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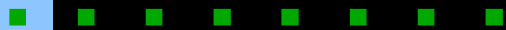
functional analysis for synthetic biology  
metabolic frustration is driving compartmentalisation

■ ■ ■ ■ ■ ■ ■ ■



antoine danchin 唐善 · 安東

amabiotics sas



cost workshop on whitefly metagenomics –  
synthetic biology & symbiosis

valencia, february 28th, 2012

# contributions

in silico

stefan engelen collaborators: gang fang eduardo rocha

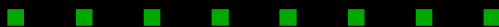
in vivo

agnieszka sekowska collaborators: undine mechold francis biville

institutions

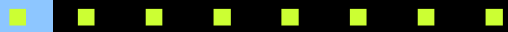
genoscope, beijing genome institute, fudan university, the university of hong kong,  
hong kong university of science and technology

financial support





p r o l o g u e



# cells as computers making computers

life requires (100 years after Alan Turing's birth):

- o a **program** (a "book of recipes": **replicated**)

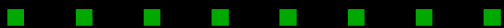
**recursive** information transfer and trapping

=> coding from one level to a second level introduces an essential **asymmetry** (conceptually **differs from feedback** or feedforward)

- o a **machine** ("compartmentalised chassis") allowing the program to be **expressed** and defining an inside and an outside (**reproduces**)

- o a **dynamic coupling process**: **metabolism** (chemical interchange)

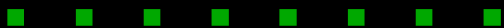
synthetic life asks that **one places the program within the chassis**



# structural frustration



it is not enough to have a dna molecule with the right sequence, it needs to be correctly folded!

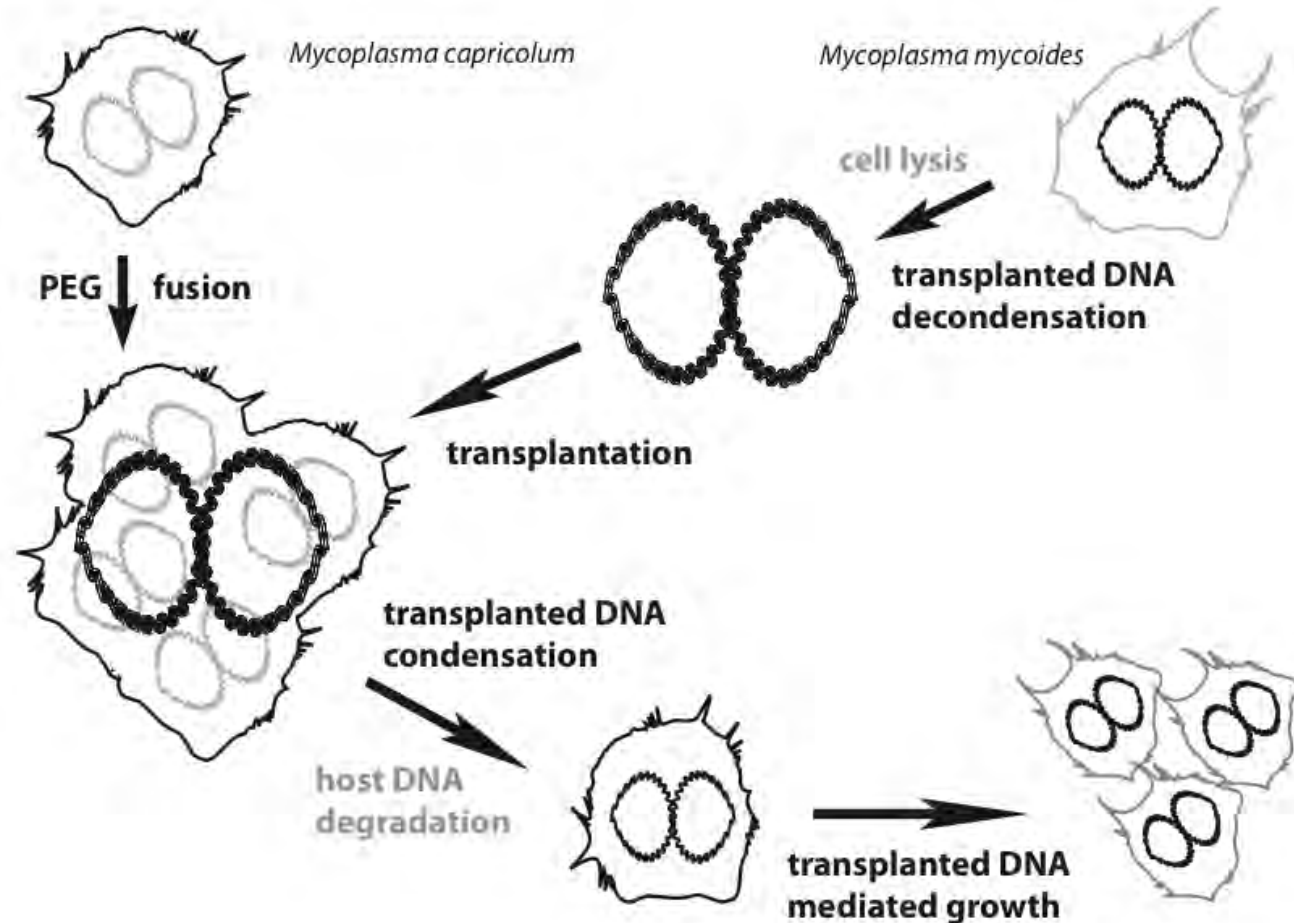


# d n a t r a n s p l a n t a t i o n

upon lysis dna is prone to expand as unavoidable nicks cut strands randomly

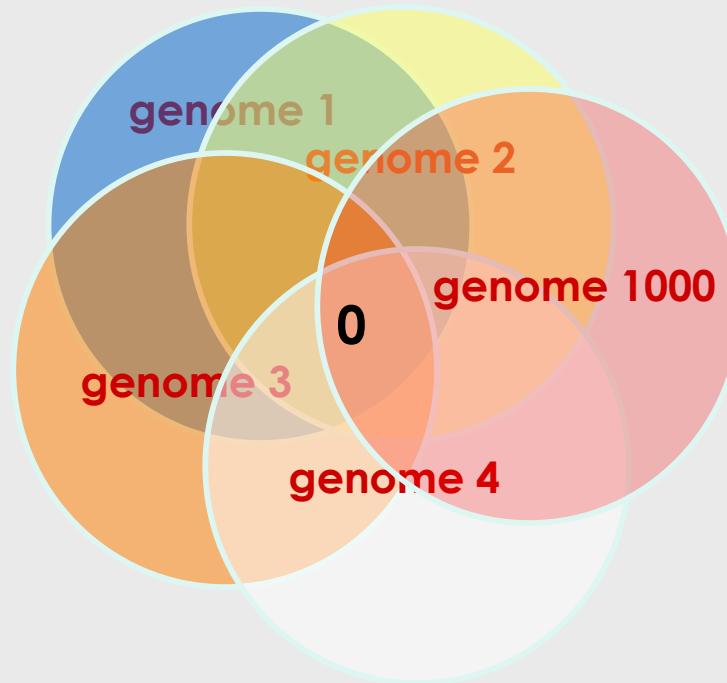
it cannot enter a single host cell

PEG makes a macro cell that can accomodate it



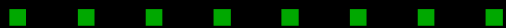
lartigue c, glass ji, alperovich n, pieper r, parmar pp, hutchison ca, 3rd, smith ho, venter jc: genome transplantation in bacteria: changing one species to another. science 2007, **317**(5838):632-638.

