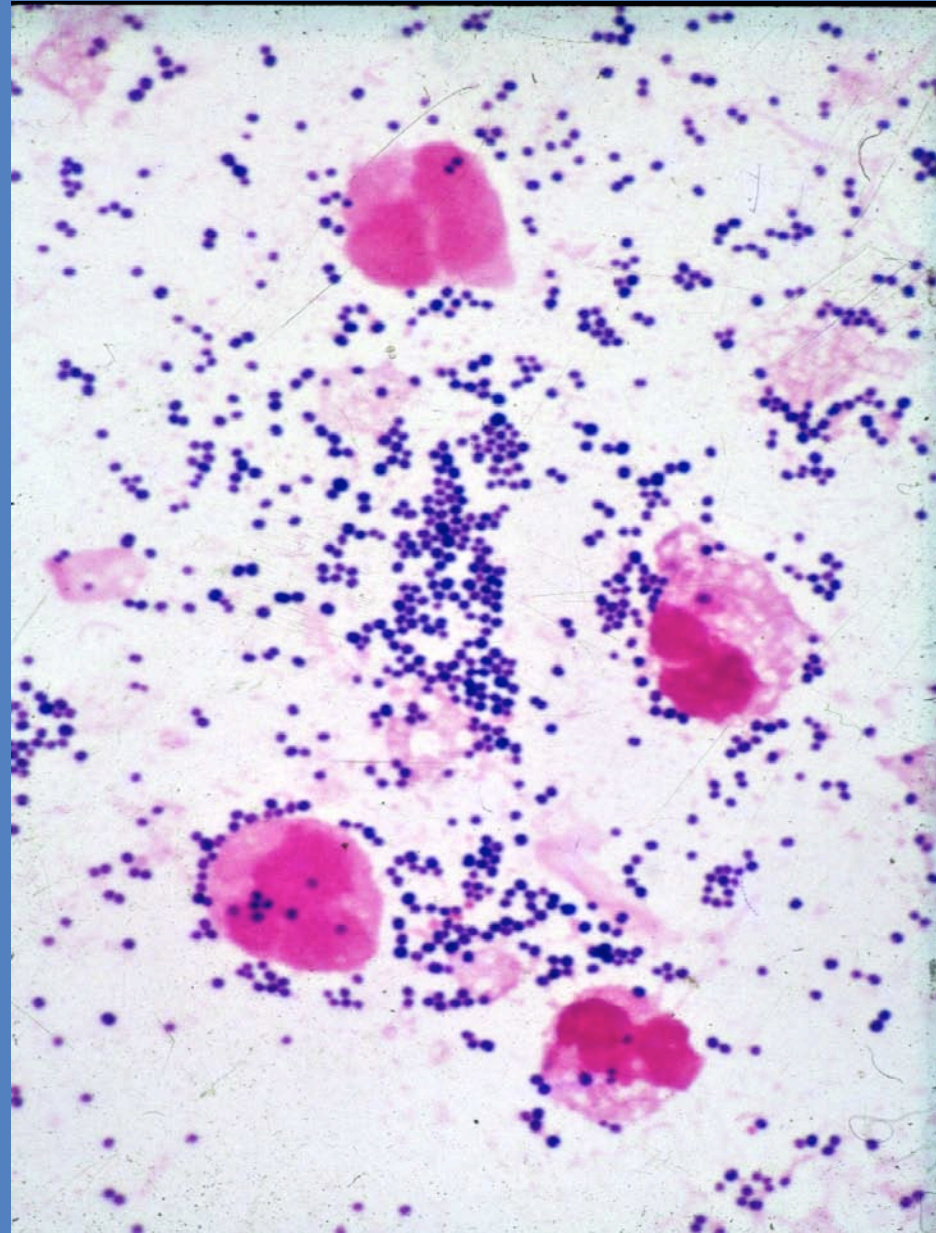


***Staphylococcus aureus* background  
and experimental model for gene  
expression analysis**

Keith T. Holland

*S.aureus* in a  
pus sample



CLOSTRIDIUM GROUP FIRMICUTES (LowG+C)

BACILLACEAE

***Staphylococcus***

## DISTRIBUTION OF STAPHYLOCOCCI

35 species

10 man

Poultry, goats, primates, dolphins, cattle, horses, pigs, sheep, dogs, cats, rodents, flies.

***S. aureus* - opportunistic pathogen of man and animals.**

*Coagulase* -ve staphylococci:- Primary mode of existence is as commensals.

# SKIN CARRIAGE %

SITE	INFANTS	YOUNG ADULTS	OVER 60
Axilla	6	4	9
Toeweb	23	9	5
Forehead	39	16	14
Perineum	40	20	13

**Much higher for skin with perturbed barrier function**

# NASAL CARRIAGE

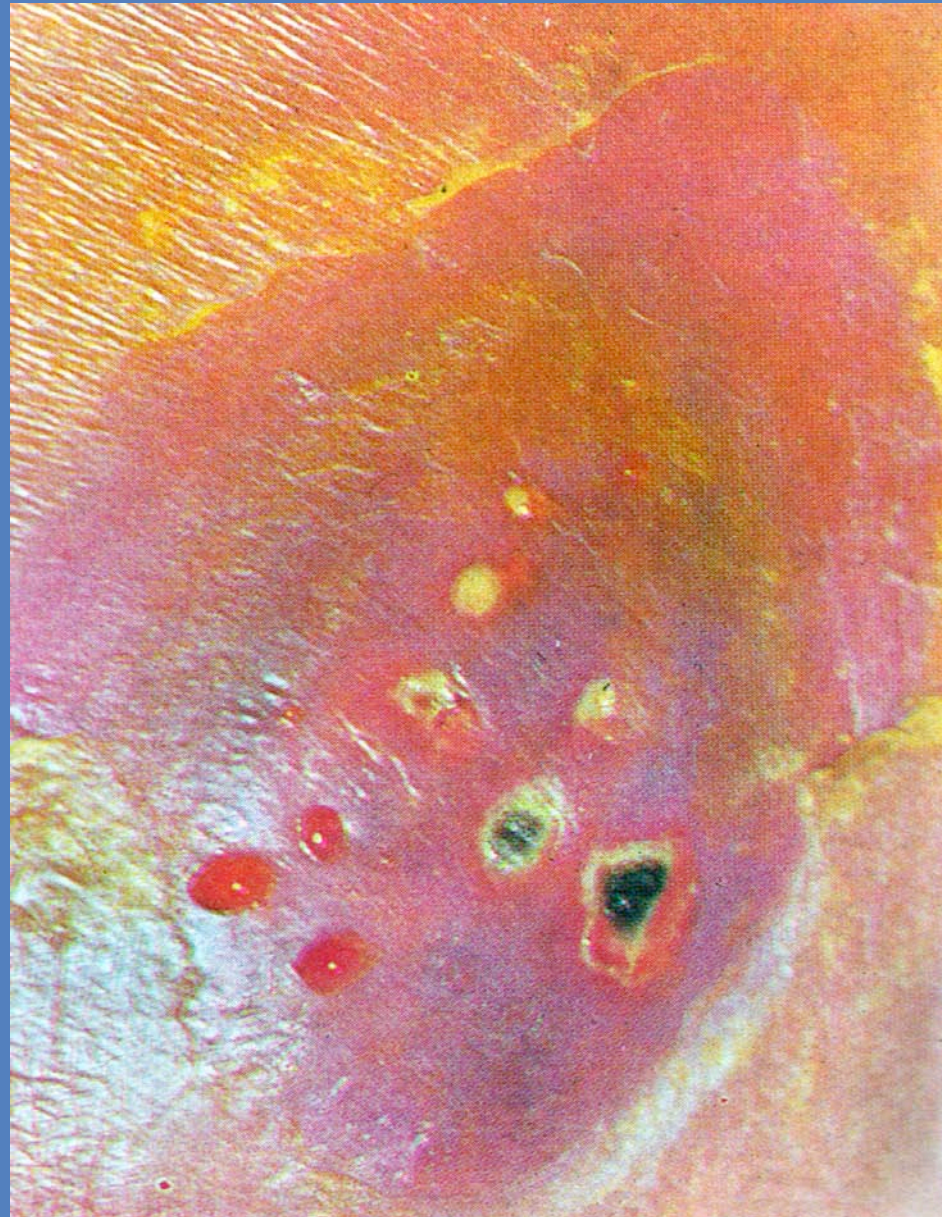
Natural habitat of *S. aureus*

	Carrier		
	Non	Intermittent	Persistent
Occasion %	0	1-10	>80
Population %	50	20	30
Risk (bacteraemia)	lower	lower	higher
Mortality	higher	higher	lower
Density	0	lower	higher

