Within-host evolution of *Staphylococcus aureus* stringent response imparts a fitness advantage under nutrient stress

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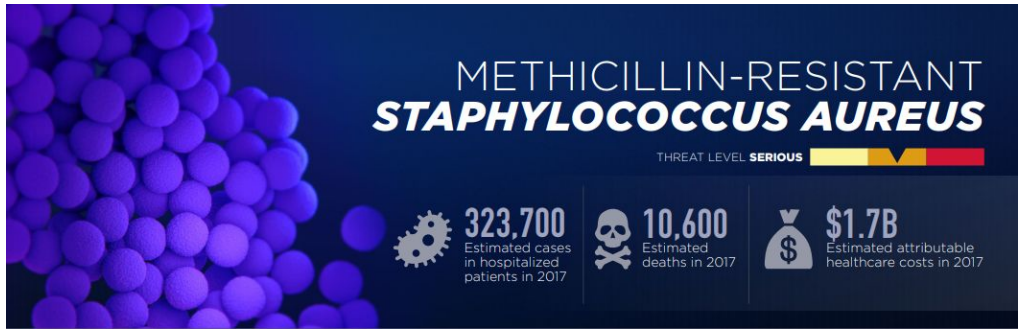
Invasive MRSA infections are a healthcare threat



Persistent MRSA bacteremia is common despite appropriate antibiotic selection (Paul 2010, Hawkins 2007, Leibovici 1998)

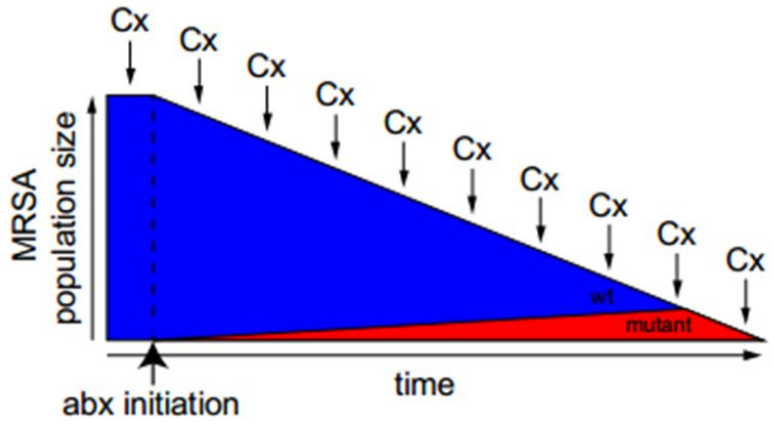
Persistent MRSA bacteremia is associated with a higher mortality:

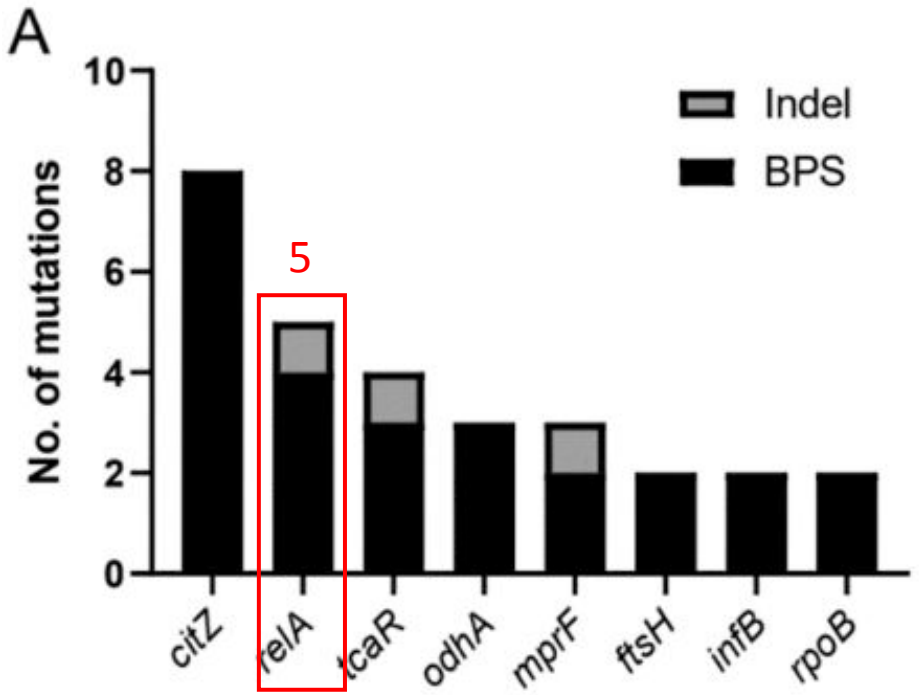
- 45% for persisters (>7 days) vs 9% for non-persisters (≤3 days) (Yoon 2010)
- 54% for persisters (>7 days) vs 31% for non-persisters (<3 days) (Hawkins 2007)

∴ There is great interest in understanding the mechanism(s) underlying persistent clinical infections.