# incomplete rosetta stones...



2010: the number of conserved genes is zero! but many functions are ubiquitous

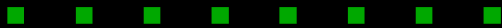
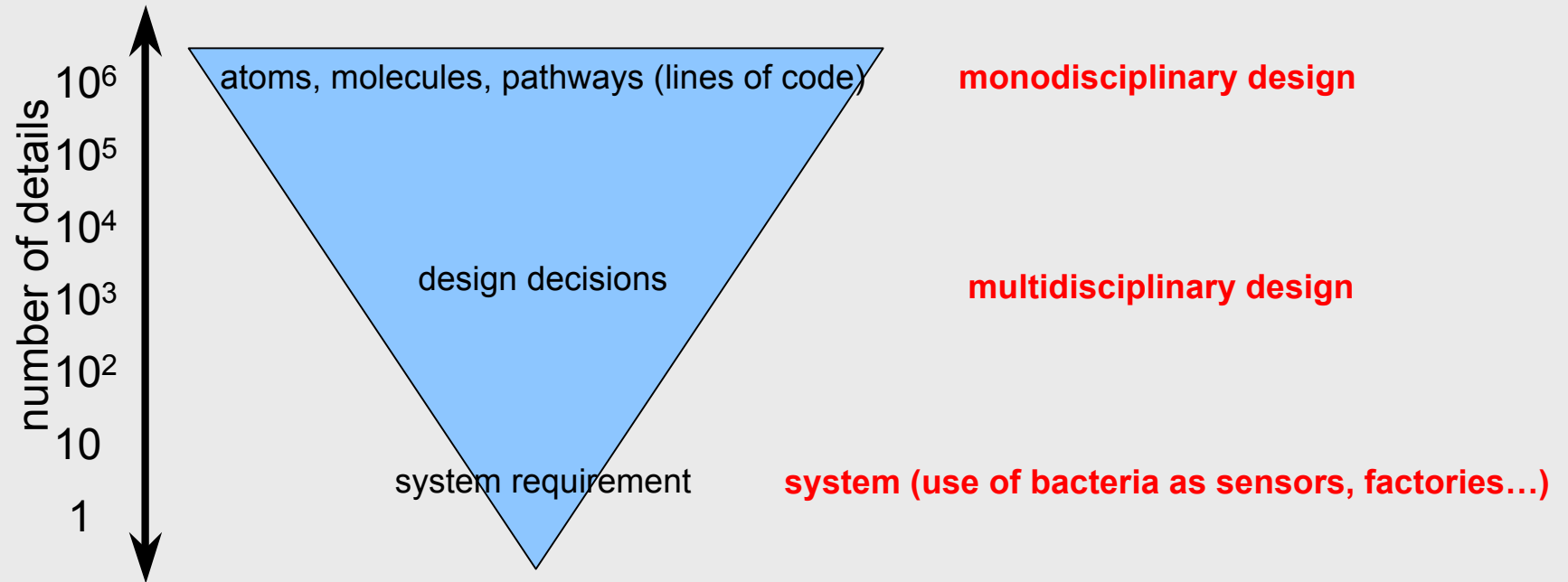
**conservation of function does not imply conservation of structure => no need for a luca**



k lagesen, dw ussery, tm wassenaar  
genome updated: the 1000th genome - a cautionary tale  
microbiology sgm 2010 156:603-608



# ...require functional analysis

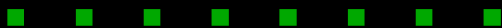


# the genome is functionally organized

some genes tend to stay close to one another:

- **persistent genes** (present in a large quorum of genomes: **no** ubiquitous genes) => **paleome**
- **rare genes** (present in specific strains of a given species) => **cenome**

the latter genes are easily accounted for, as they come from **horizontal gene transfer**; they tag the environment of the organism

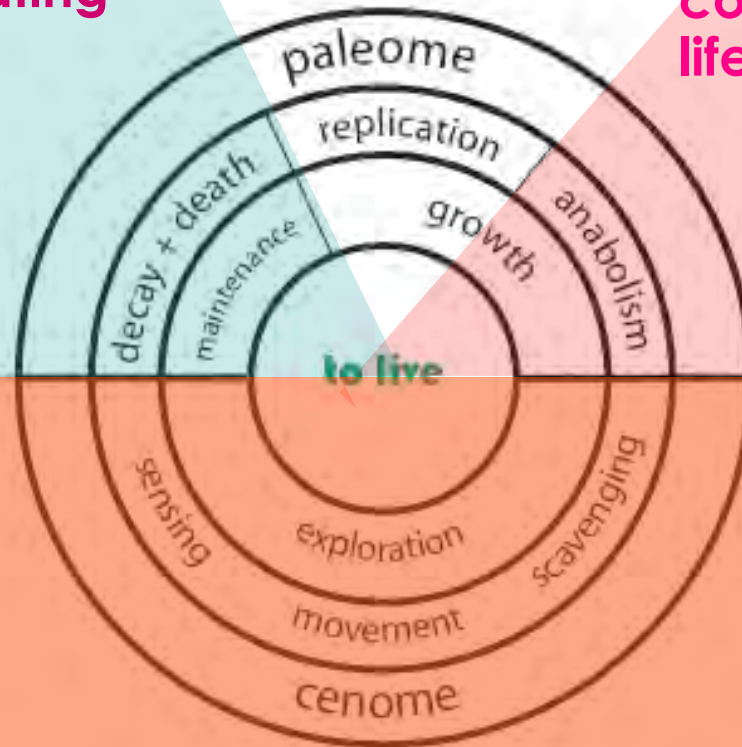


# a tale of two genomes

metabolic engineering

perpetuating  
life

constructing  
life



living in context

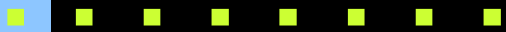
pan genome: paleome + cenome

in e. coli paleome: 1,900 genes

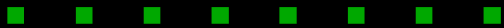
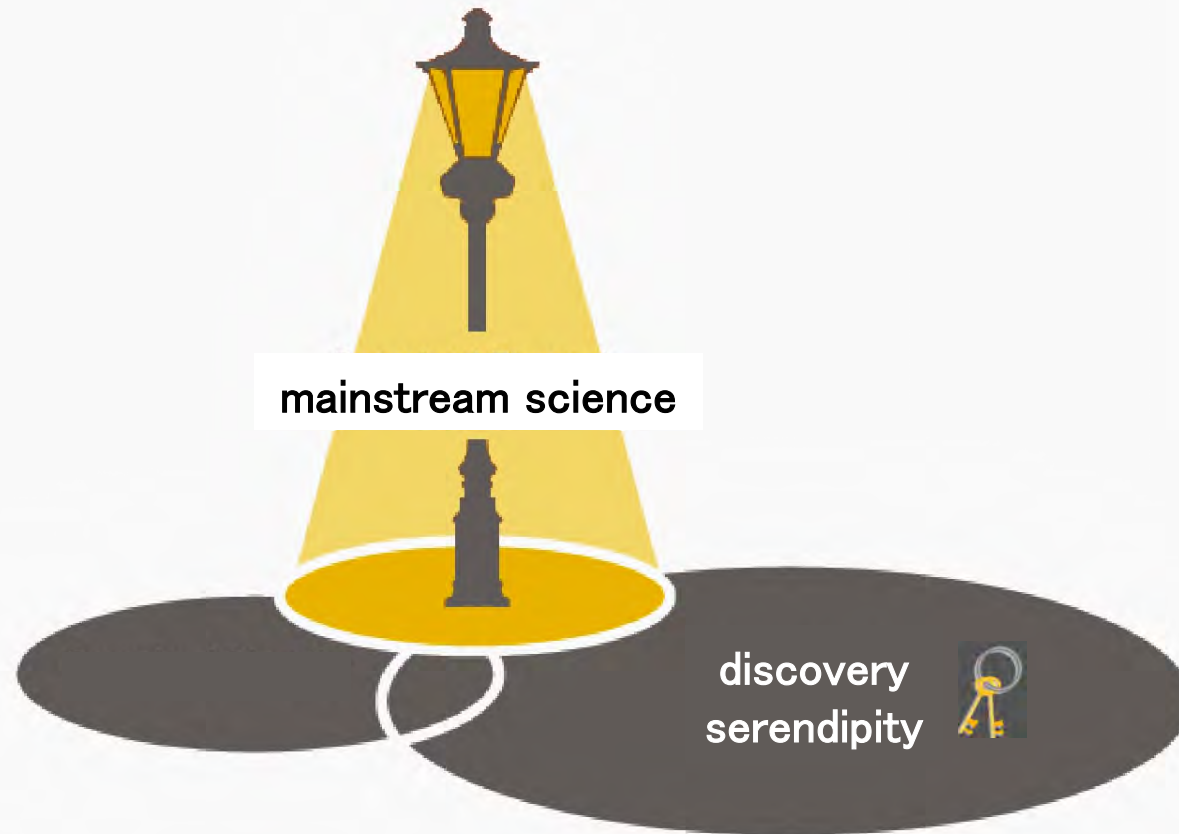
cenome > 40,000 genes



