

The Stringent Response in *Clostridioides difficile*

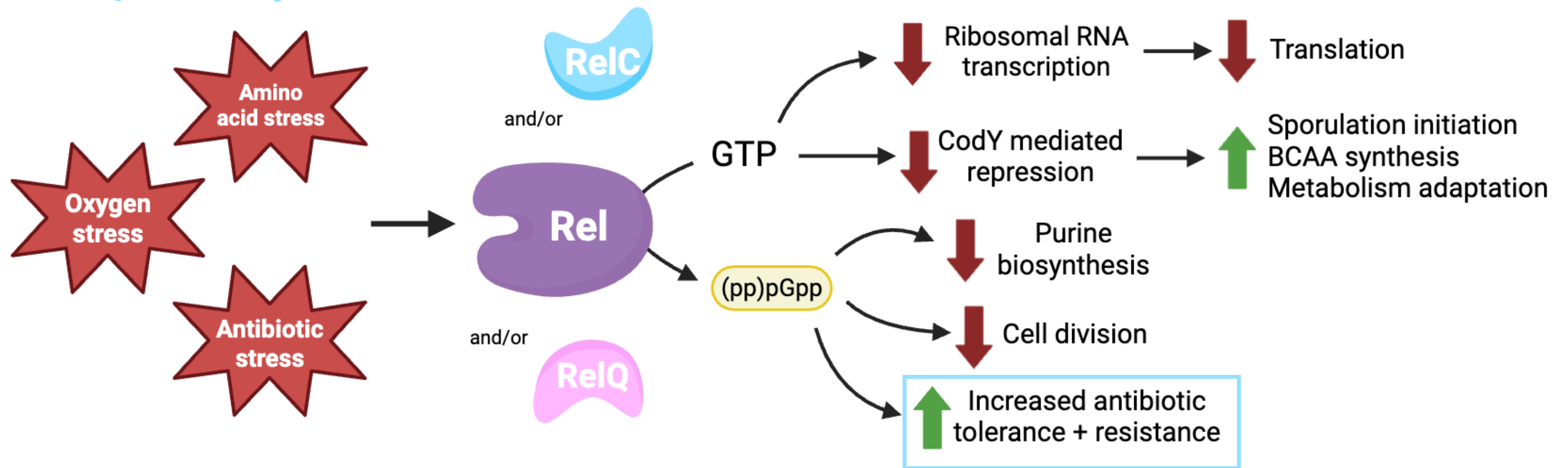
Lilia Hodge

3CdF

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What is the stringent response?

- Ubiquitous bacterial signalling mechanism required for survival under **nutrient -limiting** conditions
- Characterised by **rapid synthesis** of the alarmones (pp)pGpp
 - pGpp, ppGpp, and pppGpp
- Controlled by enzymes of the **RelA/SpoT homolog (RSH) superfamily**

