



# Fitness landscape of a metabolic pathway



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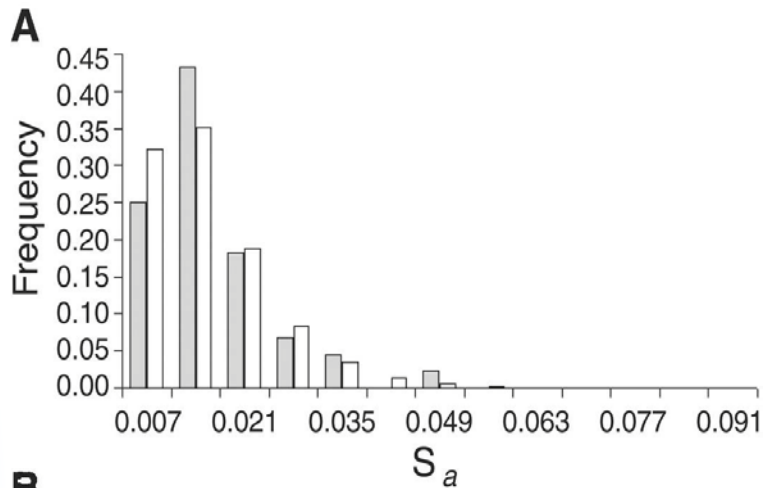
# How do phenotypes shape evolution?

- Evolution shapes phenotypes
- What is the role of phenotypes in shaping the fitness of an organism?
- How is the genetic fitness landscape different from the phenotypic?
- Can we introduce the environment in a meaningful way?

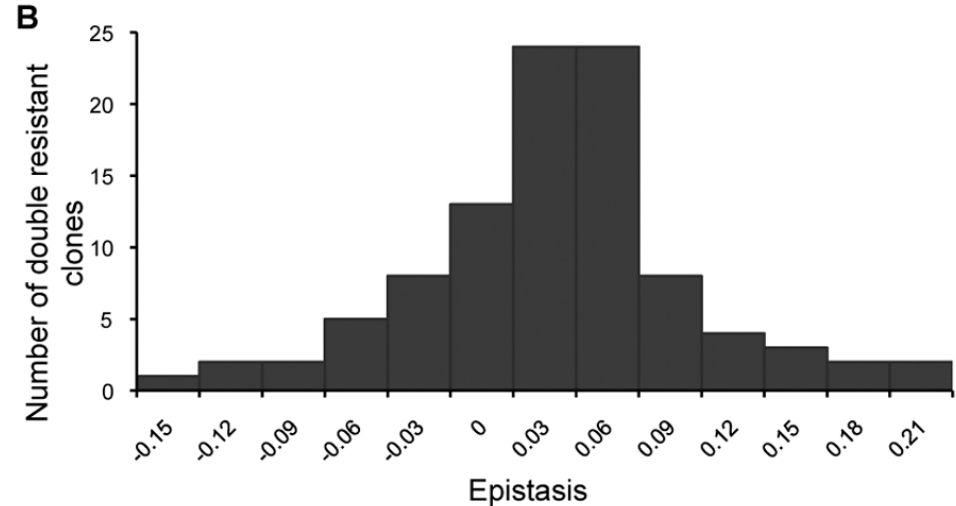
# What can we learn from phenotypes?

- Can we predict/explain the distribution of fitness effects and epistasis from mechanistic data?
- Can we predict/explain the evolution of particular phenotypes?

# Distribution of fitness effects and epistasis



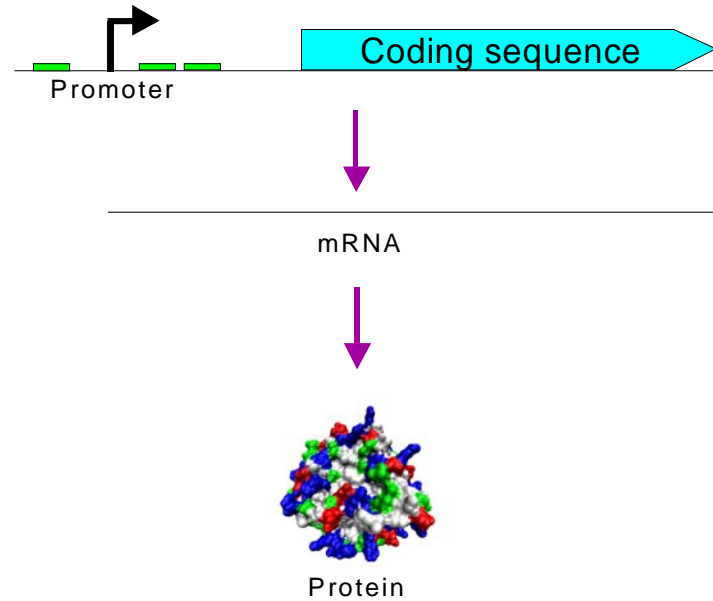
Perfeito *et al* 2007



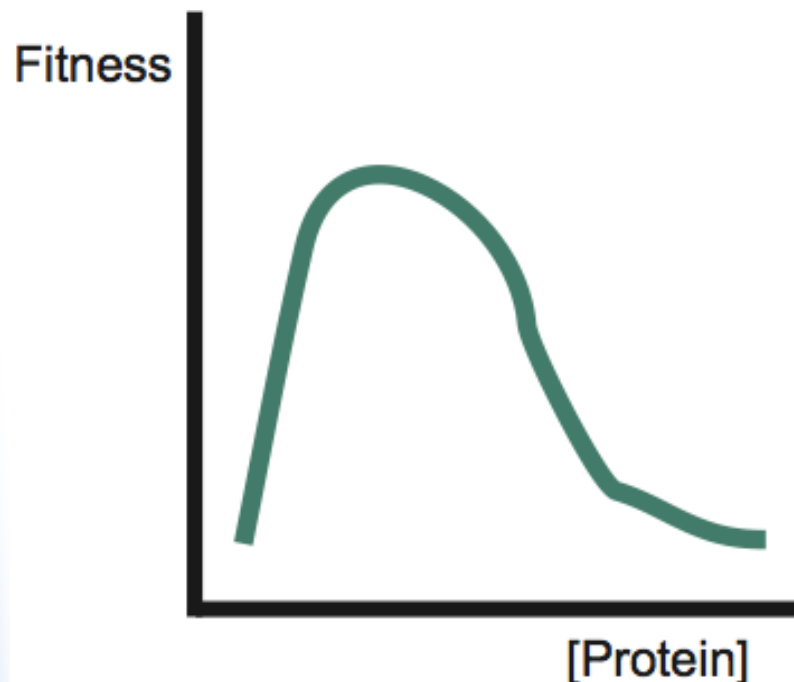
Trindade *et al* PLoS Genet. 2009

- Are all functional classes of genes represented?
- Does a particular pattern of epistasis emerge from specific mechanistic patterns? (e.g., the shape of the genetic network)

# Two classes of mutations: cis-regulatory and coding



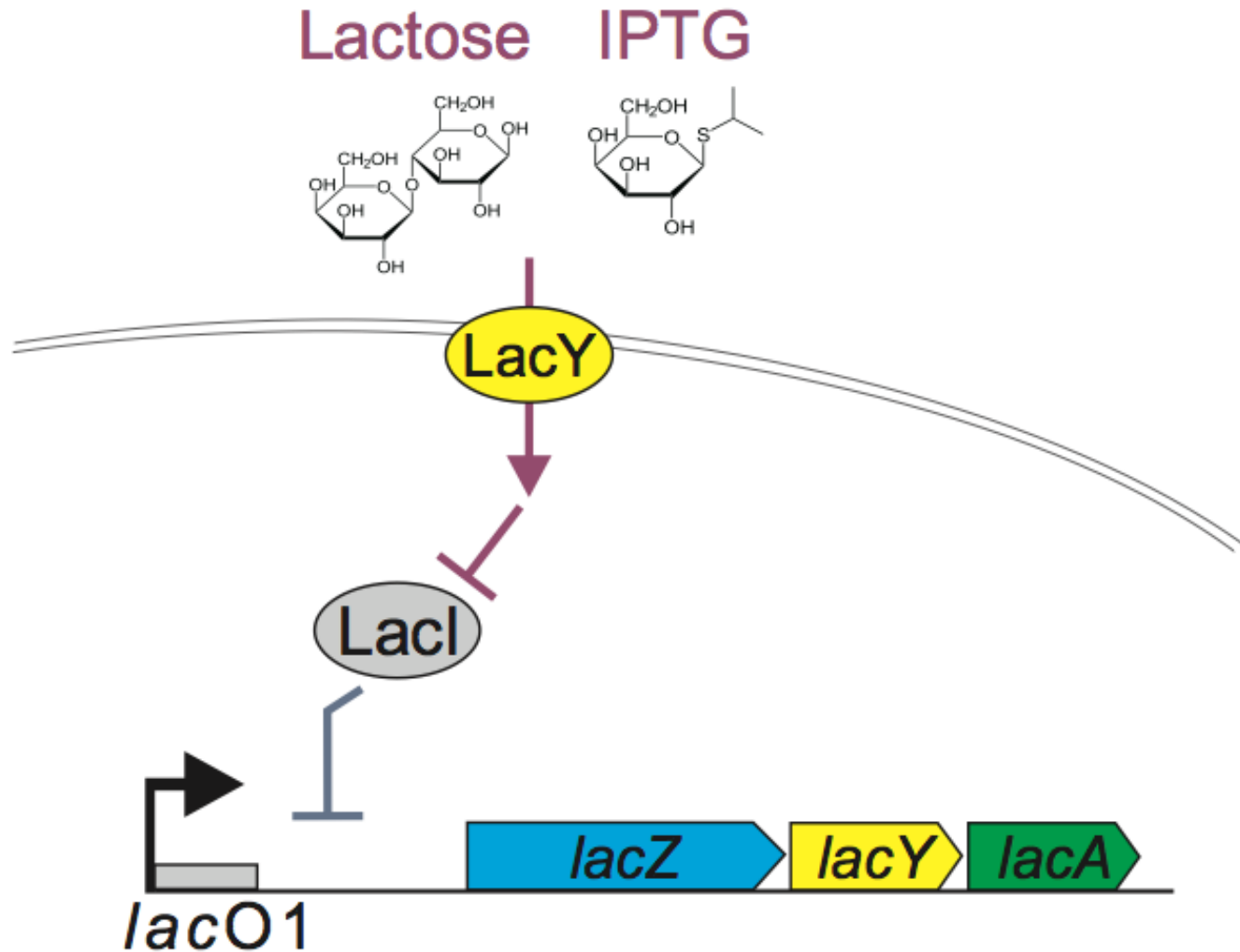
# Fitness effects of regulatory mutations