chassis' engineering

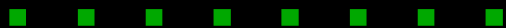
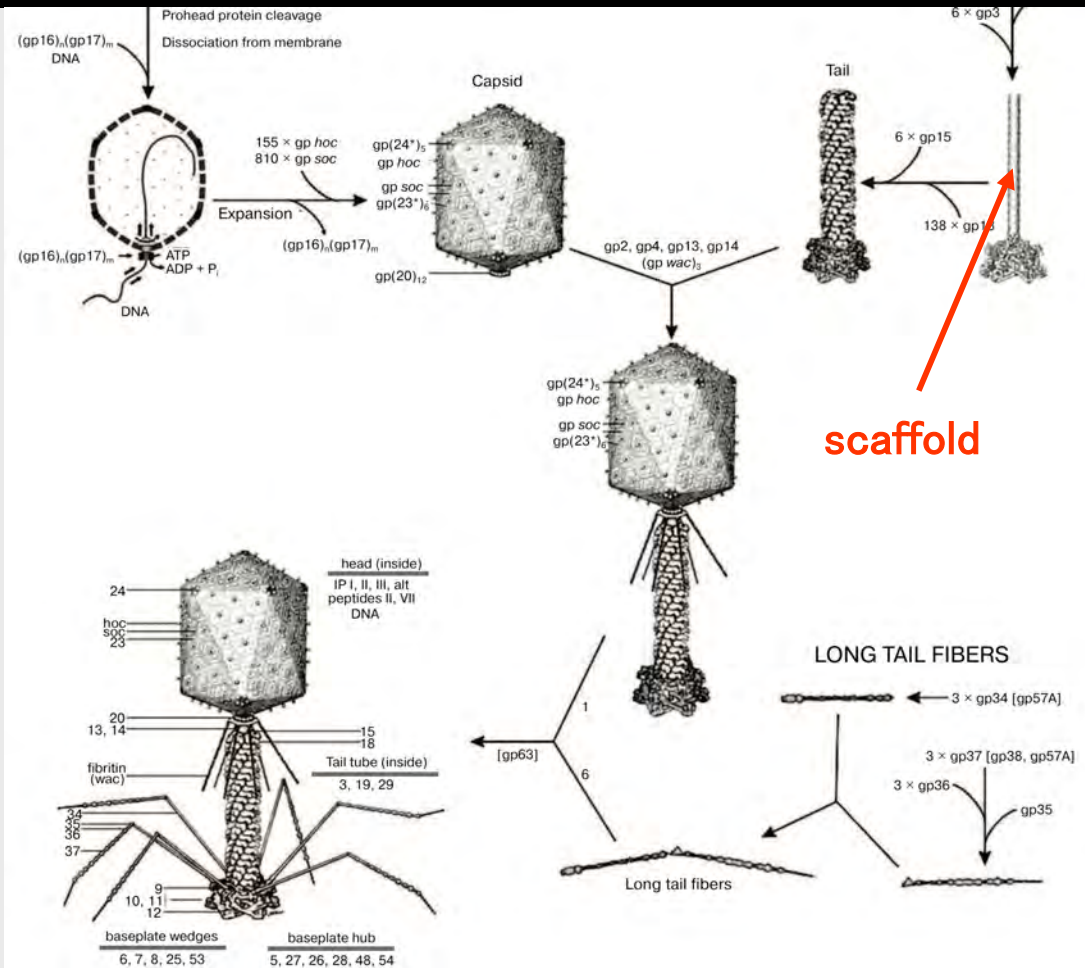


# the lamppost effect



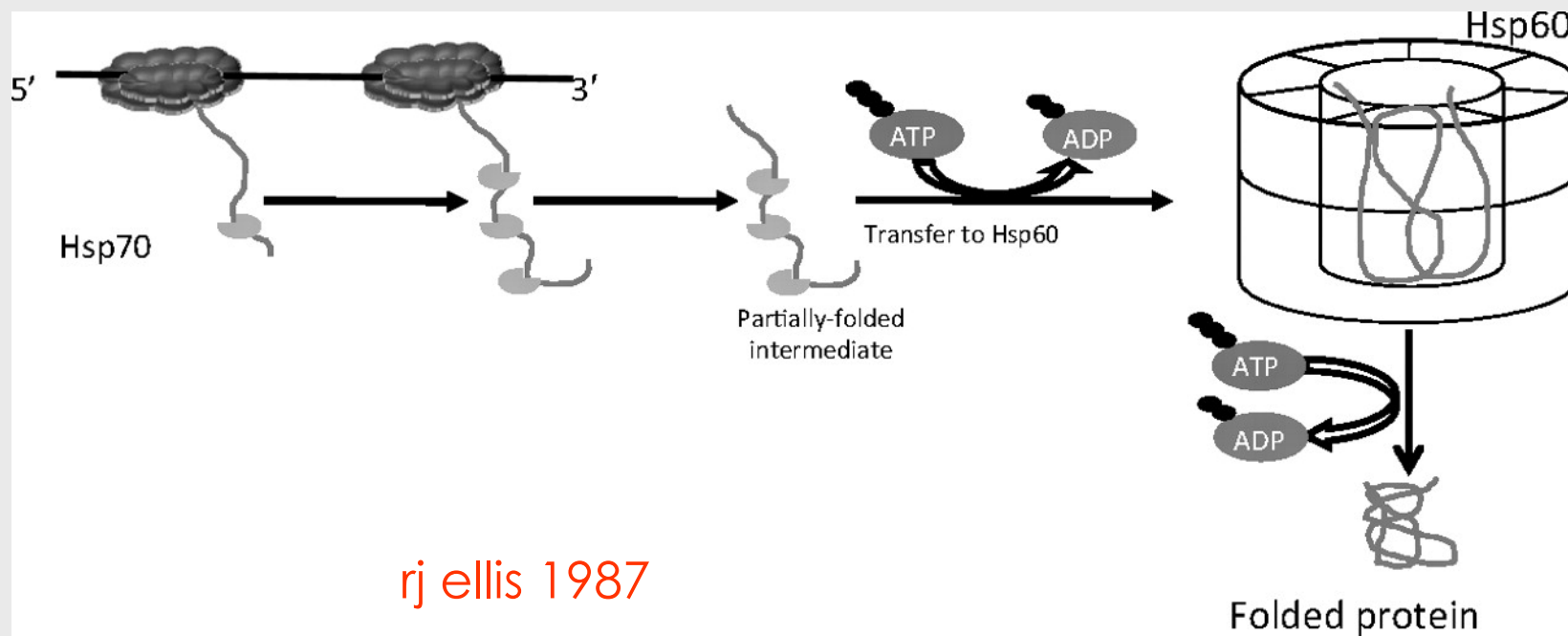
# s c a f f o l d s

to build up a bacteriophage such as phage T4 a scaffold is constructed and used as a vernier to make a tail of fixed length, with the proteins of the tail making an helix structure around the scaffold that is later disposed of

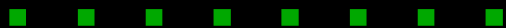


lartigue c, glass ji, alperovich n, pieper r, parmar pp, hutchison ca, 3rd, smith ho, venter jc: genome transplantation in bacteria: changing one species to another. *science* 2007, **317**: 632-638.

