

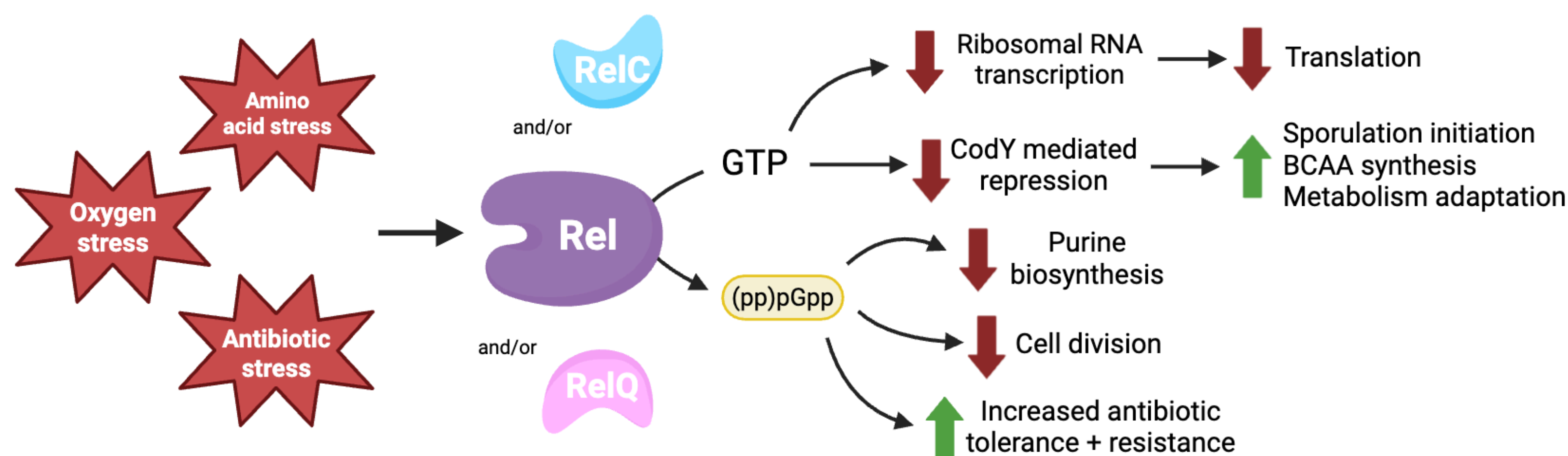
Exploring the *C. difficile* Stringent Response

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Introduction

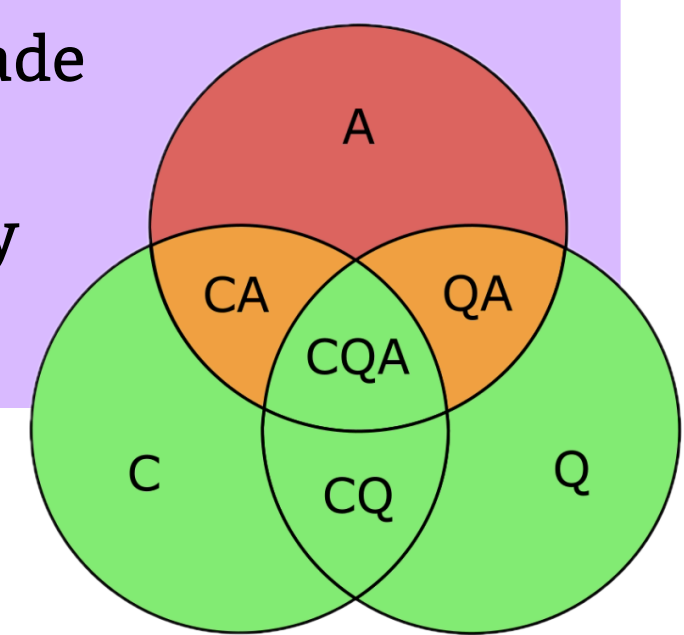
The Stringent Response is a ubiquitous bacterial stress signalling pathway responsible for survival and adaptation to nutrient stress. The response is characterised by a rapid synthesis of the alarmone (pp)pGpp by enzymes of the RSH superfamily. These enzymes also hydrolyse (pp)pGpp to prevent toxic accumulation of the alarmone.