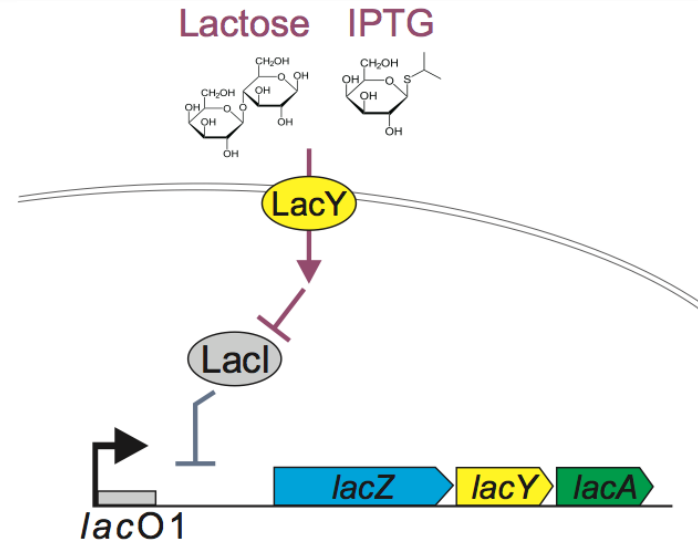
The effect of cis-regulatory mutations depends on:

- Which phenotypes are affected by protein concentration
- How these phenotypes interact with each other
- The interaction of these phenotypes with the environment (e.g., type and number of substrates)

# The *lac* operon of *Escherichia coli*



# Phenotypes affected by cis-regulatory mutations in the *lac* operon

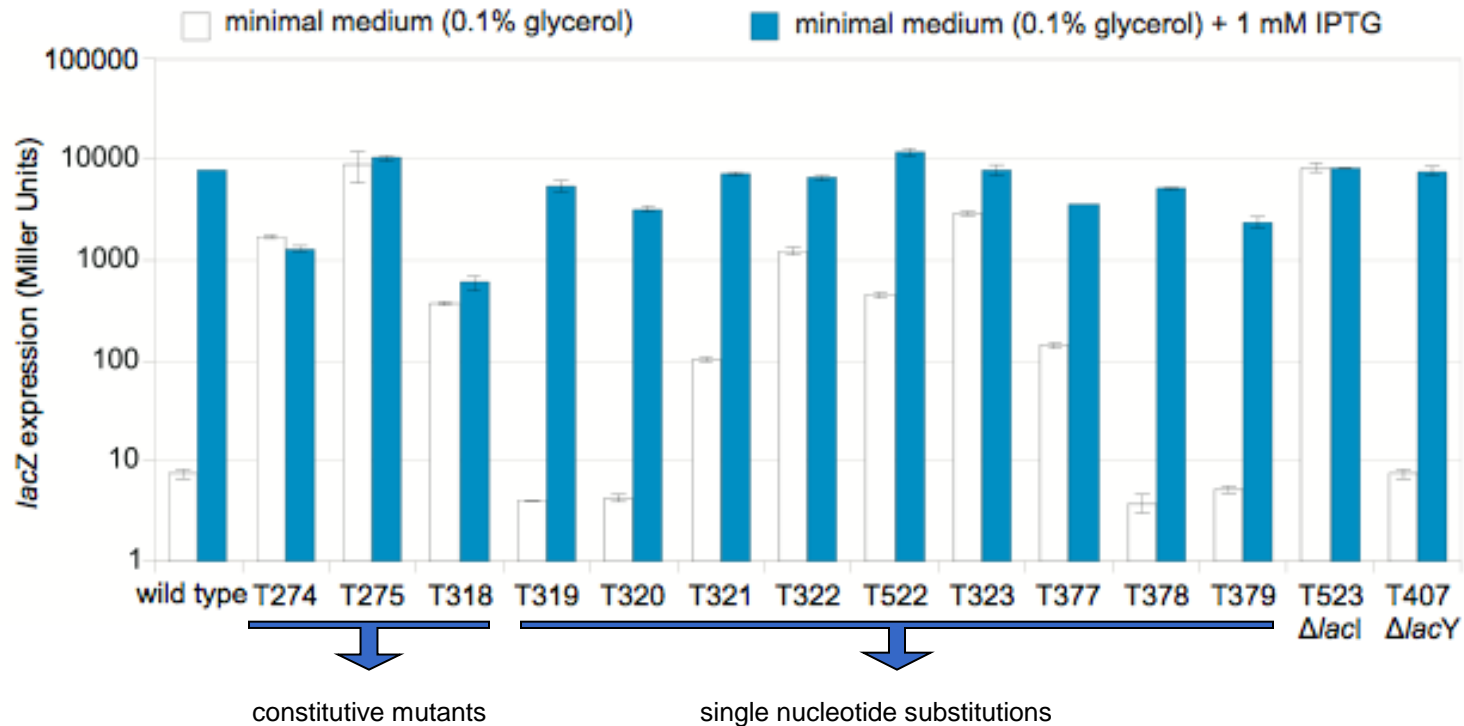


2 defined phenotypes:

- ✓ **Protein concentration** (of all the *lac* proteins)
  - manipulated by mutation in *lacO1*
  - monitored by measuring LacZ
- ✓ **Protein activity** (of LacY)
  - manipulated by external IPTG concentration



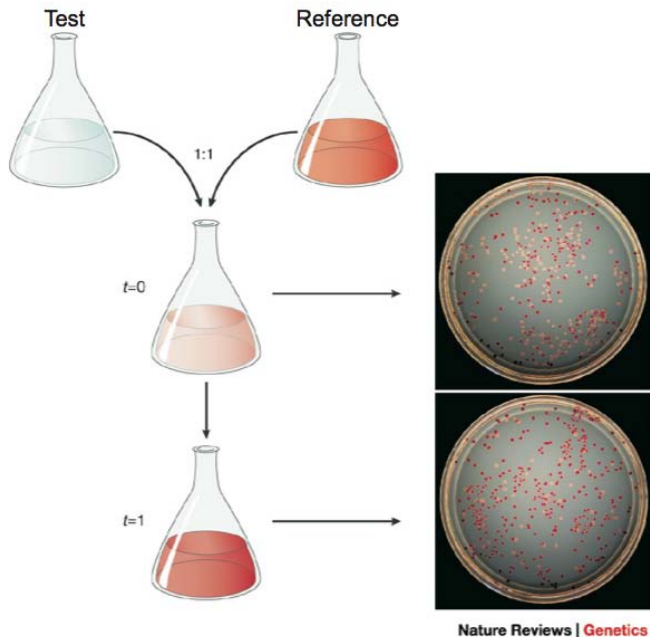
# The cis-regulatory mutants



- Different *lacO1* mutants have different expression values,
  - ✓ in the presence of the *lac* repressor
  - ✓ in the absence of the *lac* repressor