# molecular chaperones

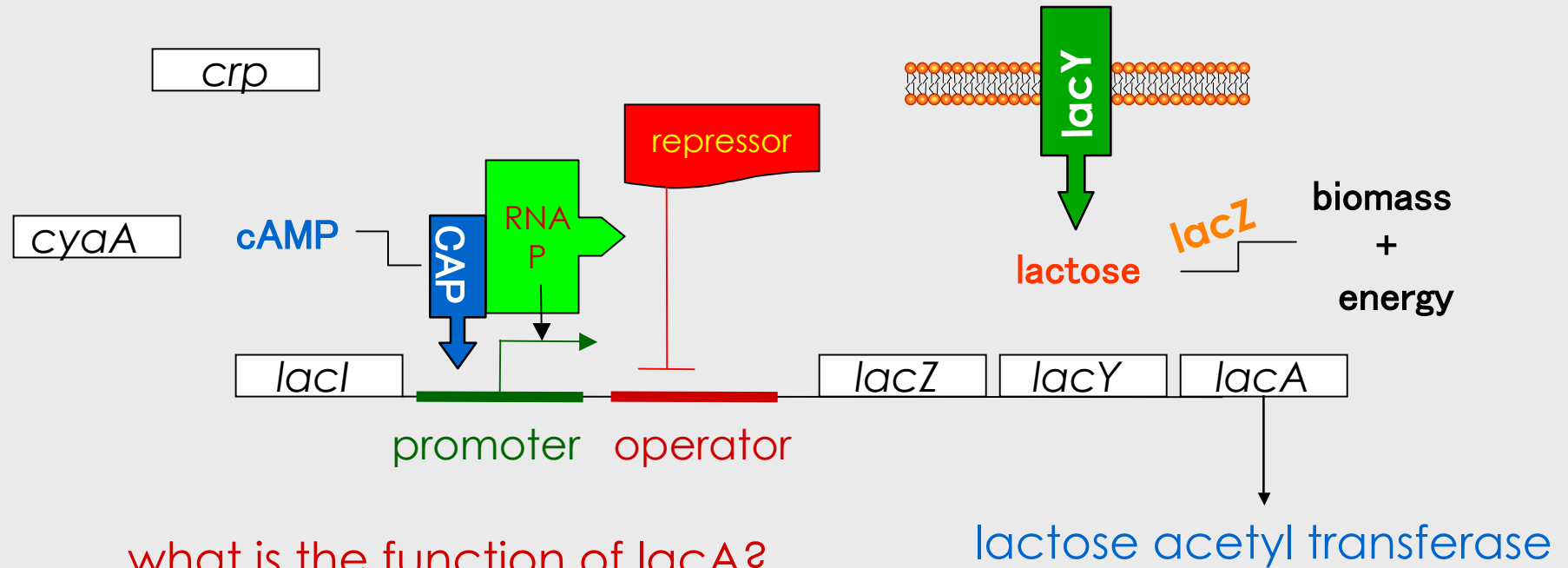


ry ellis 1987



# a chassis' engineering need

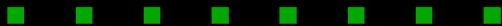
**lactose and not glucose**



what is the function of *lacA*?

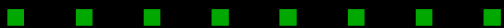
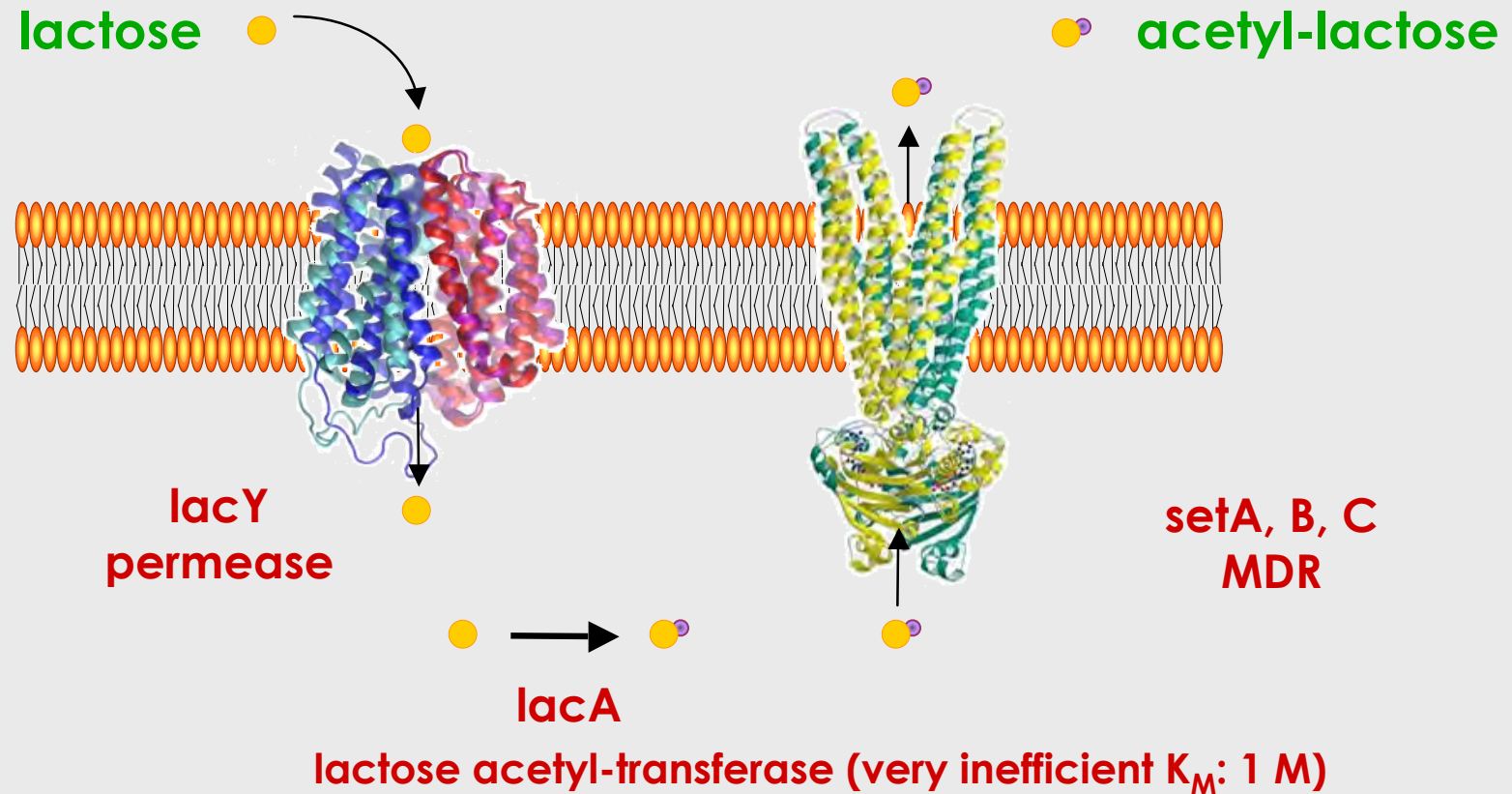
lactose acetyl transferase