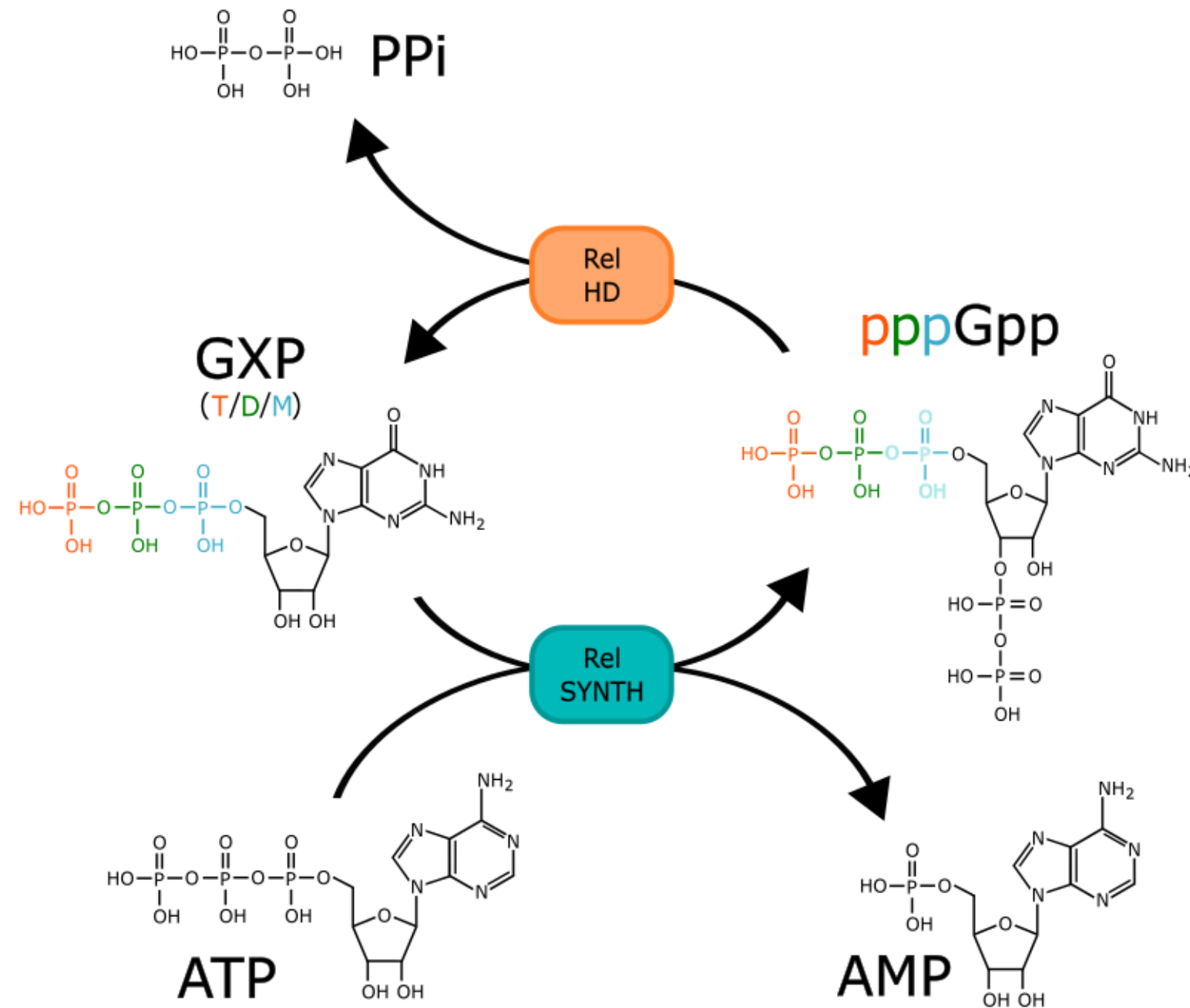




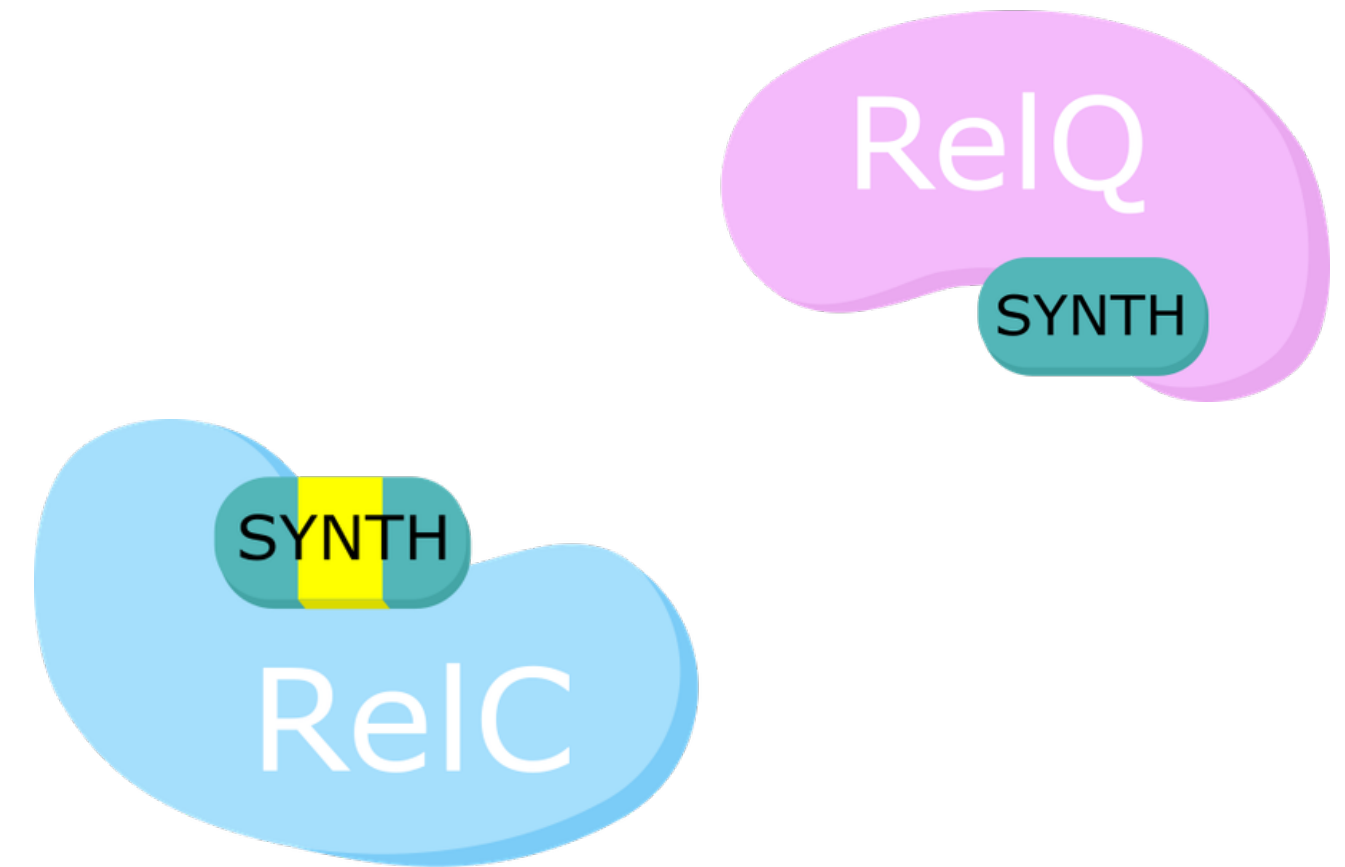
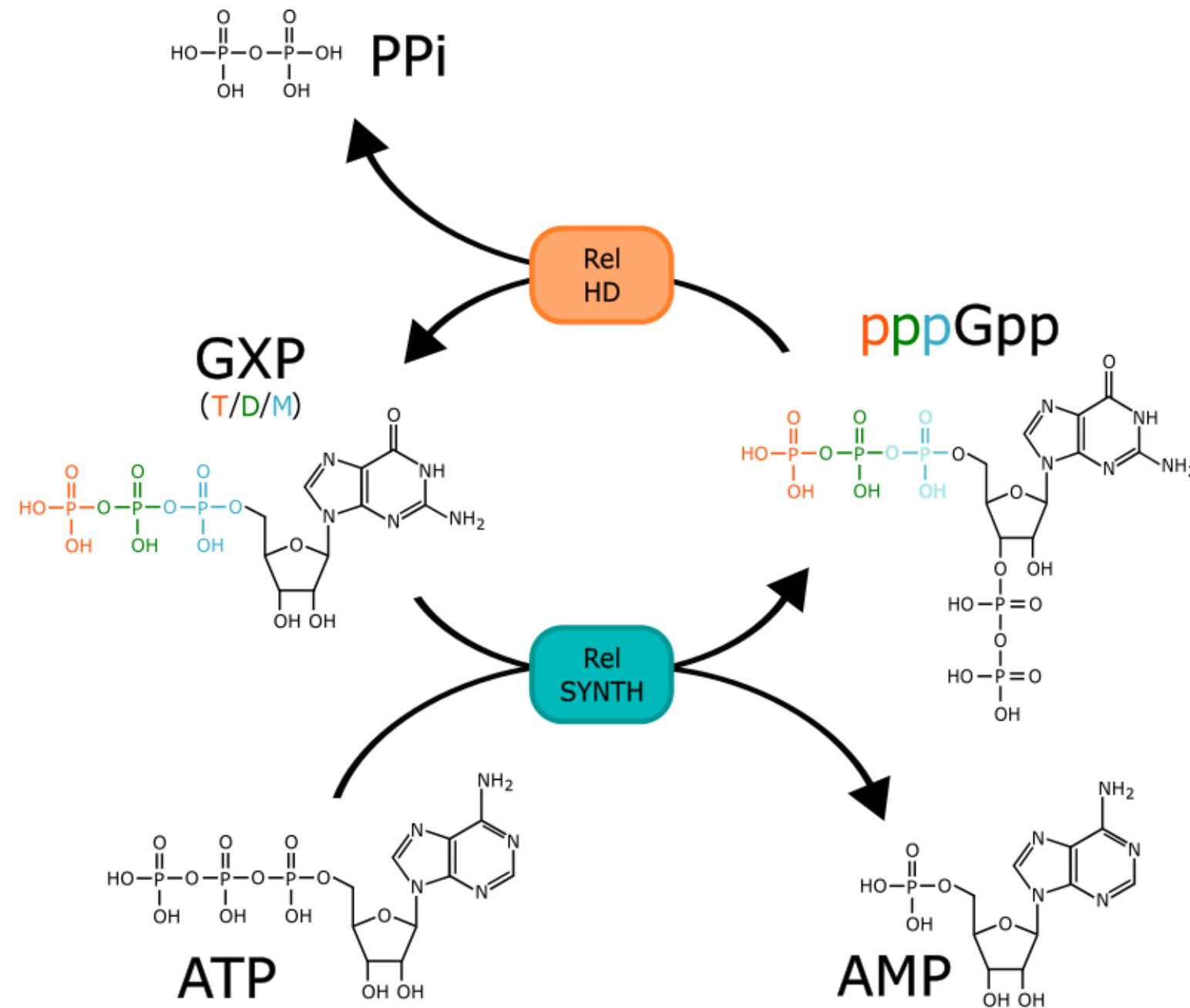
Why care about the stringent response and AMR?

- Big picture:
 - Clinical **resistance** to antibiotics is emerging