# Fitness measurement

## Competitive fitness assay

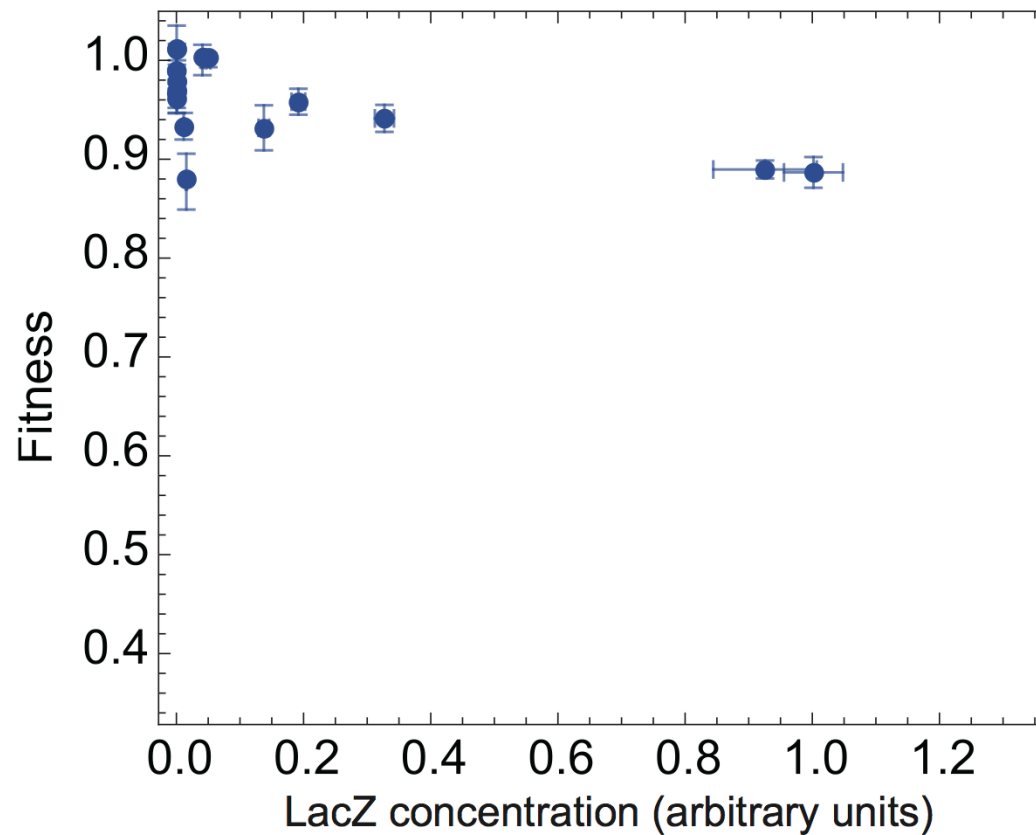


$$W_m = 1 + s_m$$

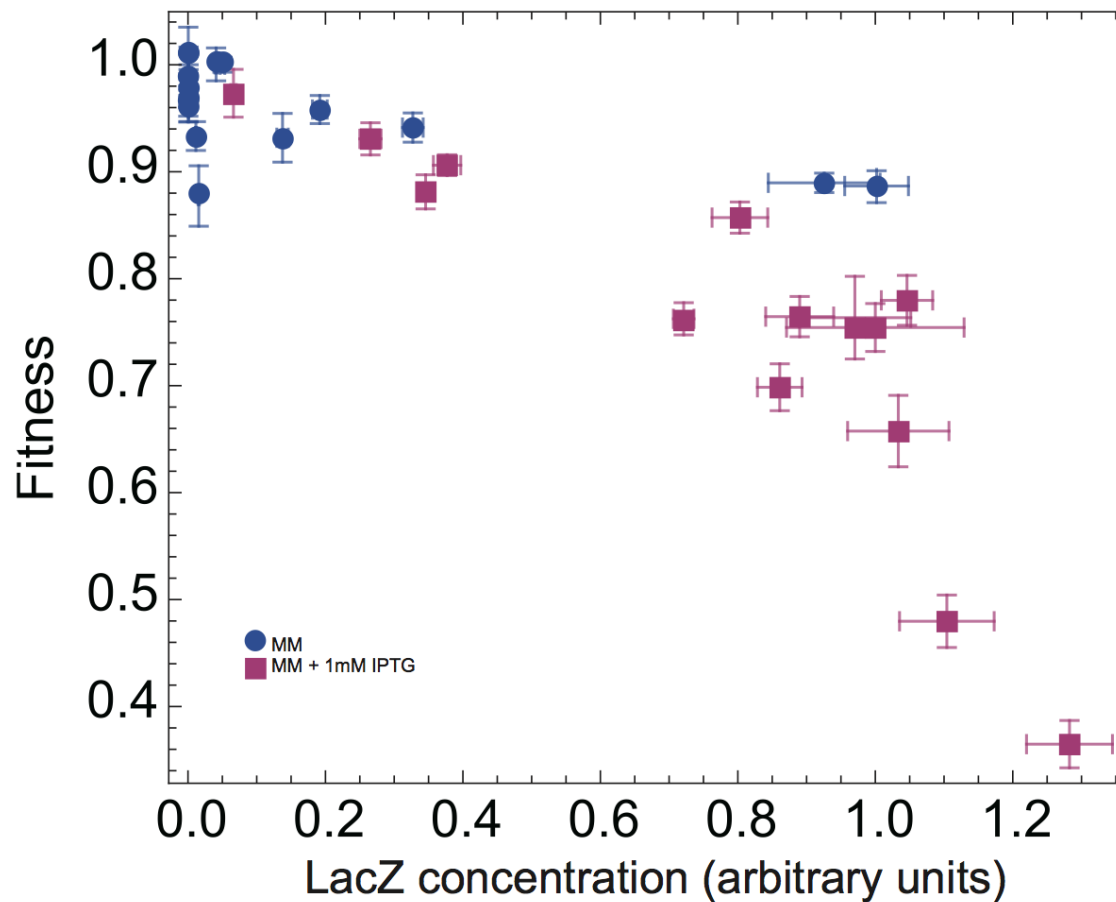
$$W_m = \ln(Nf_m/Ni_m) / \ln(Nf_r/Ni_r)$$

Reference strain (r) -  $\Delta lac/ZYA$

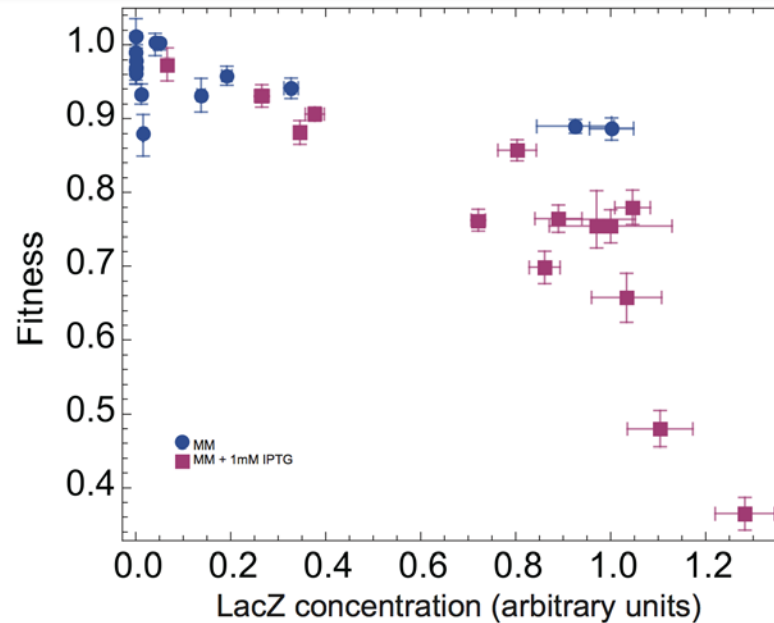
# Fitness as a function of protein expression



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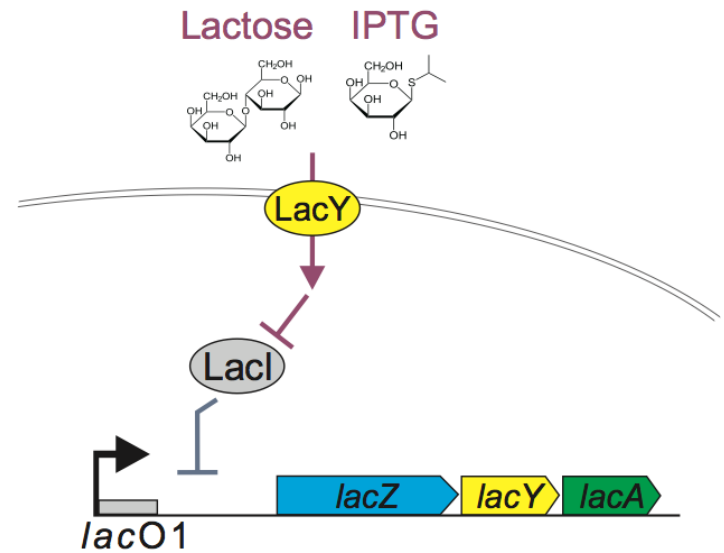
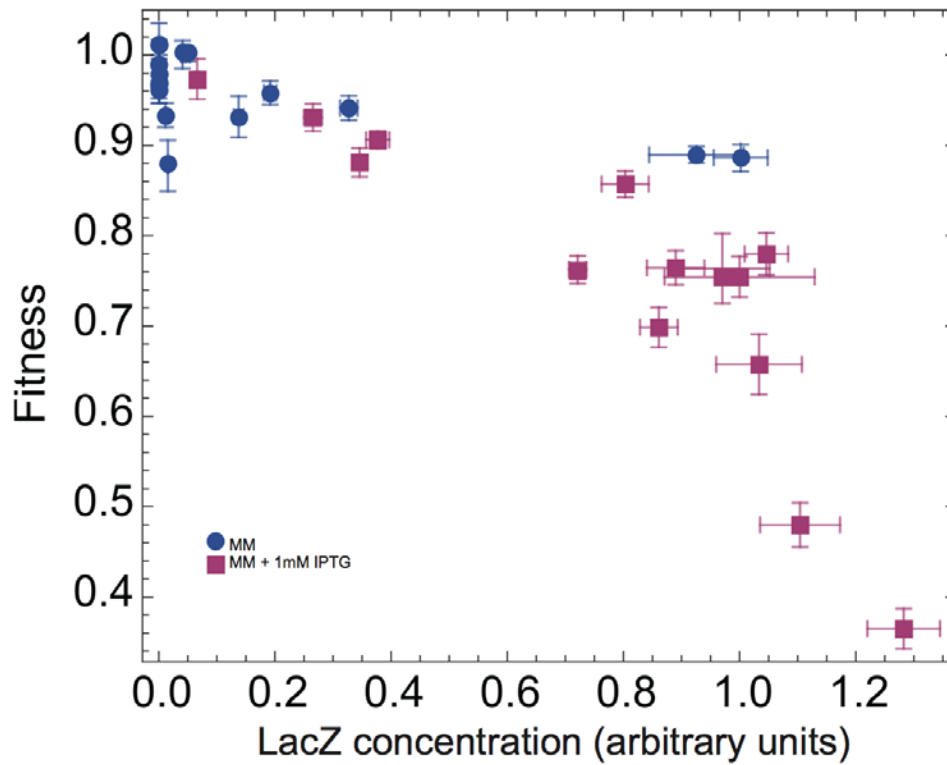