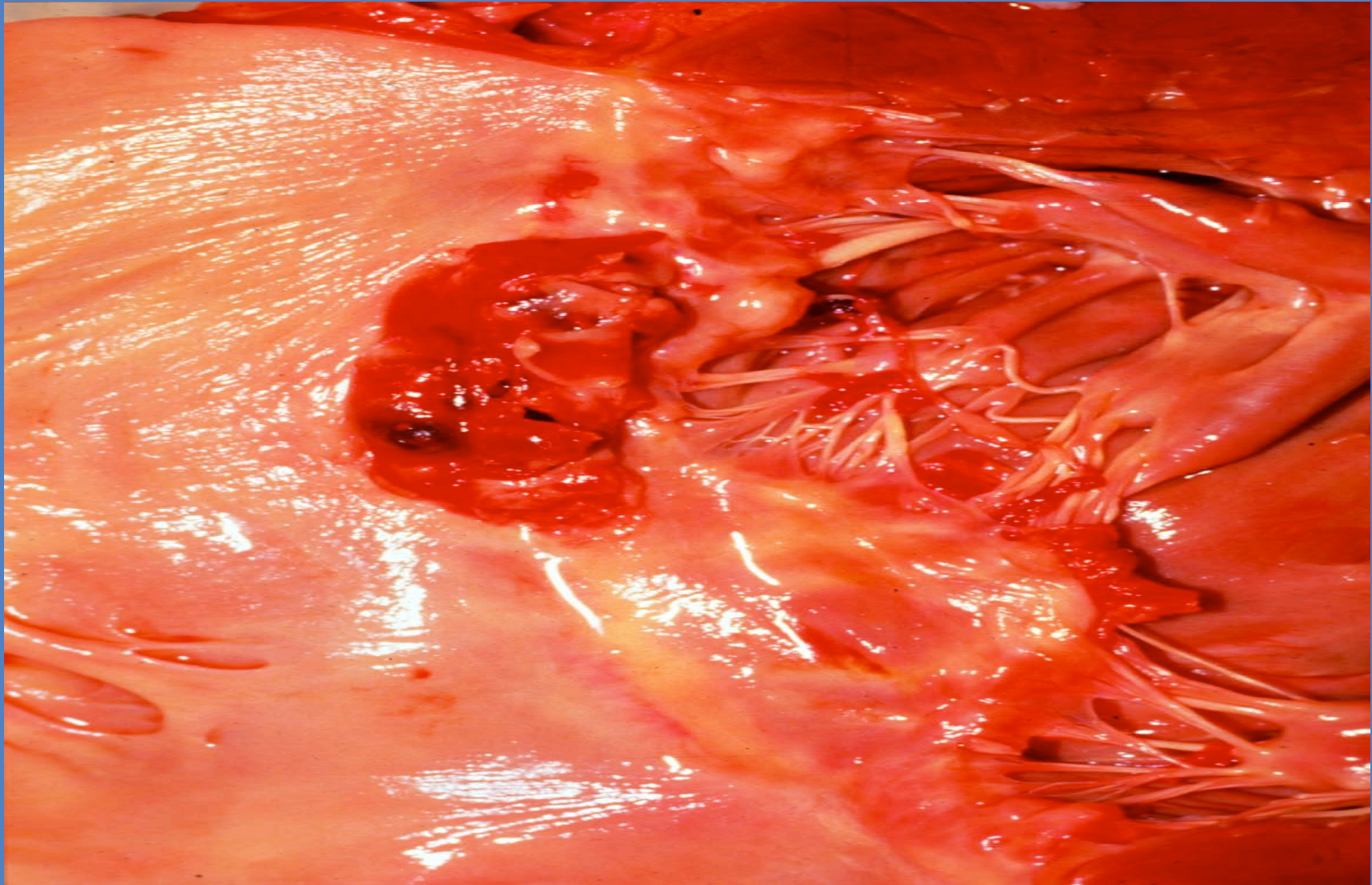
## IMPETIGO



## CARBUNCLE



## ENDOCARDITIS



## **DISEASES CAUSED BY *S. aureus***

**Skin infections, e.g. boils, impetigo, furunculosis, toxic epidermal necrolysis**

**Endocarditis (Drug addicts, open heart surgery)**

**Eye infections - conjunctivitis, styes**

**Osteomyelitis (in growing bones of children)**

**Food poisoning (ingestion of preformed toxins)**

**Toxic Shock Syndrome**

**Lung**

**Bacteraemia**

**Rarely implicated in meningitis, brain abscesses, arthritis**

## EXOENZYMES & TOXINS OF *S. aureus*

Coagulase

Phosphatase

Leucocidins

Fibrinolysin

Proteinases

Haemolysins

Lipases

Hyaluronate lyase

DNA'se

Lecithinase

Staphylokinase

Collagenase

Exfoliative Toxins

Heat-stable enterotoxins

Toxic Shock Syndrome Toxin-1

## ADHESINS

Specific molecules on the surface to bind the cell to a surface.

**Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMS)**

Strong and specific for a ligand

Fibrinogen

Collagen

Thrombospondin

Fibronectin

Elastin

Osteoparitin

Vitronectin

Laminin

Sialoprotein

## STRAINS OF *S. aureus*

Multi locus sequence typing MLST

Virulence genes variations

Antibiotic genes variations - MRSA

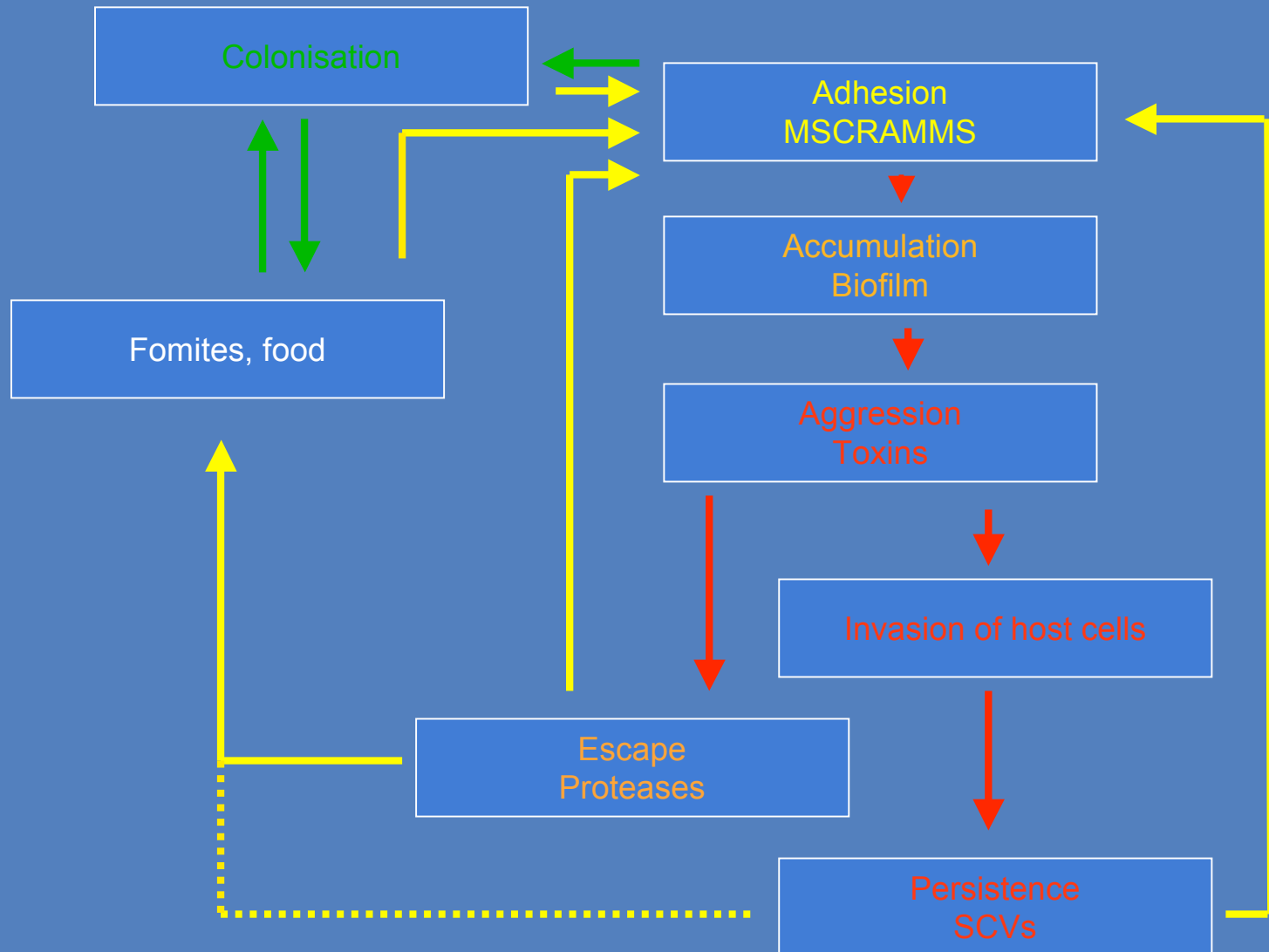
The Health Improvement and Protection Bill - Hygiene code for hospitals

