Emerging themes in bacterial cell-cell communication networks

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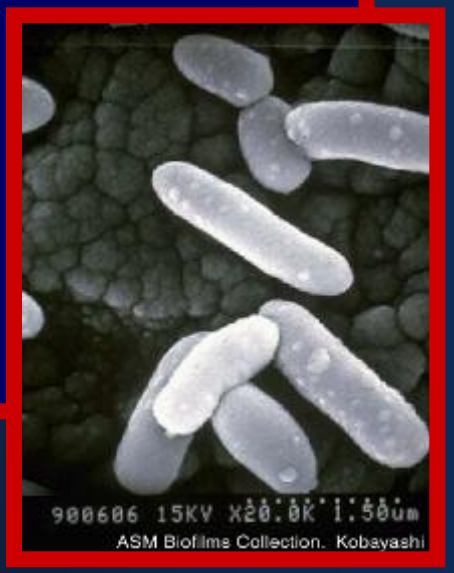
Recognition and Communication in the Unicellular Bacterial World

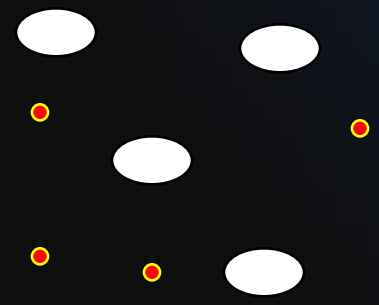
- Sexual Exchange – Conjugation
- Protecting Your Niche
- Combating Host Defences
- Population Migration

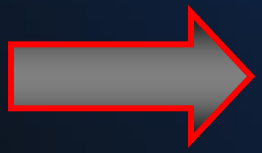


Bacteria Communication: Quorum Sensing

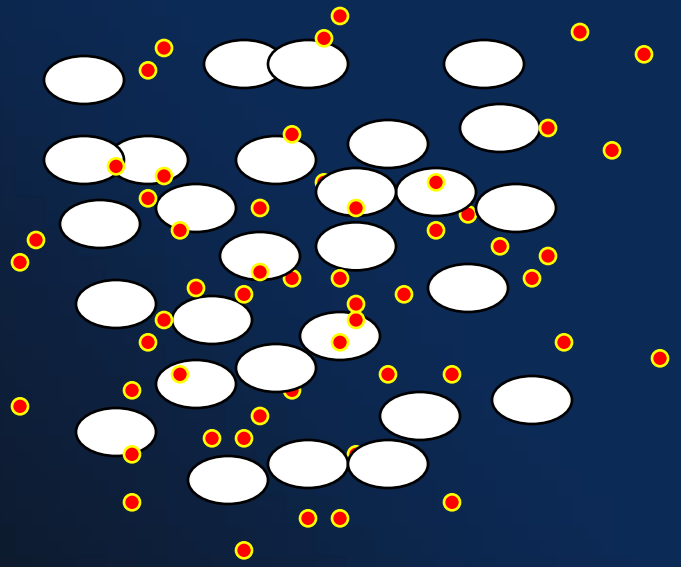
Cell-to-cell communication via a diffusible signal molecule

Low Population Density





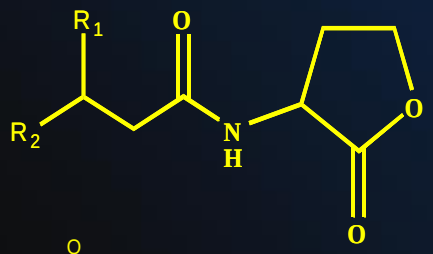
High Population Density



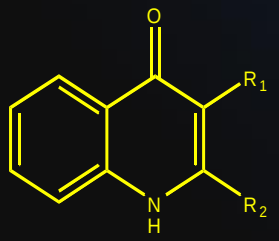
- phenotypic change

QS Signal Molecules are Chemically Diverse

***N*-Acylhomoserine Lactones (AHLs)**



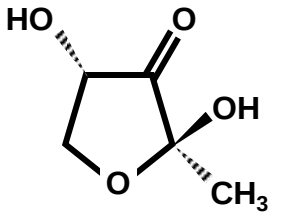
2-Alkyl-4-Quinolones



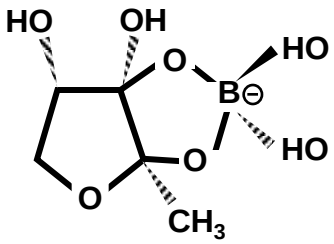
DSF

C18-FAME

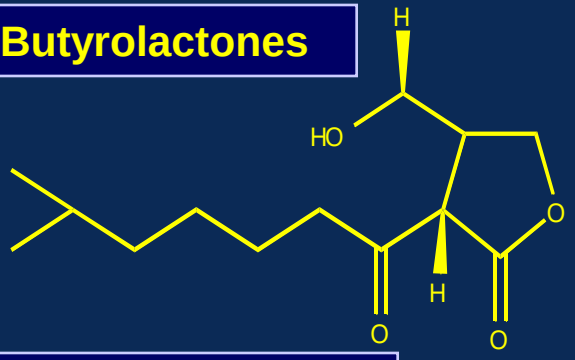
Gram-negative



AI-2?



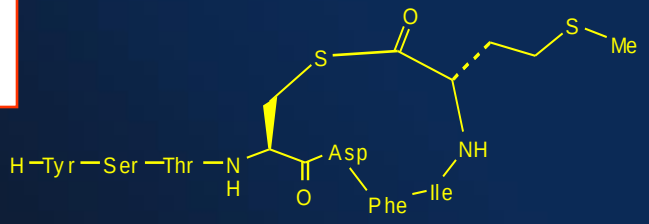
γ-Butyrolactones



Linear Peptides

$_2$HN-EMRLSKFFRDFILQRKK-COOH

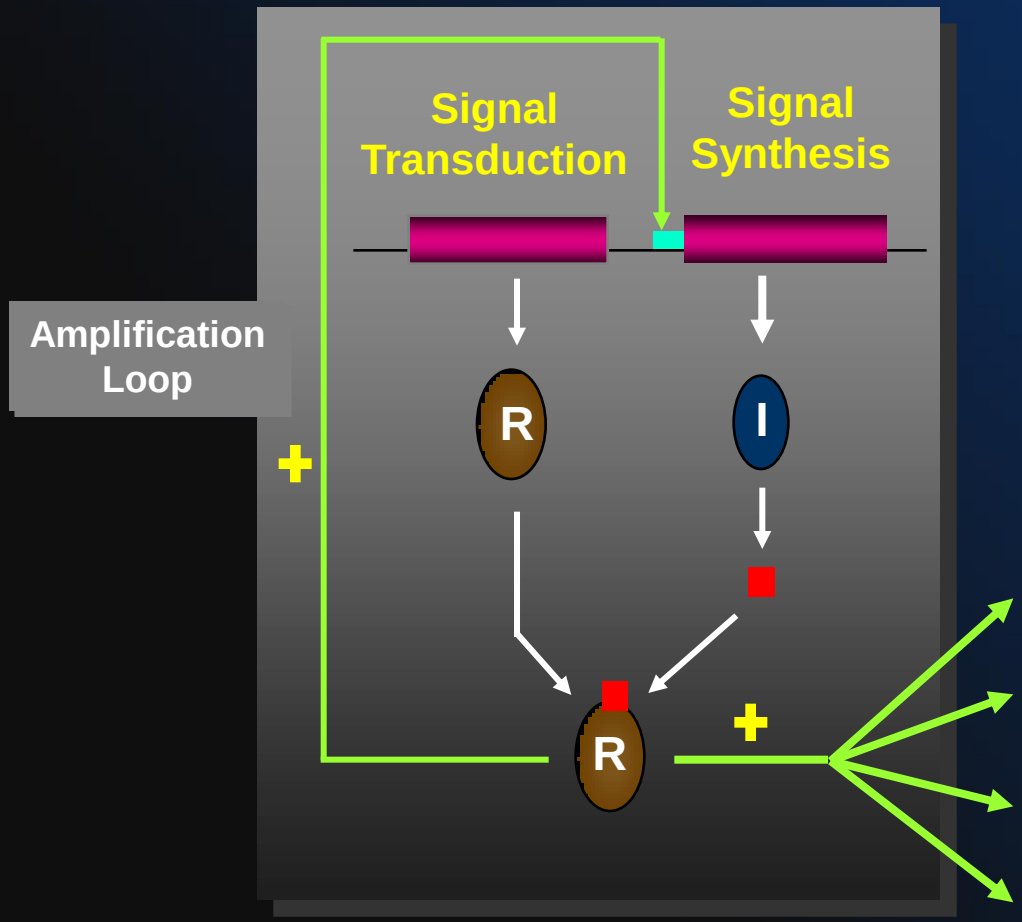
CSP-1



Cyclic Peptides

Gram-positive

Quorum Sensing Regulatory Circuits



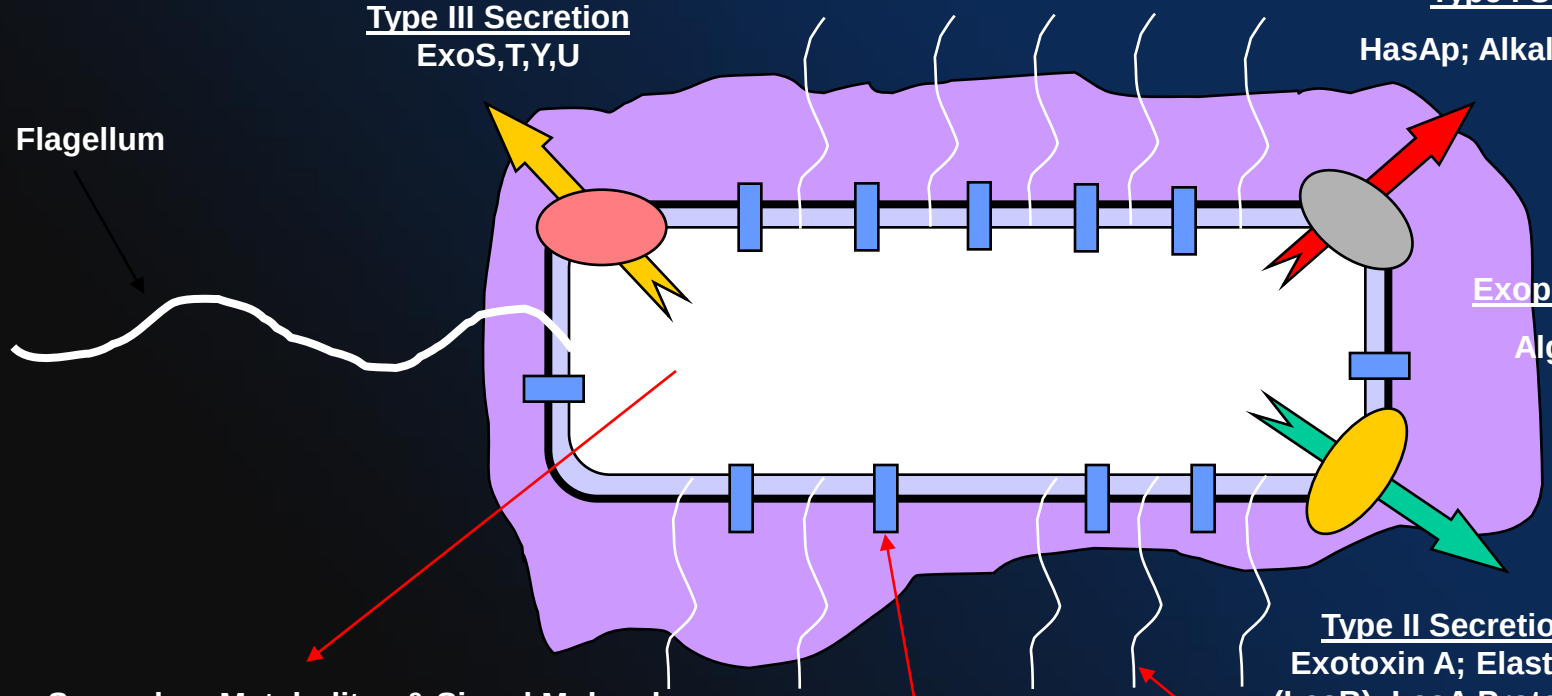
Multiple Gene Expression

- Virulence
- Secondary Metabolites
- Motility and Swarming
- Conjugation
- Biofilm Development
- Growth Inhibition

