthetic Biology

Reconstructing immunological signaling pathways in an orthogonal host

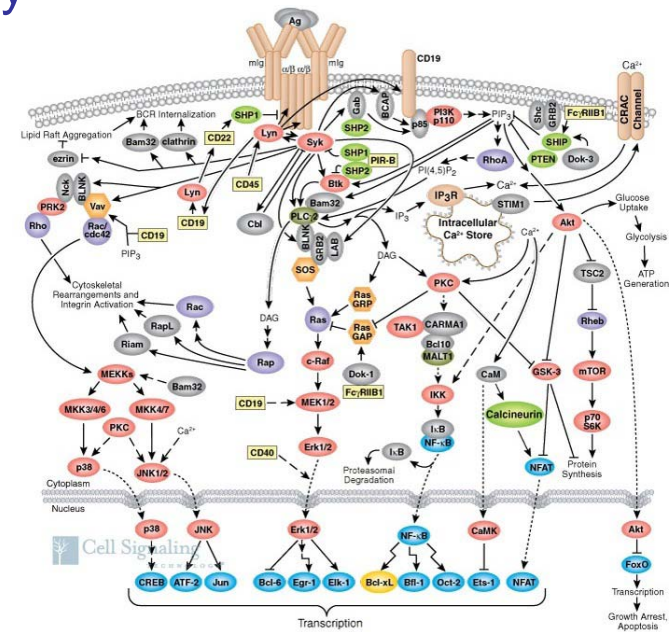
- *Drosophila* S2 cells for reconstructing B-cell signaling (EMBO J (2008) 27, 1333)
- Identifying the molecular basis of a rare form of agammaglobulinemia (J Exp Med (2007) 204, 2047)

Tracing back the SARS epidemic pathway

- Reconstruction of coronaviruses with synthetic membrane glykoproteins to understand the change in tropism from bats to humans (PNAS (2008) 105, 19944)

Understanding the virulence of influenza virus

- Reconstruction of close relatives to Spanish influenza virus to elucidate virulence factors (Science (2005) 310, 77)



Treatment: Designer phages as adjuvant for antibiotic therapy

Bacteria have protection mechanisms against antibiotics

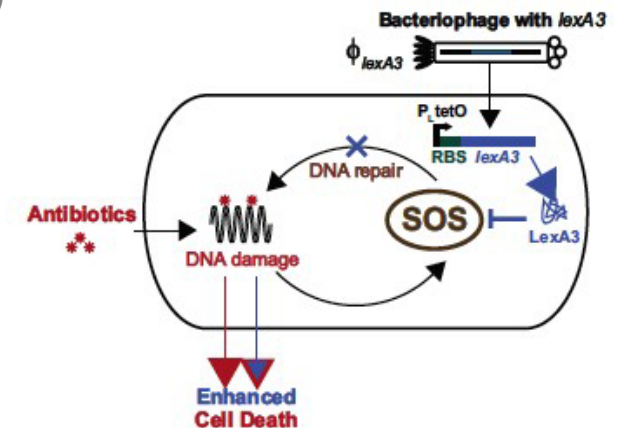
- Oxidative stress response (SOS-response), biofilm formation, low transporter availability (Cell (2007) 130, 797)
- High antibiotic concentrations required to kill bacteria

Designer phages render bacteria more susceptible to antibiotics

- Expression of regulators to attenuate SOS response (*lexA3*)
- Expression of antibiotic importers (*ompF*)
- Attenuation of biofilm formation (*csrA*)

Co-application of designer phages and antibiotics...

- ...Increase survival of infected animals (80 % vs. 20 %)
- ...Reduce the emergence of drug resistance



Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy

Timothy K. Lu^{a,b} and James J. Collins^{b,1}

PNAS (2009) 106, 4629

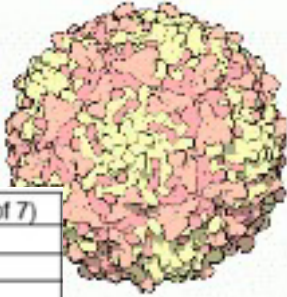
Prevention: Development of safe vaccines

Conventional polio vaccine

- Poliovirus attenuated by 5 mutations
- Virulent viruses formed by spontaneous revertants
- Mutation and recombination with Coxsackie A virus caused small epidemics of poliomyelitis