Mutants

Small colony variants

*agr* negative

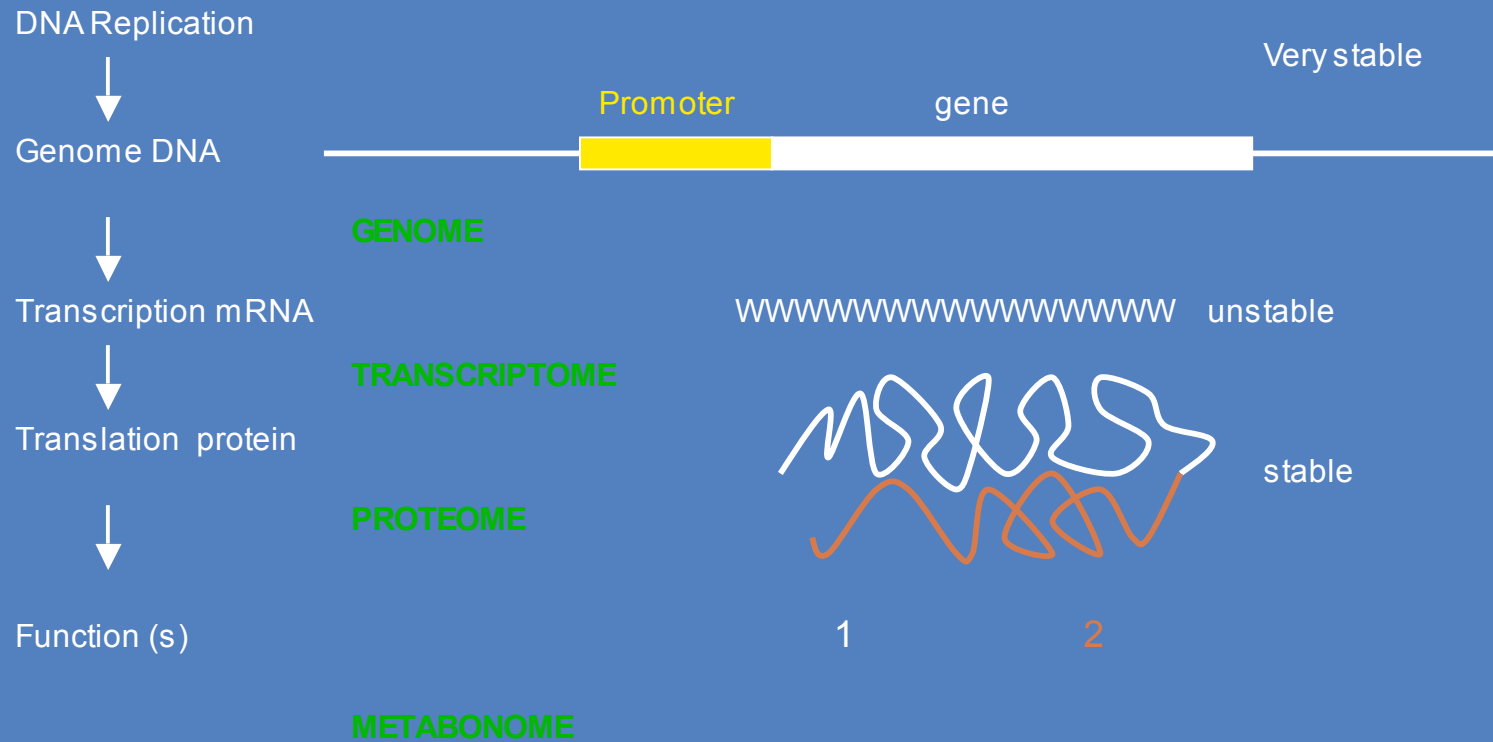
### LIFE CYCLE



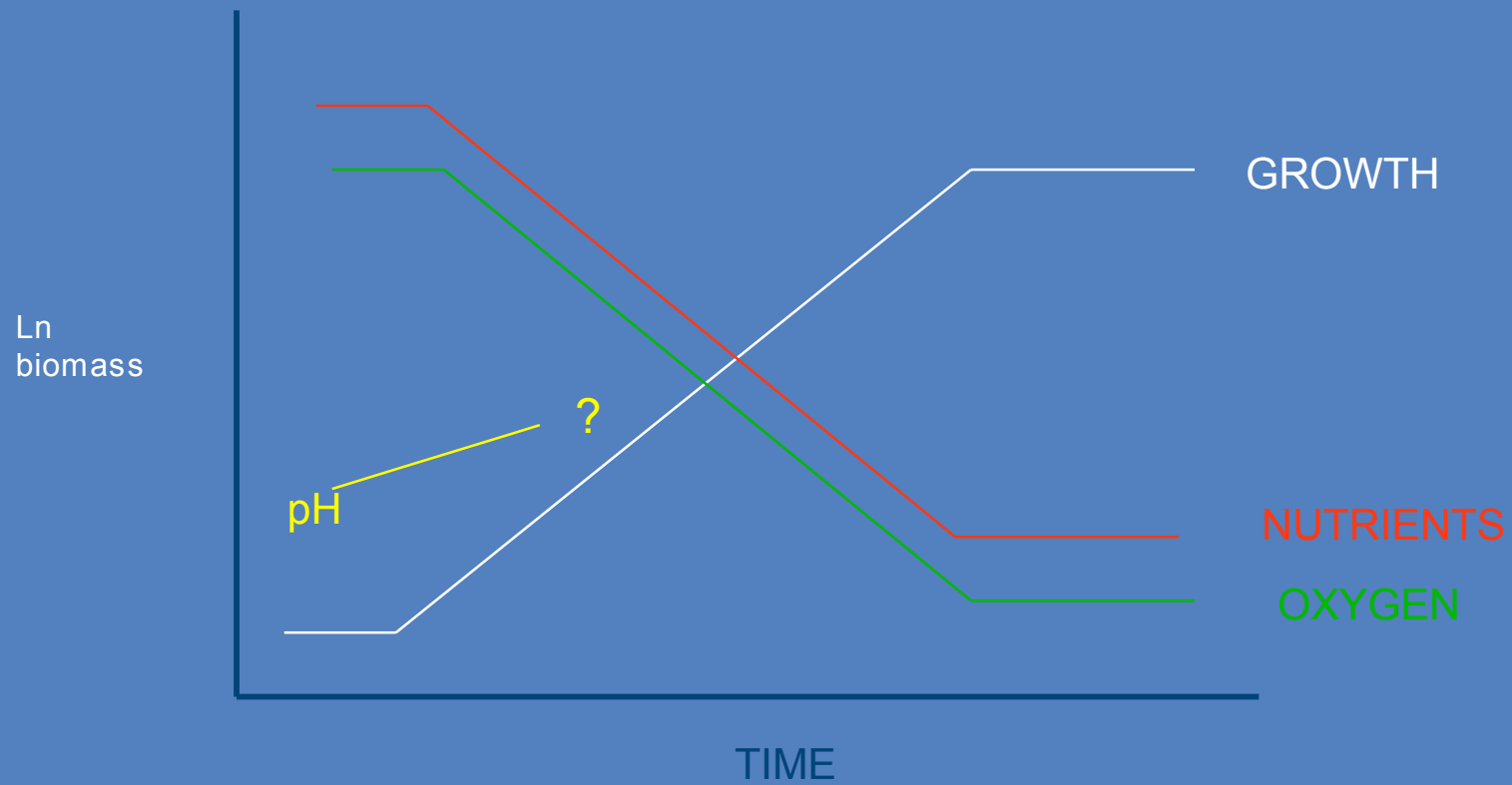
## SUMMARY

- Relevant and important microorganism
  - hospital acquired infections
  - community acquired infections
  - antibiotic resistance (new targets)
- Complex interactions
  - Pathogen- many diseases
  - coloniser - many sites
- Complex array of virulence/ colonisation factors (genes)
- Complex intelligence network, endogenous and exogenous signals
- Complex regulatory network
- Not **ONE** *S.aureus*