**why did we need 50 years to ask the question?**



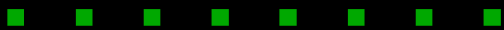
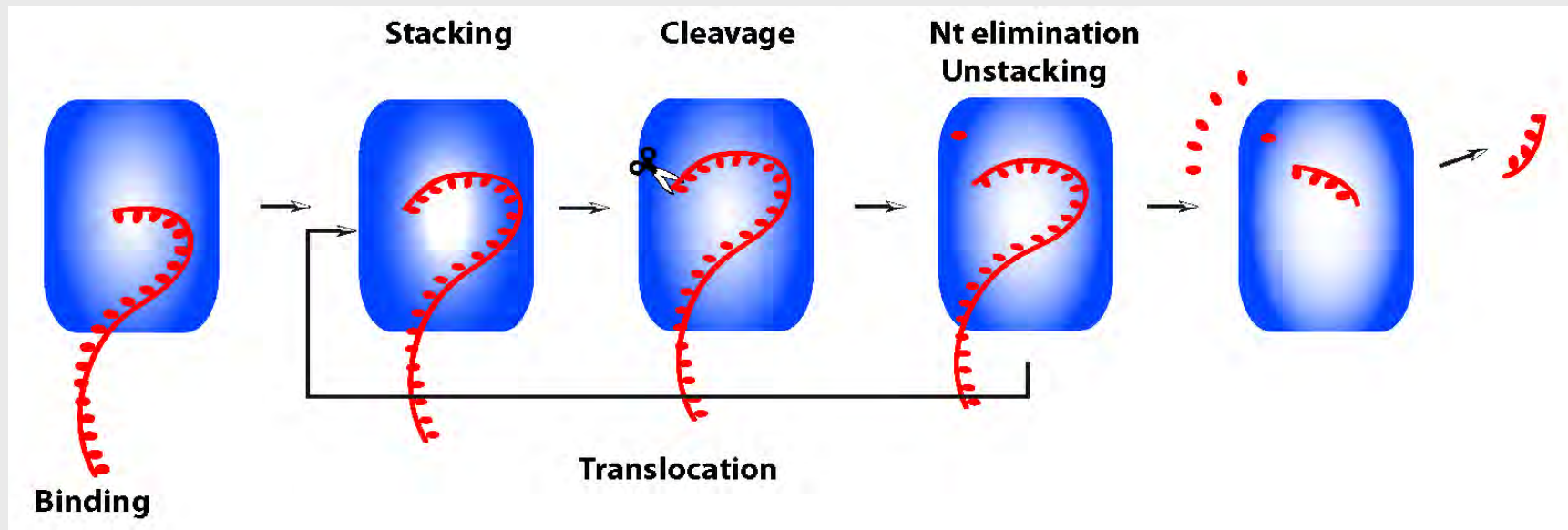


# cells need safety valves, not leaks

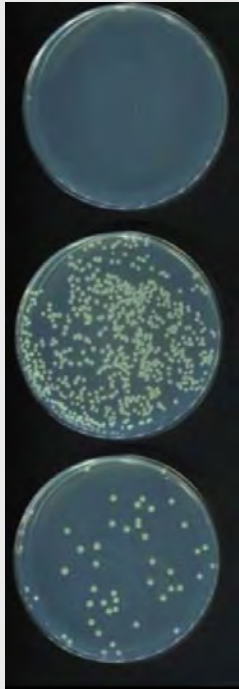


# coping with leftovers

nanornase is an essential function



# nano-rnases: functional, not structural ubiquity

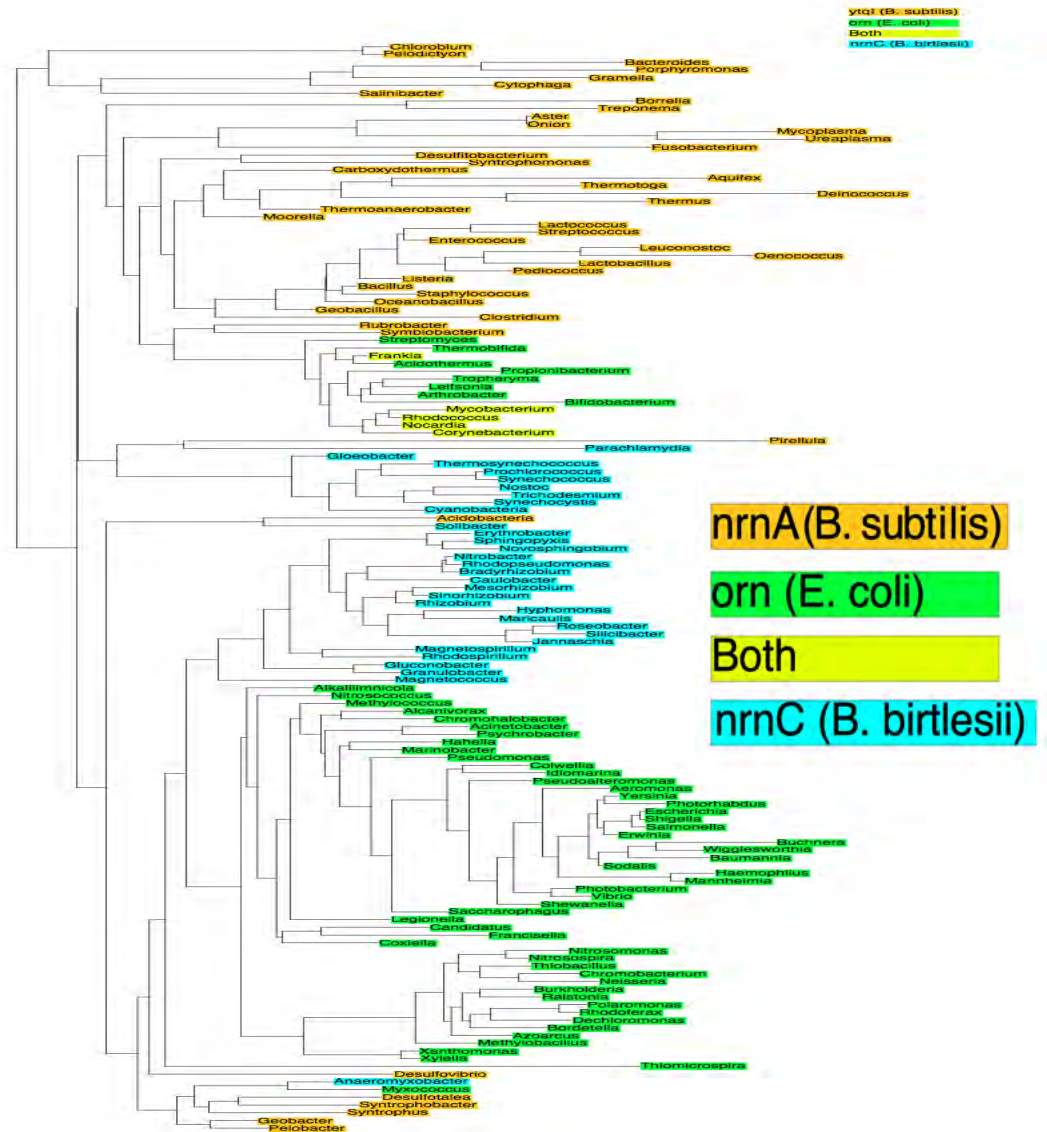


vector

bb\_nrnC

ec\_orn

complementation  
in vivo



# a proprietary new functional activity

sb engineering reasoning allowed us to identify metabolic pathways that are essential to cope with repeated stresses and extend the concept of vitamins

**AMAbiotics**

Saturday, February 25th, 2012 | our aims | our publications | direction | our values | our method | lectures | news | media

### AMAbiotics SAS - Metabolic bioremediation

#### News

Blow up | Titles

- ▶ The University of Wageningen withdraws a statement promoting milk against cardiovascular diseases.
- ▶ Recent patents on the use of antioxidant agents in food.
- ▶ AMAbiotics participates to the effort of the Beijing Genome Institute to sequence the genome of *Escherichia coli* EHEC serotype O104:H4.
- ▶ A protein from royal jelly is necessary and sufficient to trigger the development of bees into queens.
- ▶ Selenium does not prevent cancer.
- ▶ Sulfite, widely used as a food preservative, is toxic for neurons involved in memory.
- ▶ Choline is essential to humans, but it promotes cardiovascular diseases.
- ▶ Even pathogens communicate.
- ▶ Chromium is a poison.
- ▶ Rapid time fluctuations of the ileon microbiota.
- ▶ Brain lactate increases with aging.
- ▶ Copolymerization = disease

#### The company

AMAbiotics is a research company focused on the link between microbial metabolism, nutrition and health. Using cutting-edge techniques in genomics and modelling, AMAbiotics develops for its own account or with partners a portfolio of know-how, patents and applications.