- 3 RSH enzymes in *C. difficile*:
 - Rel** - bifunctional (pp)pGpp synthetase + hydrolase
 - RelQ** - (pp)pGpp synthetase
 - RelC** - putative (pp)pGpp synthetase

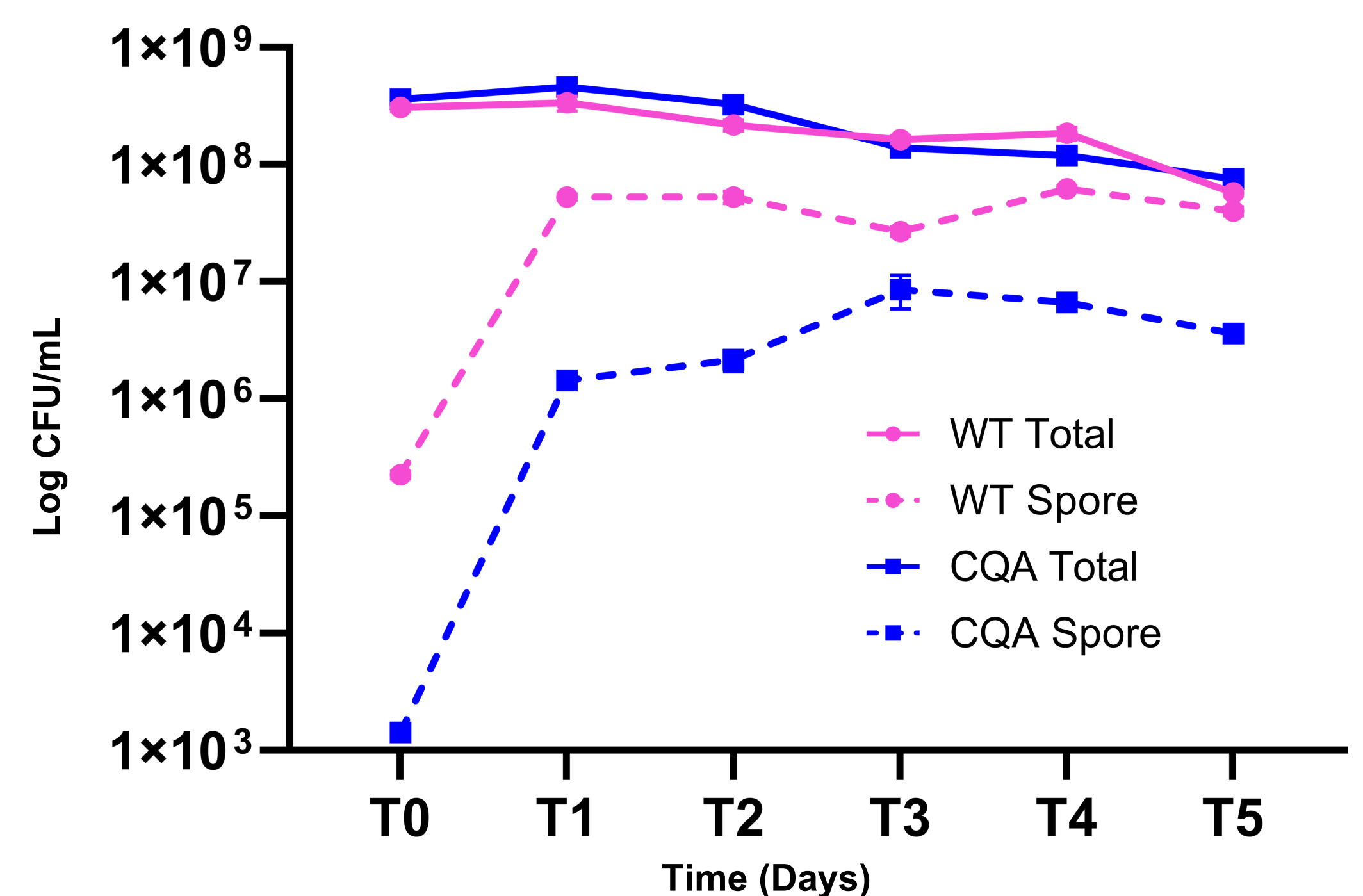
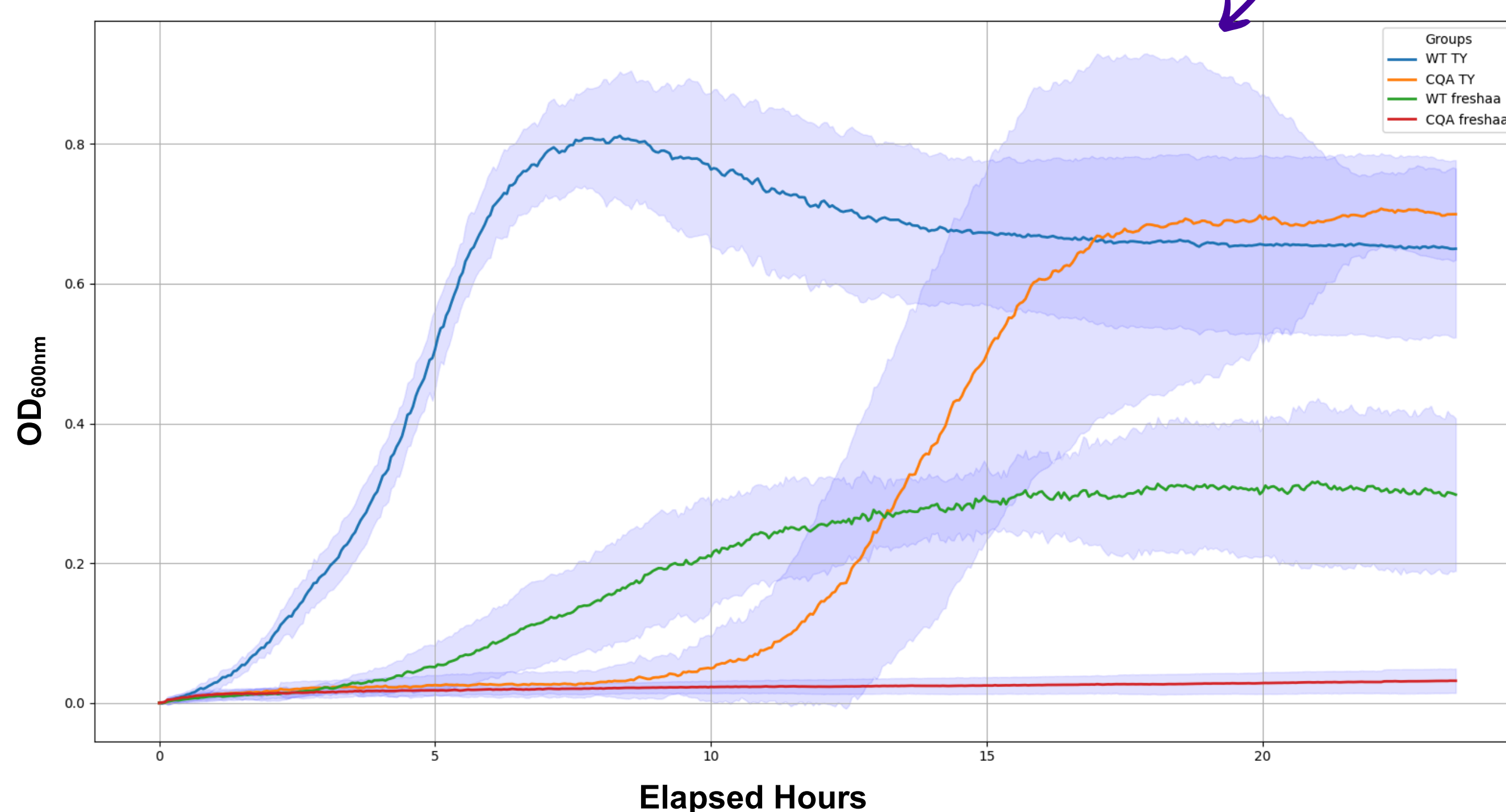
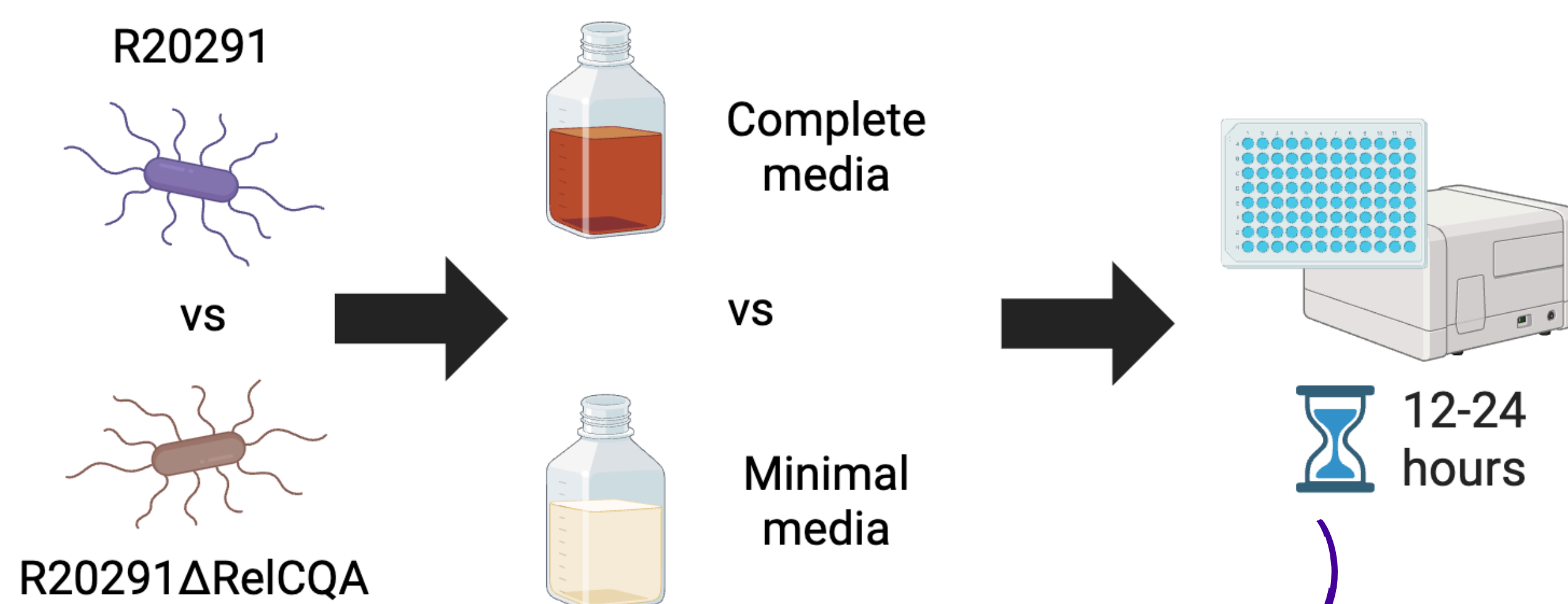
- Existing mutants: $\Delta relC$, $\Delta relQ$, $\Delta relCQ$, $\Delta relCQA$