# GENE TO FUNCTION



## CLOSED CULTURE GROWTH MODEL



## GENE REGULATORY NETWORKS

- Closed culture
- Virulence
- Gene knock-outs

accessory gene regulator - endogenous signals, cell density

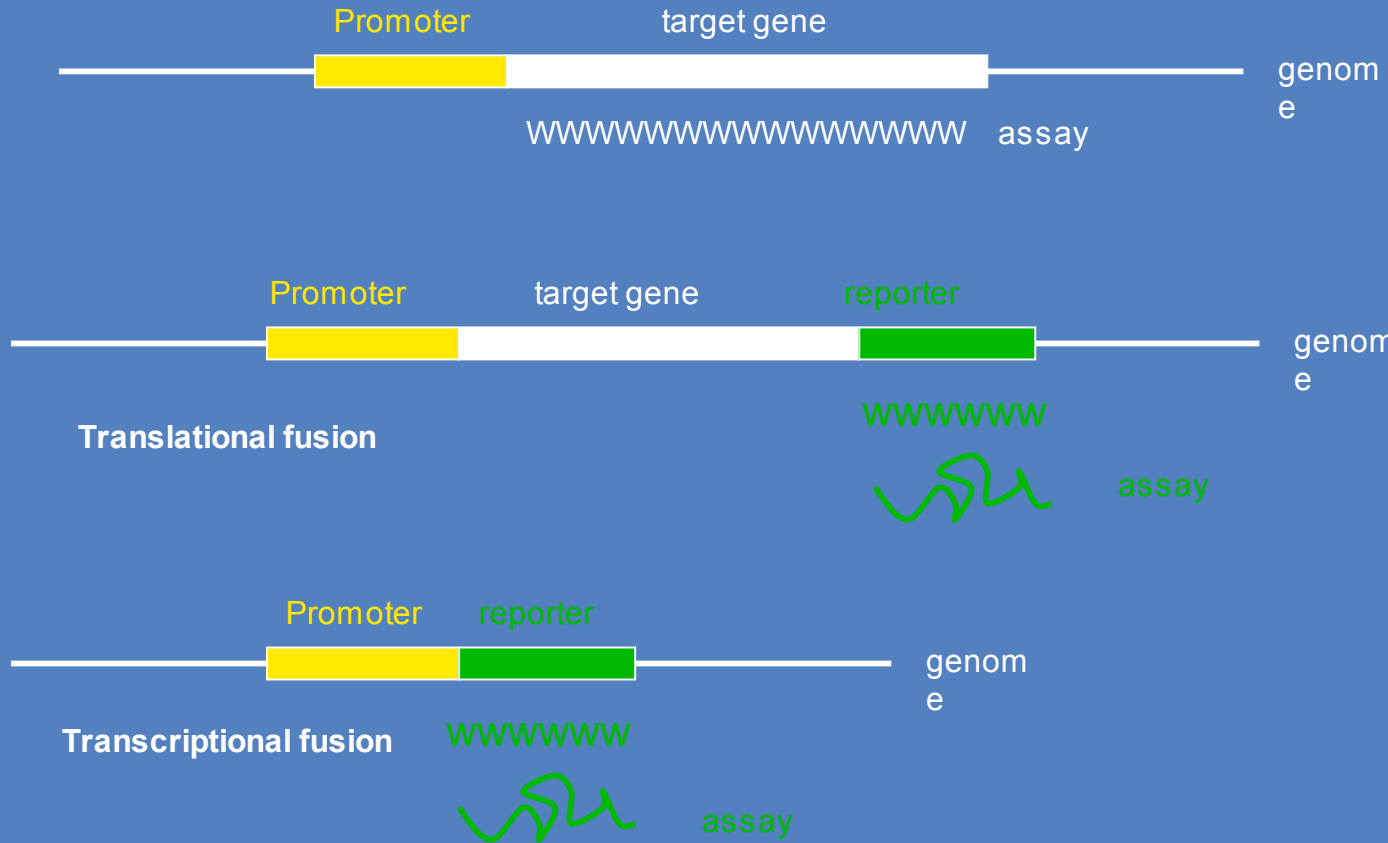
staphylococcal accessory regulator - exogenous signals?

sigma factor B - endogenous, metabolic stress

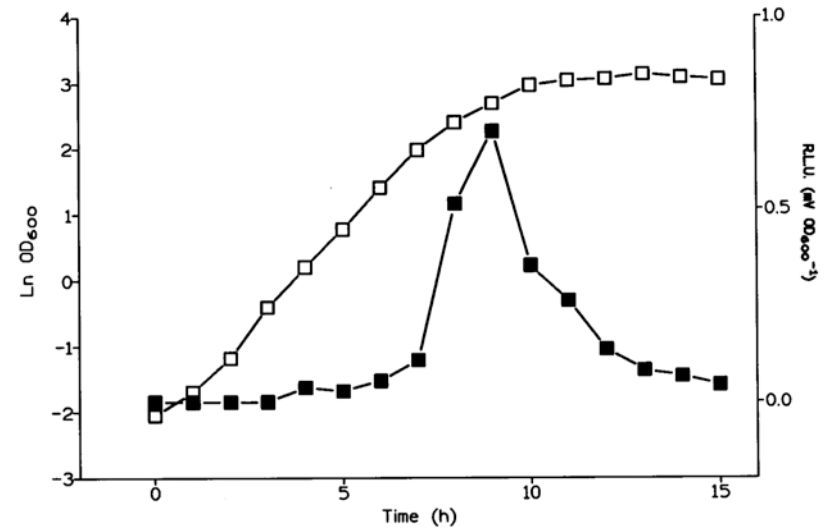
- exogenous, environmental stress

- Hierarchy?
- Others?
- Integration of signals?

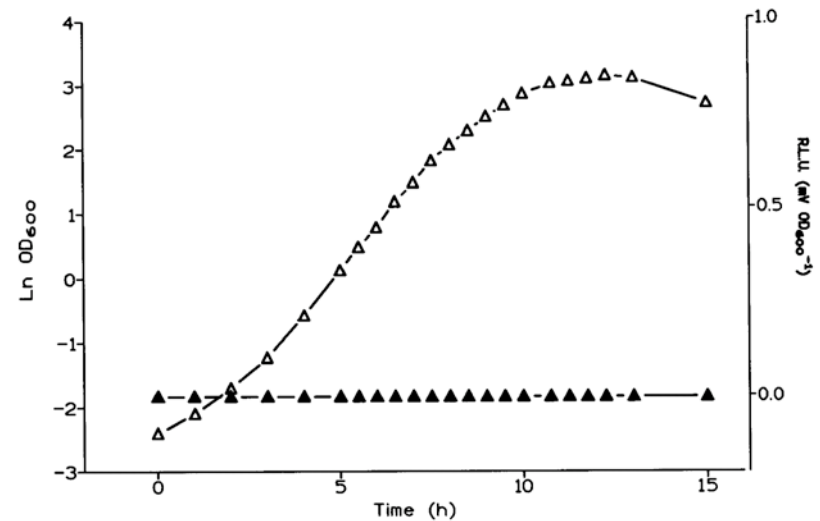
## TRANSCRIPT ANALYSIS



EXPRESSION OF *TST* IN *S. AUREUS*  
AS REPORTED BY *LUX* ON A PLASMID



CONTROL INVERTED *TST* PROMOTER



# REGULON MUTANTS - EFFECT ON VIRULENCE GENE EXPRESSION IN CLOSED CULTURE

*agr*

*hys* down regulation +

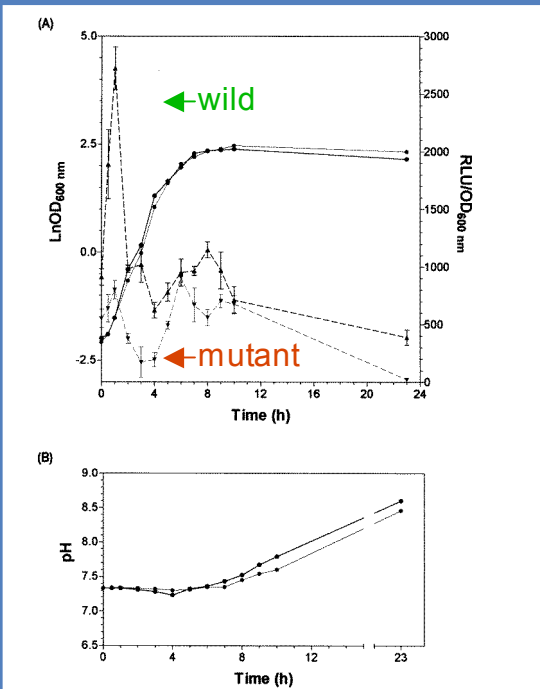


Figure 5.4 Role of *agr* in the regulation of *hysA* expression during batch culture. (A) *S. aureus* w.t. (●, ▲) and WA250 (●, ▼) carrying plasmid pGMLUX01 were grown in CDML supplemented with chloramphenicol (10 $\mu\text{g ml}^{-1}$ ); 2L baffled flasks, 160rpm, 37°C. (●, ●) In optical density reading at 600nm; (▲, ▼) Relative Light Units unit OD<sub>600</sub><sup>-1</sup> (B) pH of w.t. (●) and WA250 (●) cultures during growth.