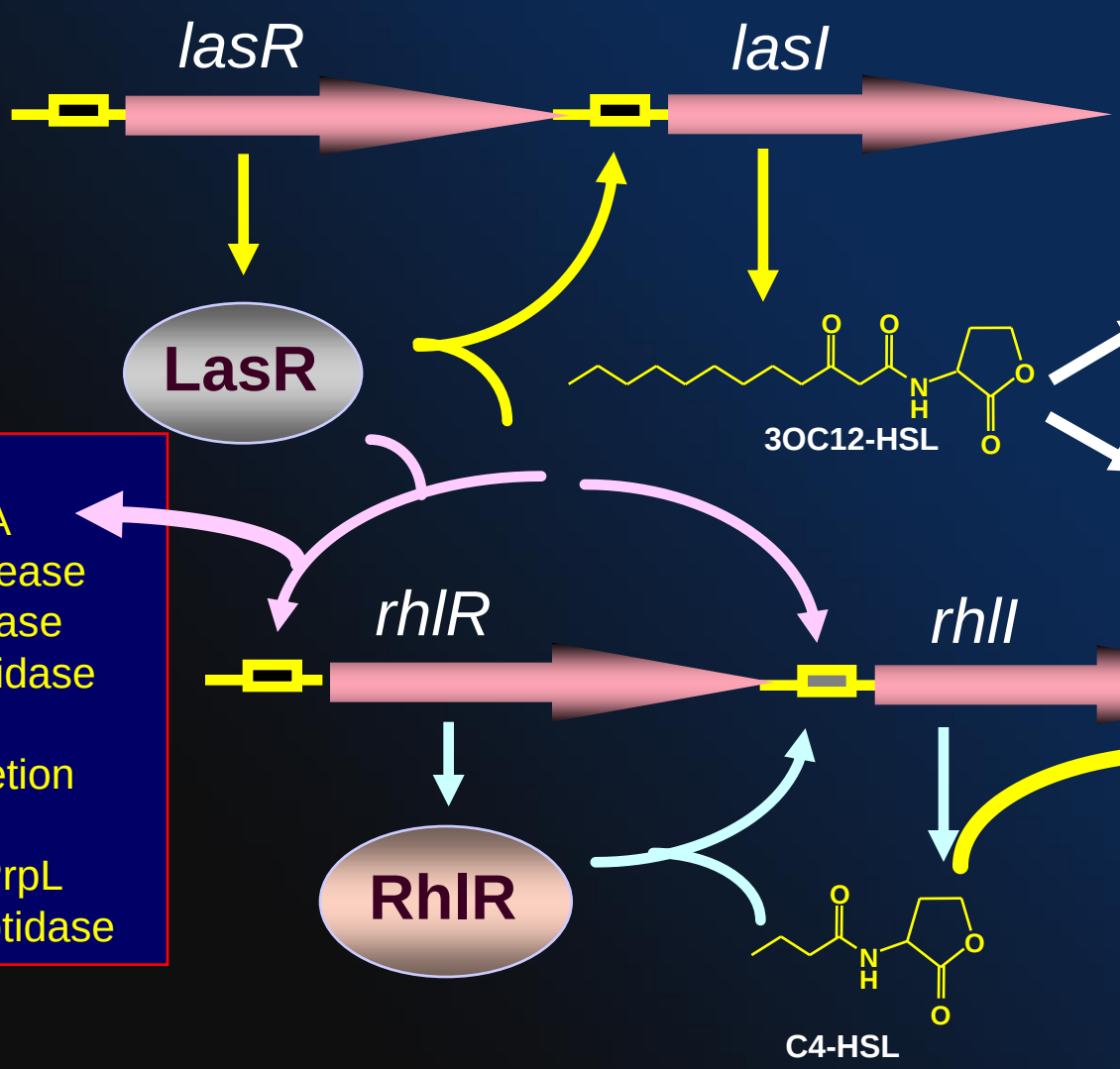
The Lifestyle of *Pseudomonas aeruginosa*

- Gram-negative - Ubiquitous in soil and water
- Environmentally highly adaptable
  - *– 6.3 MB genome*
  - *– 5,570 predicted ORFs*
  - *– 521 Putative Regulatory Genes*
- Opportunistic pathogen
  - *-Wide spectrum of infections in humans*
  - *- Cystic fibrosis*
- Intrinsically antibiotic resistant
- Multiple surface-associated and extracellular virulence determinants

Virulence Determinants of *P. aeruginosa*



A Hierarchical Quorum Sensing Cascade in *P. aeruginosa*



Microarray studies indicate QS regulon consists of ~10% of genome

QS controls community behaviour in *P. aeruginosa*

1.Swarming Motility

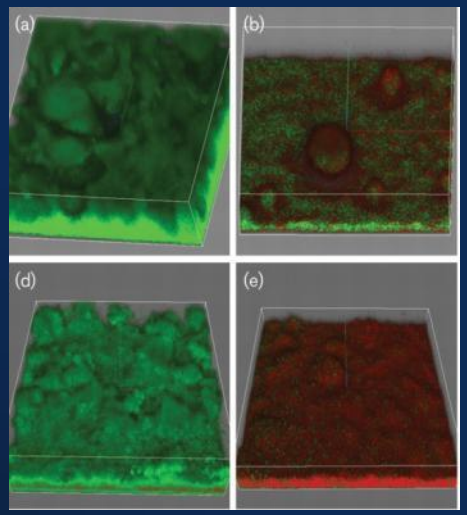
WT

rhlR-

2.Biofilms Antibiotics

WT

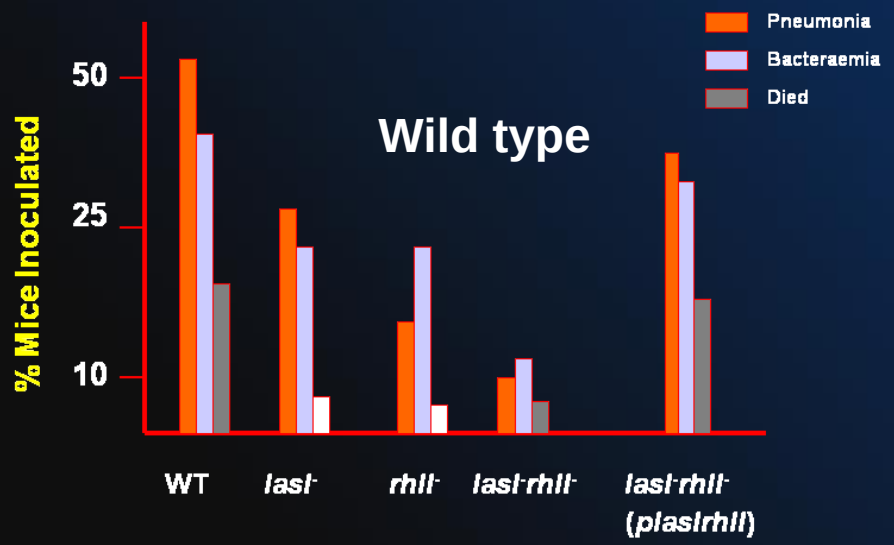
lasR- *rhlR*-



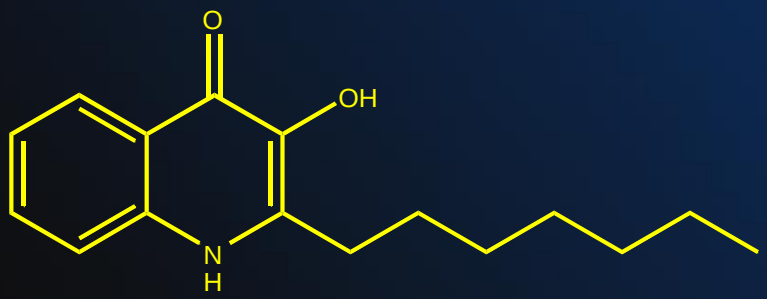
WT + Tobramycin

lasR- *rhlR*- +Tobramycin

3.Virulence in mouse pneumonia model

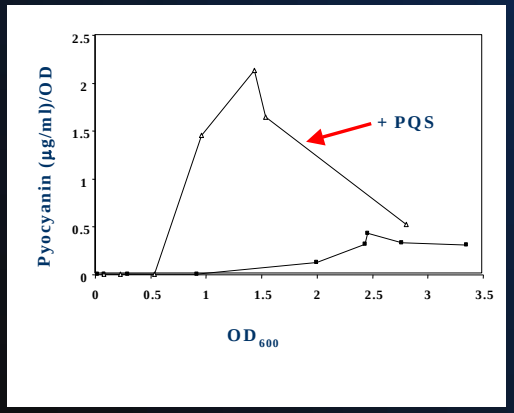


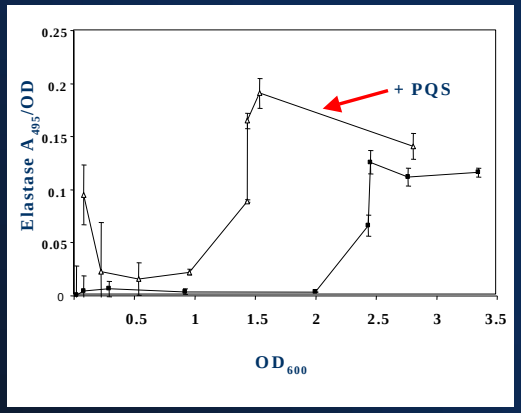
P. aeruginosa is bilingual



2-heptyl-3-hydroxy-4-quinolone (PQS)