# Fitness as a function of protein expression

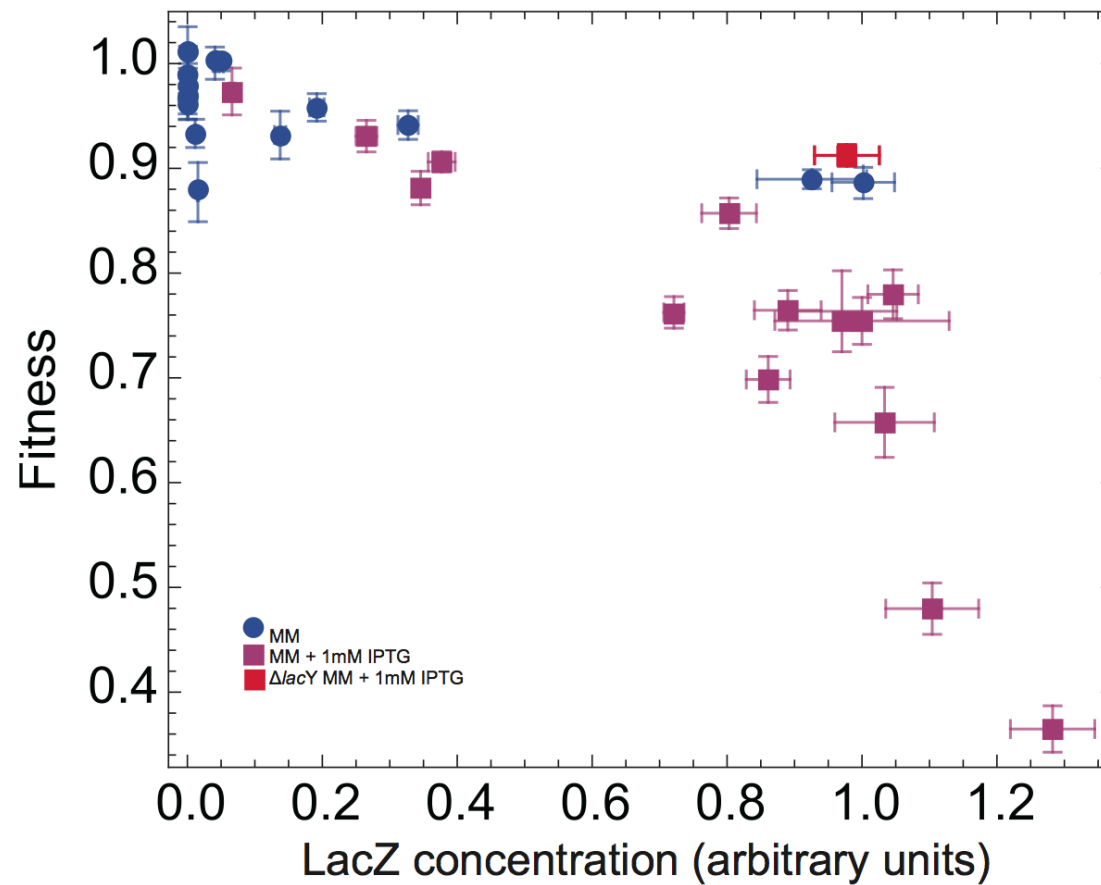


- ✓ Fitness cost of producing the lac proteins
  - linear in the absence of IPTG
  - nonlinear in the presence of IPTG

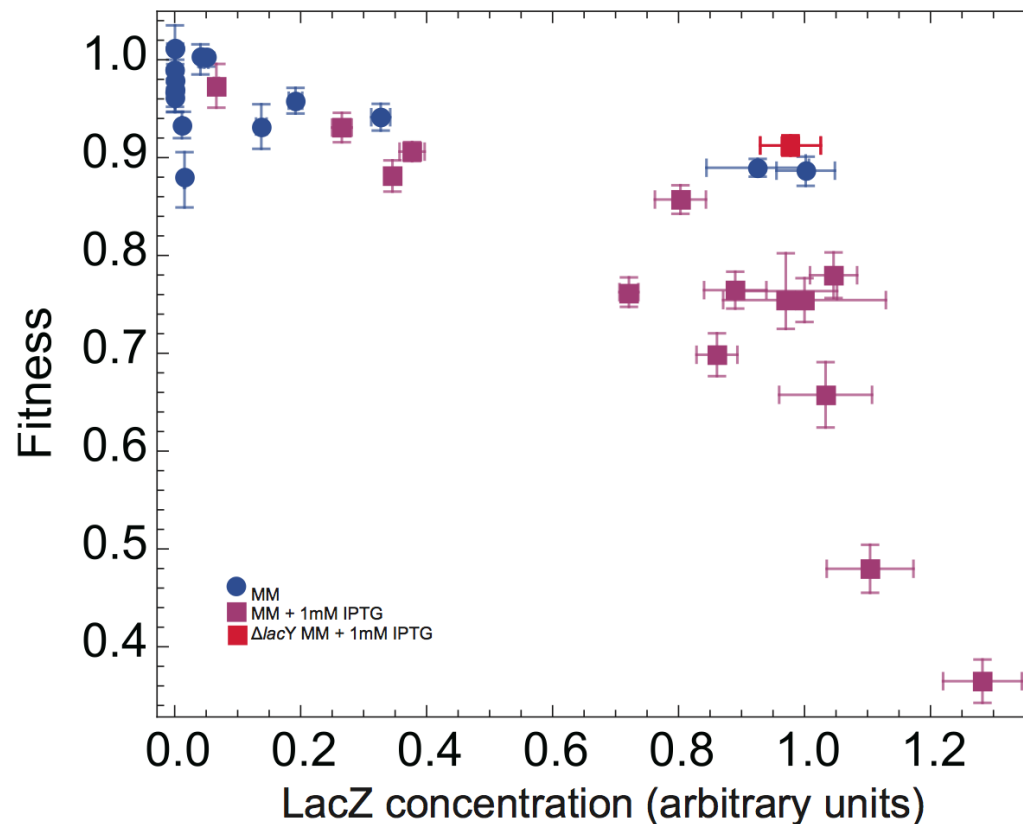
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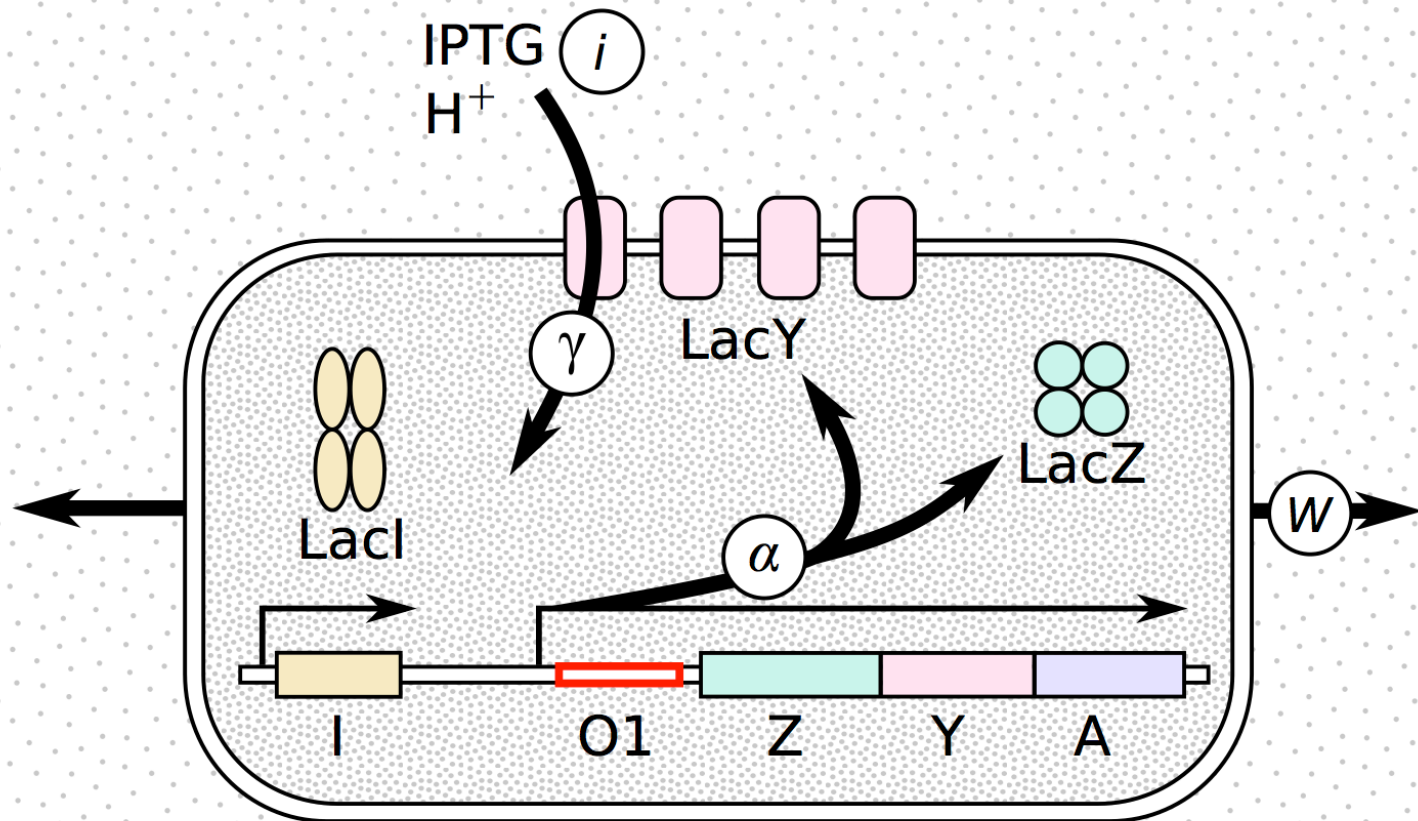
# Fitness as a function of protein expression



- ✓ The extra fitness cost in IPTG is related to the presence of the *lac* permease

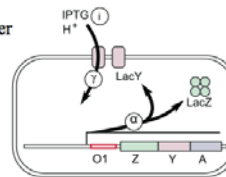


# The relevant parameters



# How phenotype depends on growth

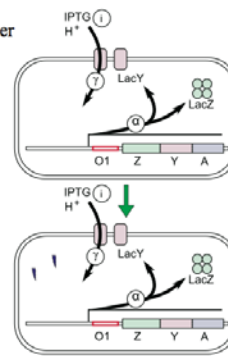
Fast growing mutant  
protein production: 2 permeases per cell  
growth rate: 1 division per time unit  
rate of transport: 1 molecule per permease per  
time unit



1 internal IPTG molecule

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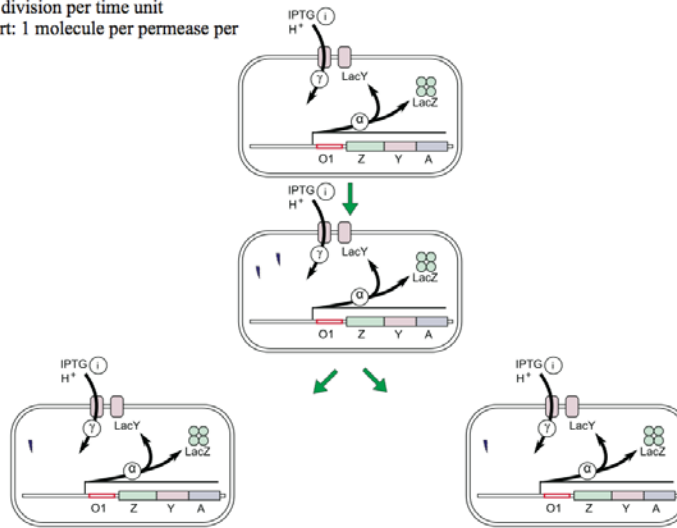