*sarA*

*hys* up regulation -

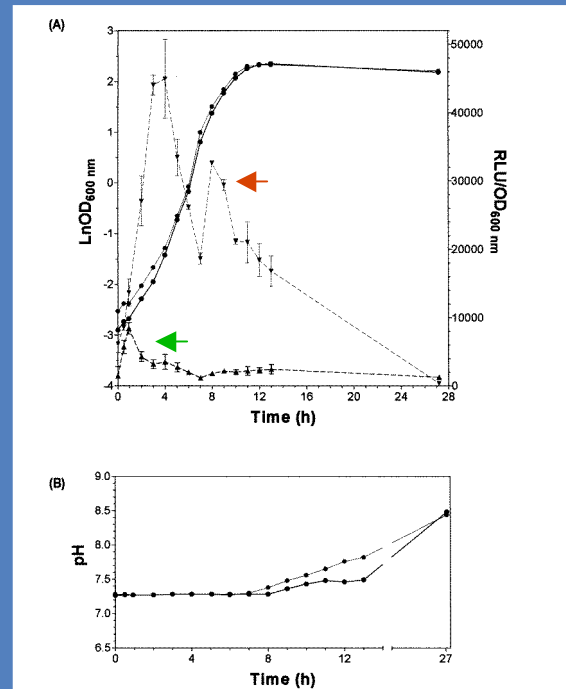


Figure 5.5 Role of *sarA* in the regulation of *hysA* expression during batch culture. (A) *S. aureus* w.t. (●, ▲) and PC1839 (●, ▼) carrying plasmid pGMLUX01 were grown in CDML supplemented with chloramphenicol (10 $\mu\text{g ml}^{-1}$ ); 2L baffled flasks, 160rpm, 37°C. (●, ●) In optical density reading at 600nm; (▲, ▼) Relative Light Units unit OD<sub>600</sub><sup>-1</sup> (B) pH of w.t. (●) and PC1839 (●) cultures during growth.

*agr/sarA*

*hys* up regulation -

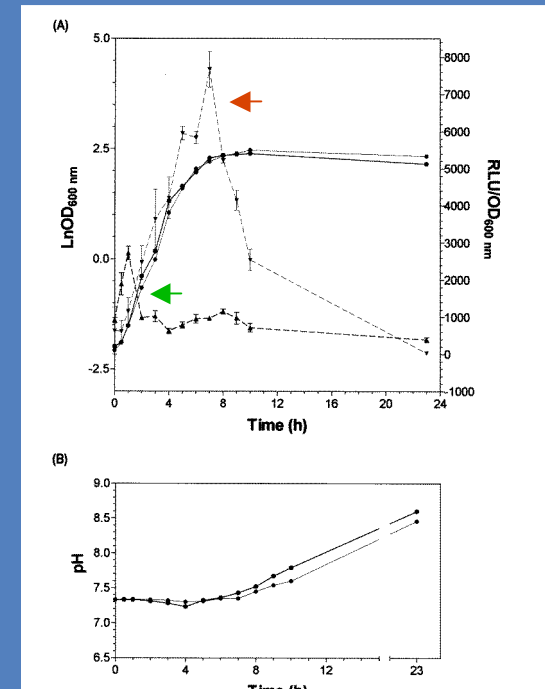


Figure 5.6 Temporal expression of hyaluronate lyase in an *agr/sarA* background. (A) *S. aureus* w.t. (●, ▲) and an *agr/sarA* strain (●, ▼) carrying plasmid pGMLUX01 were grown in CDML supplemented with chloramphenicol (10 $\mu\text{g ml}^{-1}$ ); 2L baffled flasks, 160rpm, 37°C. (●, ●) In optical density reading at 600nm; (▲, ▼) Relative Light Units unit OD<sub>600</sub><sup>-1</sup> (B) pH of w.t. (●) and strain *agr/sarA* (●) cultures during growth.



## FUTURE

- Ideal

individual cells *in vivo*, bacterium and host cells  
assay all transcripts

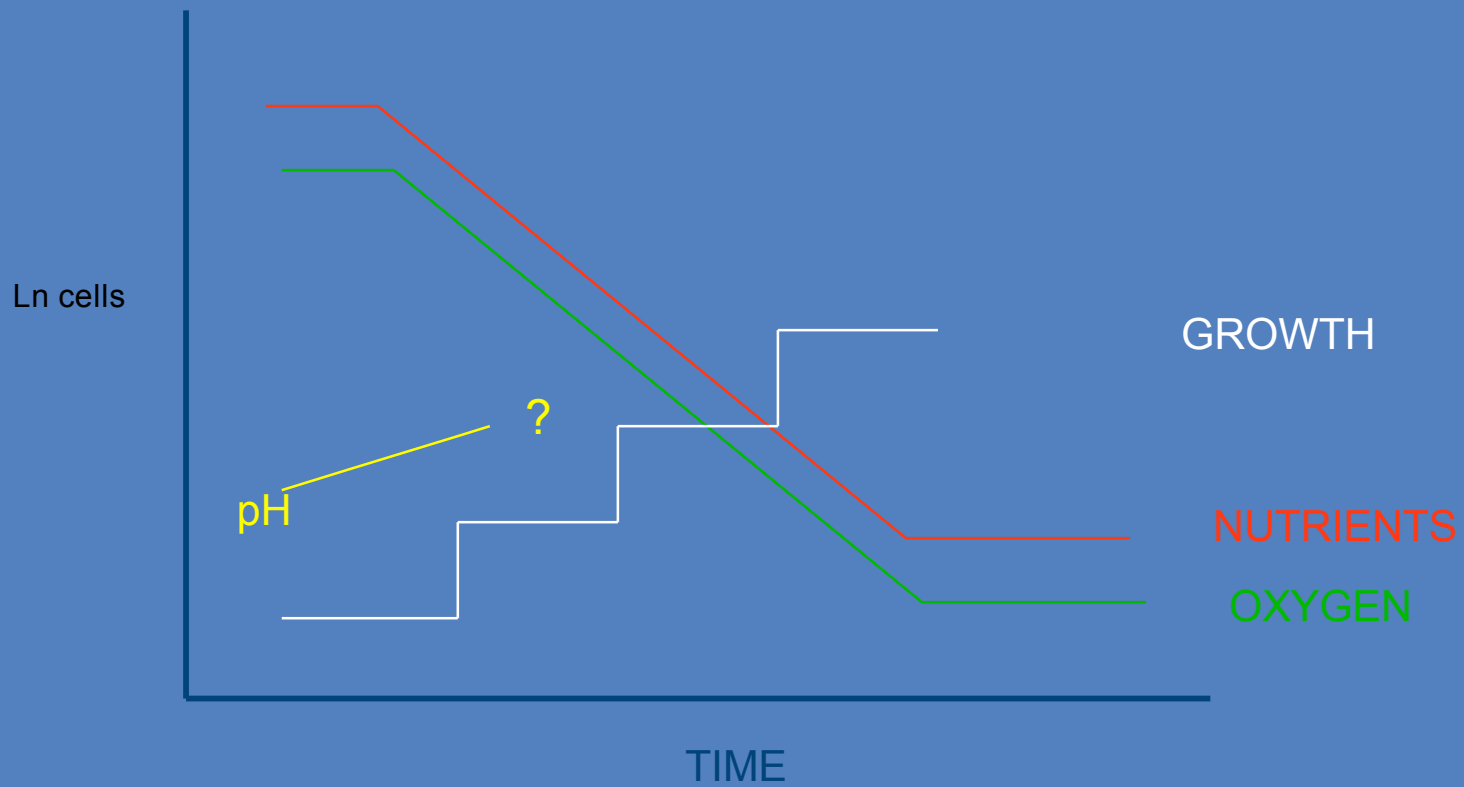
- Possible?

large cell numbers in the same phenotype *in vitro*, no host cells  
assay all transcripts

open culture (chemostat), growth conditions under control of experimenter



## CLOSED CULTURE SYNCHRONOUS GROWTH MODEL



## OPEN CULTURE

Nutrient reservoir with a  
Limiting growth factor

Flow meter

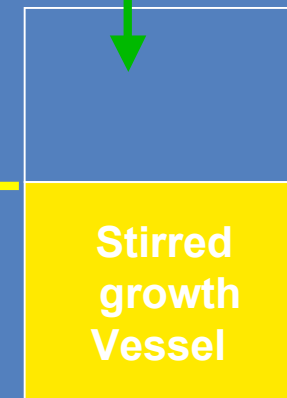
Pump

Steady state: specific growth rate = dilution rate

all cells in the same phenotype

Specific growth rate divorced from pH, T°C

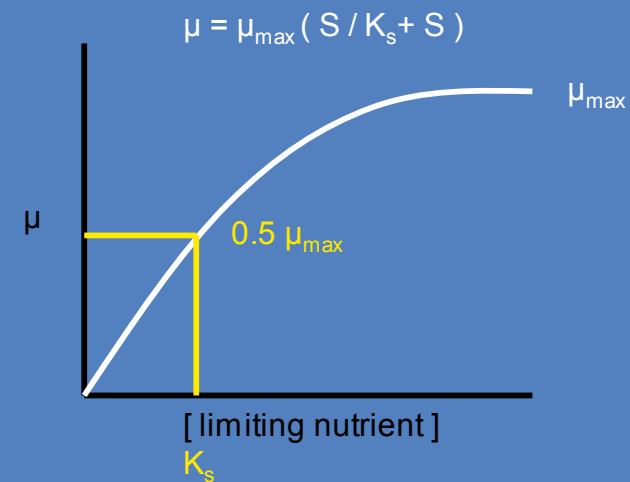
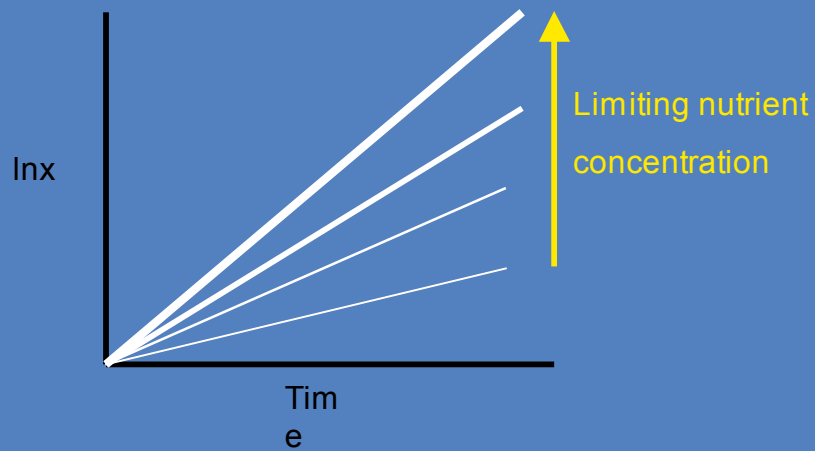
Highly selective environment: mutants