 - **Characterisation** of the pathway may help with eventual treatment
- More importantly:
 - Huge gap in knowledge!

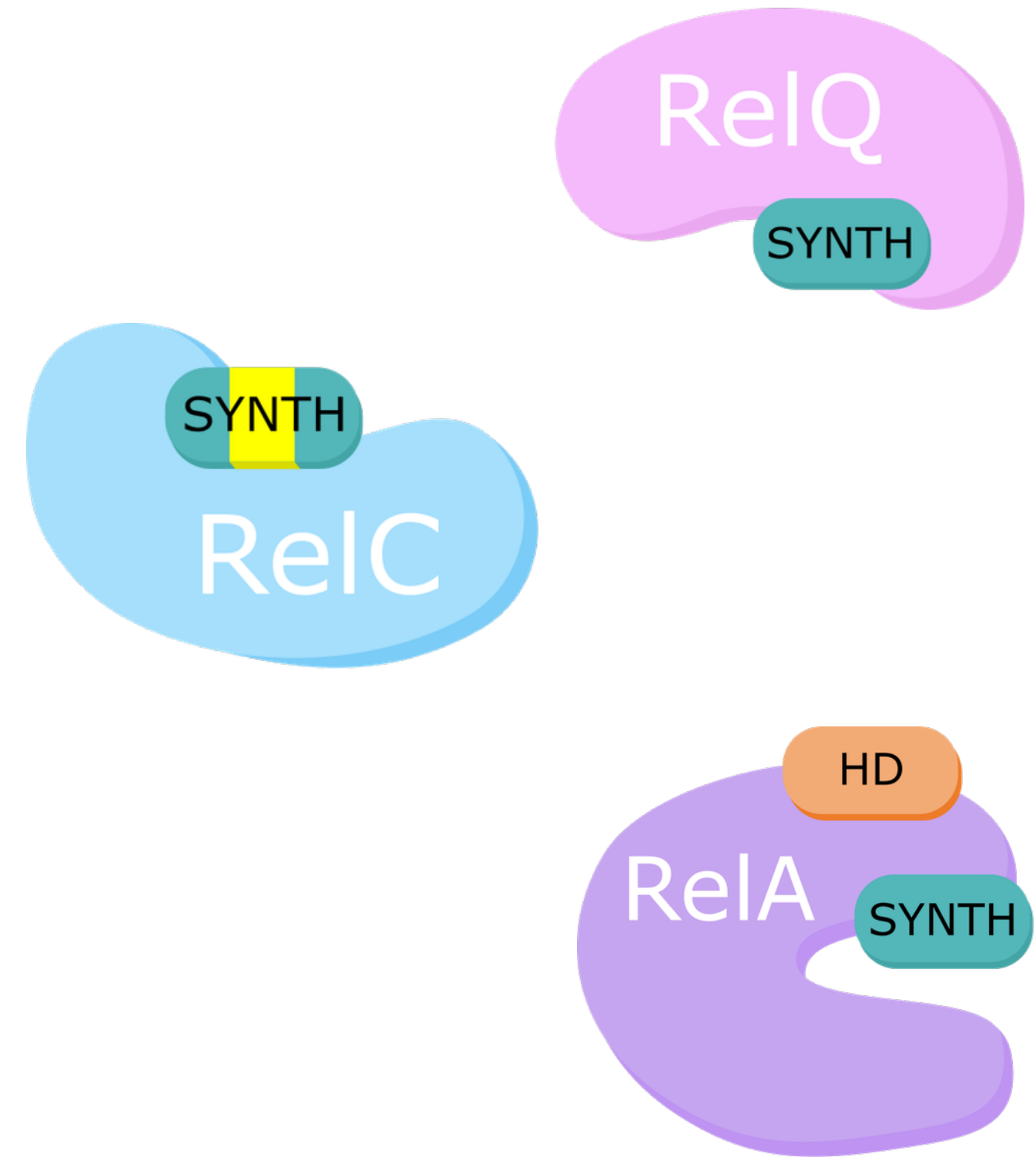
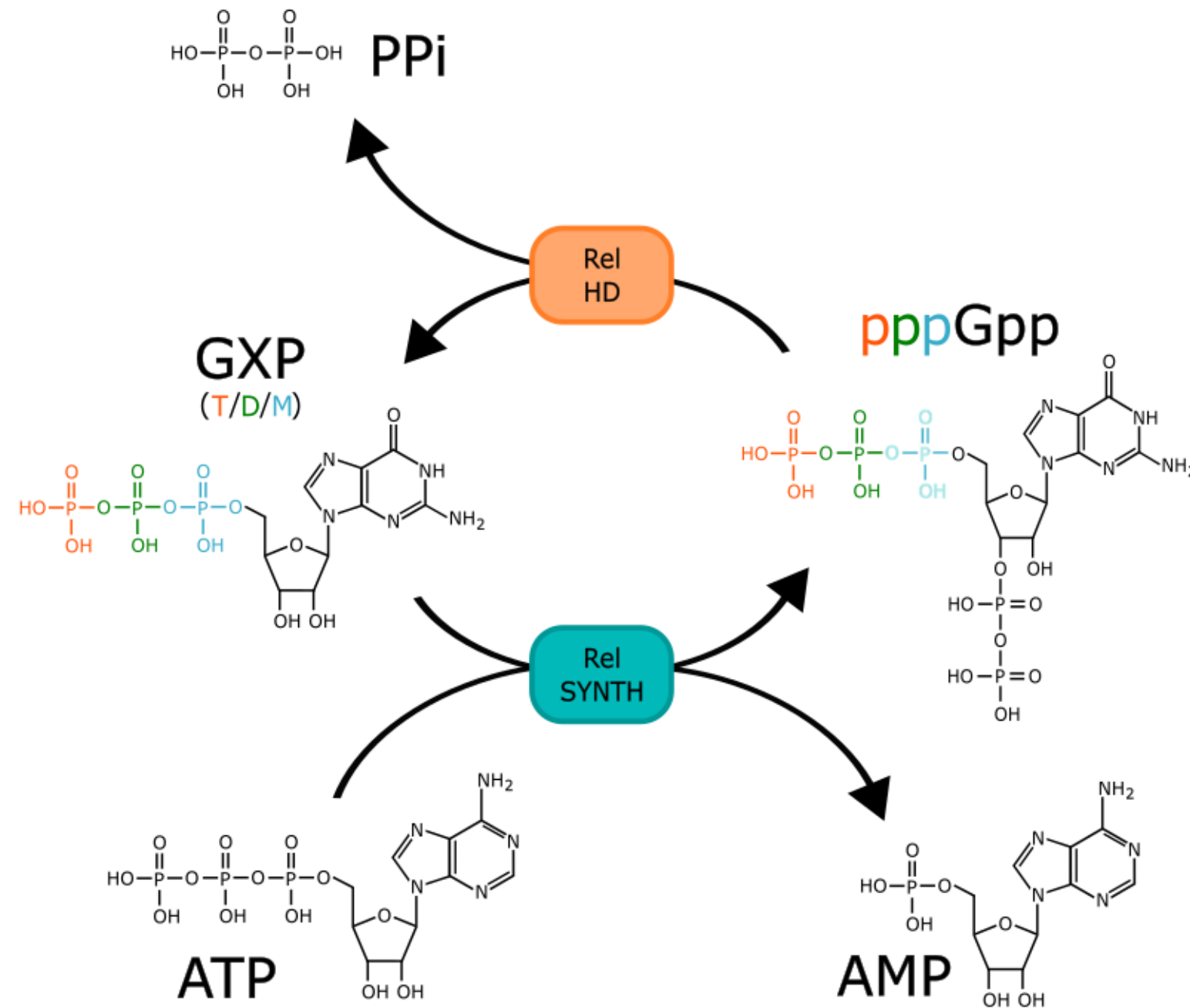
The *C. difficile* stringent response