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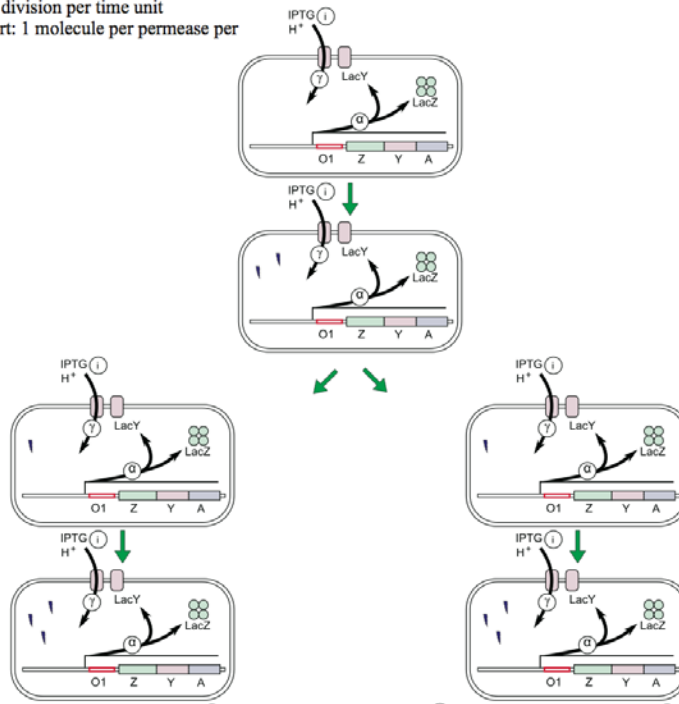
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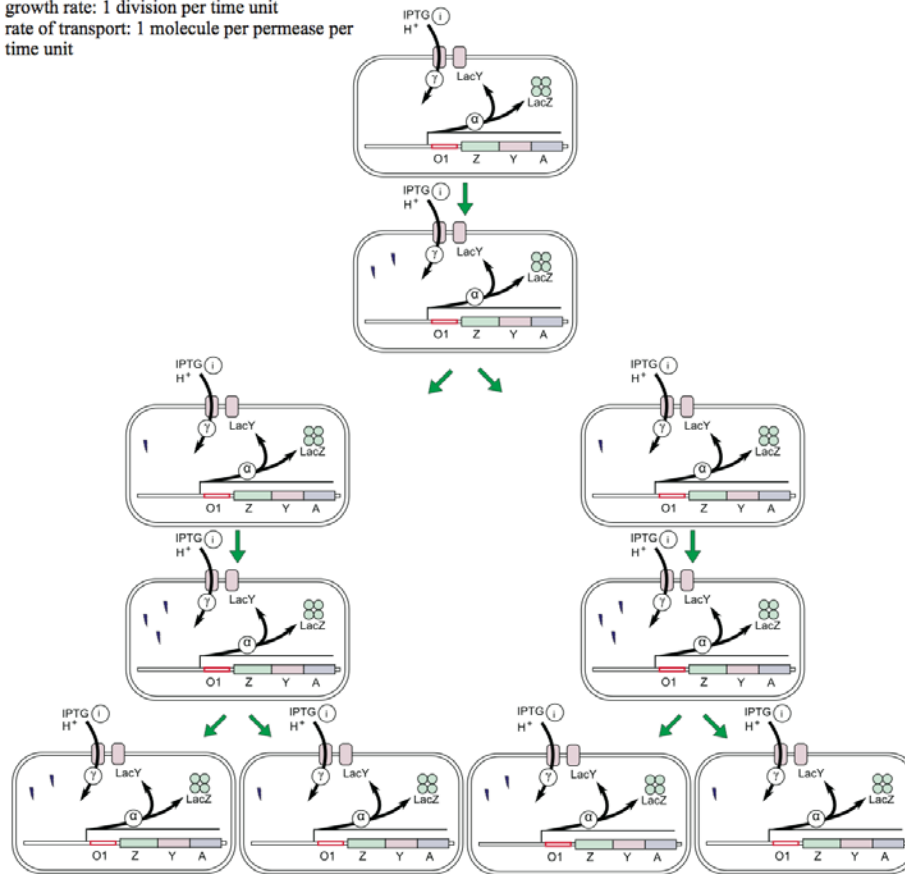
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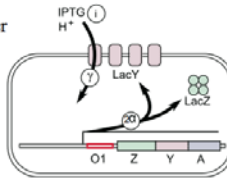
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# How phenotype depends on growth

## Slow growing mutant

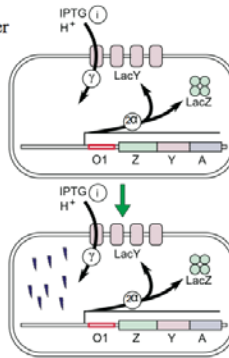
protein production: 4 permeases per cell  
growth rate: 1 division per 2 time unit  
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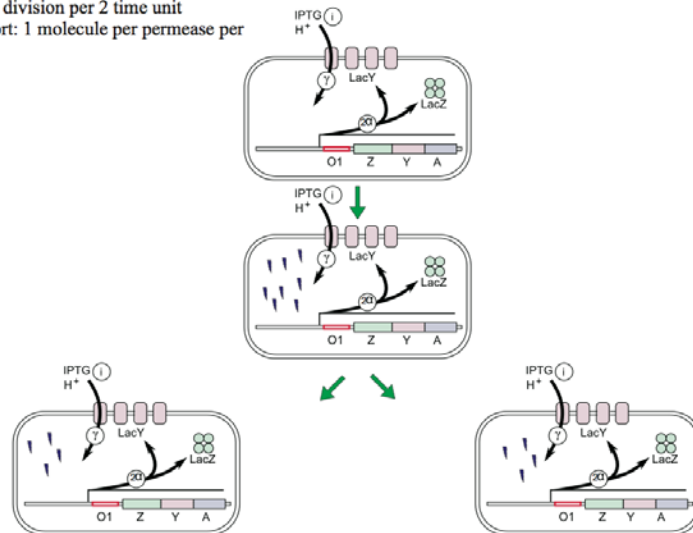
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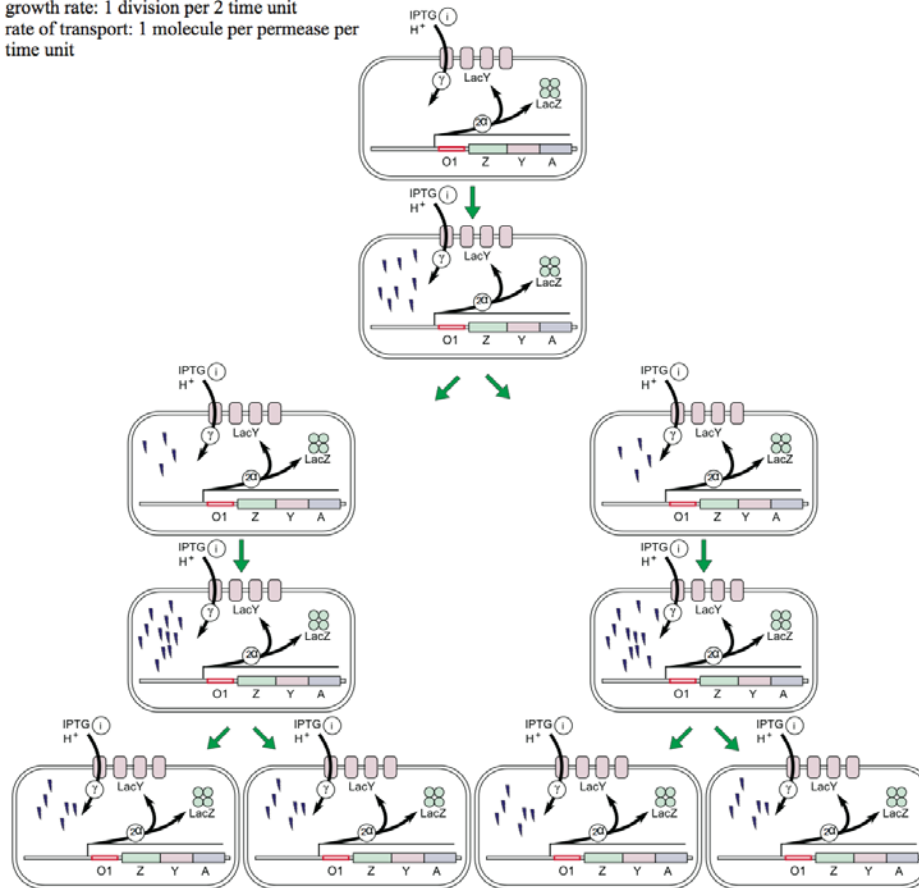
1 internal IPTG molecule



# How phenotype depends on growth

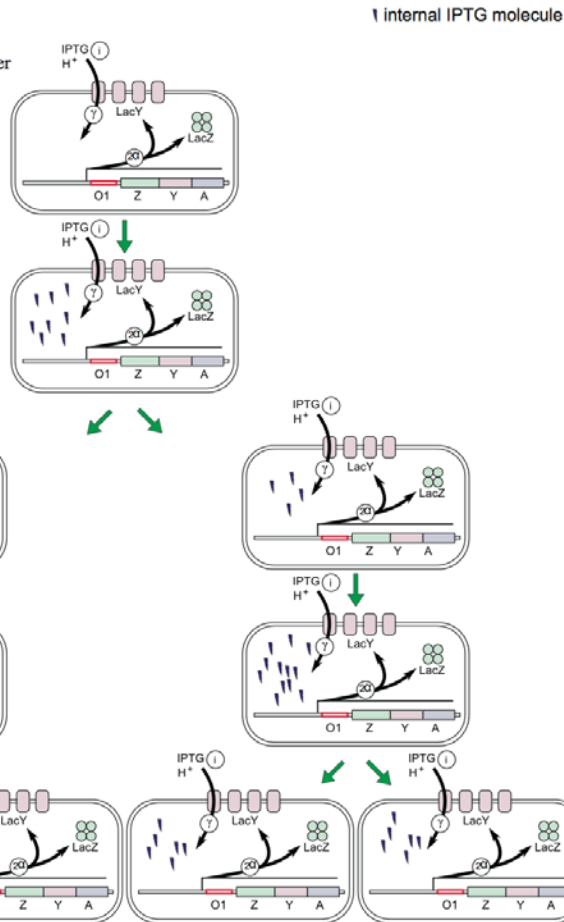
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1 internal IPTG molecule