## THEORY ?

### Closed Culture

$\mu = \ln x / t = \text{specific growth rate (h}^{-1}\text{)}$



### Open Culture

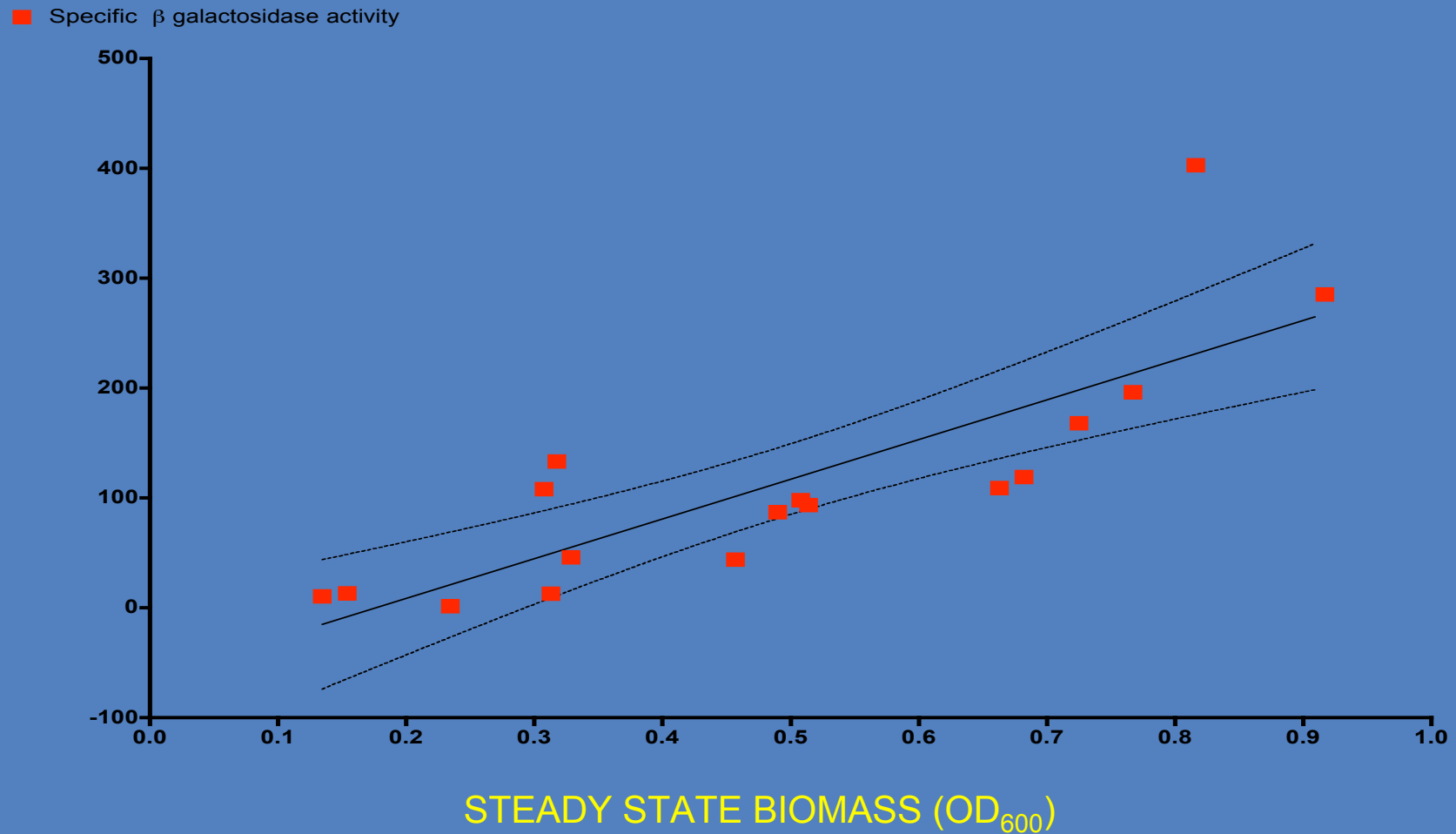
Biomass change = growth - loss

$$dx/dt = \mu x - Dx$$

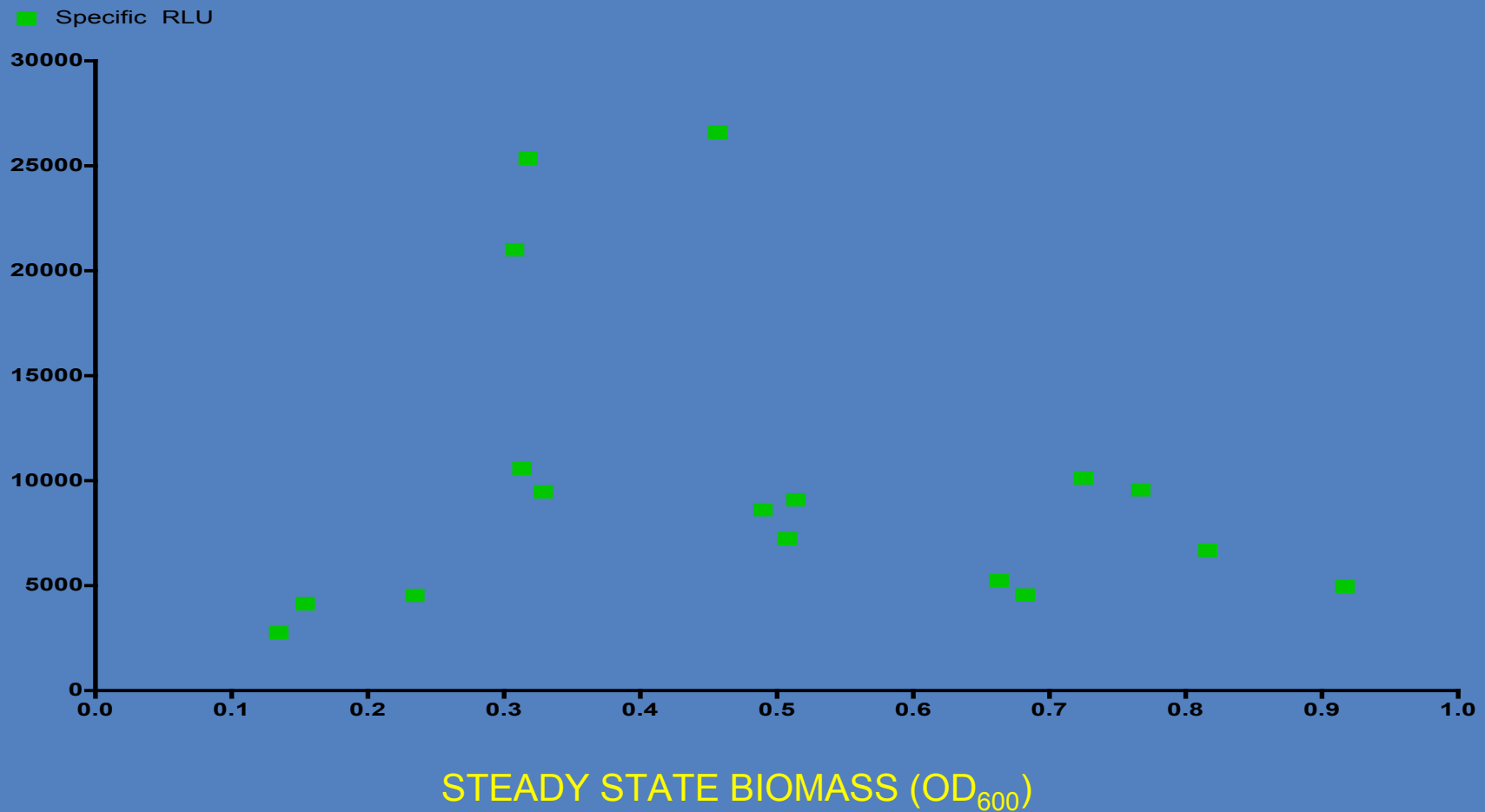
$$0 = \mu - D \text{ steady state}$$