#### In brief

Living beings make communities where each has its own place, from indifference to collaboration, competition and even aggression. The invisible part of these communities, that made of microbes, is most often ignored. Yet it is an essential asset, and the equilibrium of the whole is what makes the well-being of each member of these complex communities, man included. This equilibrium results from the exchange of chemical compounds that come either from the outside or from synthesis and degradation of compounds specific to the different species making the community. Understanding this metabolism, in each particular situation, permits us to propose solutions to make individual organisms stay at equilibrium, or to come back to equilibrium. This is the goal of the research developed at AMAbiotics.

#### Collaborations

AMAbiotics is hosted at the Genopole Ile de France and it collaborates with the University of Evry. The goals of the Fournentin-Guilbert Foundation lead us in the selection of relevant features of what makes the heart of the living

#### Collaborations

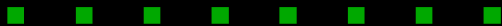
Search:  go

#### Links

- EU project Microme
- EU project BioSaplens
- EU project TARPOL
- Antoine Danchin's home page

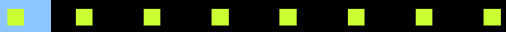
#### Liens bibliographiques

- Arsenic in our plates
- Arsenic and heart troubles
- Secondary effects of cortisone
- An example of sulfur-mediated protection
- A kin of the natto bacterium
- Sulfur metabolism





metabolic frustration



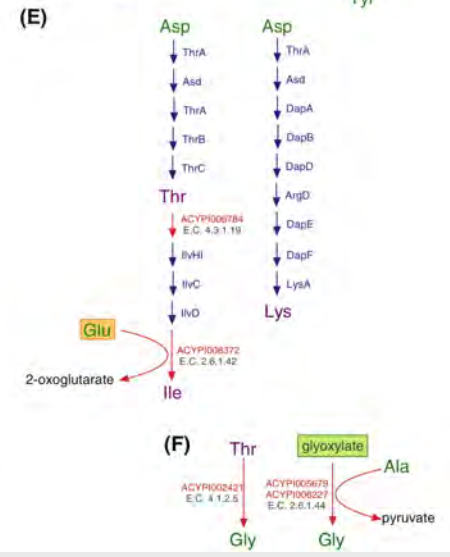
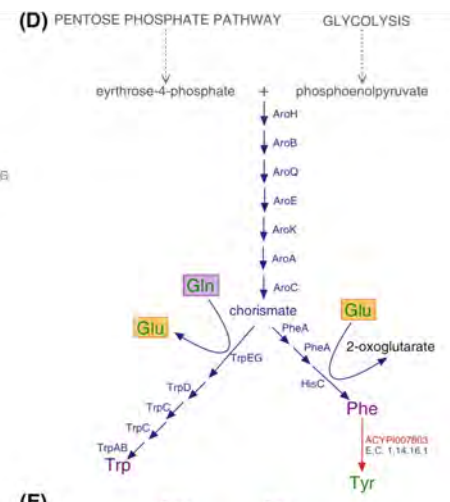
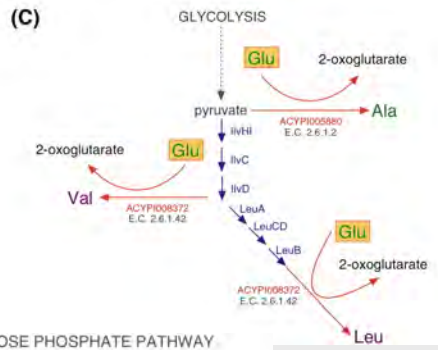
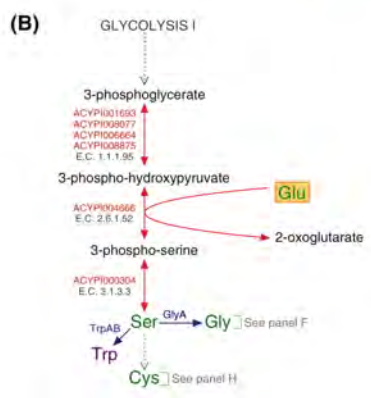
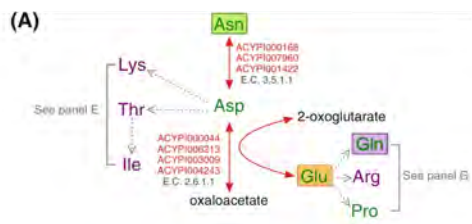
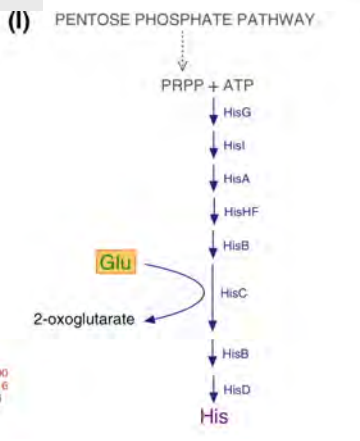
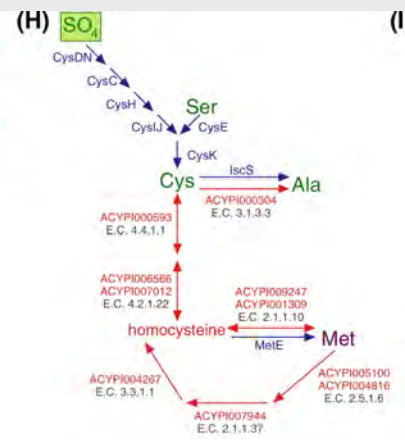
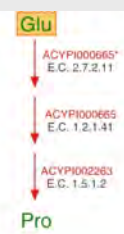
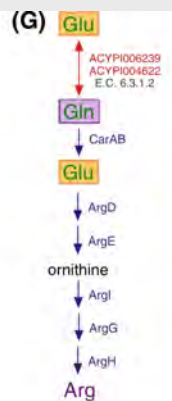
# serine toxicity