A forward genetic screen to characterize within-host evolution of *S. aureus*

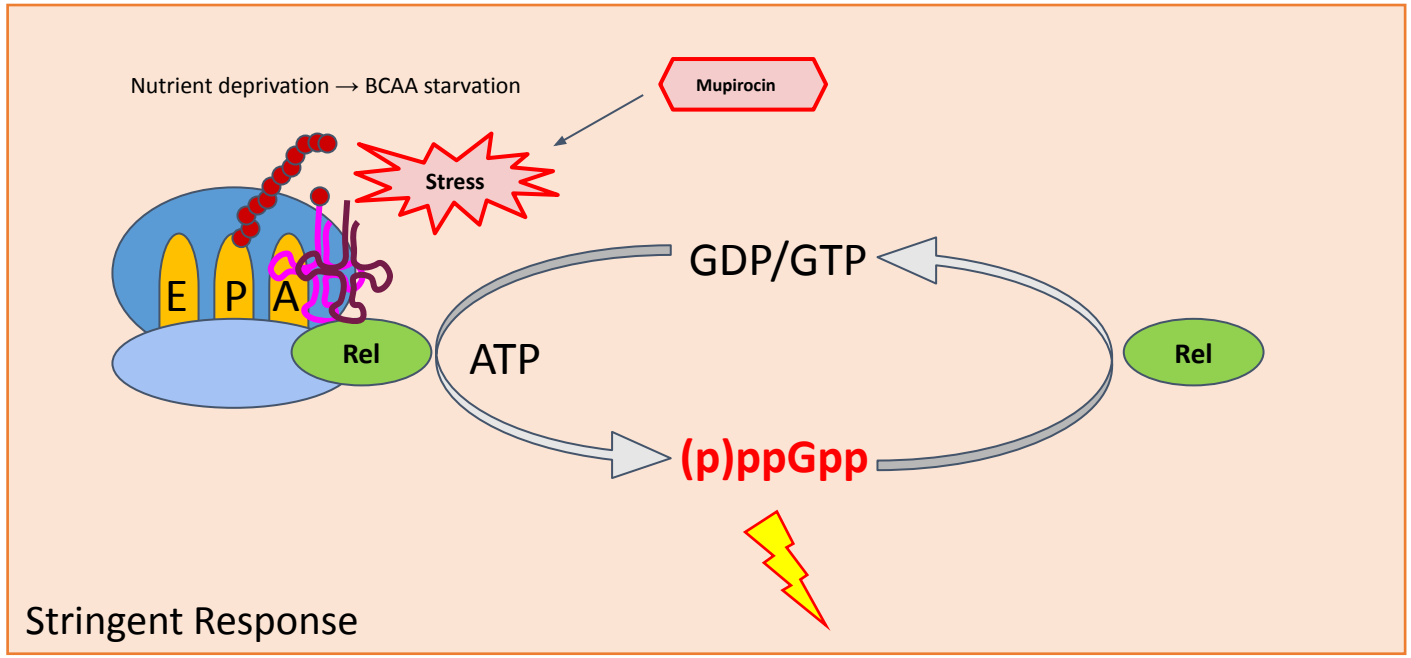
Whole-genome sequenced 206 serially positive MRSA blood cultures from 20 patients with persistent clinical infections to identify evidence of within-host evolution.