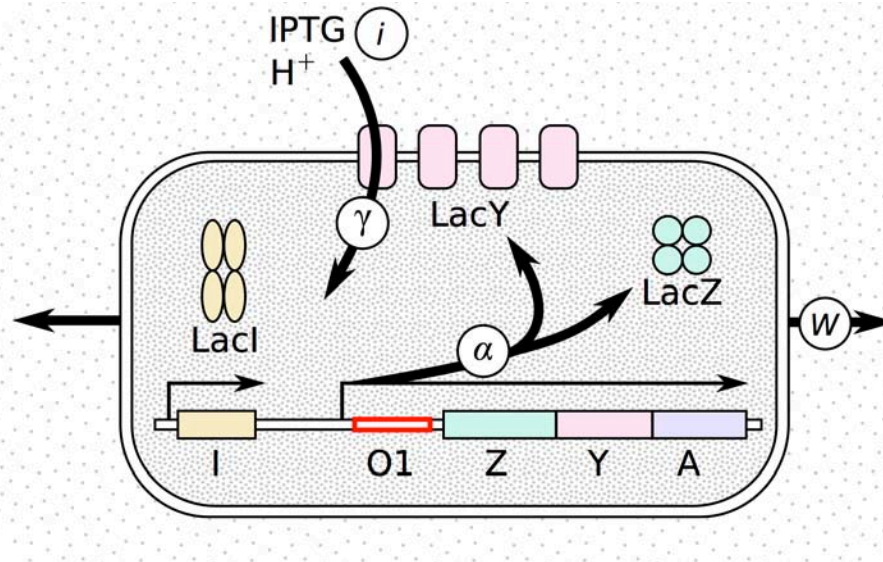
# How phenotype depends on growth

Slow growing mutant  
protein production: 4 permeases per cell  
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- ✓ The concentration of internal molecules depends on growth rate
- ✓ An increase in production/uptake leads to a nonlinear increase in concentration
- ✓ Growth rate and phenotype interact

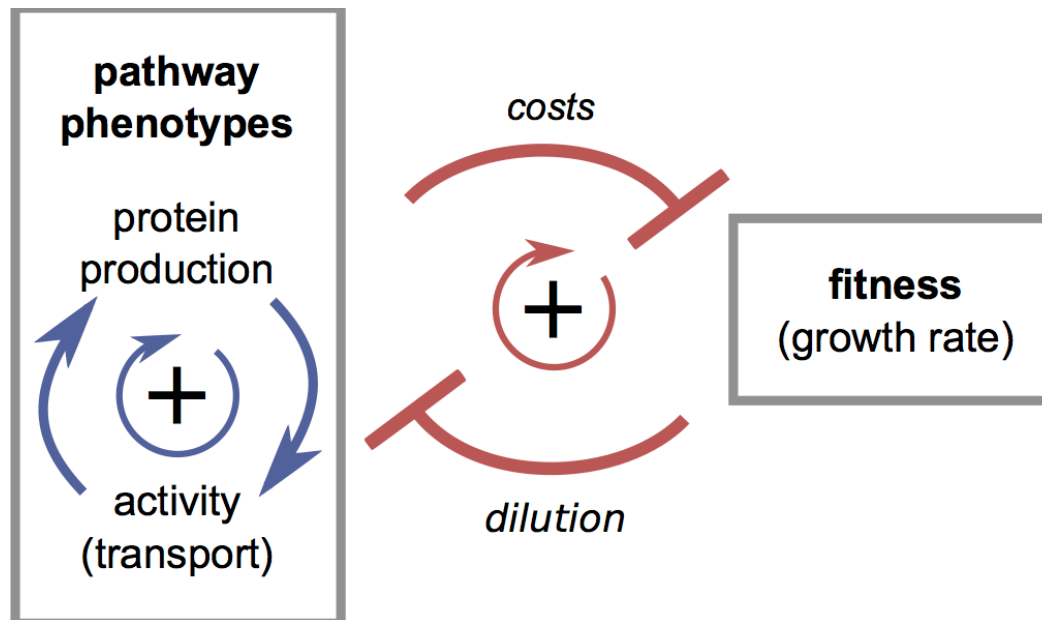
# The relevant parameters



Phenotypes affecting fitness in the lac operon:

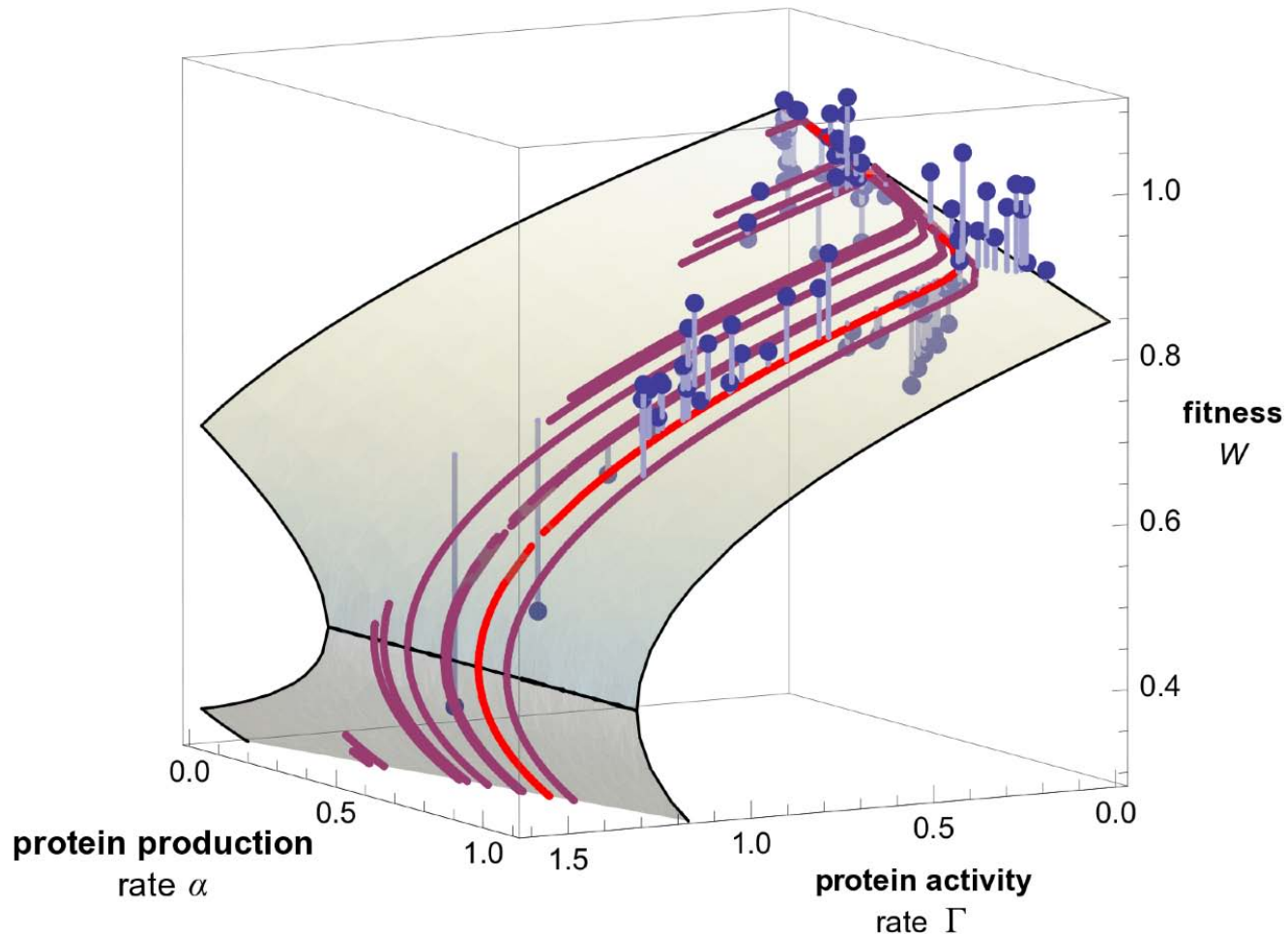
- ✓ Protein production ( $\alpha$ )
- ✓ Protein activity
  - Transport via the lac permease ( $\Gamma = \gamma \times \alpha / W$ )
  - Concentration of IPTG inside the cell ( $\Pi W = \gamma \times \alpha / W^2$ )