serine is made by the host

isoleucine, leucine and valine are made by the symbiont

**this matches with serine toxicity demonstrated in bacteria**

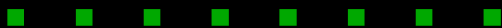
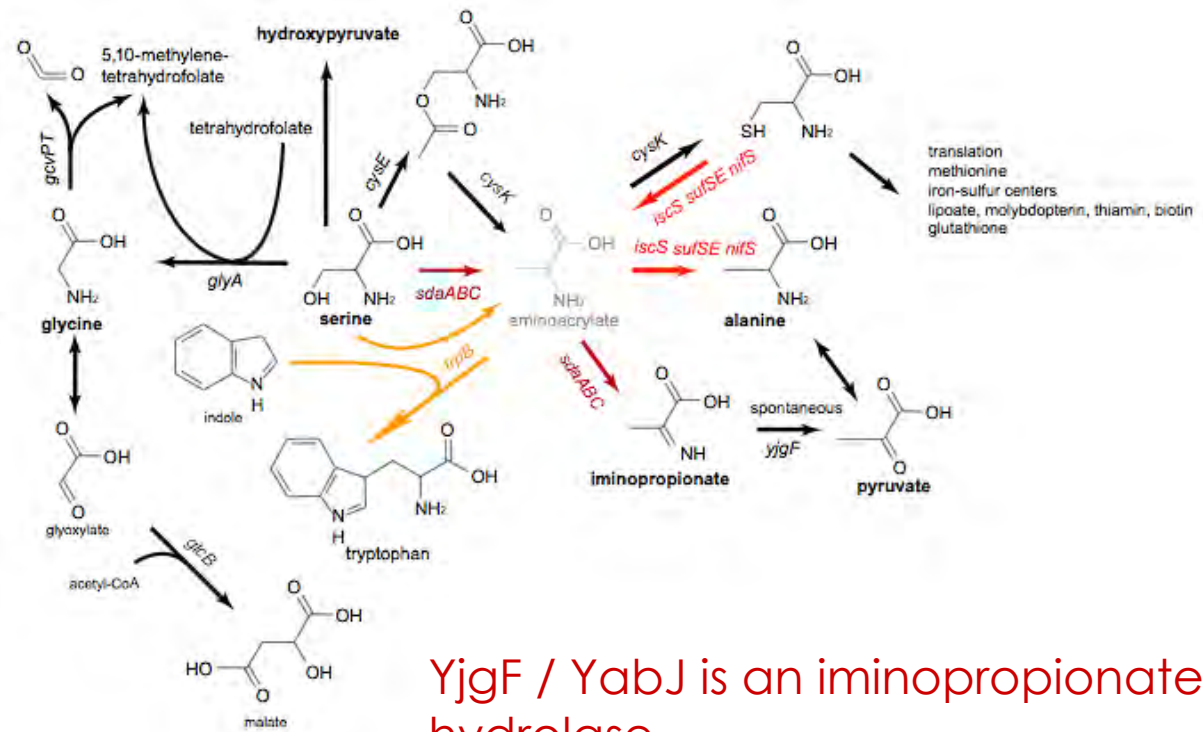
serine concentration must be tightly controlled in the presence of bcaa biosynthesis enzymes  
**serine dehydratase belongs to persistent genes**



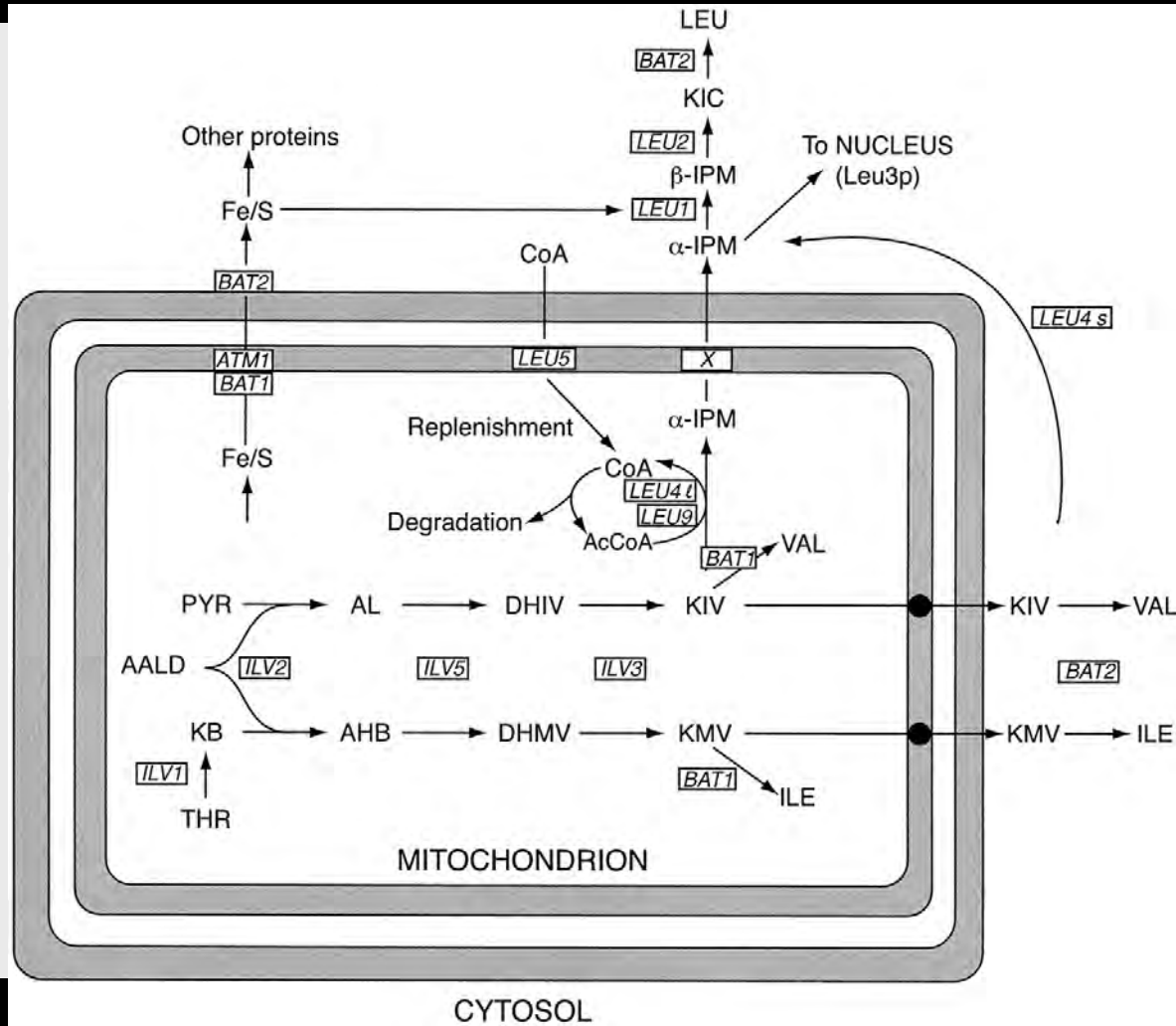
# hydroxypyruvate and aminoacrylate / iminopropionate

serine is transaminated into hydroxypyruvate, that leads to dead-end compounds with thiamine

Excess serine goes in pathways such as cysteine and tryptophan biosynthesis, creating a reactive intermediate 2-aminoacrylate / iminopropionate