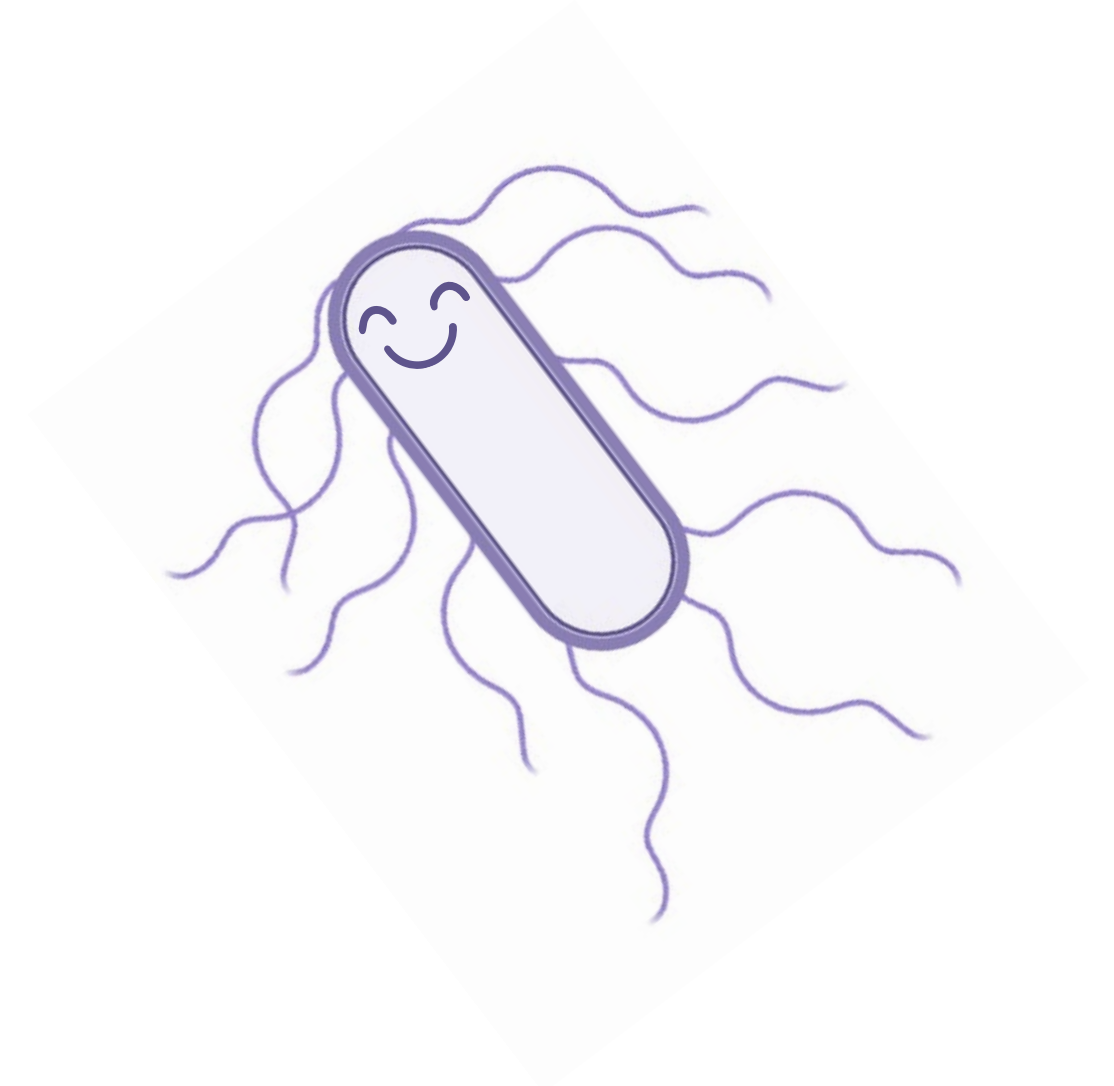
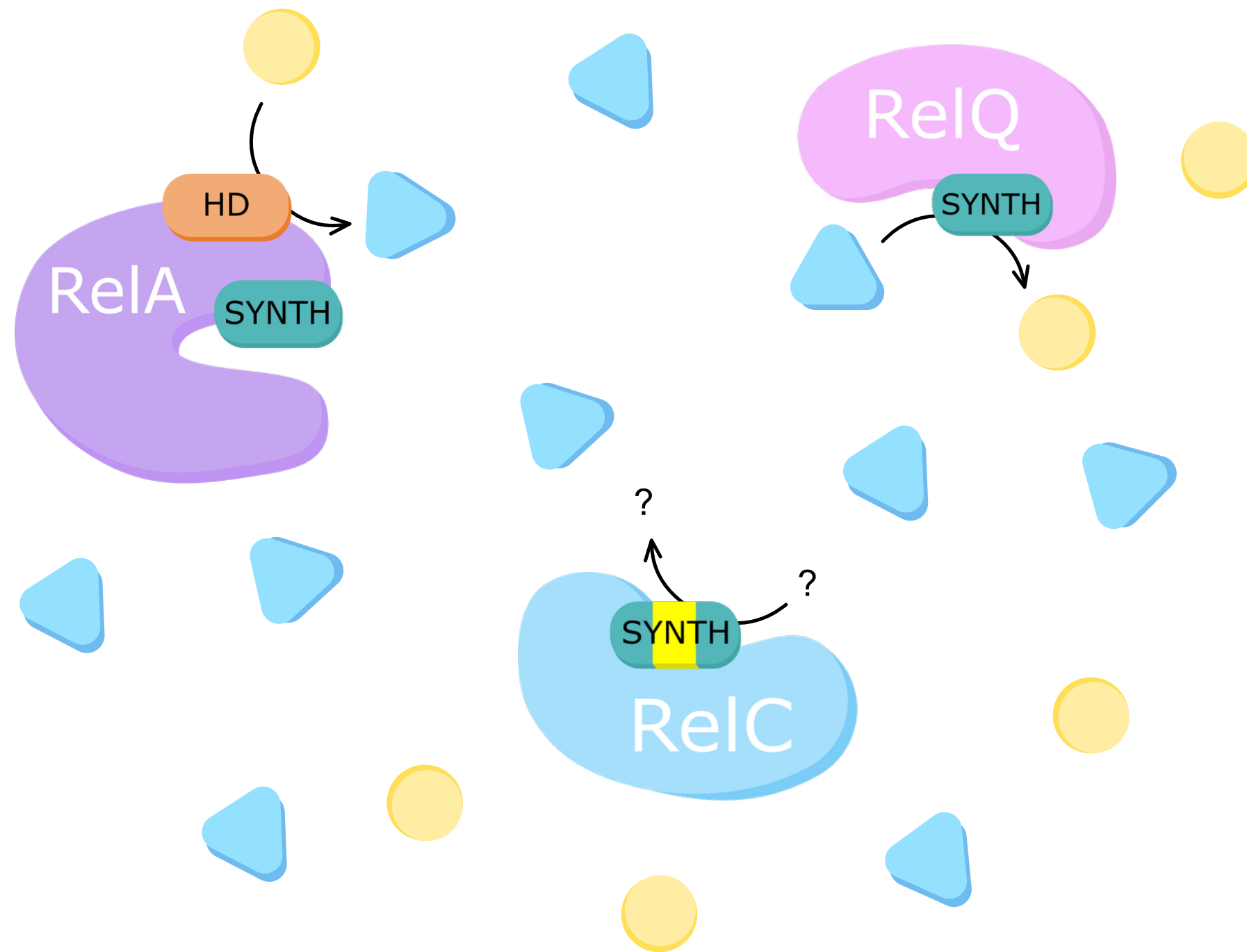
The *C. difficile* stringent response