# The phenotype-fitness model

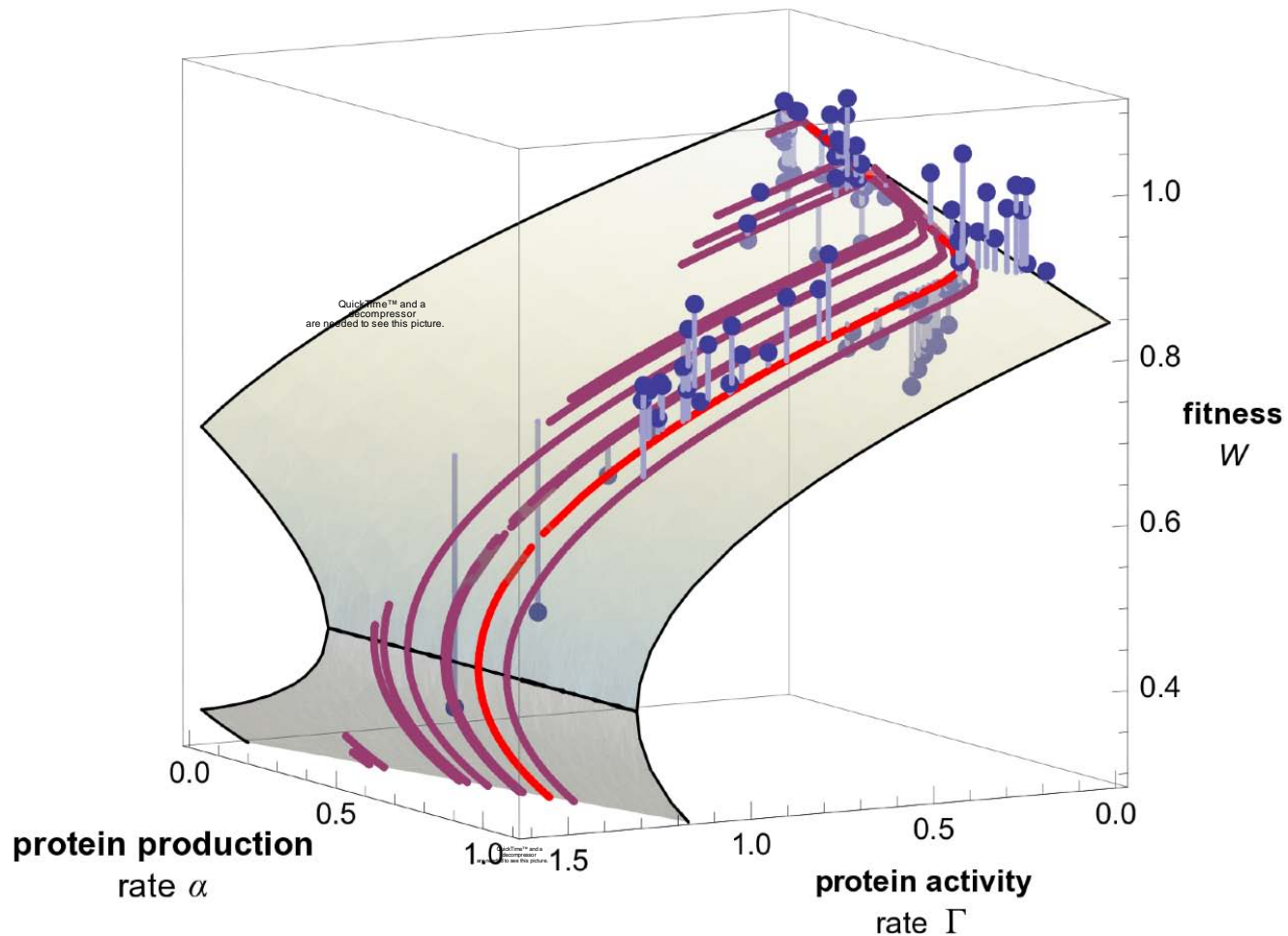


$$W = 1 - \left( a \times \alpha + b \times \frac{\Gamma}{W} \right)$$

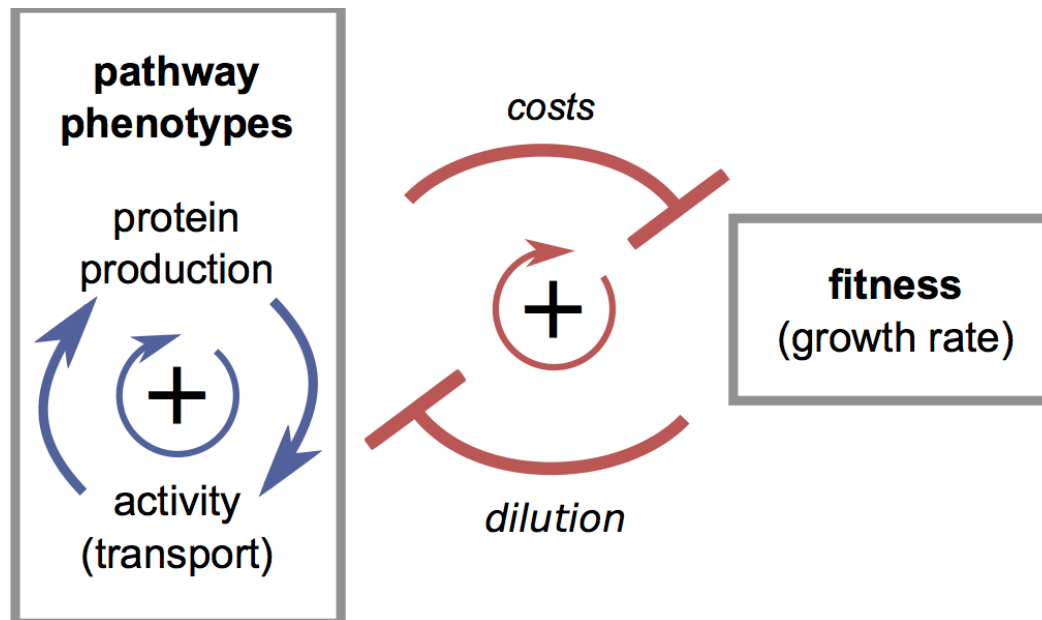
# Fitness as a function of phenotype



# Prediction of an extinction threshold



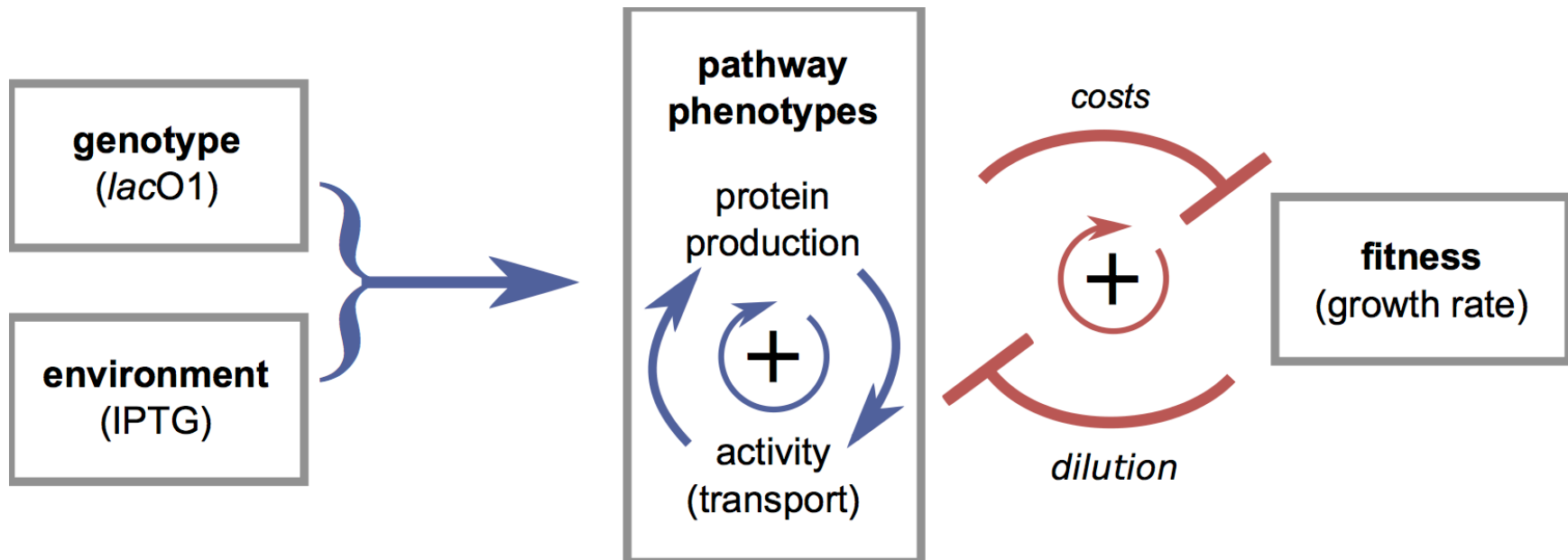
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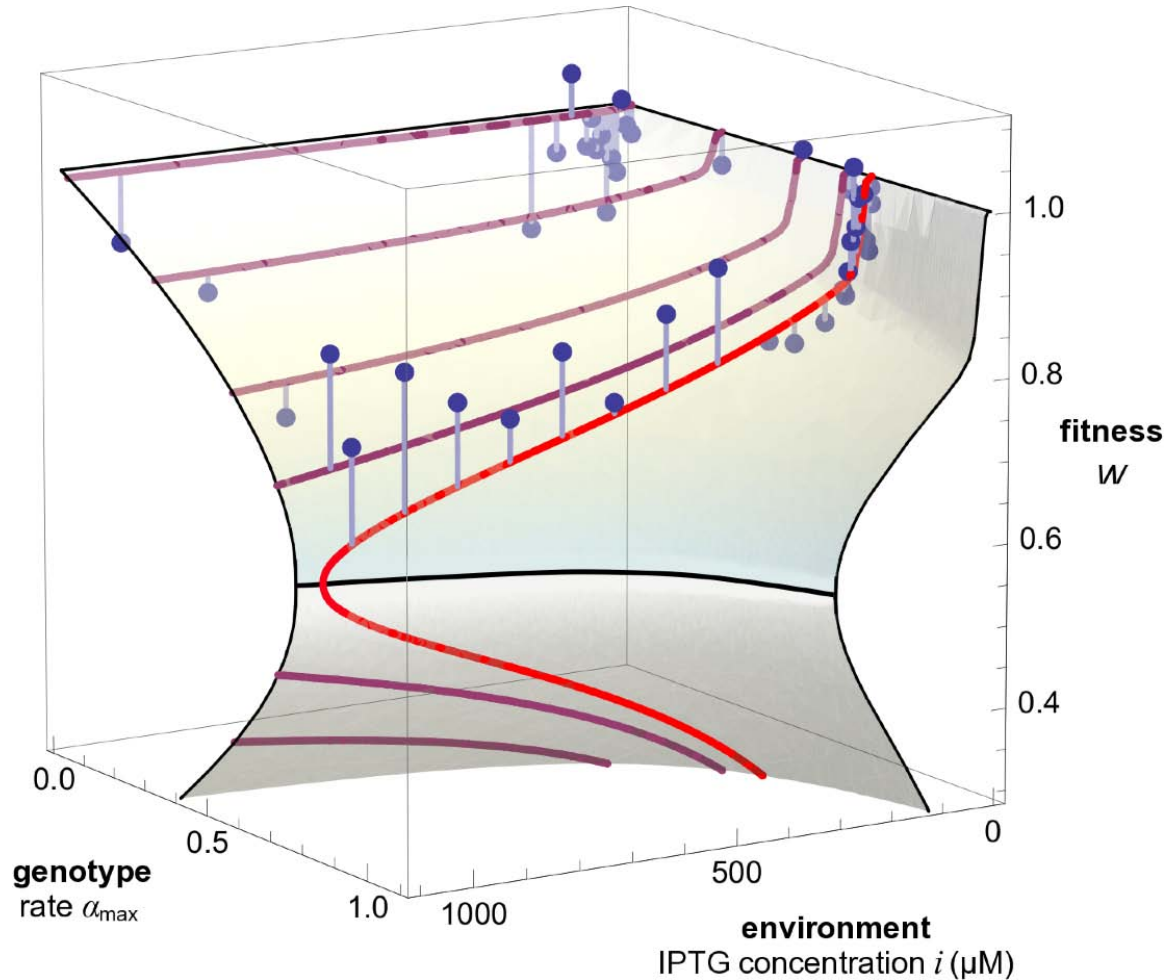