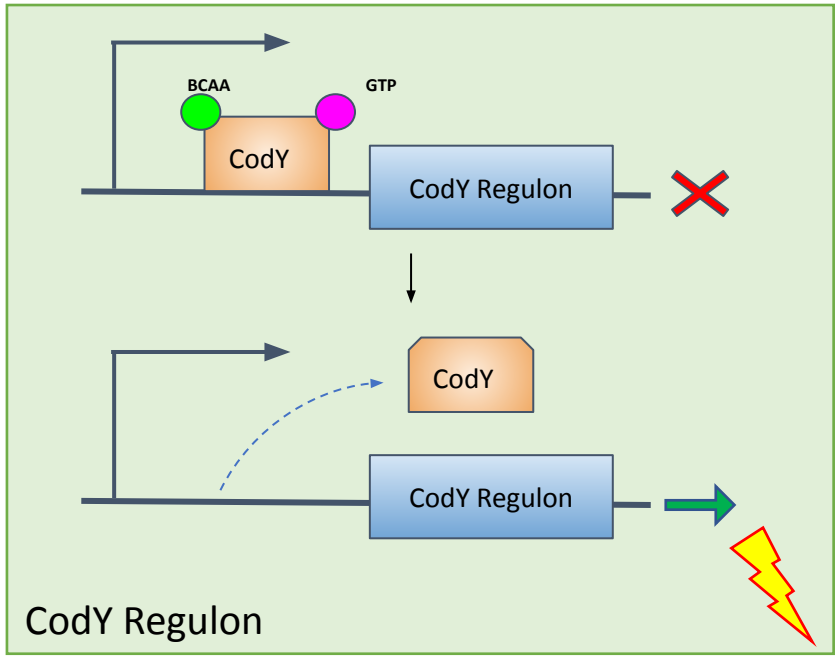




Elgrail, Chen 2022

The stringent response is a conserved bacterial stress response

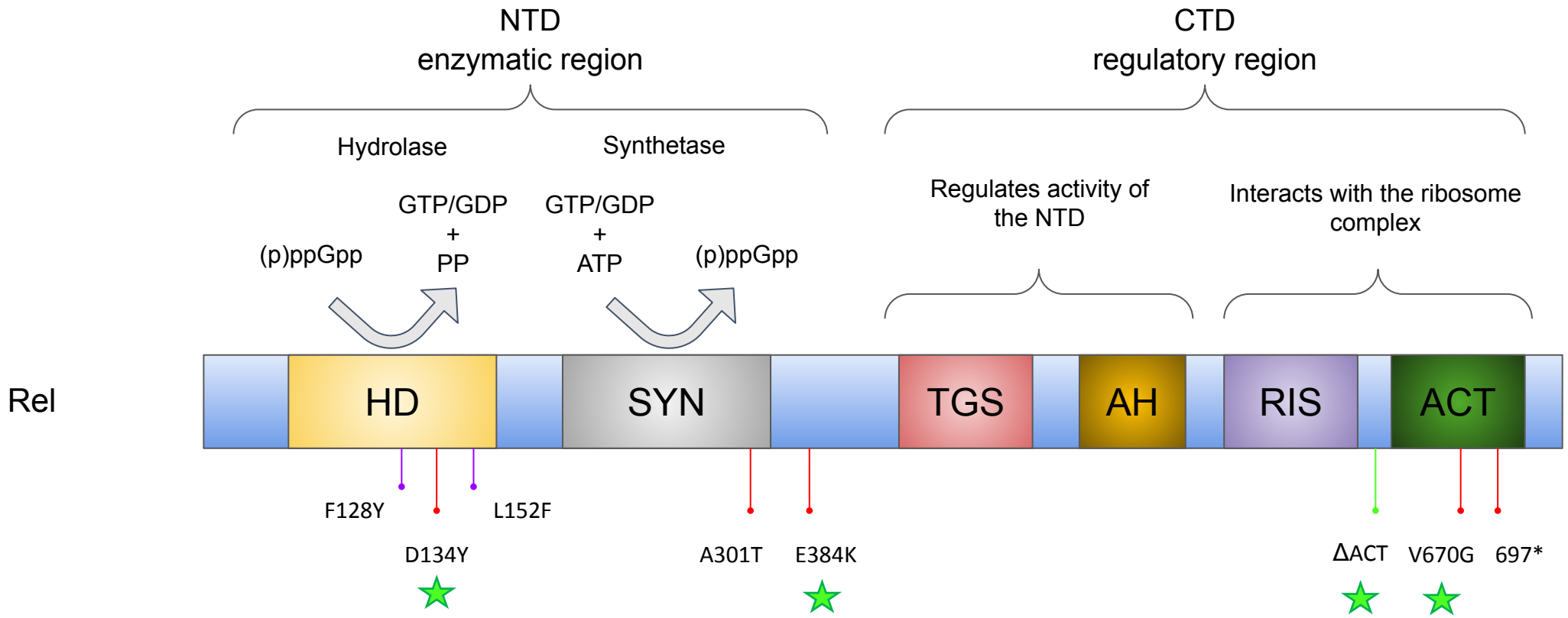




Antibiotic tolerance
Virulence
Phagosomal escape
Transcriptional regulation

Metabolism and growth
Protein translation
DNA replication
Transcriptional regulation