$\mu = D$  experimenter controls D and therefore controls  $\mu$

## CORRELATION BETWEEN CELL DENSITY AND AGR EXPRESSION



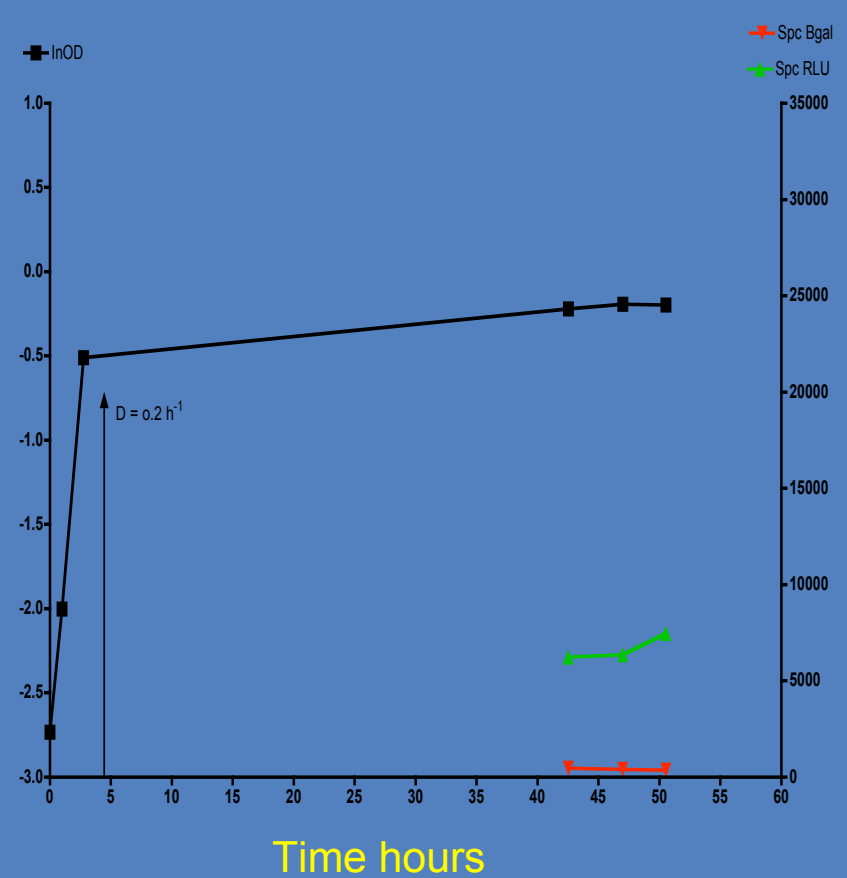
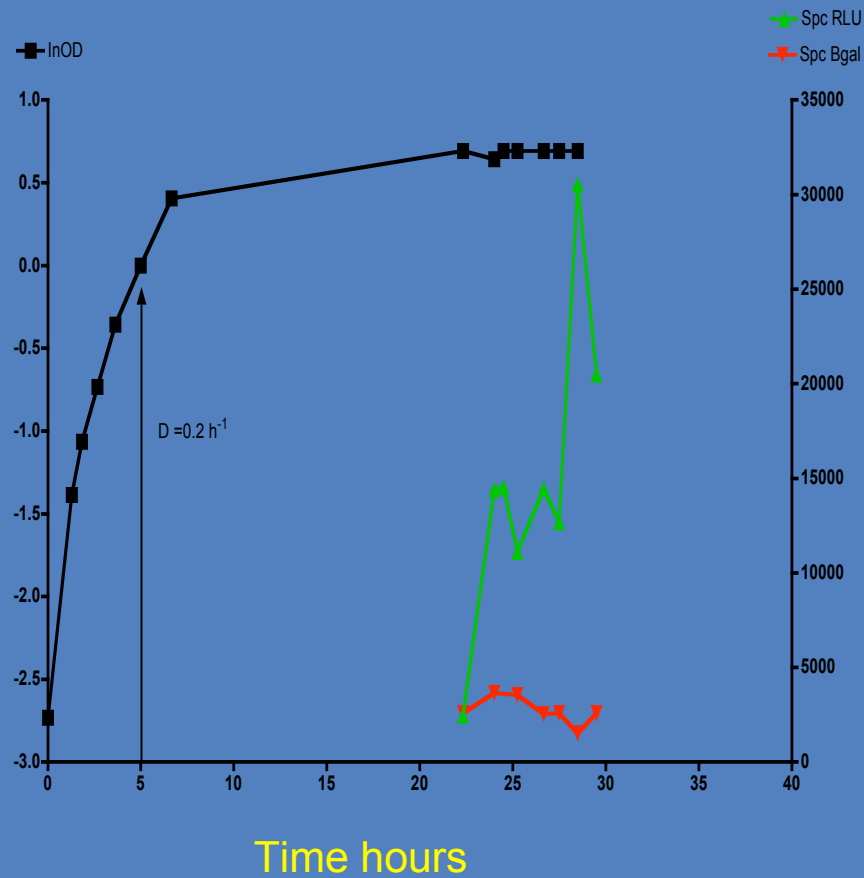
## CORRELATION BETWEEN CELL DENSITY AND TST EXPRESSION