# The phenotype-fitness model



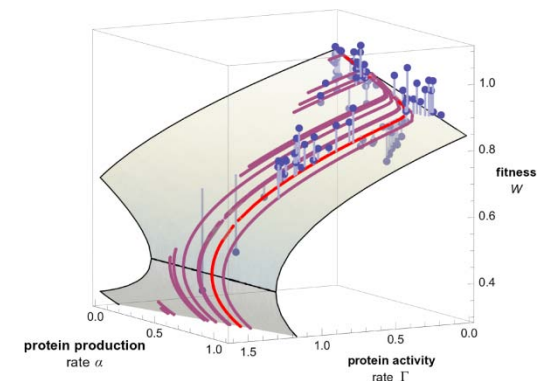
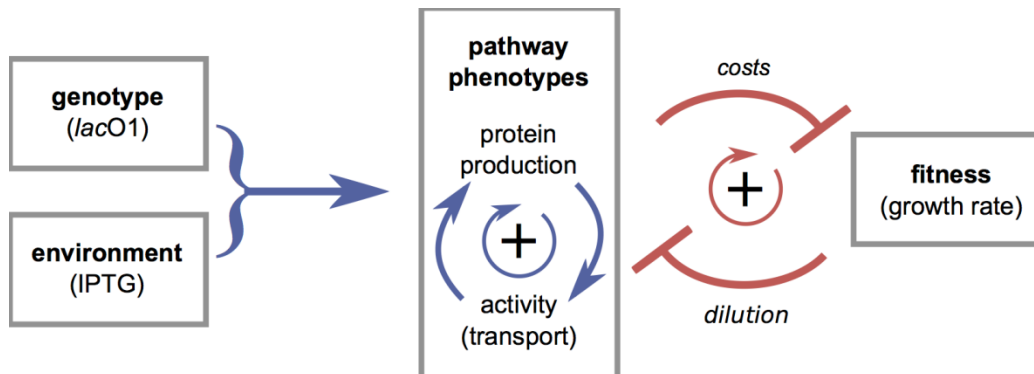
$$W = 1 - \left( a \times \alpha + b \times \frac{\Gamma}{W} \right)$$

# Fitness as a function of the genotype and the environment



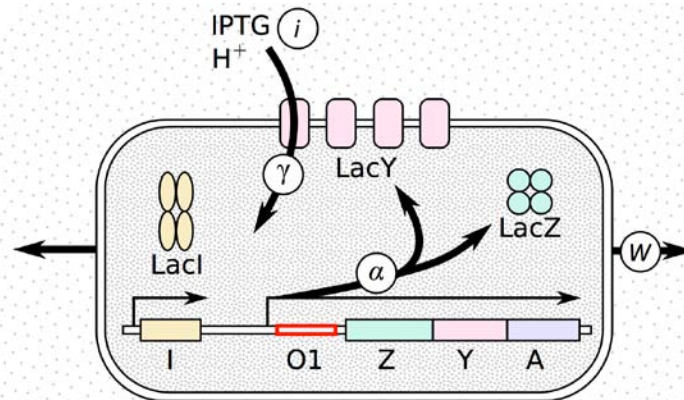
# Summary I - Phenotypic fitness landscapes

- Make predictions beyond measured genotypes (even those that are not viable)
- Show epistasis between phenotypes
- Predict how the environment changes fitness and in which direction
- Smoother, with less dimensions but still with striking features such as extinction cliffs



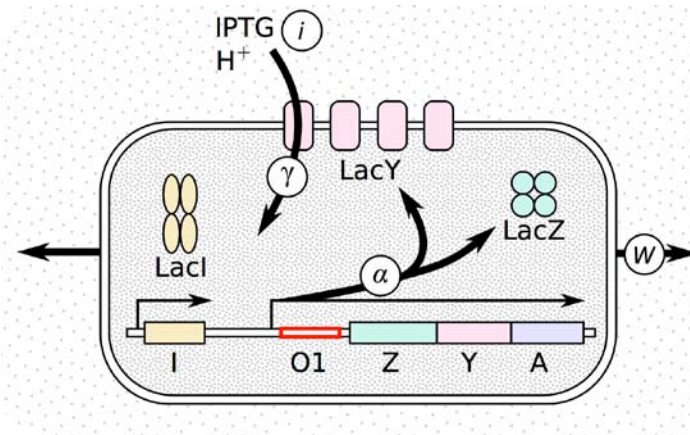
# Summary II - How much can we generalise?

- Lactose has the same cost as IPTG (can show you the data later)
- The benefit brought by lactose adds another dimension to the landscape
- The full landscape still has cliffs but they are in different places in parameter space - there is no optimum expression value
- Bacteria have several transporters like LacY

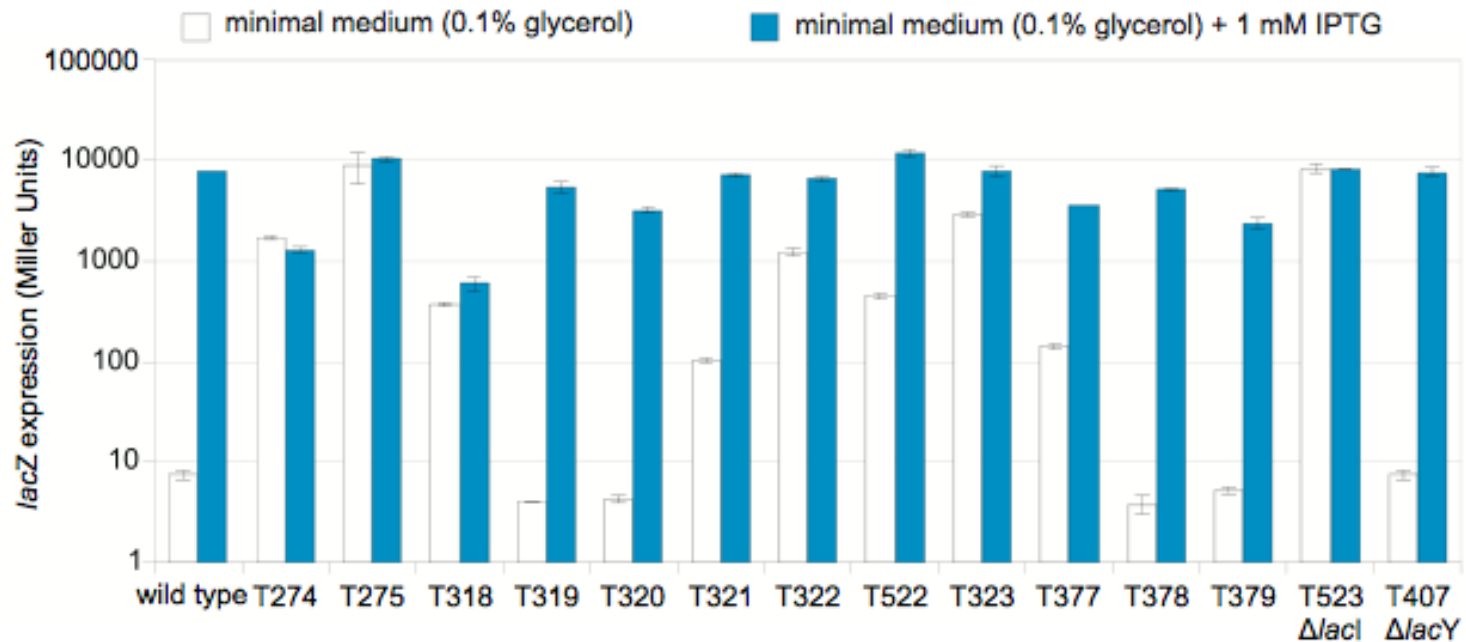


# Summary III - Population features

- Amplification and maintenance of phenotypic variability
- Strong epistasis between mutations (coupled by growth rate), leading to lethality of some combinations



# The mutants



Strain	<i>lacO1</i> allele	Sequence
BW30270	<i>lacO1</i> (wild type)	AATTGTGAGCGGATAACAATT
T274	<i>lacO1</i> -20R	ATCGCGACTGTCCACTGTGCA
T275	<i>lacO1</i> -20GCW	AGTGTCAATATACATCGAATG
T318	<i>lacO1</i> -20GCI	AATGCCACAGTCGCTCACCGG
T319	<i>lacO1</i> -SN2	AATTGTGAGCGGATAACAATT
T320	<i>lacO1</i> -SN3	AACTGTGAGCGGATAACAATT
T321	<i>lacO1</i> -SN4	AATGGTGAGCGGATAACAATT
T322	<i>lacO1</i> -SN5	AATTCTGAGCGGATAACAATT
T522	<i>lacO1</i> -SN7	AATTGTΔAGCGGATAACAATT
T323	<i>lacO1</i> -SN8	AATTGTGCGCGGATAACAATT
T377	<i>lacO1</i> -SN9	AATTGTGATCGGATAACAATT
T378	<i>lacO1</i> -SN12	AATTGTGAGCGGATAACAATT
T379	<i>lacO1</i> -SN19	AATTGTGAGCGGATAACAATT

Chromosomal mutants (method adapted from Datsenko&Wanner2000)

# Lactose is also associated with a cost