Rel, the central regulator of the stringent response



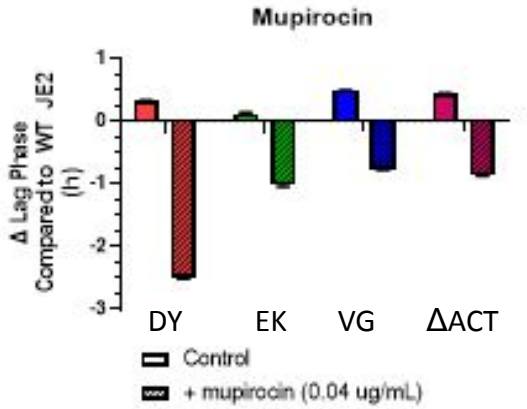
Previously identified clinical *Rel* mutations in persistent Gram(+) infections, found to impart **multidrug tolerance**

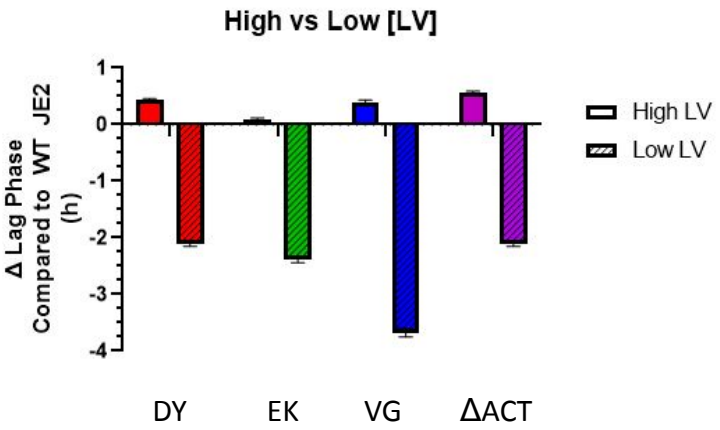
Our newly identified clinical *Rel* mutations

Allelic exchange mutants in a MRSA JE2 background

What role do our mutations play in persistent clinical infections?

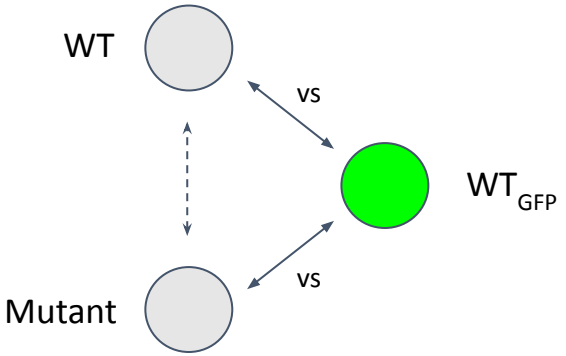
Clinical *Rel* mutations alter growth



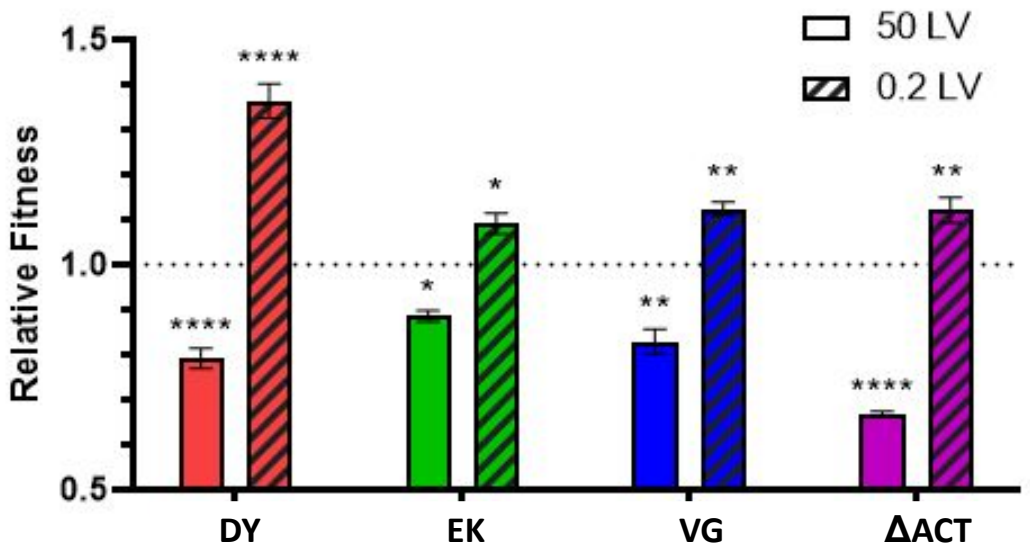


Clinical *Rel* mutations result in an abnormal stringent response phenotype and alter bacterial growth kinetics.