- $\Delta relA$ mutant cannot be made due to essentiality of RelA (pp)pGpp hydrolase activity



Growth Defects

- Tested growth differences between WT *C. difficile* (R20291) and Stringent Response mutants ($\Delta relCQA$) in minimal vs nutrient replete media
- Cultures were grown and maintained using the Don Whitley A35 Anaerobic workstation to ensure repeatable and consistent growth conditions
- Python pipeline developed to normalise, blank, and graph growth curves
- Stringent response mutants cannot grow in minimal media and display a profound lag period upon subculture from stationary phase cultures into complete media



Delayed Sporulation

- Tested the ability of $\Delta relCQA$ mutants to sporulate over a 6 day period
- WT and $\Delta relCQA$ cultures grown for 6 days using the Don Whitley A35 Anaerobic workstation, with total and spore counts obtained at 24h intervals by plating onto BHIS + Tch
- Spore counts were obtained after heat treatment at 65°C for 30 minutes
- $\Delta relCQA$ mutants display delayed sporulation and an approximately tenfold reduction in spore number compared to WT cultures

Conclusion and Future Directions

- R20291 $\Delta relCQA$ mutants display distinctly altered growth and sporulation phenotypes, suggesting fundamental defects in multiple essential cellular processes.
- In progress:
 - Antibiotic resistance/tolerance assays
 - Complementation of $\Delta relA$ mutation
 - Generating $\Delta relCA/\Delta relQA$ mutants
- Future directions:
 - (pp)pGpp pulldown using capture compound developed for *S. aureus*
 - Luciferase assay to test promoter strength of *relC*, *relQ*, *relA* promoters
 - LC-MS based measurement of *in vivo* alarmone concentrations