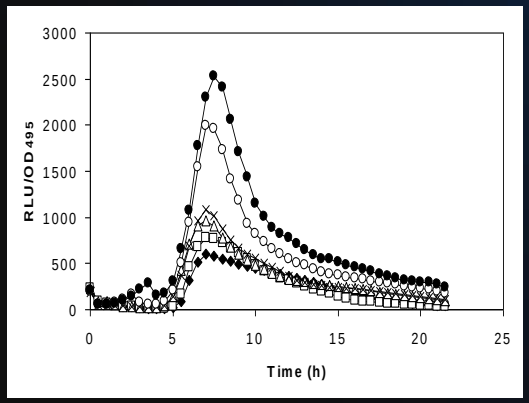
Pyocyanin

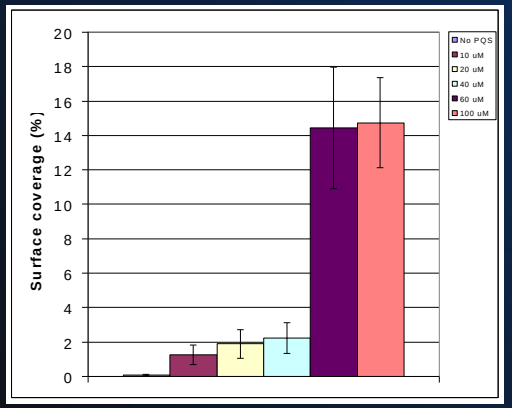




Elastase

lecA expression

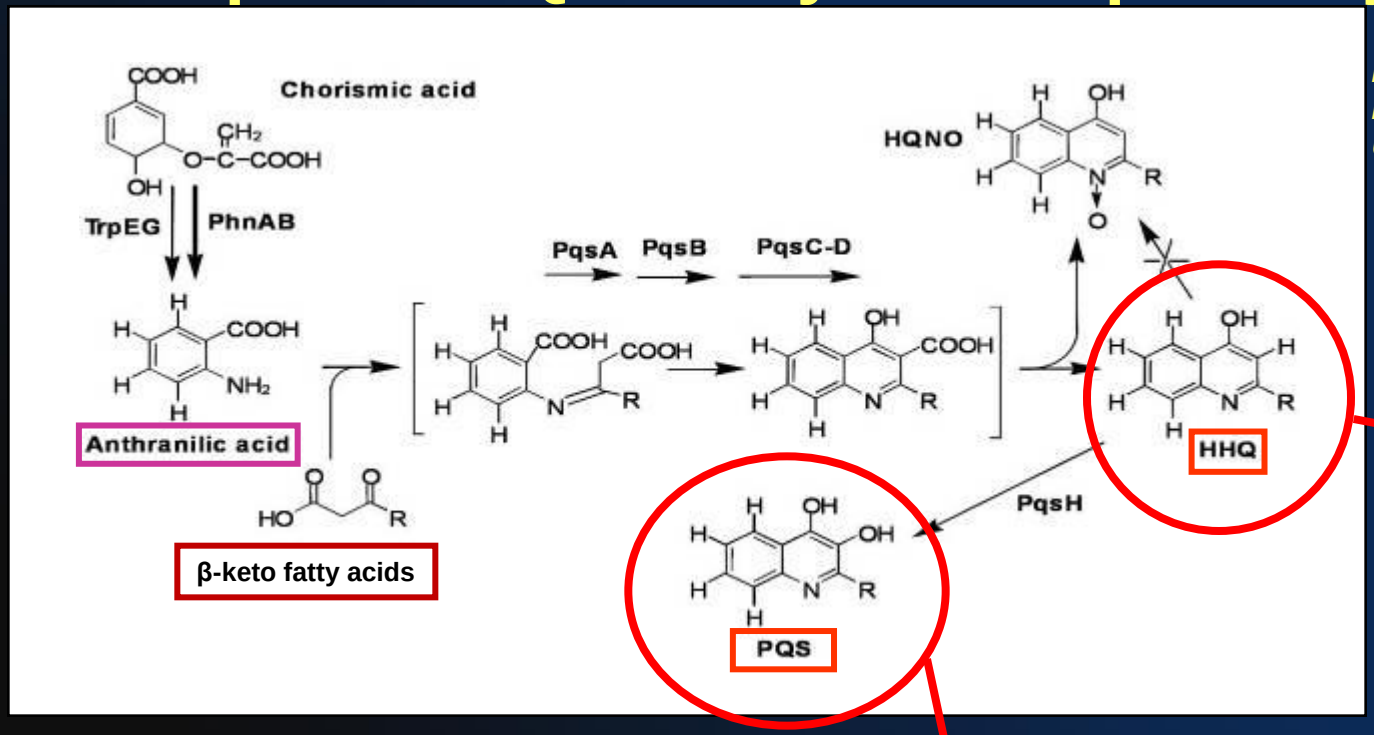




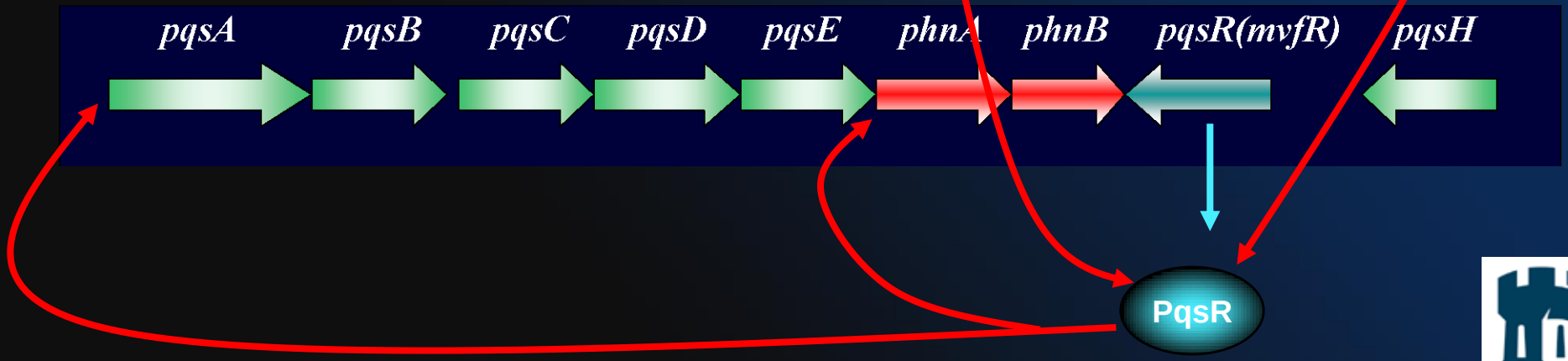
Biofilm surface coverage

Diggle (2003) Mol Micro ***50****:29.*

Proposed PQS biosynthetic pathway



Déziel et al. (2004) Proc. Natl. Acad. Sci. USA 101, 1339-1344

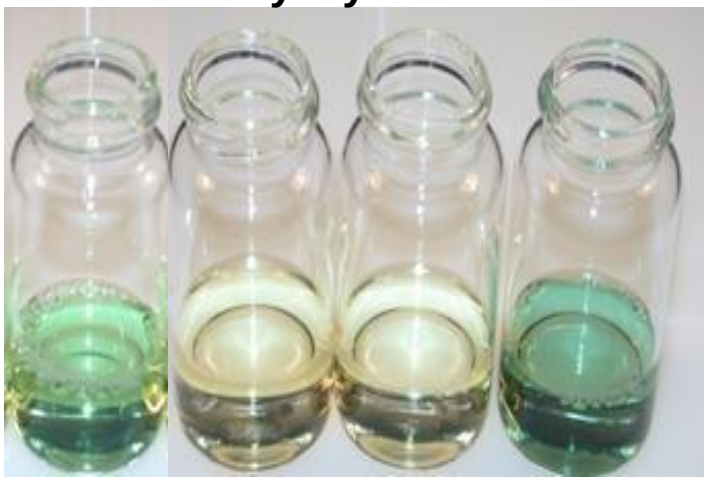


Fletcher (2007) Env. Micro

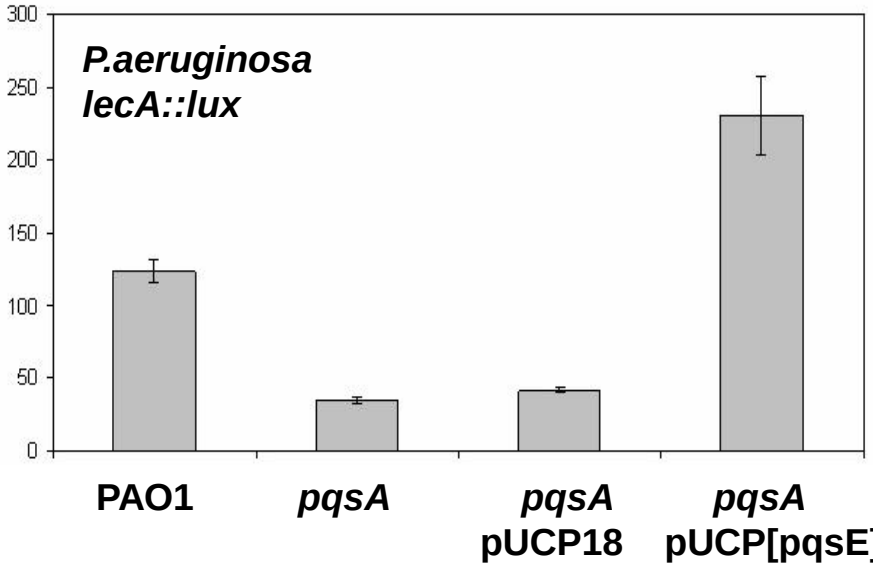
*Xiao et al (2006) Mol Micro **6**:1689.*

PqsE restores virulence independent of PqsR and PQS

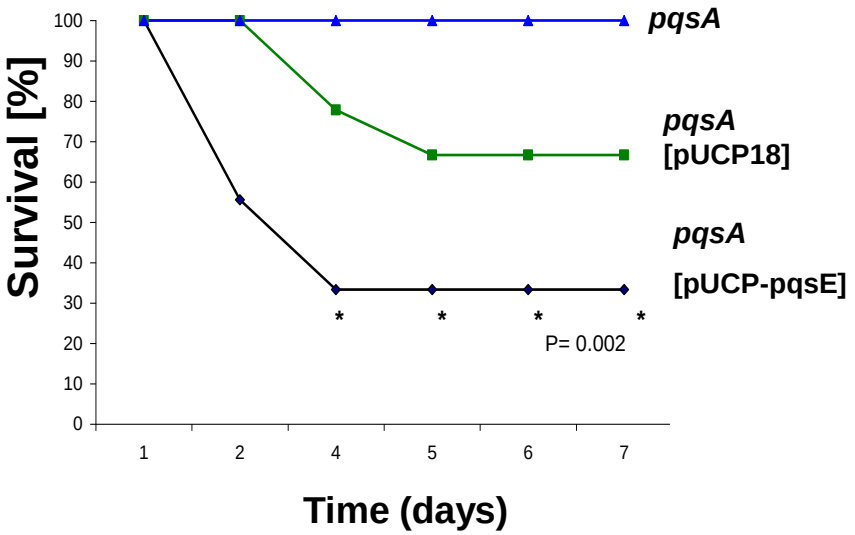
Pyocyanin



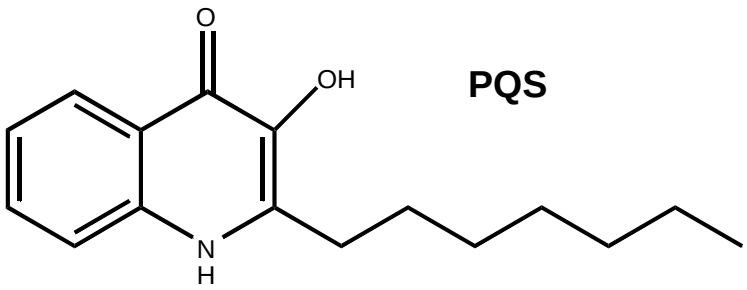
PAO1 | *pqsR* | *pqsR* pUCP18 | *pqsR* pUCP[pqsE]



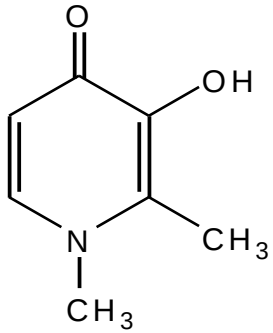
Mouse Wound Infection Model



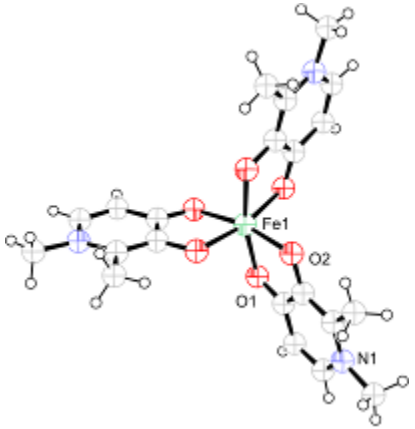
P. aeruginosa PQS

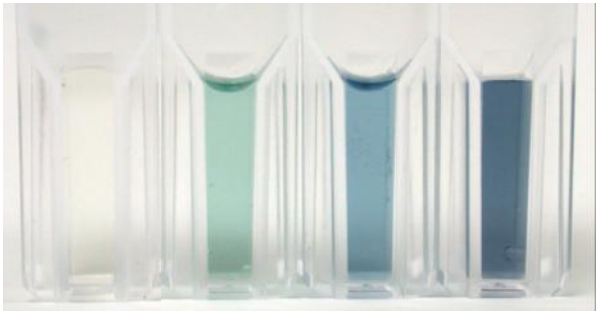


PQS



4-Pyridone

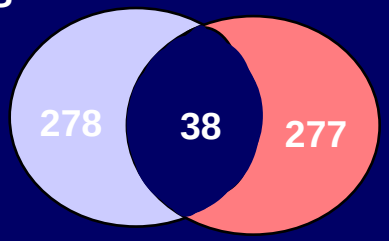




Fe^{3+} Fe^{3+} + PQS

Genes upregulated upon addition of PQS (316)

Genes upregulated upon addition of 3O-C12-HSL/ C4-HSL (315)