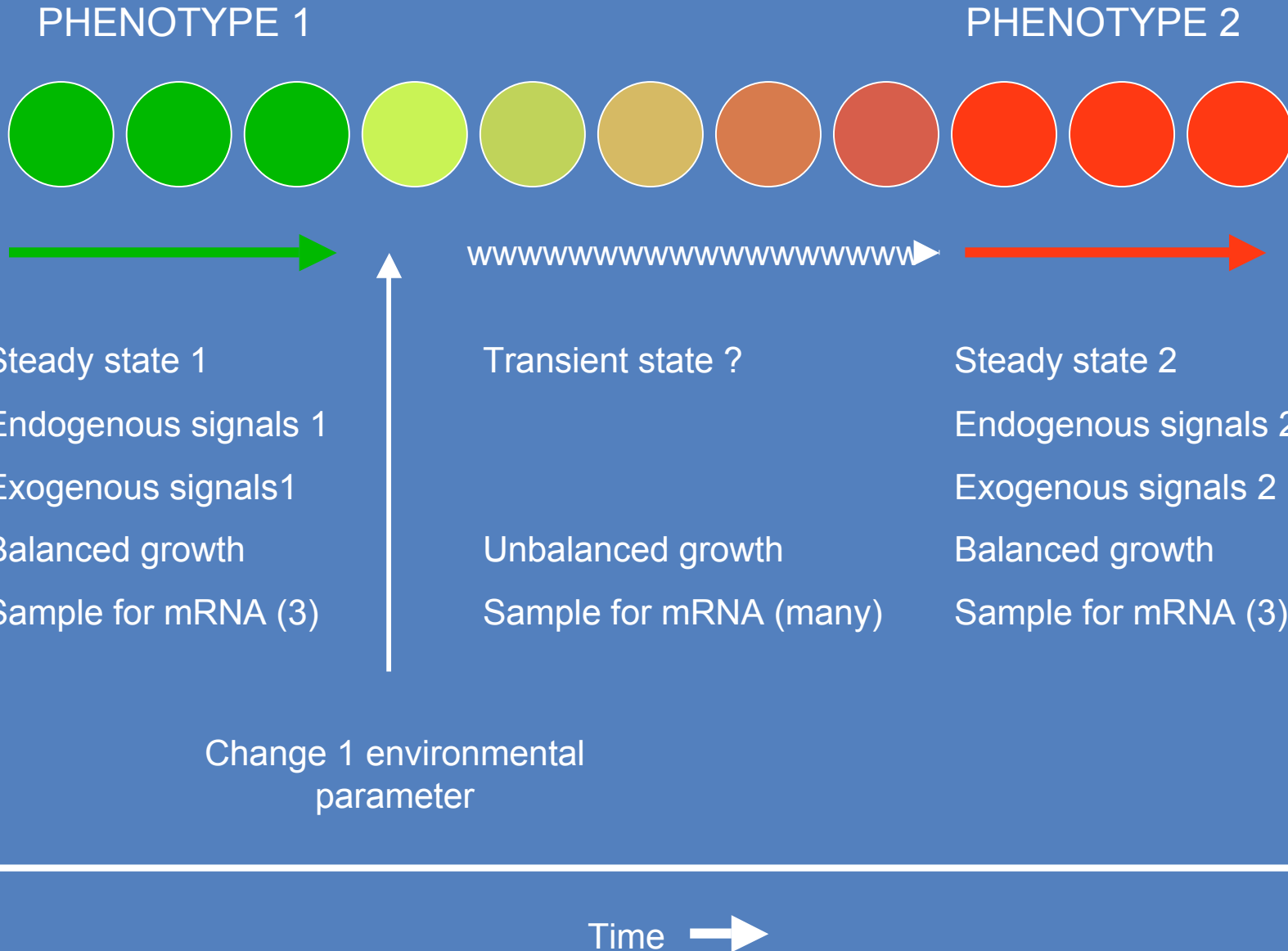


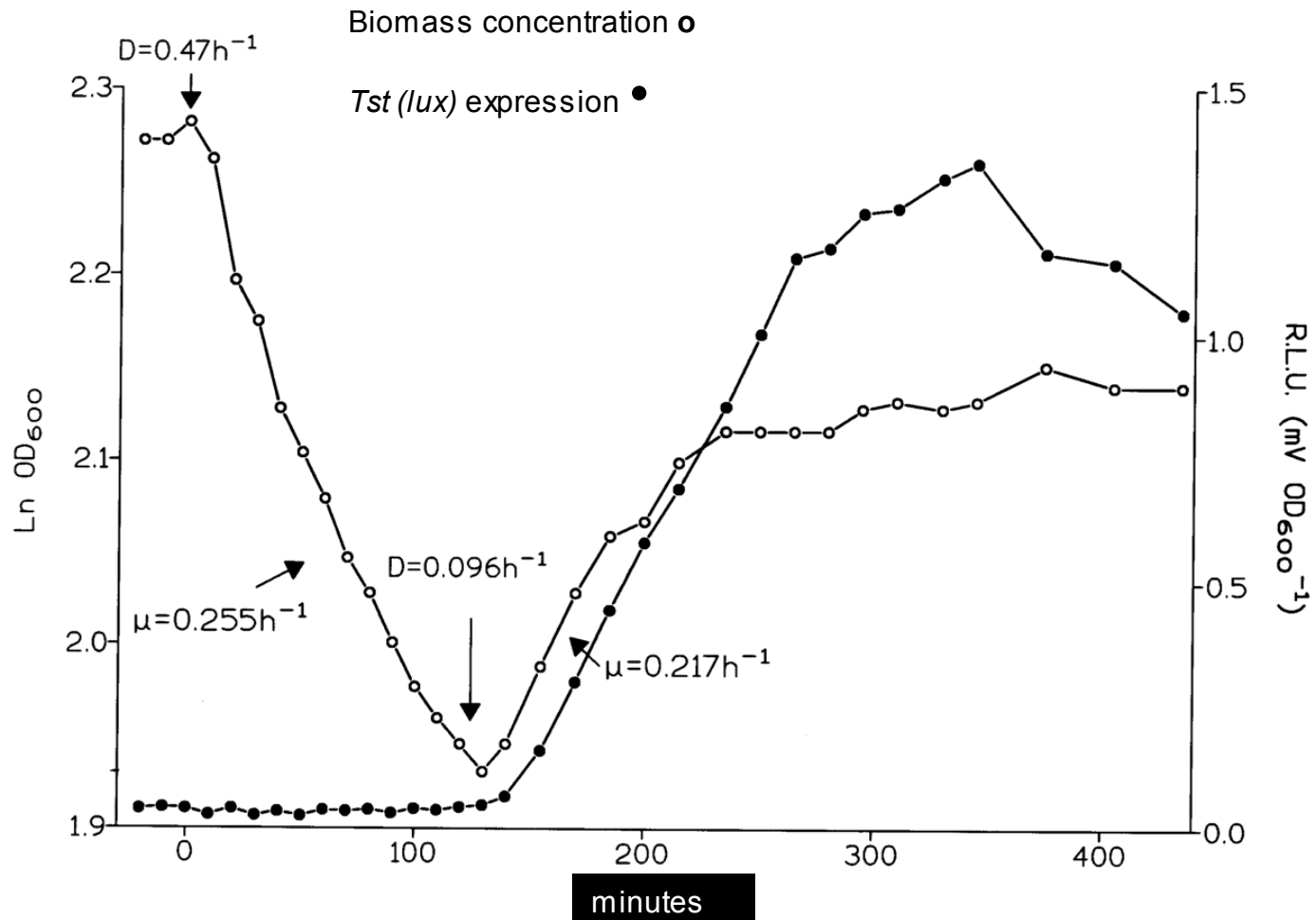
## TECHNICAL PROBLEMS

- Growth model
  - stability, reproducibility, mutations
- Sampling
  - perturbation (volume), speed
- Transcript analysis
  - sensitivity, reproducibility

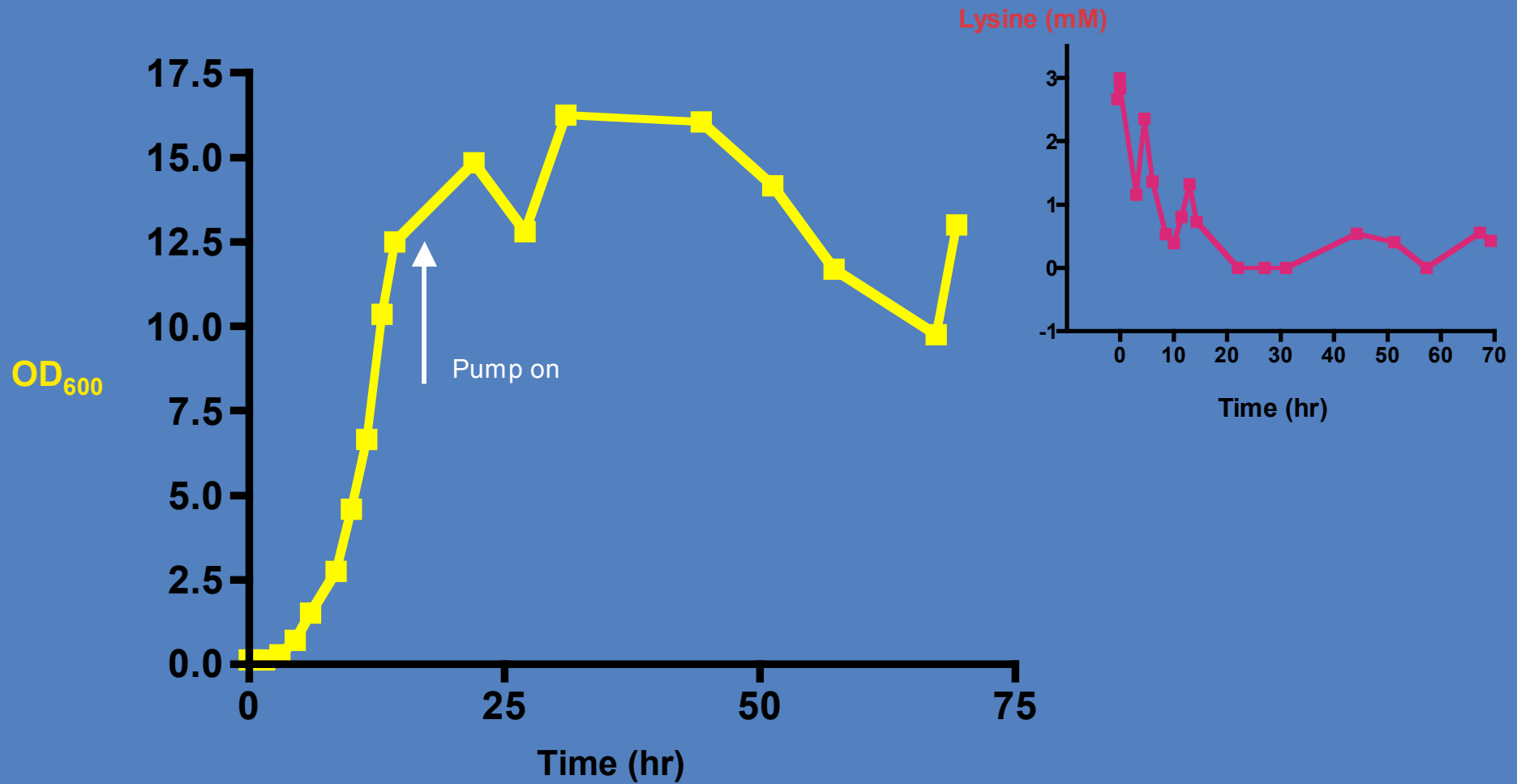
## EFFECT OF FREQUENCY OF SAMPLING







## STEADY STATE PROBLEM IN SYNTHETIC MEDIUM



## SUMMARY

- Goal

To predict behaviour/interactions of *S.aureus* with its environment

- Required

Gene regulatory network knowledge then.....

Finance

Cooperation of modellers and 'wet' experimentors

- Technical obstacles

Transcript analysis - microarray

Robust *in vitro* system

Chemostat

Steady state????

Sample volume and high biomass???

Rapid sampling with mRNA stabilisation?

## SKIN EQUIVALENT MODEL