The *C. difficile* stringent response



Generating mutants



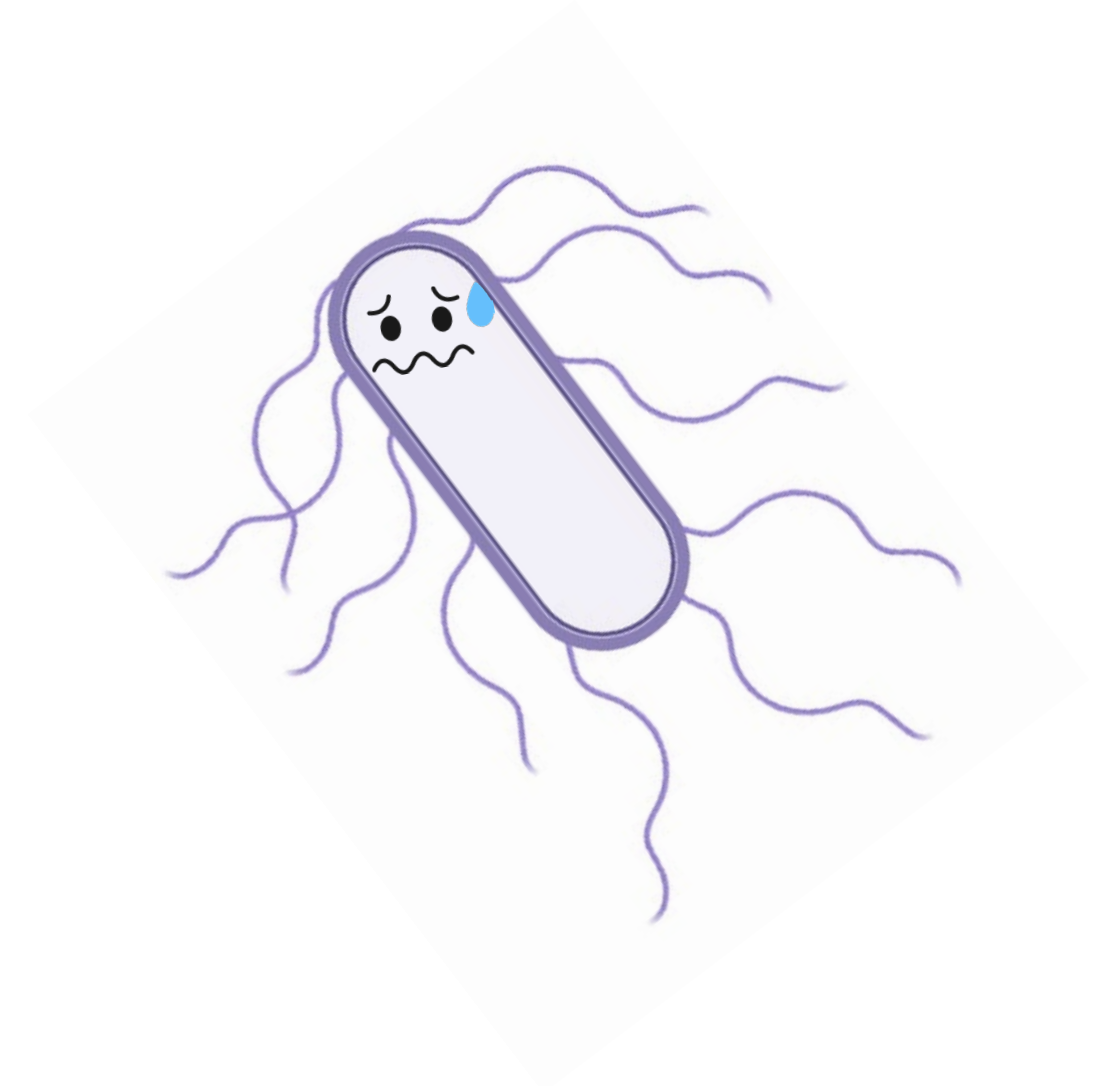
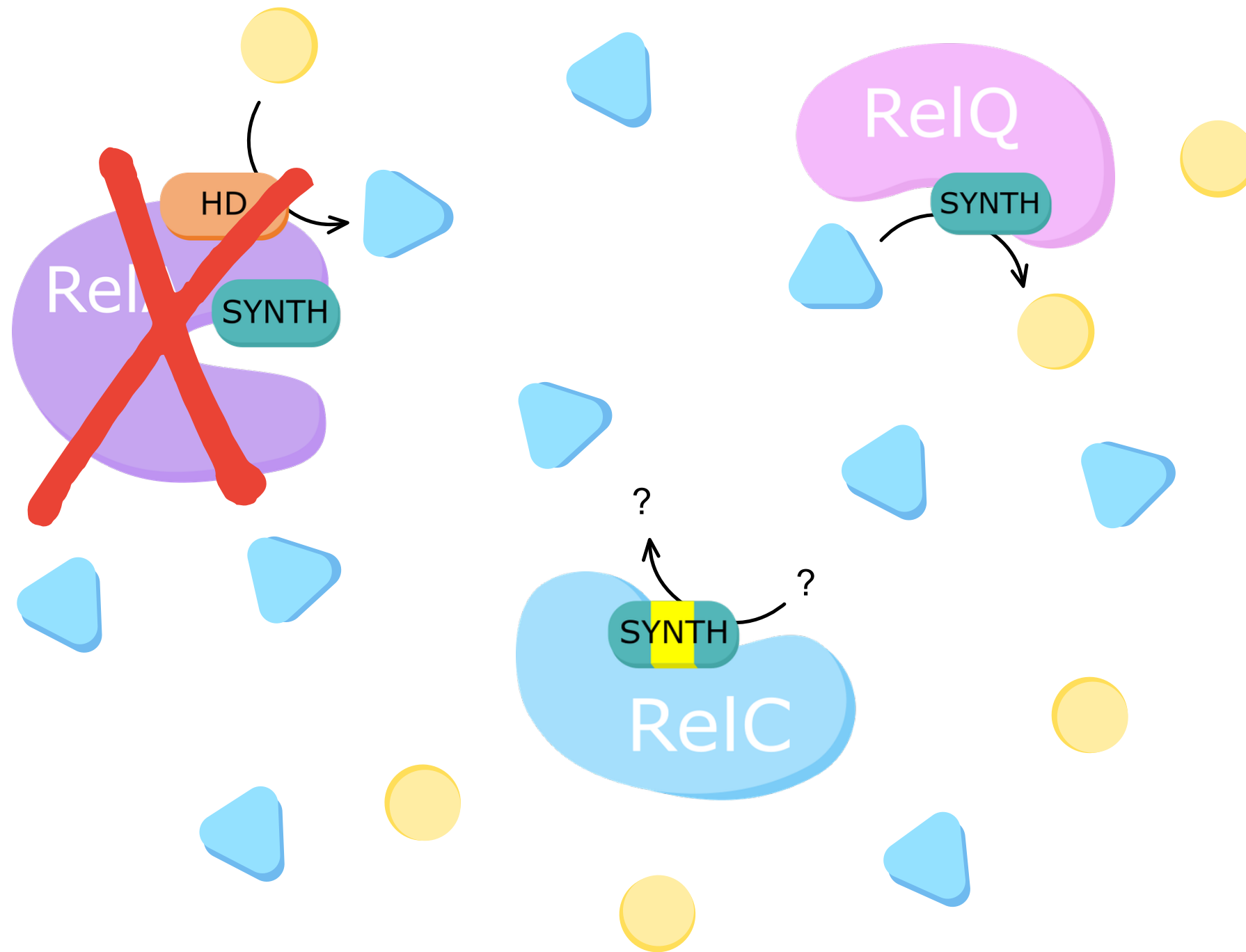
GTP