Synthetic Biologic polio vaccines

- *De novo* synthesis of poliovirus genome with unfavorable codon bias (631 mutations)
- Virus strongly attenuated, no cytopathic effect
- No revertants observed in prolonged passaging, revertants extremely unlikely
- Protection of mice against poliovirus-mediated paralytic poliomyelitis
- Very likely applicable to the attenuation of other viruses for vaccine production



Virus	Mice Protected (out of 7)
PV-MinZ	7
PV-MinXY	7
Mock vaccinated	0

Virus Attenuation by Genome-Scale Changes in Codon Pair Bias

J. Robert Coleman,¹ Dimitris Papamichail,^{2*} Steven Skiena,² Bruce Fitcher,¹
Eckard Wimmer,^{1†} Steffen Mueller¹

Science (2008) 320, 1784

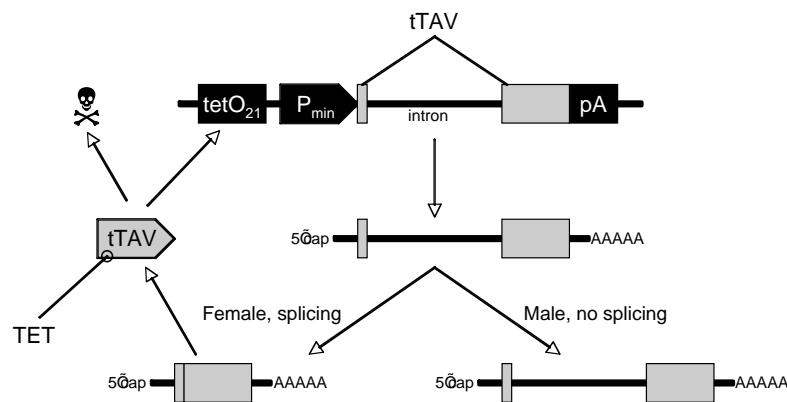
Prevention: Reducing pathogen prevalence

Elimination of insects as agricultural pest or disease transmitters

- Insects transmit widespread diseases like malaria and dengue fever
- Insects are responsible for significant agricultural losses (e.g. Mediterranean fruit fly)

Inducible female-specific lethality

- Release of male insects that carry a female-specific dominant lethality determinant
 - Mating with wildtype females will produce no female progeny
 - For production of male insects, lethality must be suppressed
- ⇒ Design of a synthetic gene switch for inducible, female-specific lethality
- ⇒ Release approved by the US Department of Agriculture (May 2009).



www.oxitec.com

Female-specific insect lethality engineered using alternative splicing

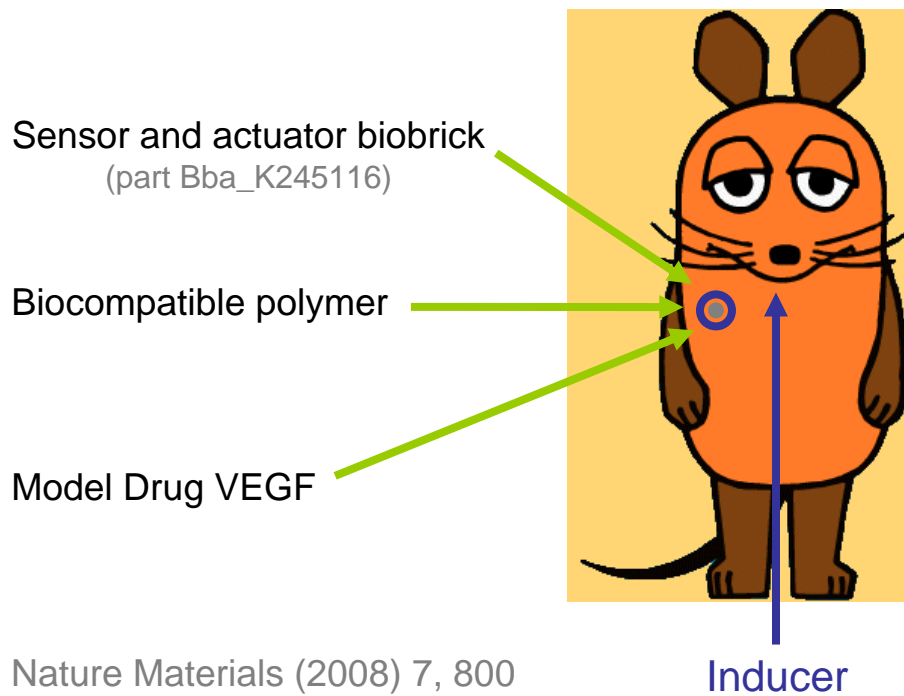
Guoliang Fu^{1,3}, Kirsty C Condon¹⁻³, Matthew J Epton², Peng Gong², Li Jin¹, George C Condon¹, Neil I Morrison^{1,2}, Tarig H Dafa'alla¹ & Luke Alphey^{1,2}