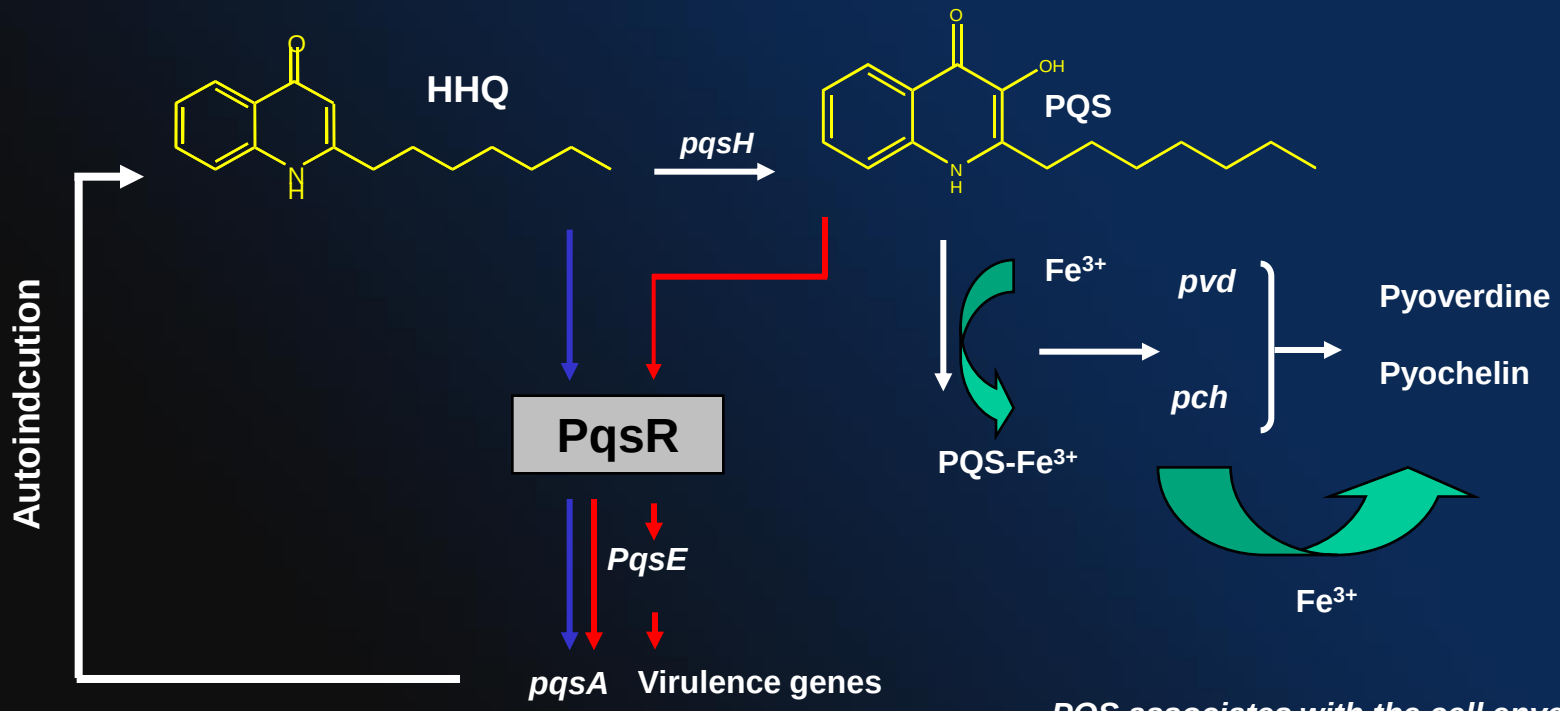


Transcriptome comparison

- Chelator properties
- Positive regulator of iron uptake mechanisms
- Autoinduction

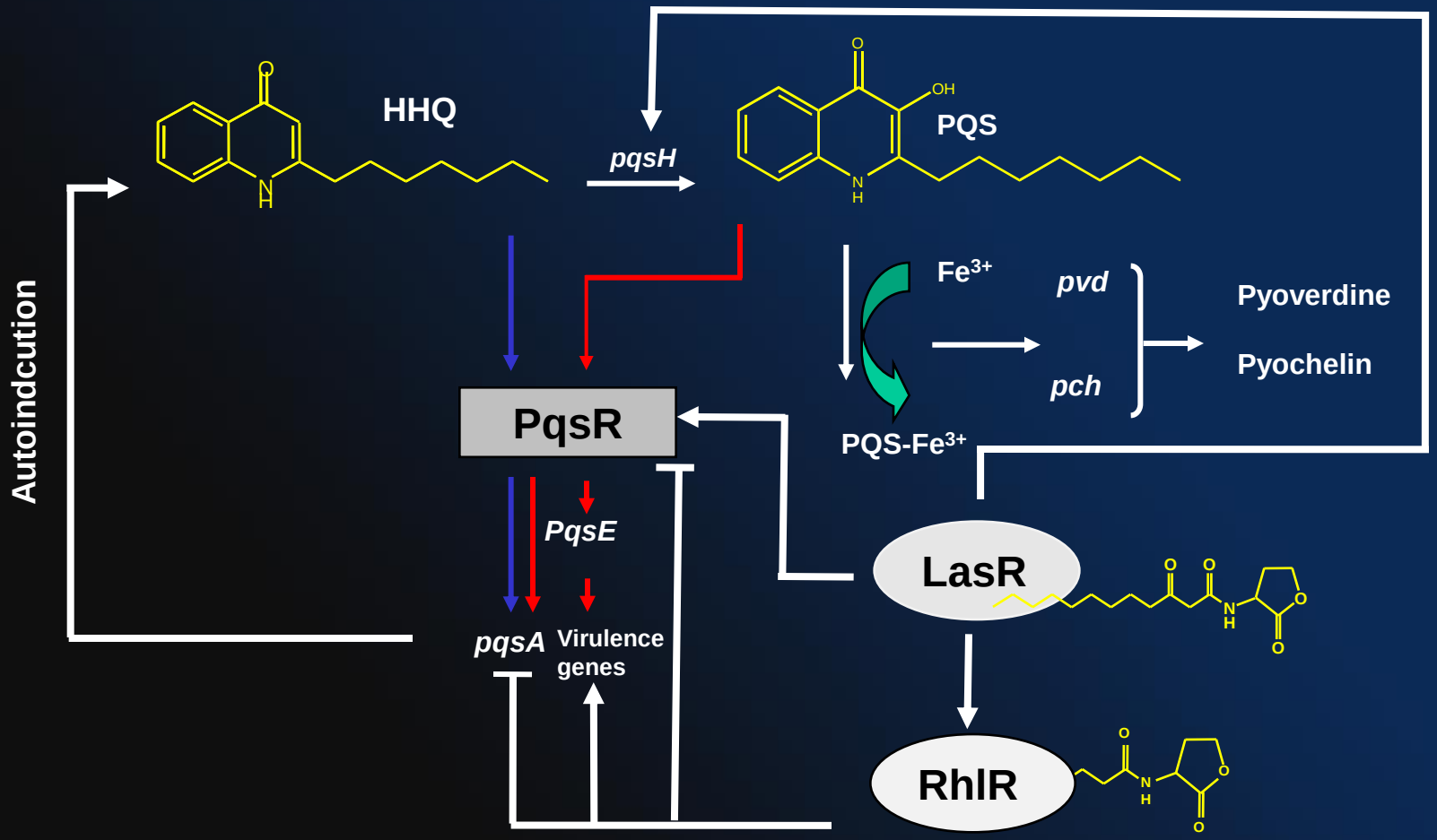
Diggle et al (2007) Chem. & Biol 14:97

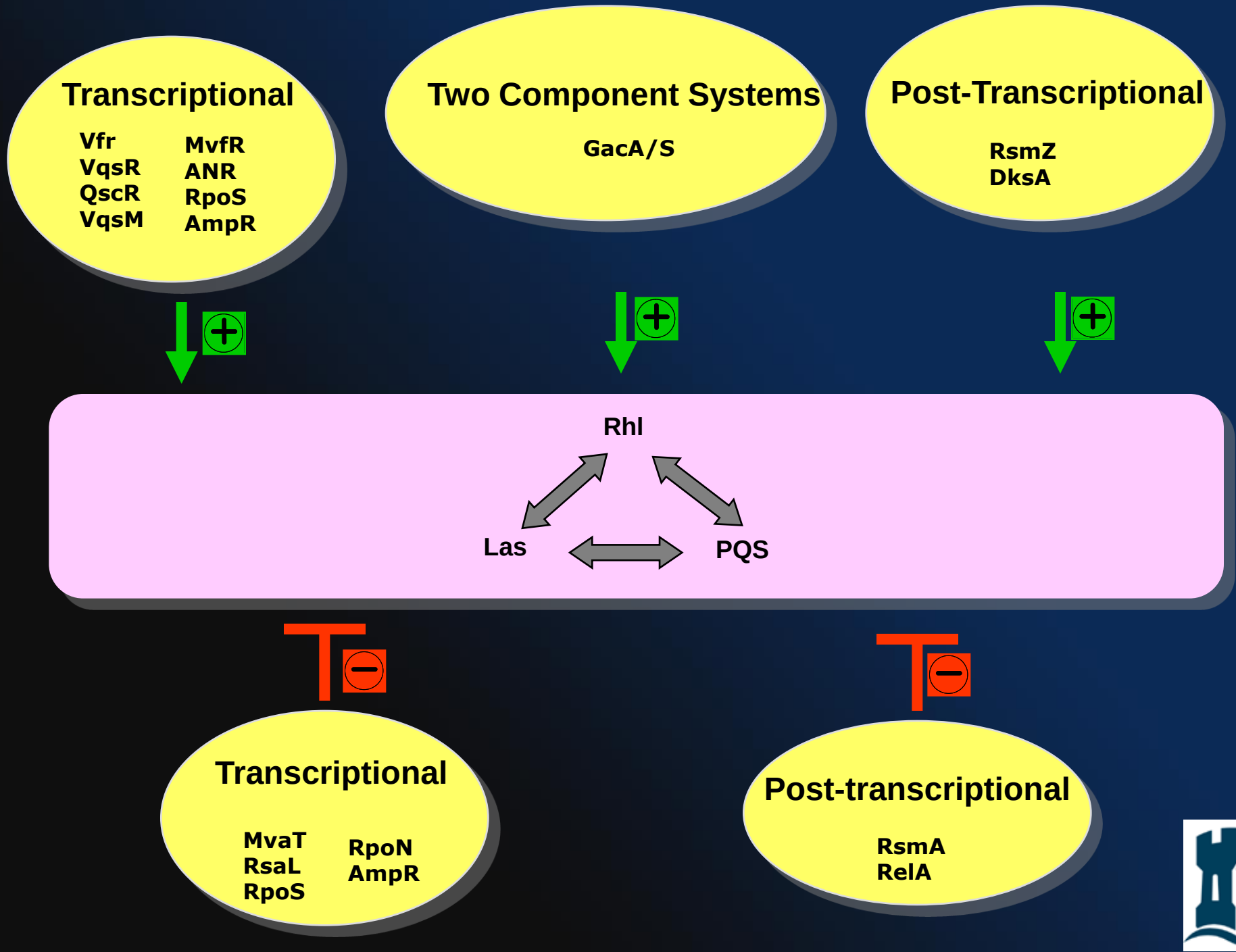
PQS and HHQ play multi-functional roles in signalling and iron entrapment