(pp)pGpp



Generating mutants



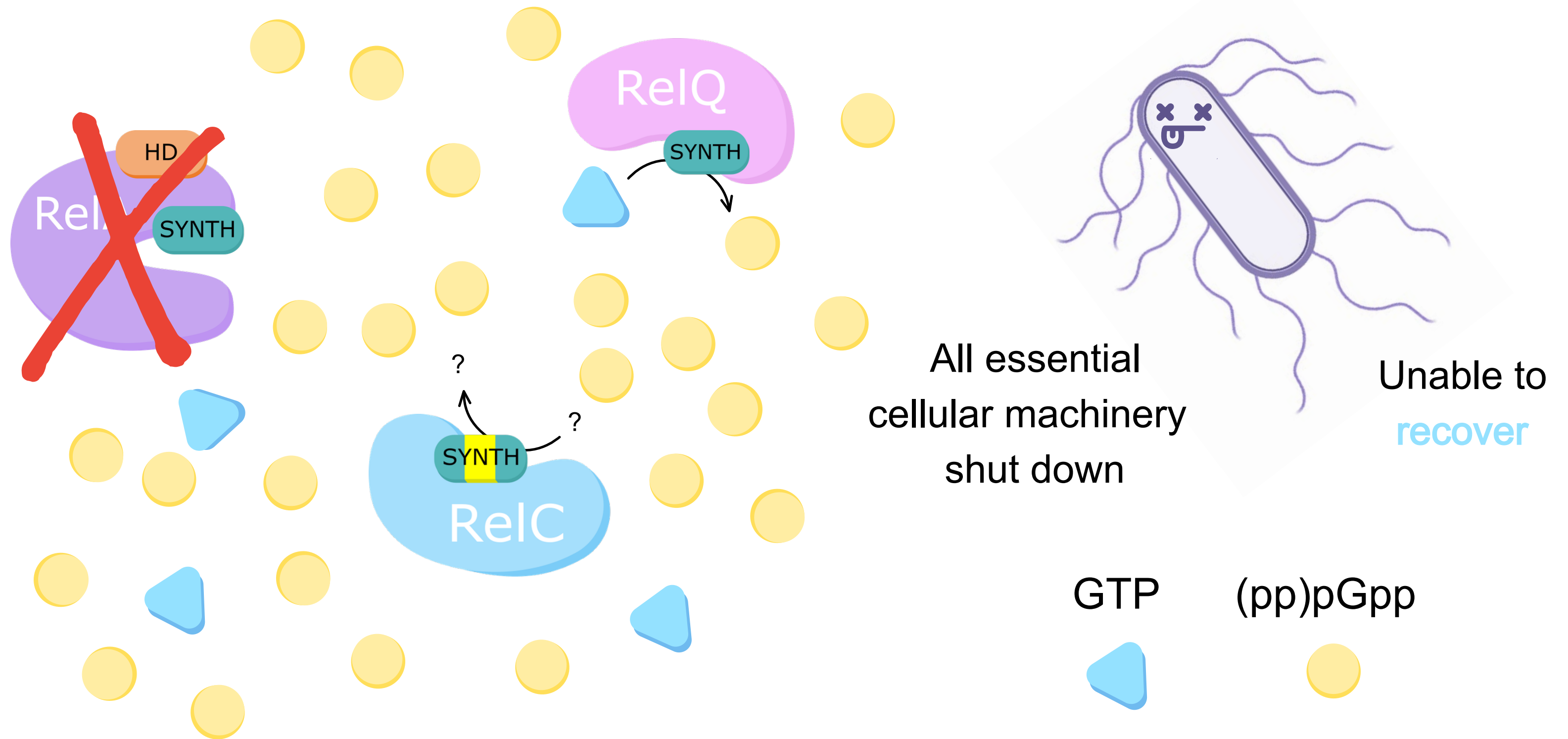
GTP

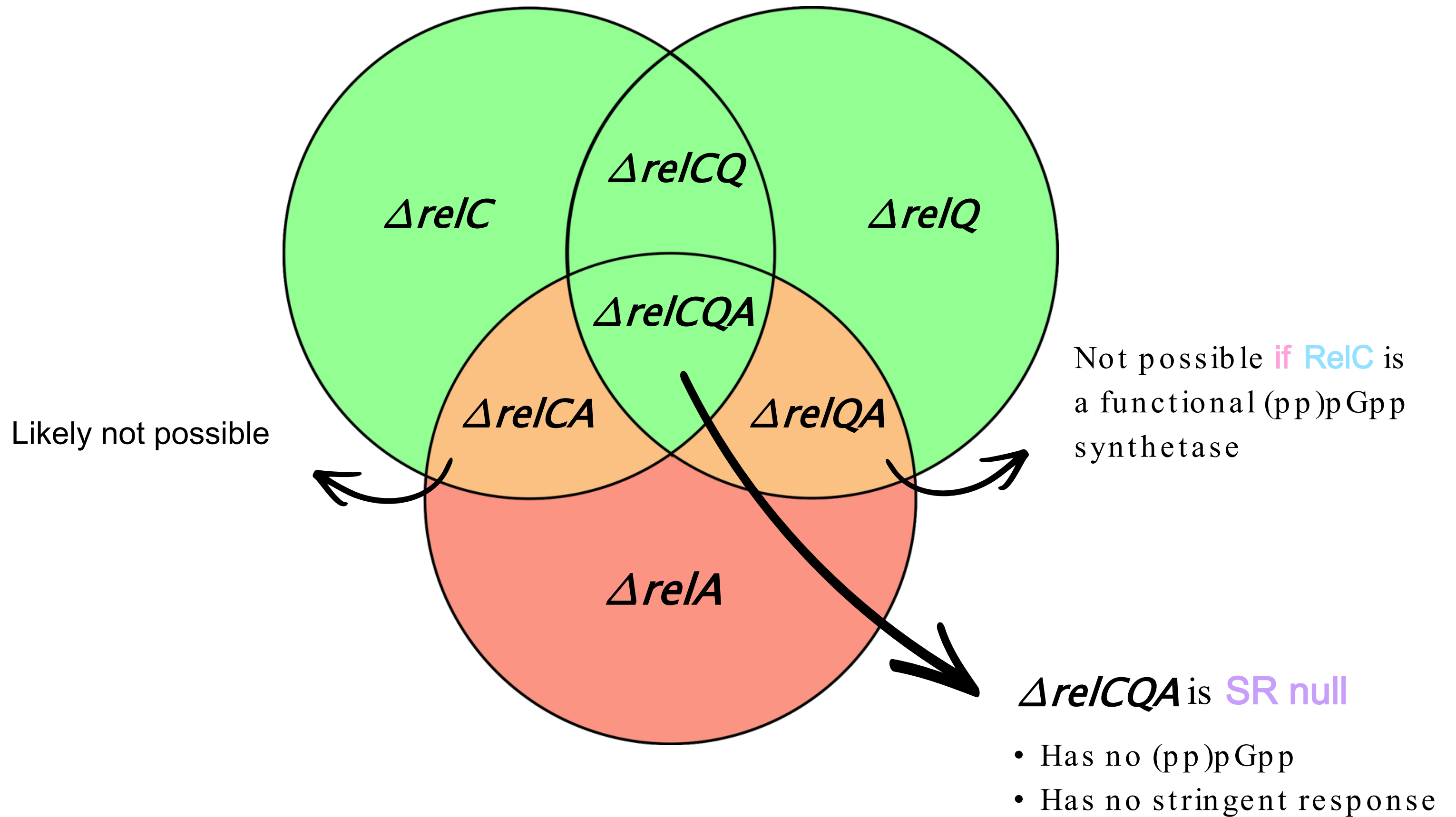


(pp)pGpp

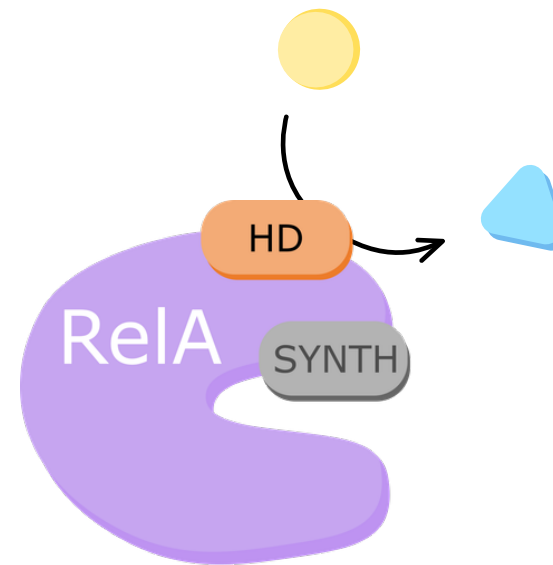
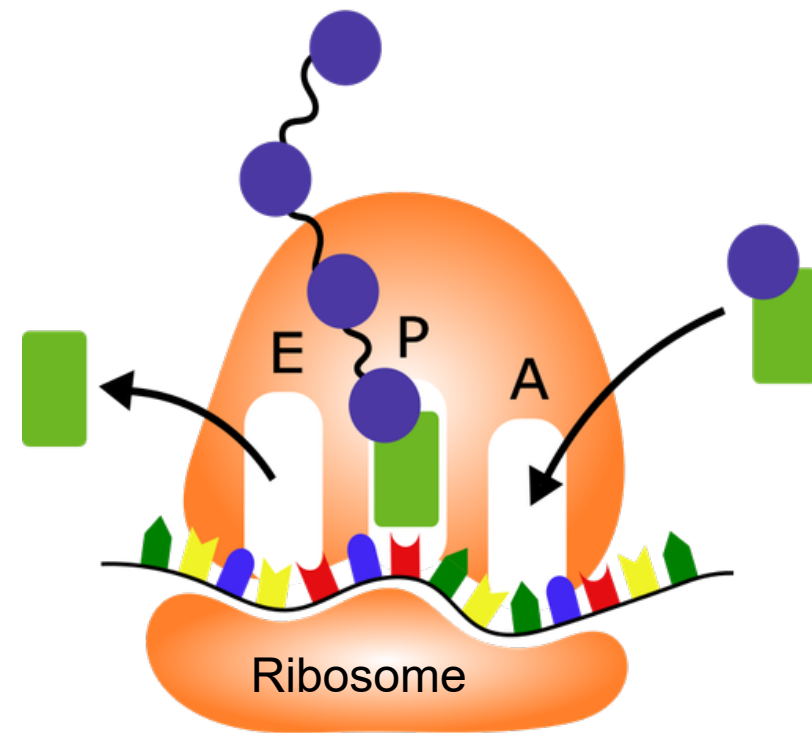


Generating mutants





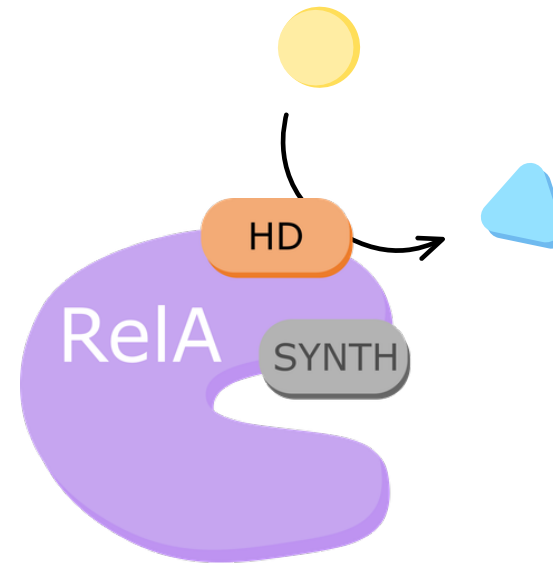
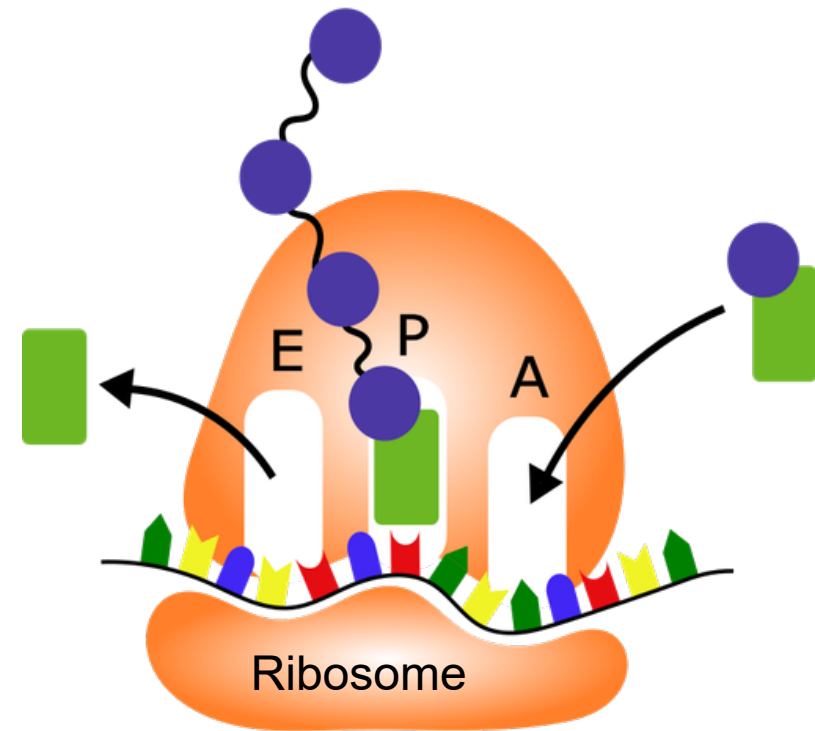
Amino acid starvation activates RelA



Nutrient replete conditions

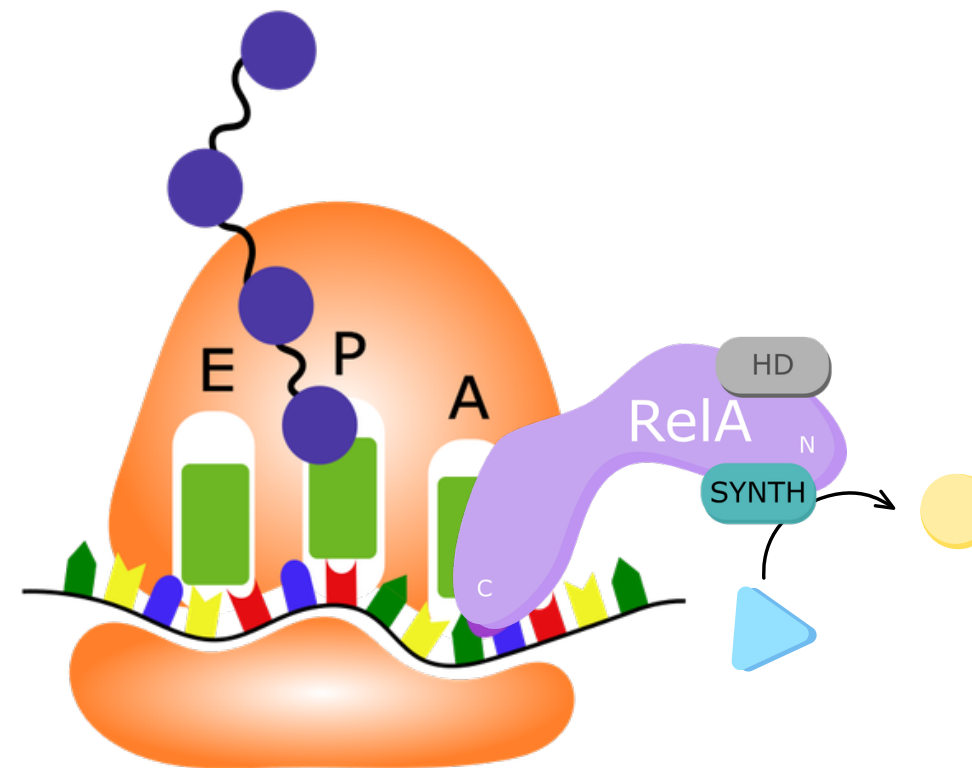
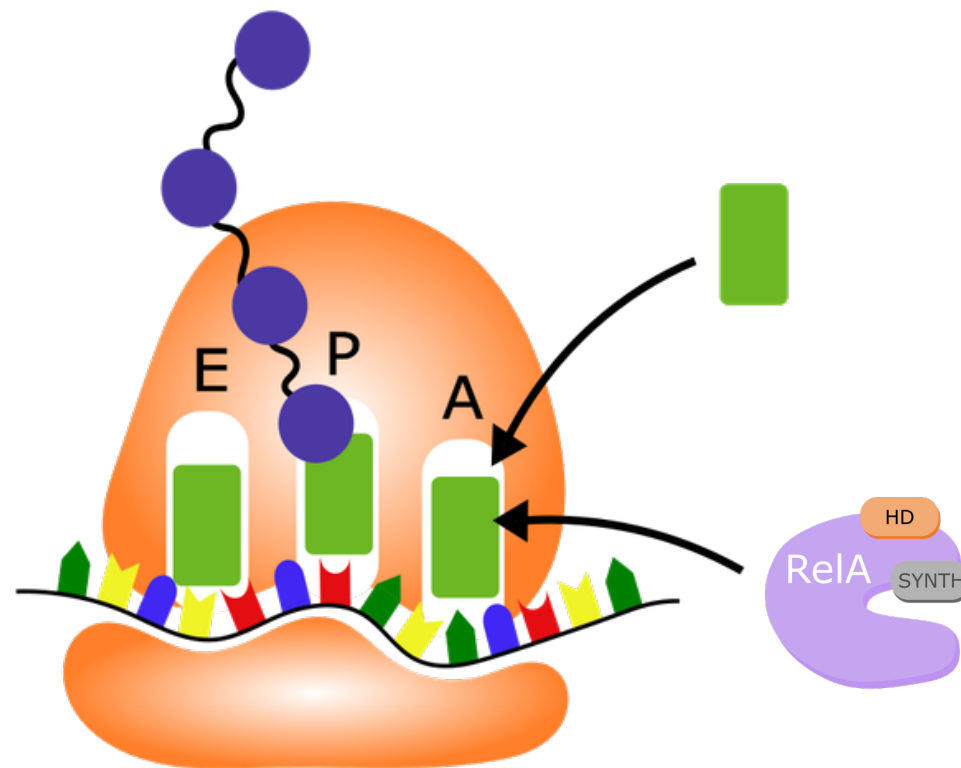
- Charged tRNA
- Amino acid transferred to peptide chain
- RelA in 'closed' conformation, synthesis OFF