Nat. Biotechnol (2007) 25, 353

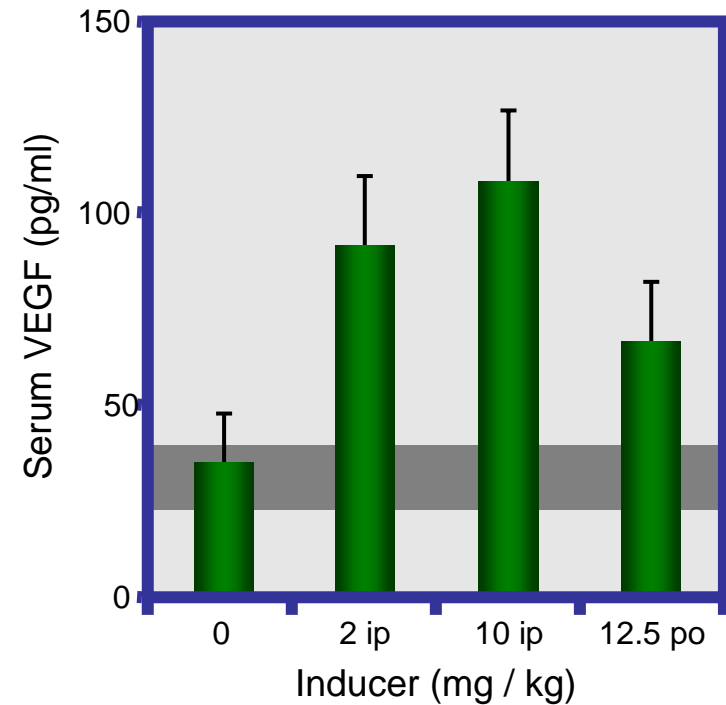
Compliance: Smart drug depots

Design of an implantable drug depot that releases a defined drug dose in response to an orally available inducer molecule

- Release of the model drug VEGF in mice in response to a clinically validated inducer



Nature Materials (2008) 7, 800

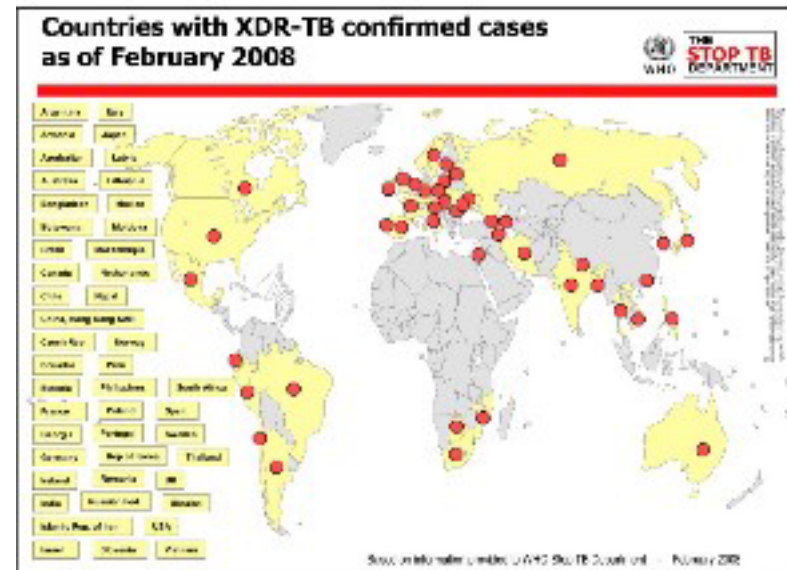
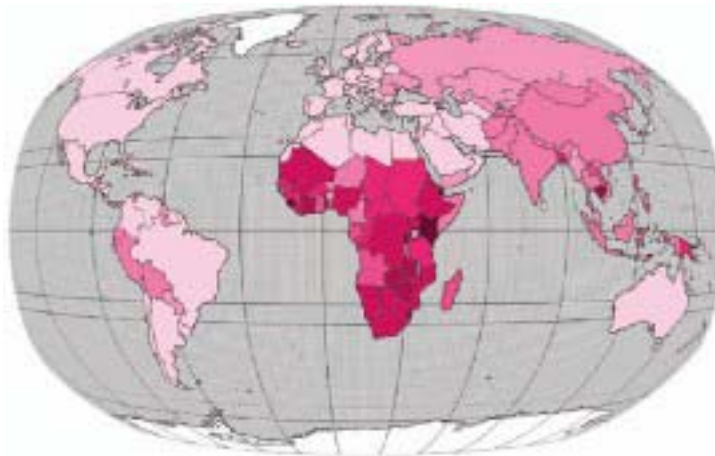