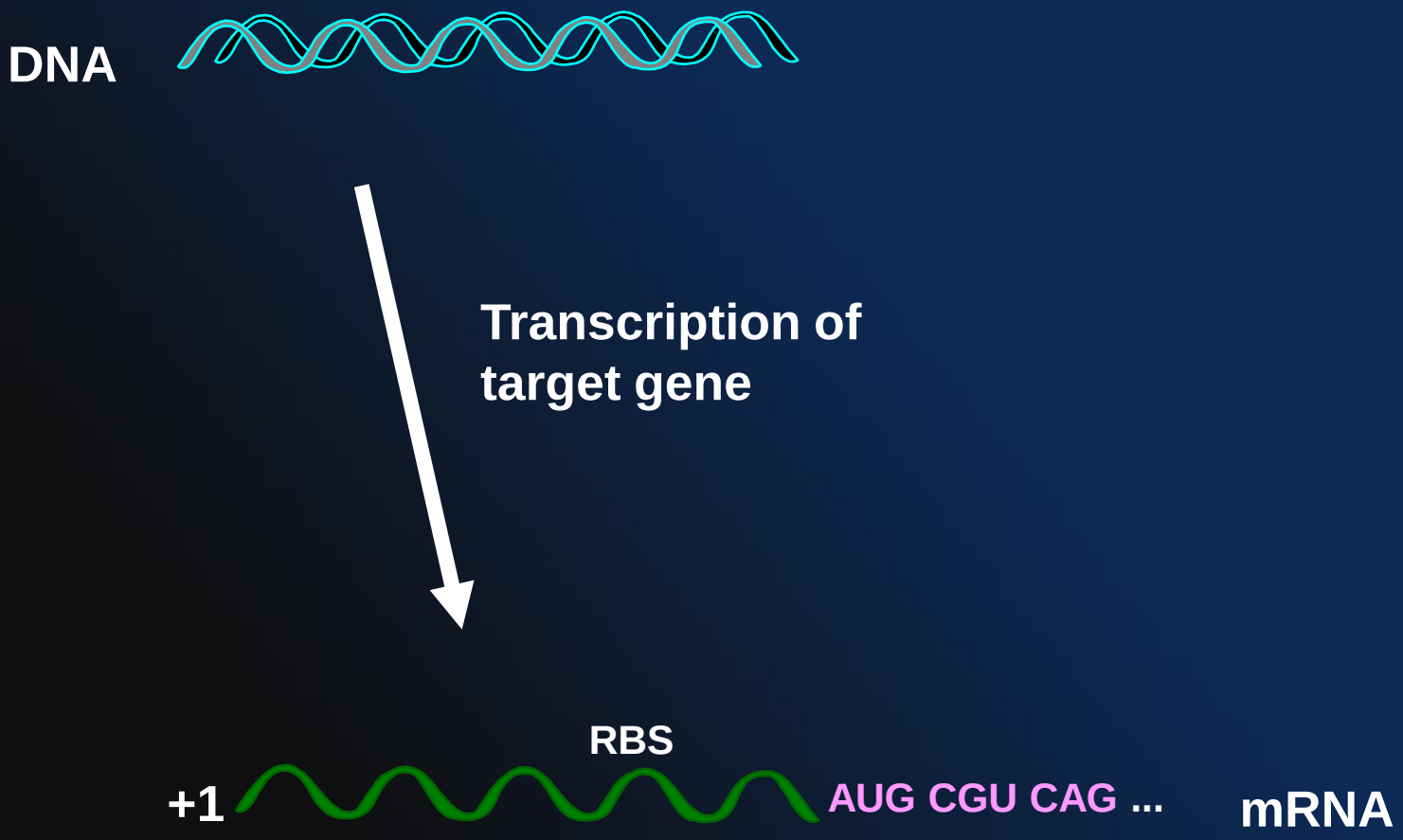
PQS associates with the cell envelope and appears to act as an iron trap delivering iron to receptor associated pyochelin and pyoverdin

An integrated quorum sensing transcriptional network in *P. aeruginosa*




Transcriptional
Vfr
VqsR
QscR
VqsM
MvfR
ANR
RpoS
AmpR
Two Component Systems
GacA/S
Post-Transcriptional
RsmZ
DksA
Rhl
Las
PQS
Transcriptional
MvaT
RsaL
RpoS
RpoN
AmpR
Post-transcriptional
RsmA
RelA