Amino acid starvation activates RelA



Nutrient replete conditions

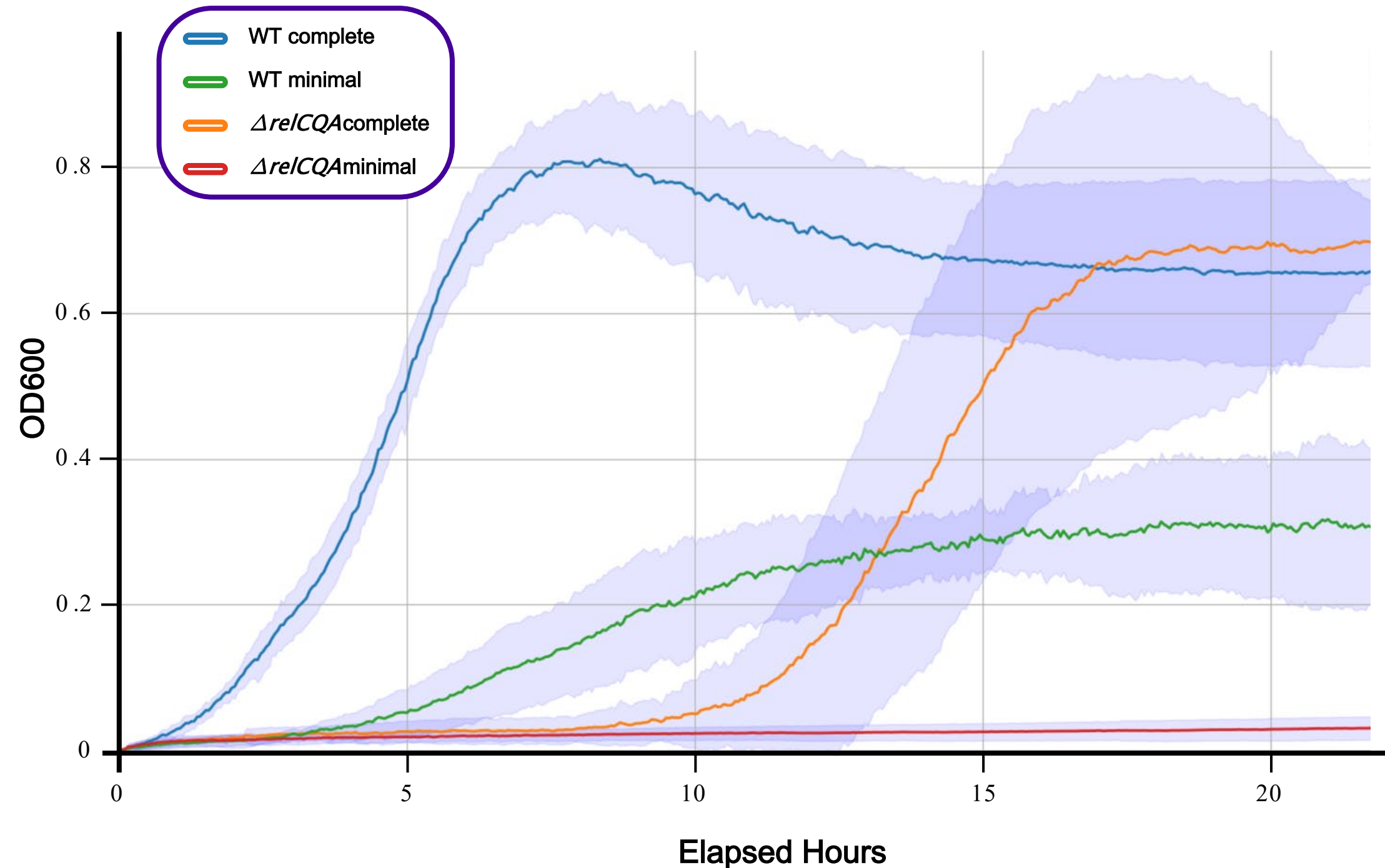
- Charged tRNA
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Amino acid starvation

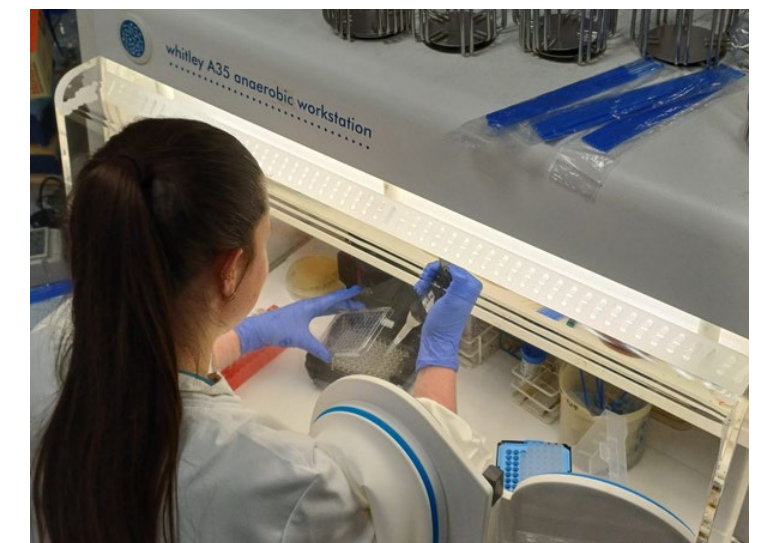
- Uncharged tRNA
- RelA CTD interacts with tRNA and ribosome
- RelA 'Open' conformation, (pp)pGpp synthesis ON

Growth



- $\Delta relCQA$ mutants display a ~15hr lag in **complete media** compared to **WT** in **complete media**
- **Minimal media** causes a survivable growth defect in **WT**
- $\Delta relCQA$ mutants cannot grow in **minimal media**

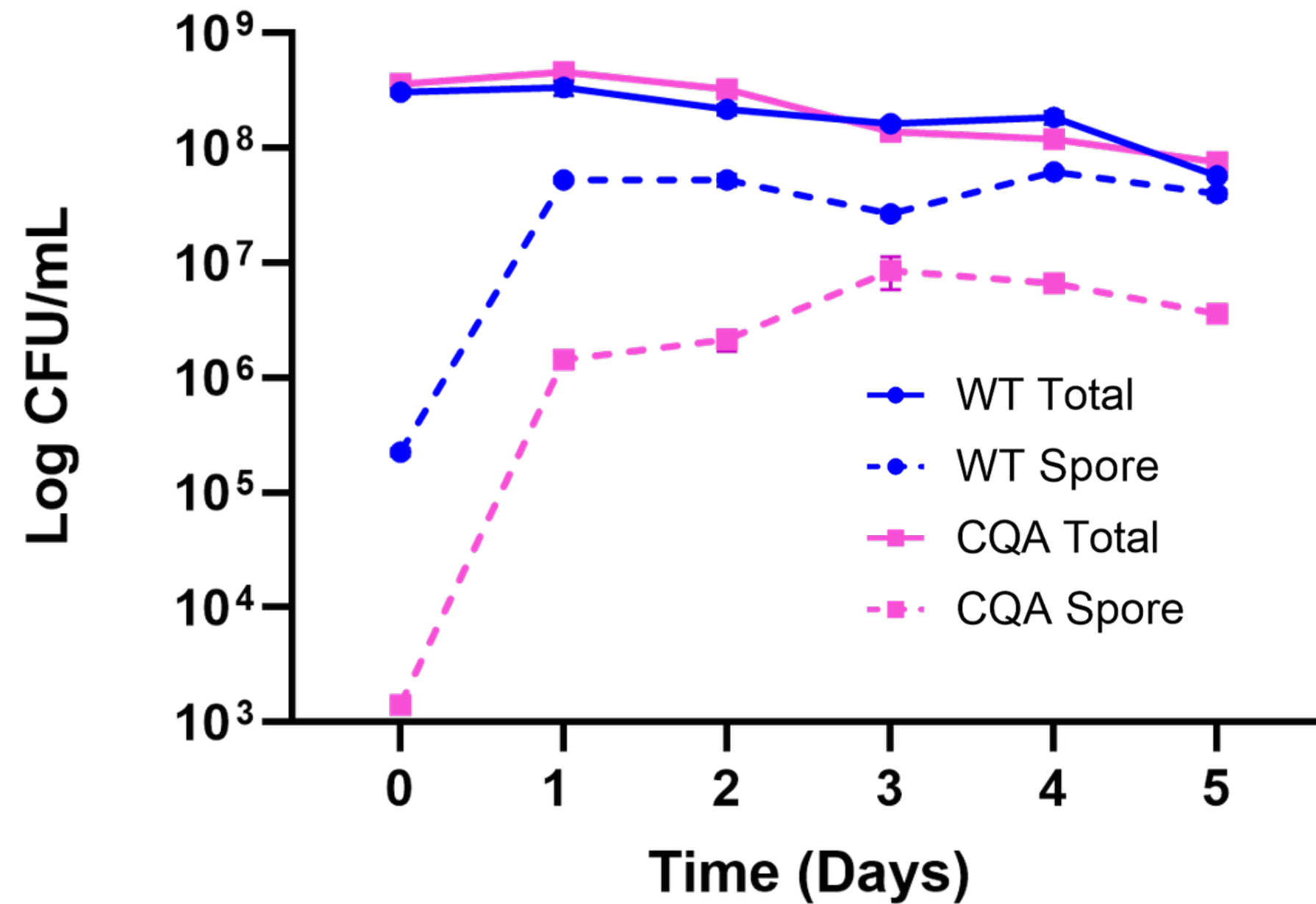
All my experiments are performed in the Whitley A35 Anaerobic Workstation!