Tuberculosis

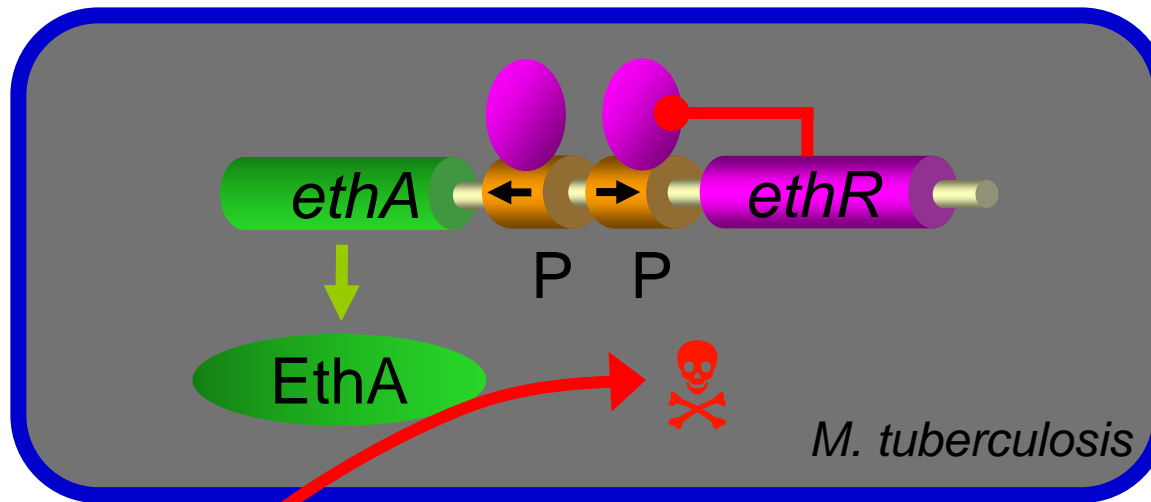
30 % of world population infected
 1'800'000 deaths per year
 Multidrug and extremely drug resistant strains emerging

The New York Times
THURSDAY, SEPTEMBER 14, 2006
 Tuberculosis is outrunning us. The accelerated pace of resistance comes from the world's neglect of tuberculosis. Stinginess created this problem. Generosity is needed to fix it.
 - editorial - 14-Sep-2006

Cases per 10⁵ people
 >900
 0



Mycobacterium tuberculosis: intrinsic drug resistance against ethionamide



Ethionamide

WANTED

New Molecule:

- Inhibits EthR
- Cell-permeable
- Non-cytotoxic to human cells

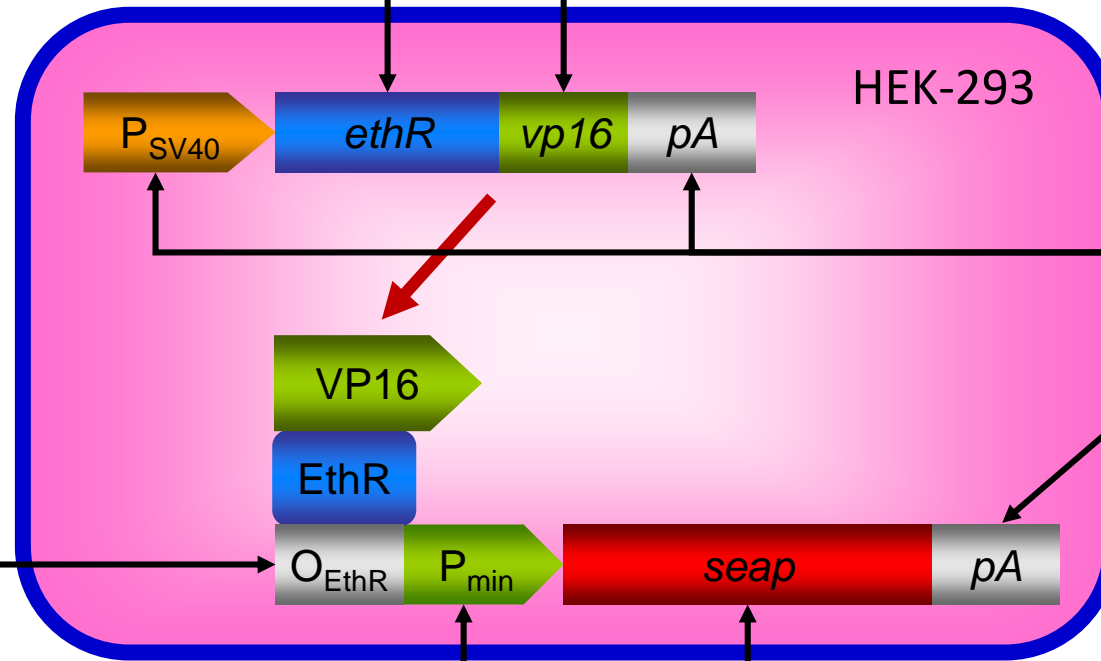
Baulard et al. (2000) *J. Biol. Chem.* 275: 28326