Post-transcriptional regulation by RsmA

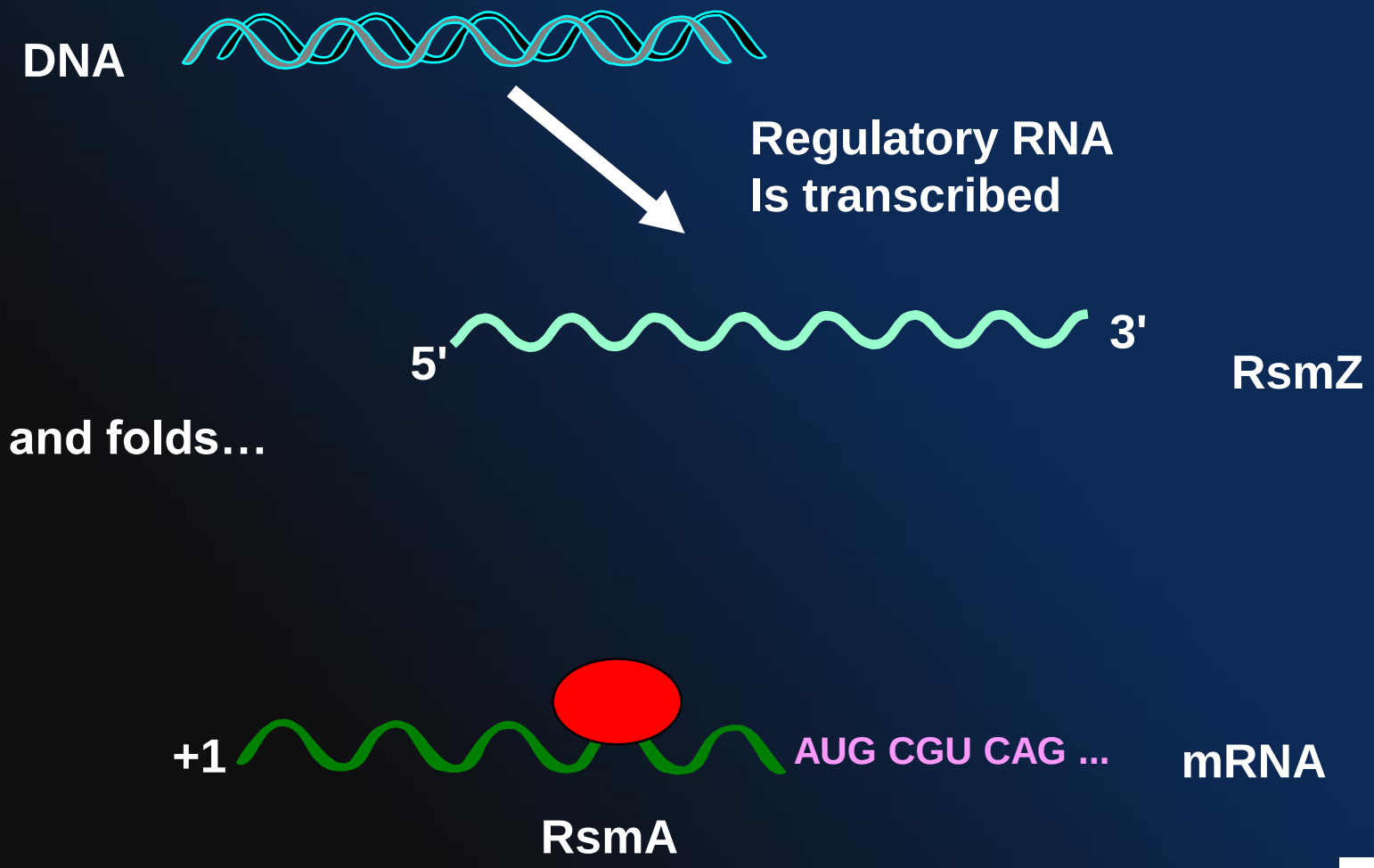


Post-transcriptional regulation by RsmA

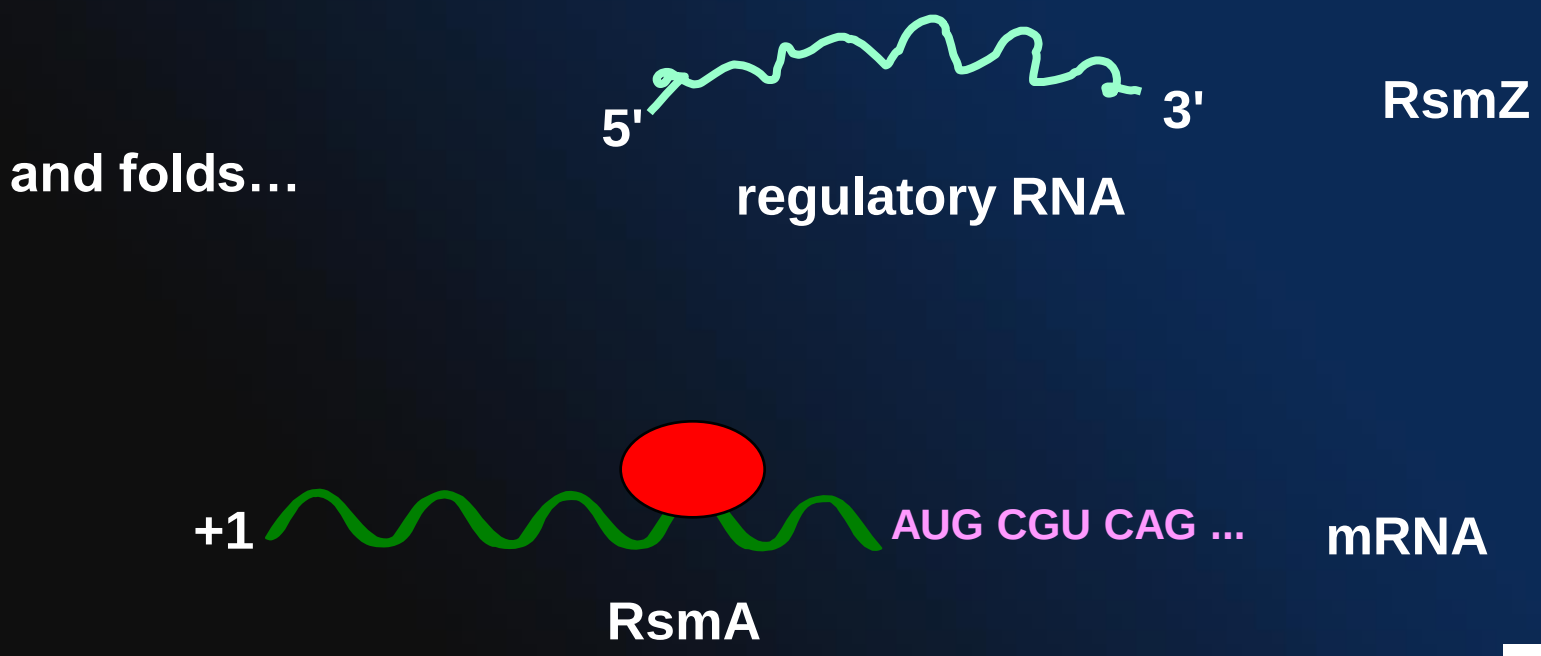
DNA

RsmA binds near RBS
and prevents translation

Post-transcriptional regulation by RsmA



Post-transcriptional regulation by RsmA



Post-transcriptional regulation by RsmA

and folds…

regulatory RNA

RsmZ

RsmA

mRNA

Post-transcriptional regulation by RsmA

RsmZ

and folds…

regulatory RNA

mRNA

RsmA

Post-transcriptional regulation by RsmA

RsmZ

and folds…

regulatory RNA

mRNA

RsmA

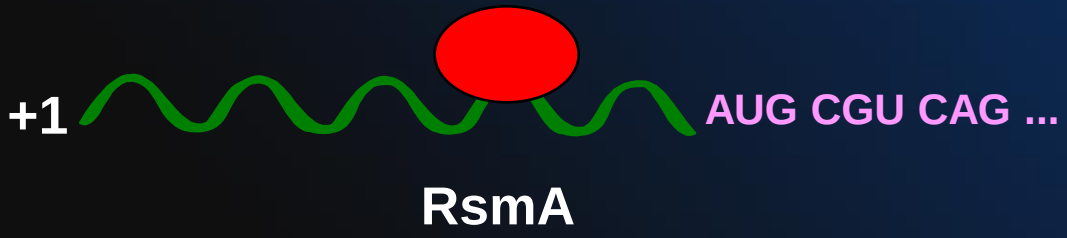
Post-transcriptional regulation by RsmA

RsmZ

regulatory RNA

and folds…

to titrate RsmA
from the RBS…



mRNA

Post-transcriptional regulation by RsmA

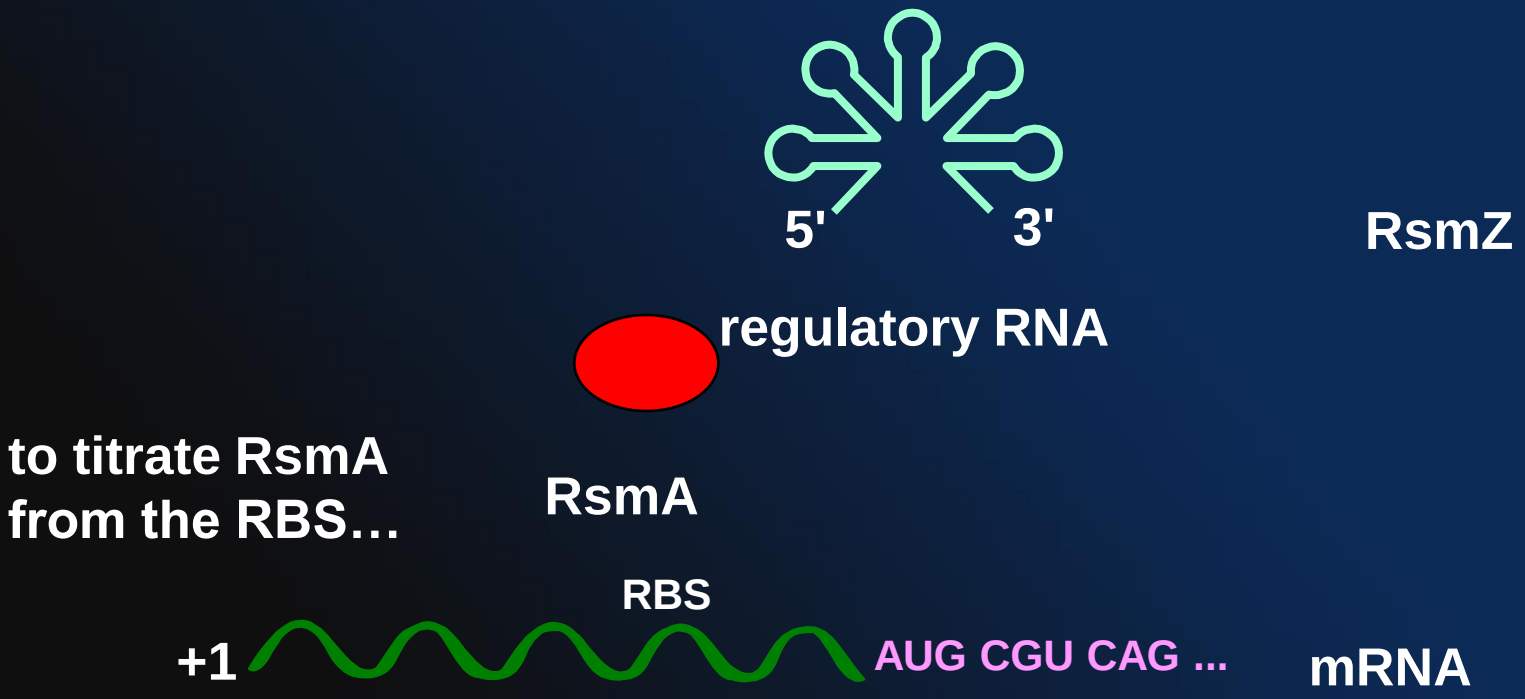
RsmZ

regulatory RNA

to titrate RsmA
from the RBS…

mRNA

Post-transcriptional regulation by RsmA



Post-transcriptional regulation by RsmA

RsmZ

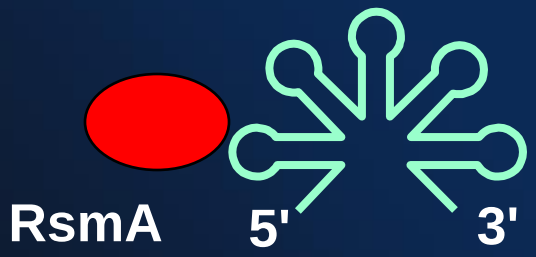
regulatory RNA

RsmA

to titrate RsmA
from the RBS…

mRNA

Post-transcriptional regulation by RsmA



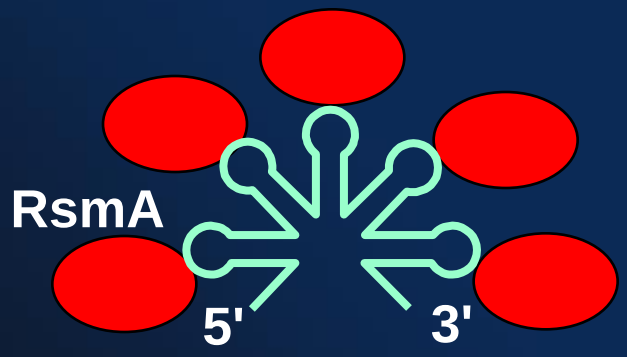
RsmZ

regulatory RNA

to titrate RsmA
from the RBS…

mRNA

Post-transcriptional regulation by RsmA



RsmZ

regulatory RNA

to titrate RsmA
from the RBS…

protein

RBS

+1

AUG CGU CAG ...

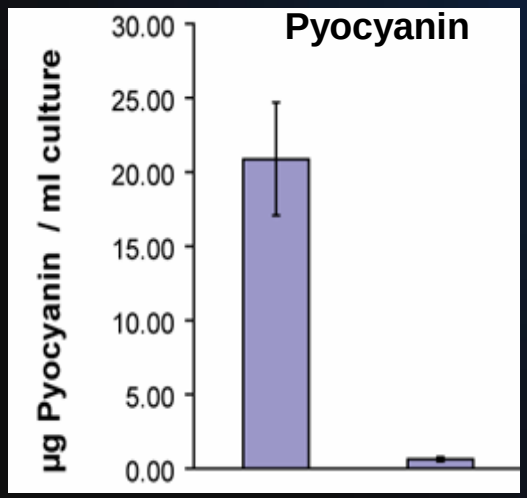
mRNA

…allowing translation

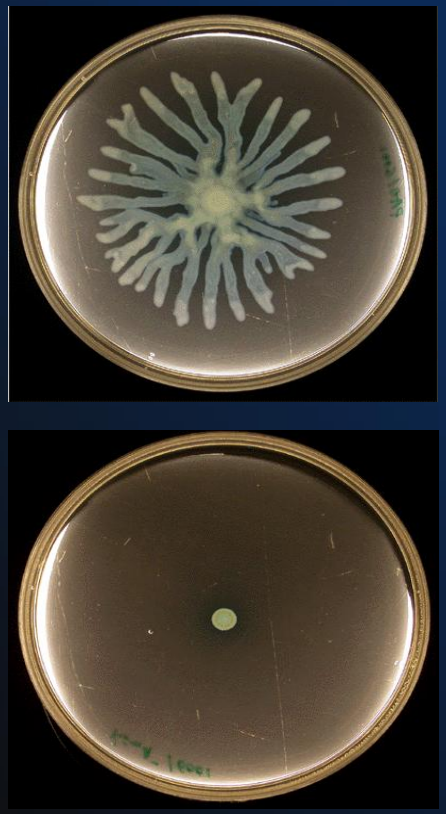
RsmA-regulated phenotypes in *P. aeruginosa*

Negative: Exoprotease, elastase, cyanide, lectin, pyocyanin, staphylolytic activity

Positive: swarming motility, lipase

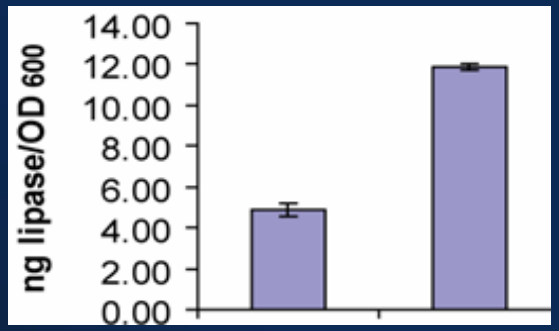


PAO1 (WT)



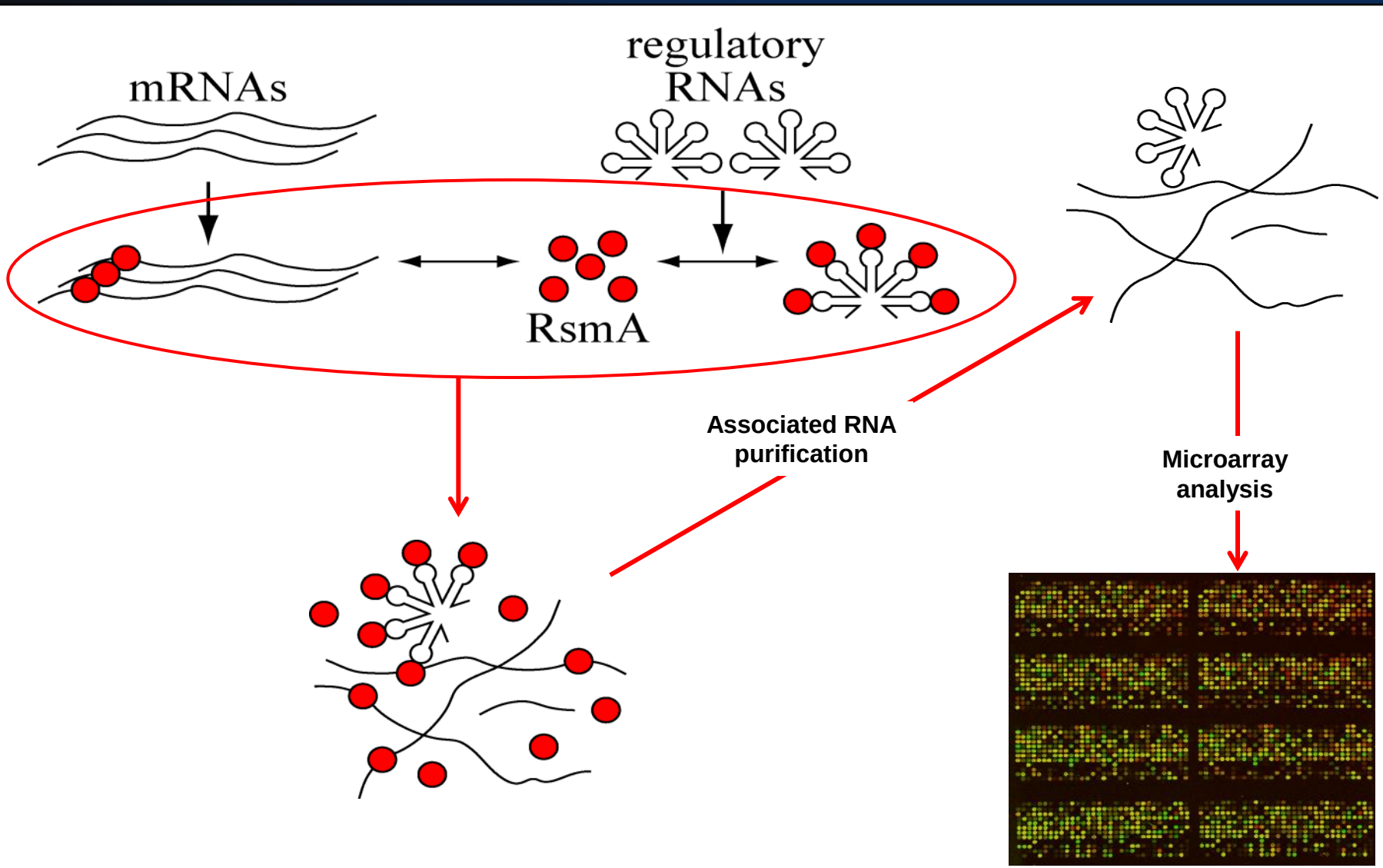
rsmA mutant

Lipase

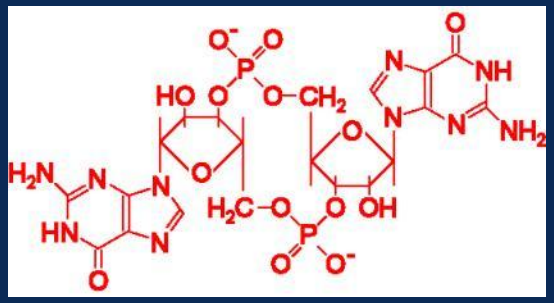


These are all QS AHL-regulated phenotypes

What is the nature of the RsmA regulome?