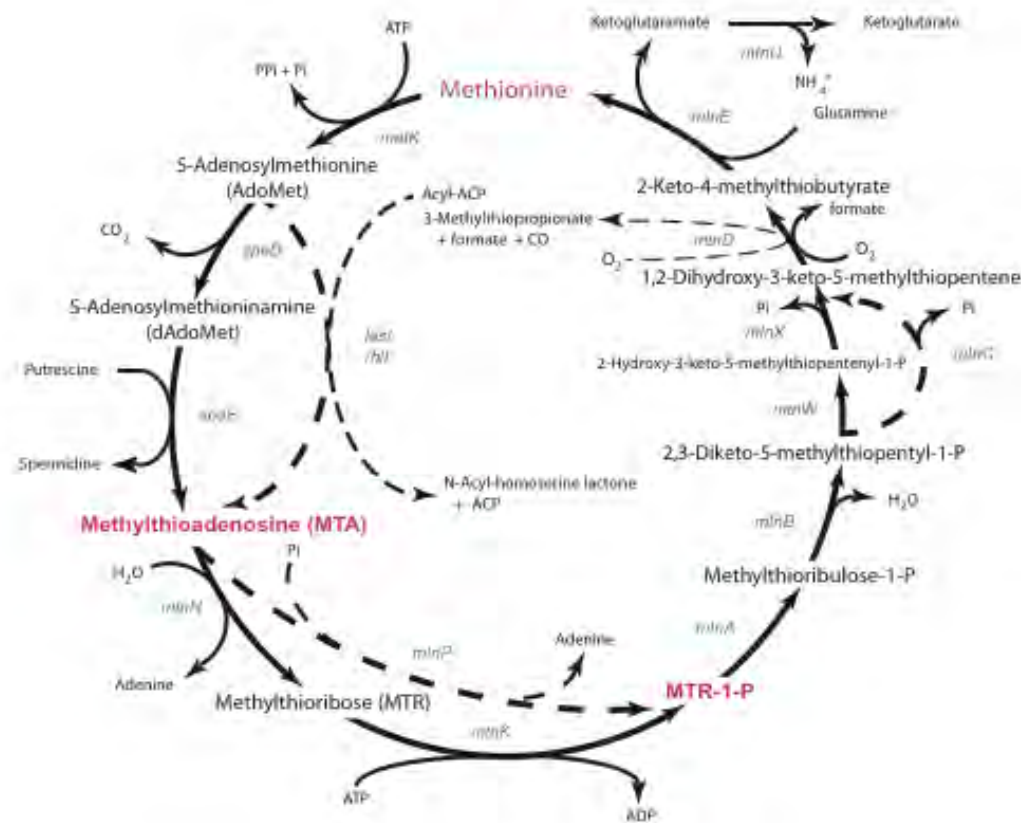


# bc aa compartmentalisation in yeast



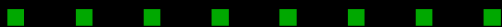


# methionine salvage



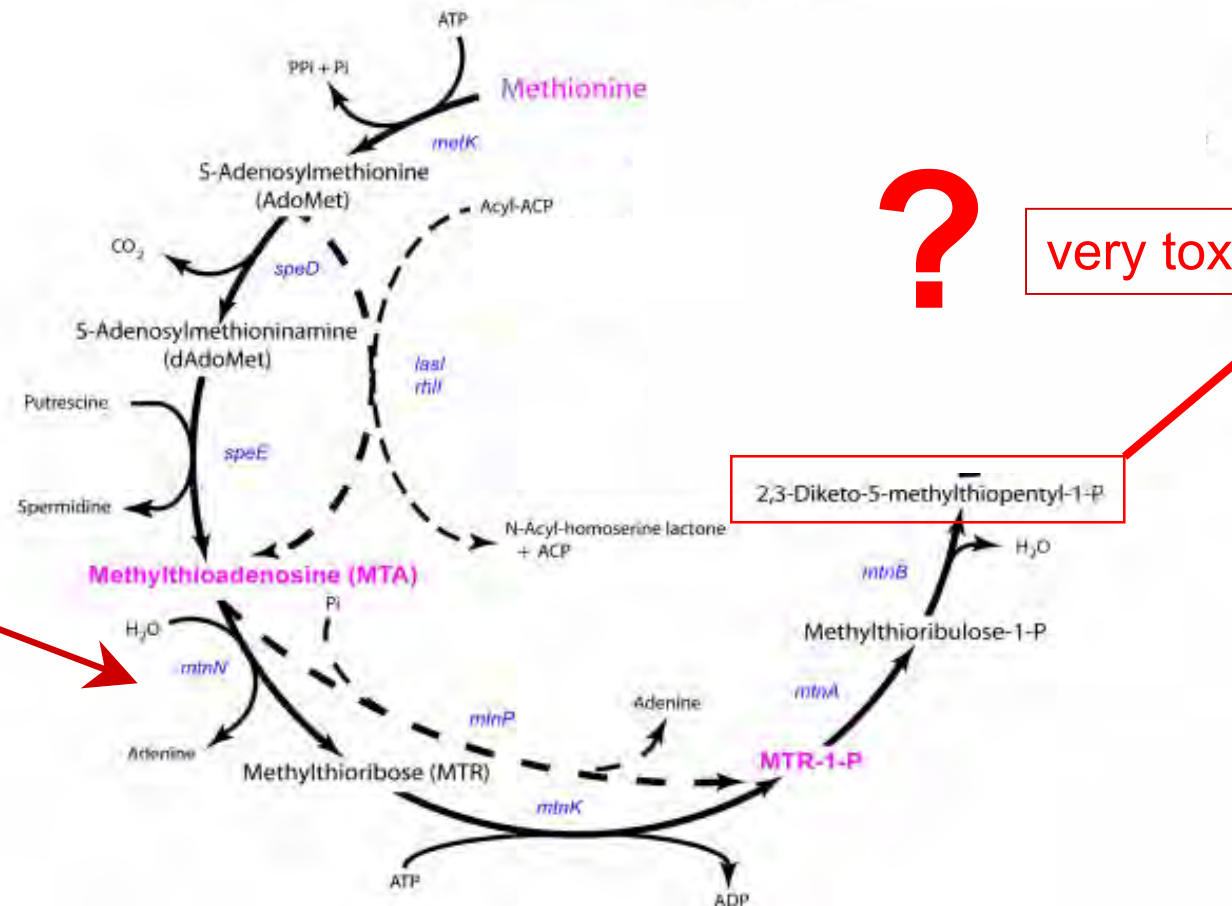
sekowska a, déneraud v,  
ashida h, michoud k, haas d,  
yokota a, danchin a.

bacterial variations on the  
methionine salvage  
pathway.  
bmc microbiol. 2004 4:9.



# an overlooked pathway

metabolism of polyamines and of menaquinone from amino-futalosine!



sekowska a, dénervaud v, ashida h, michoud k, haas d, yokota a, danchin a.

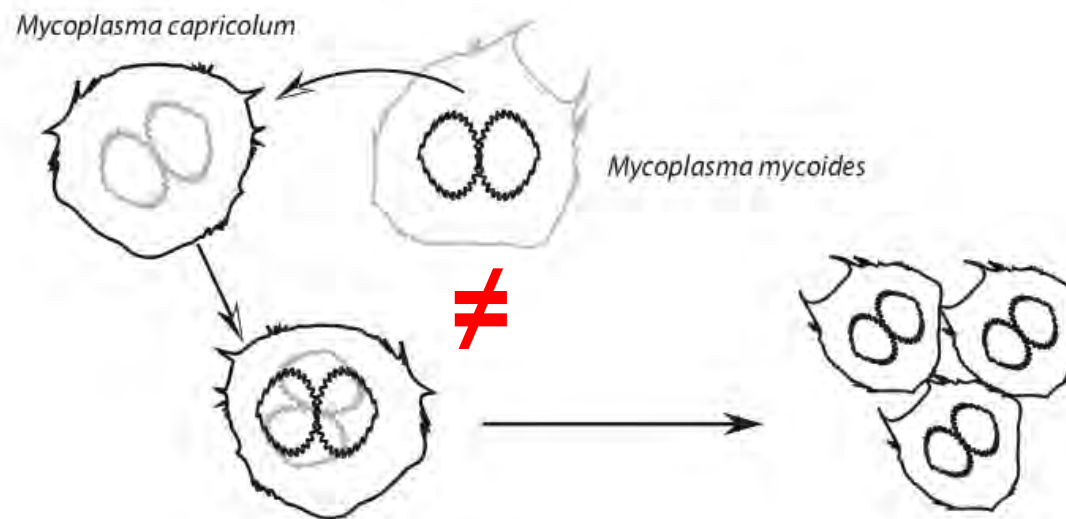
bacterial variations on the methionine salvage pathway.  
bmc microbiol. 2004 4:9.

# final caveat: reproduction $\neq$ replication

the program  
**replicates**  
(makes an  
identical copy)

the cell  
**reproduces**  
(makes a similar  
copy)

this split is the  
basis of  
evolution



# the enigma: babies are born very young!

but ageing is sometimes positive

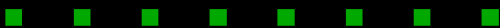
contrary to intuition, mixing a population of young bacteria with an old culture, the old one outgrows the young one (gasp phenotype : "growth advantage in stationary phase");

**is this compatible with scaling up in synthetic biology?**

**making an egg requires getting rid of aged components and dilution is far too slow to allow it to proceed**

which process underlies this phenomenon?

which genes allow information to accumulate?





g r a c i a s