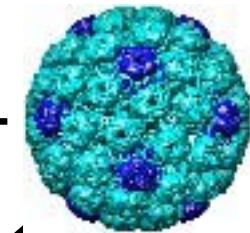
Mycobacterium tuberculosis



Herpes simplex



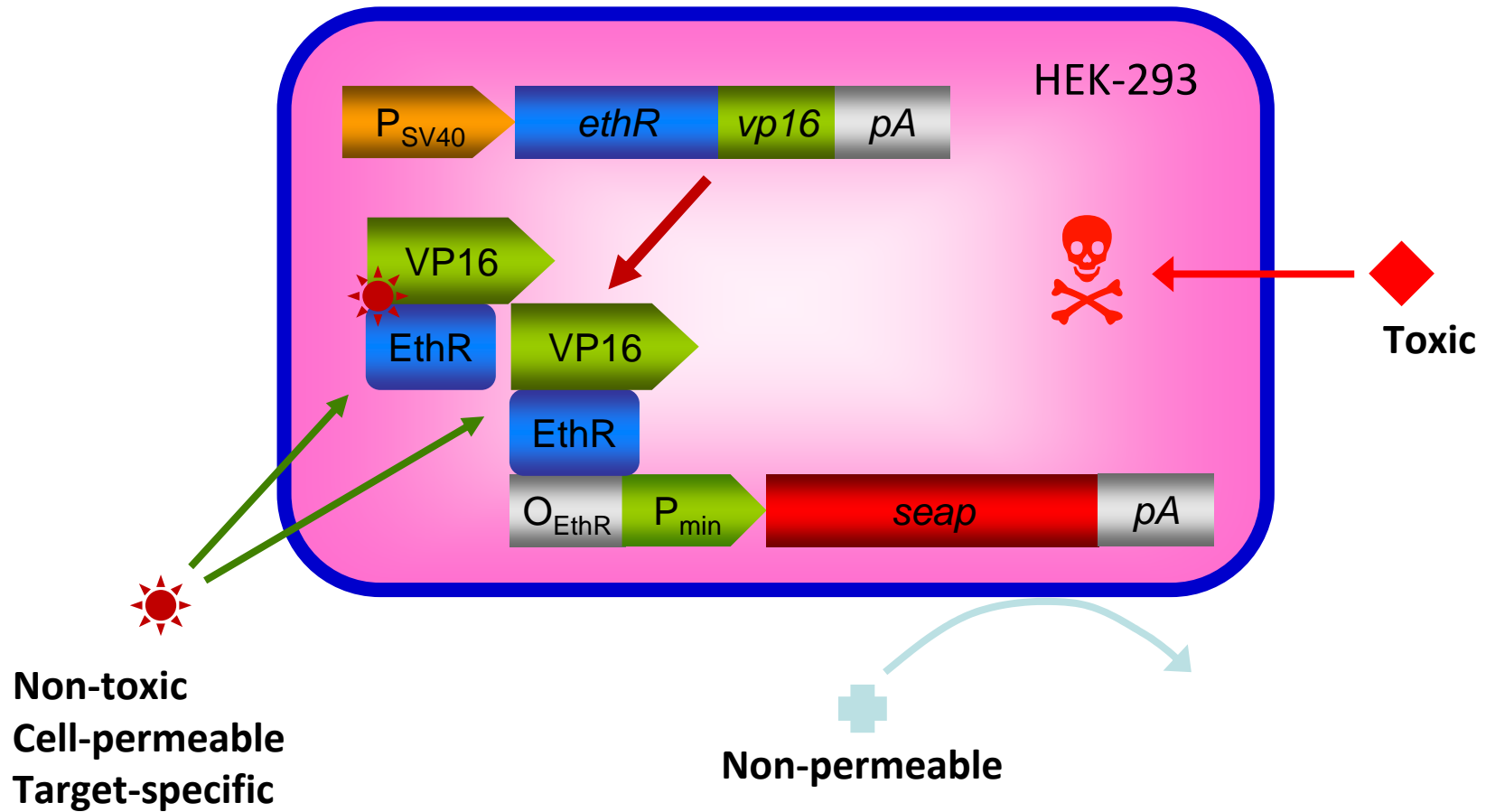
SV40 Virus



Drosophila

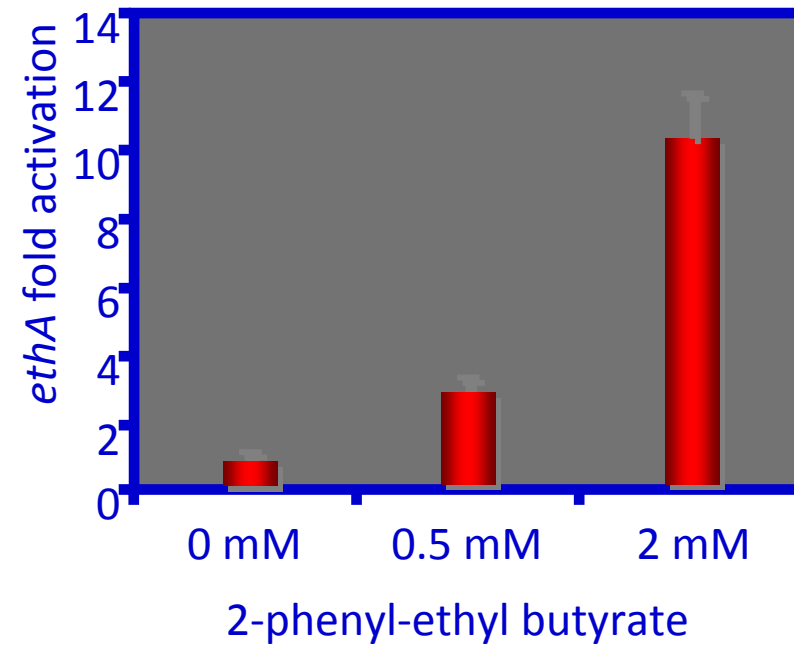
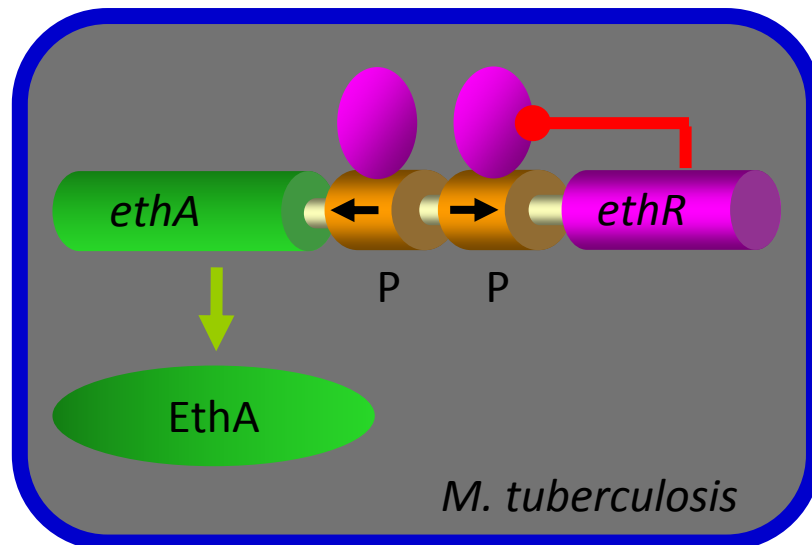