Cyclic-di-GMP signalling

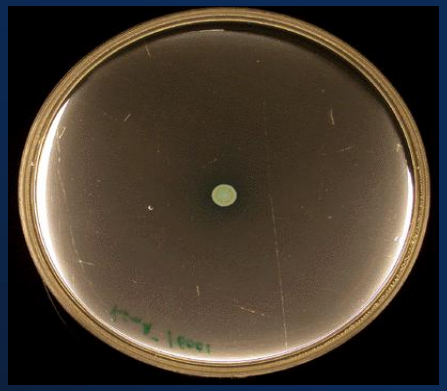


- Universal bacterial secondary messenger – intracellular signalling
- Involved in reciprocal control of motile or sessile growth
  - ↓ c-di-GMP → motility
  - ↑ c-di-GMP → biofilm
- GGDEF (cyclase), EAL (phosphodiesterase) and HD-GYP (phosphodiesterase) domain proteins produce and turnover c-di-GMP
- PilZ and PelD domain proteins are c-di-GMP binding proteins
- *P.aeruginosa* has 33 GGDEF, 16 EAL, 3 HD-GYP and 9 c-di-GMP receptors

Restoration of swarming in an RsmA mutant

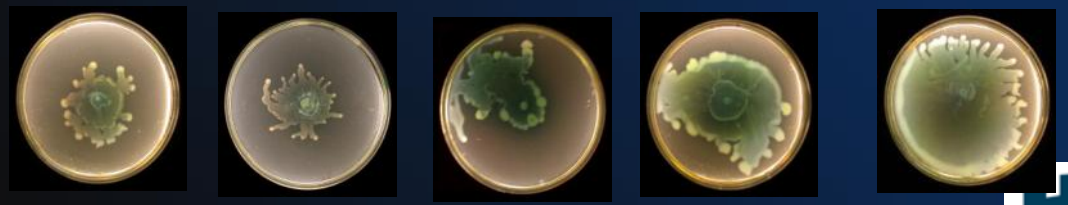
PAO1 (WT)