- Minimal media: 11 essential amino acids at lowest concentration for growth

Karasawa *et al.*, 1995, Microbiology

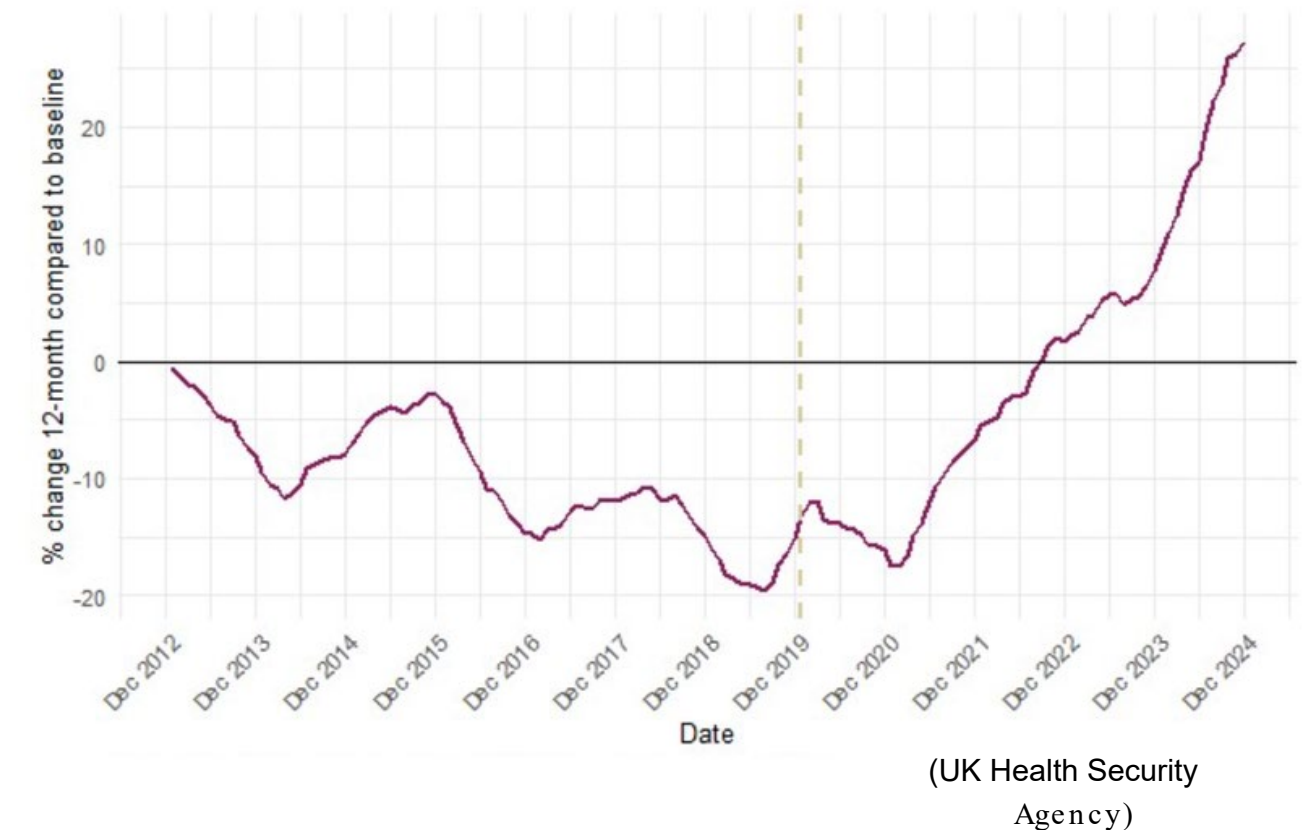
Sporulation



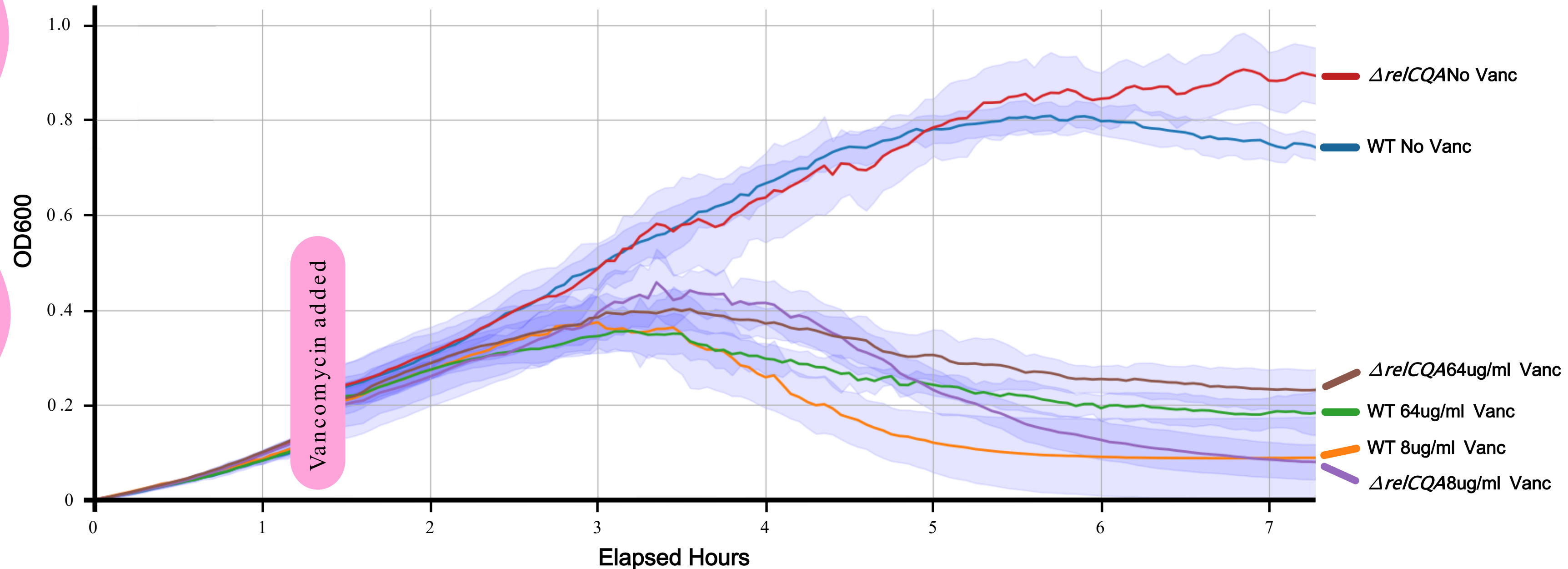
- $\Delta rel/CQA$ displays **delayed** and overall **lower** sporulation
- Stringent response activation causes rapid **decrease** of cellular **GTP** pool
 - CodY derepression $\rightarrow$ sporulation
 - Shown in *B. subtilis*

Antibiotic persistence

- Cases are rising rapidly in the UK
 - 33% increase from 2020 to 2024
- Vancomycin is first line antibiotic
 - 20-30% of cases recur
- Antibiotic persistence
 - Ability for a subpopulation of bacteria to survive through antibiotic treatment, with no change in MIC



Response to vancomycin



- Strains grow normally for ~1hr post vancomycin treatment, then lyse
- CQA takes slightly longer to halt growth
 - But overall quite similar

A big thank you to:

My supervisors

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Biotechnology and
Biological Sciences
Research Council



don whitley
scientific
culturing innovation

Plus everyone else who has
helped and supported me!



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