Homo sapiens

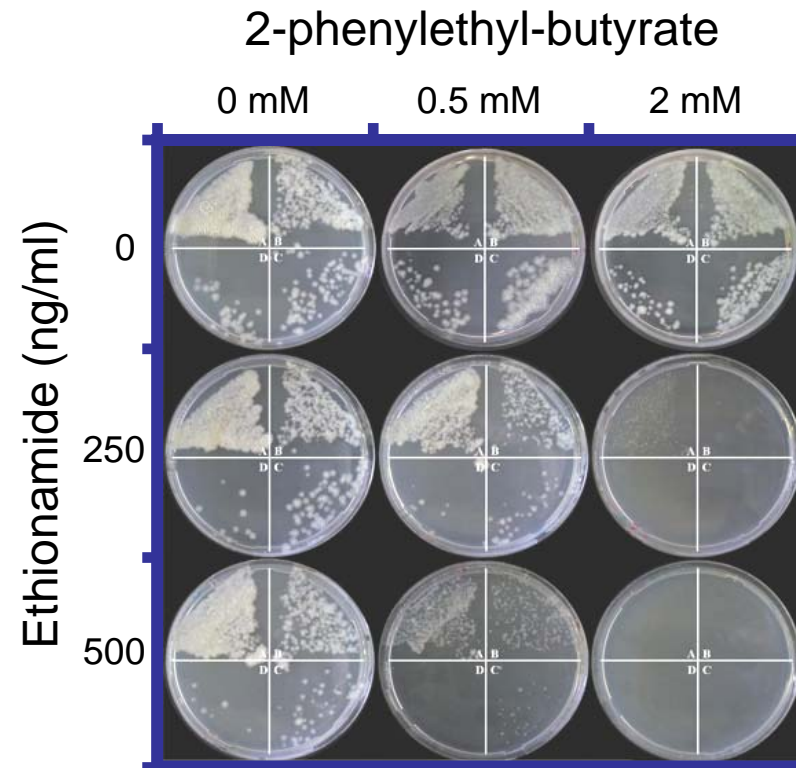


Hit validation

Identification of the food additive 2-phenyl-ethyl butyrate as cell-permeable, non-toxic inhibitor of EthR



2-phenylethyl-butyrate shuts off drug resistance



PNAS (2008) 105, 9994

Summary & Outlook

Synthetic Biology provides new insight into disease mechanisms, facilitates the discovery of conventional small molecule compounds and enables the design of biopharmaceuticals with unprecedented function and safety to treat and prevent diseases and to overcome drug resistance.

Innovations in Synthetic Biology-based drug discovery are currently achieved in universities and SMEs. Supporting these activities will stimulate creativity and entrepreneurial spirit to expand SynBio drug discovery concepts to other disease areas.

Substantial efforts in SynBio drug discovery are directed towards diseases prevalent in developing countries thus requiring ongoing support from the public sector.

As drug development (clinical trials) is a long-term but high-reward business, first drugs discovered using Synthetic Biology are expected to enter the market in 6-10 years with a highly attractive economic potential.

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