Gene library

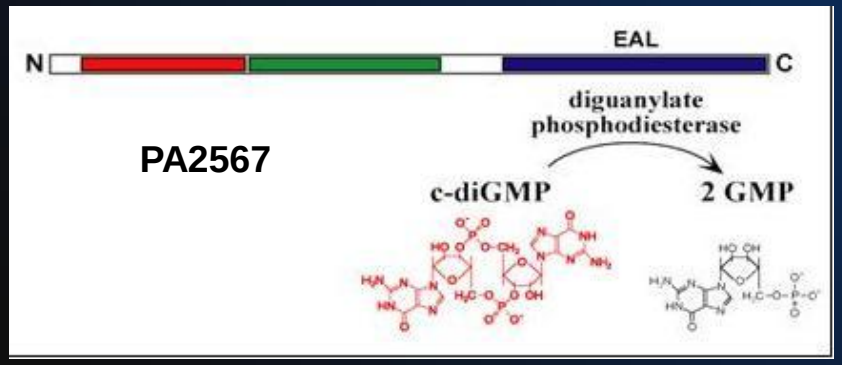


rsmA mutant

Screen for restoration of swarming



RsmA, PA2567 and cyclic-di-GMP metabolism



RsmA

↓

PA2567

phosphodiesterase ↓

decreased c-di-GMP

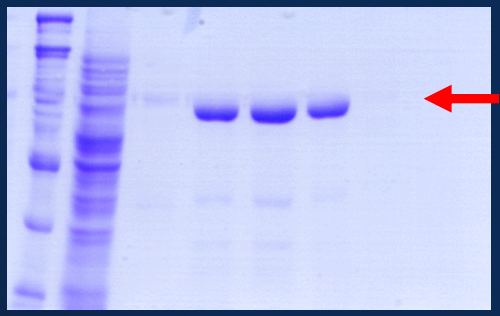
↓

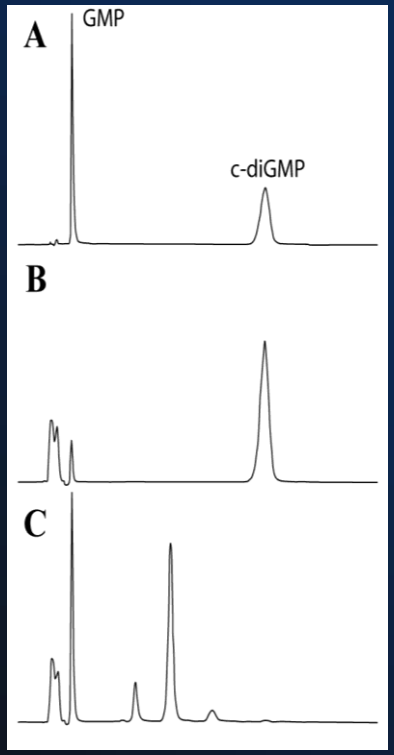
↓

↓

Swarming

Purification of PA2567



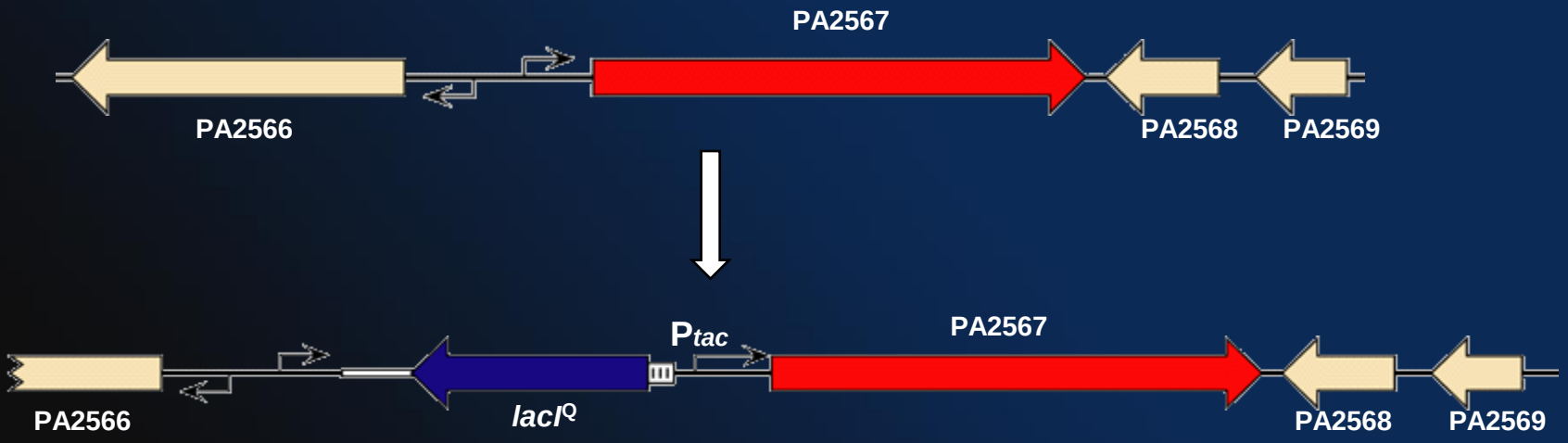


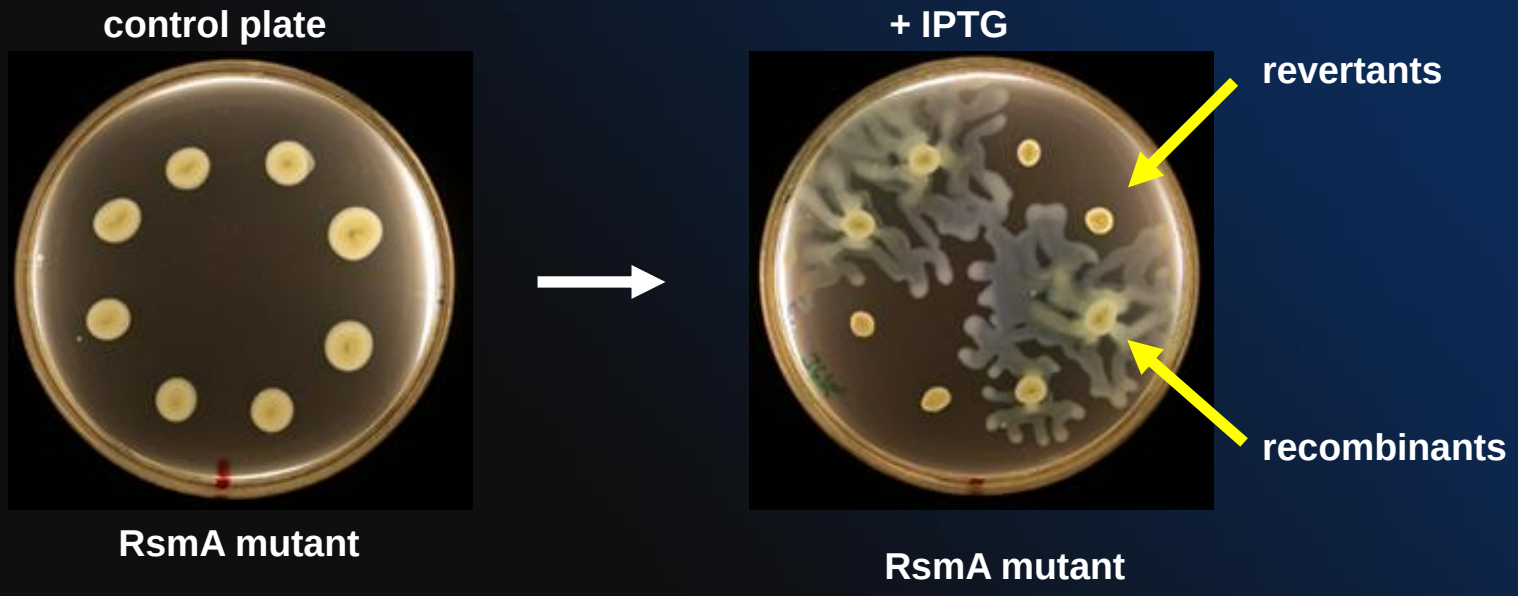
Standards

c-diGMP + buffer

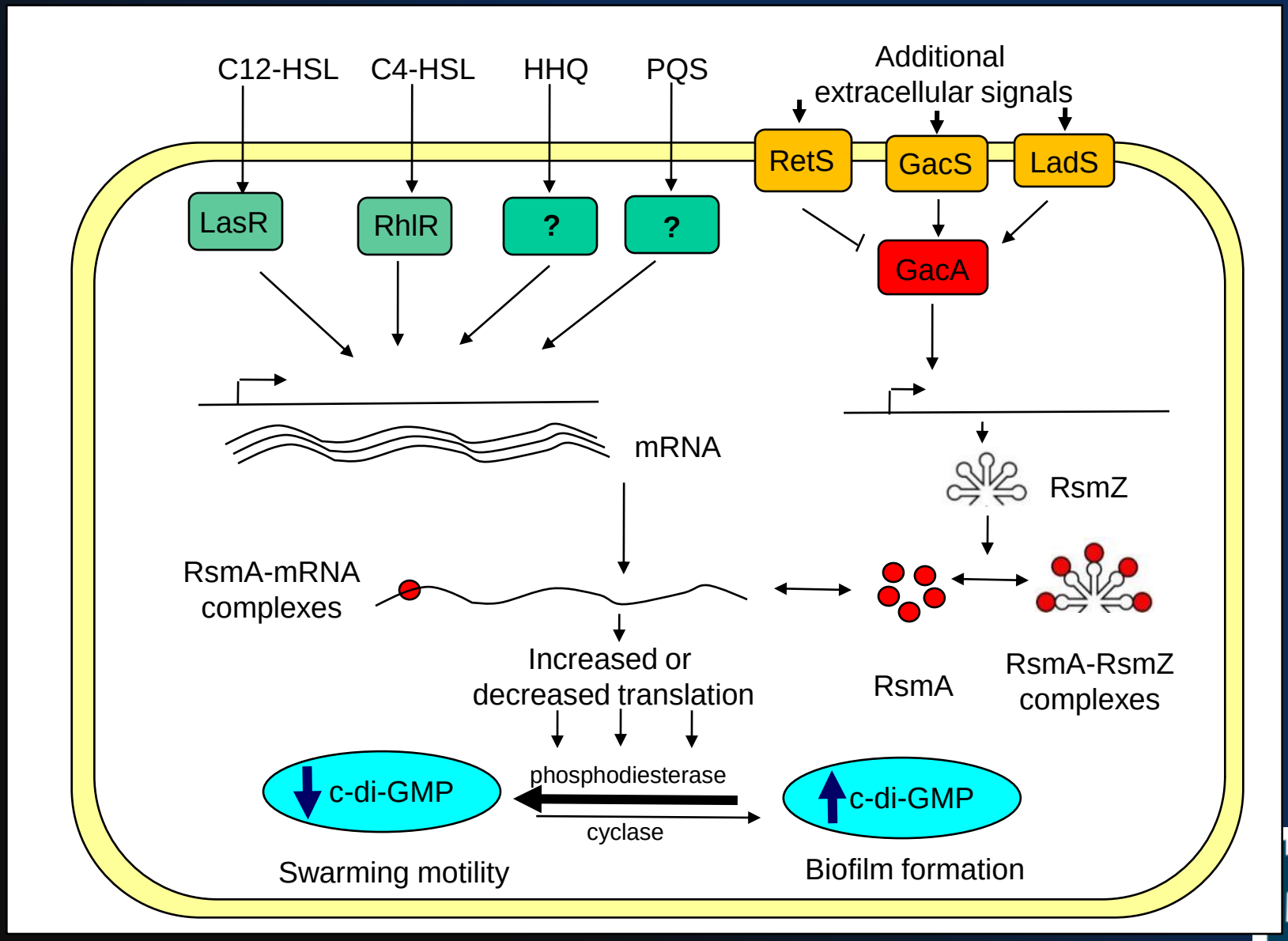
c-diGMP + buffer
+ PA2567

Inducible expression of PA2567





Global Transcriptional & Post-Transcriptional QS–dependent Regulatory Networks in *P.aeruginosa*

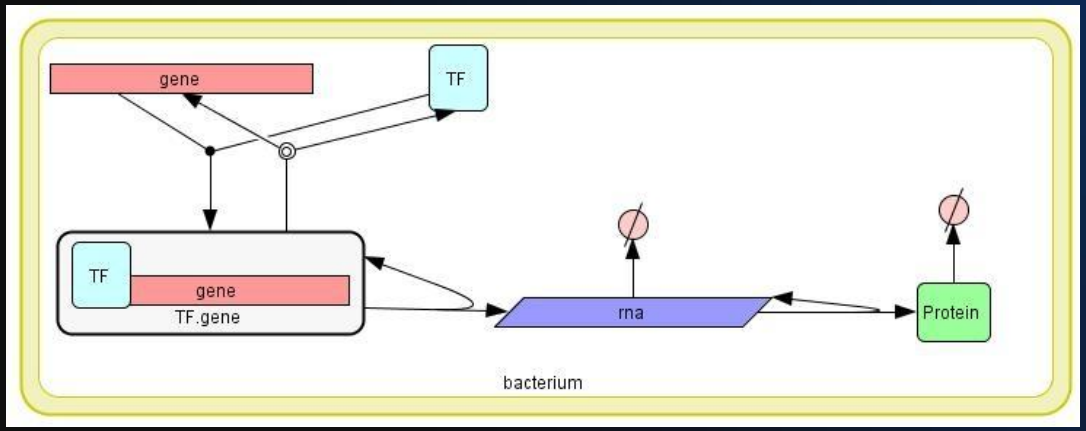


Modelling transcriptional regulation by quorum sensing

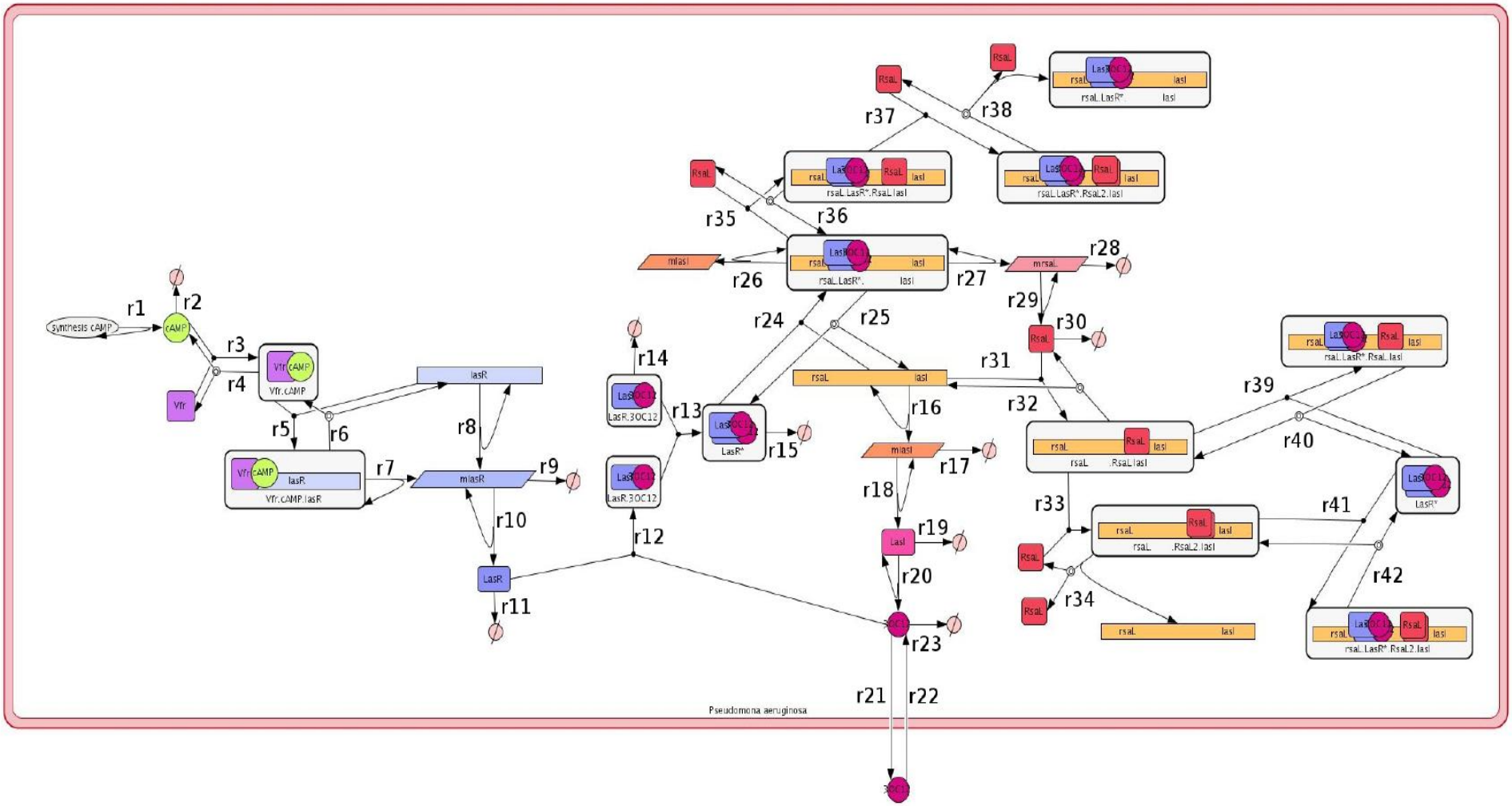
- Modelling is about making the ***correct simplifications***:

  Gene Regulation = Transcriptional Regulation

- For every gene is the model we considering the following parameters:
  - Affinity of the different transcription factors.
  - Transcription rates for the possible promoter states.
  - Messenger RNA and protein half lives.
  - Synthesis rates
  - Diffusion rates



Modelling the las system



Conclusions

- Bacteria use quorum sensing signal molecules to coordinate changes in population behaviour.
- QS regulatory cascades are tightly interlinked to other transcriptional and post-transcriptional regulatory networks.
- We are starting to dissect the relationships between these networks using computational approaches.

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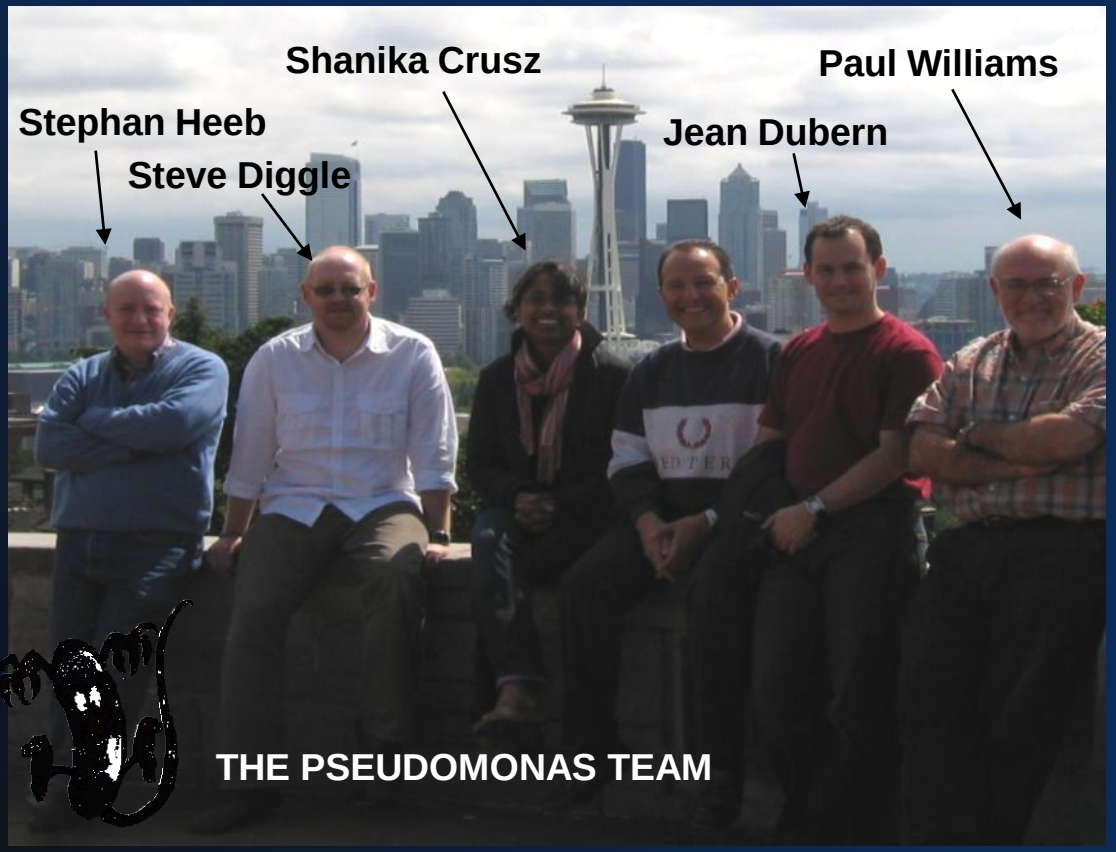

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The quorum sensing site

www.nottingham.ac.uk/quorum