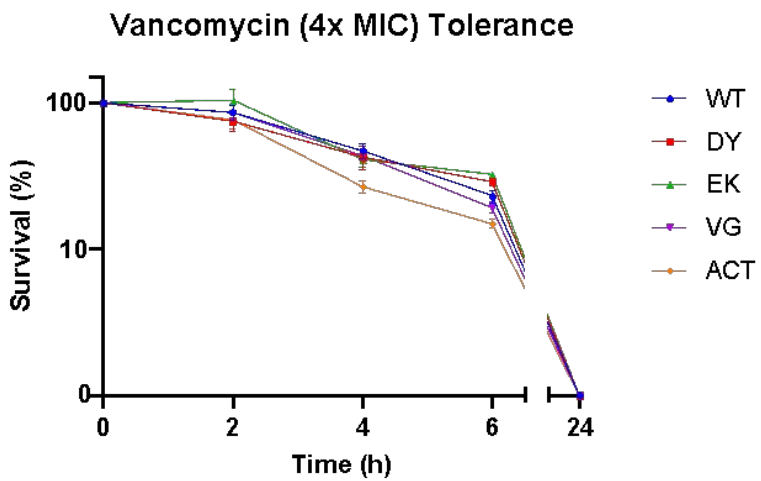
Clinical *Rel* mutations have increased fitness under stringent conditions

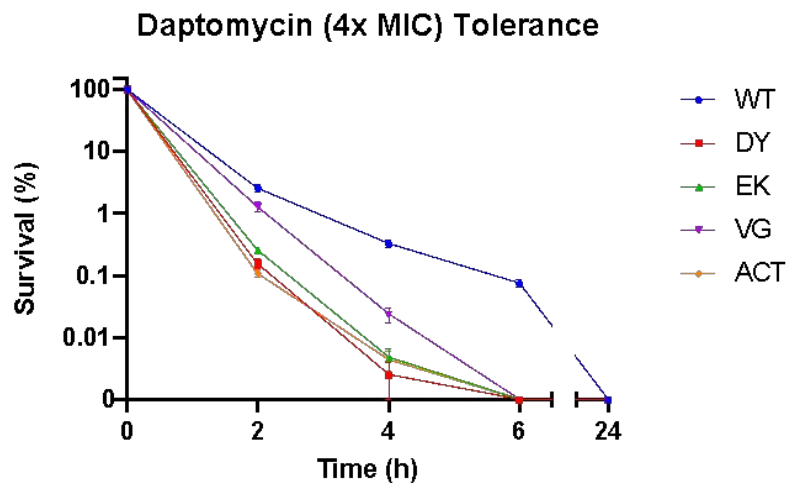


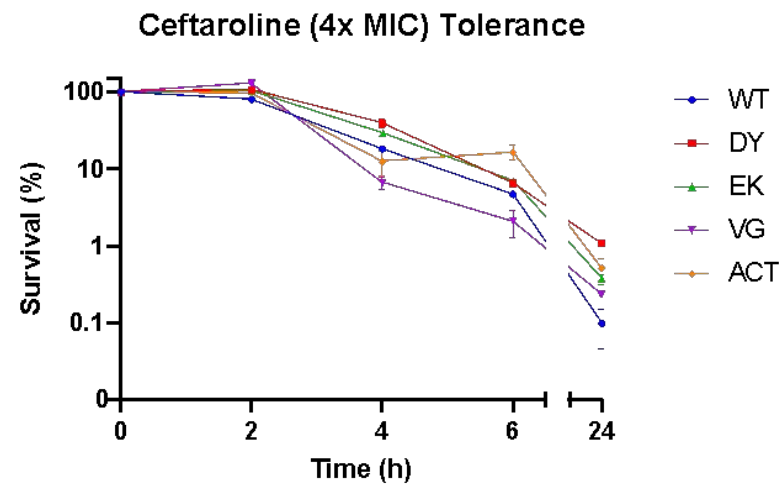
$$W = \ln\left(\frac{N_f^{dark}}{N_i^{dark}}\right) / \ln\left(\frac{N_f^{bright}}{N_i^{bright}}\right)$$



Clinical *Rel* mutations do not impart multidrug tolerance







Conclusions

1. Within-host evolution of the stringent response occurs during persistent MRSA bacteremia.

2. We have identified several novel mutations localized throughout the different domains of Rel.

3. Our *Rel* mutations impart a competitive fitness advantage under nutrient limiting conditions.

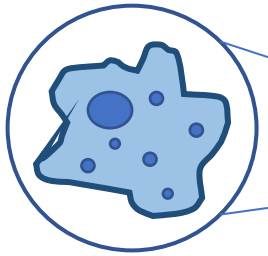
4. Our *Rel* mutations results in a diverse tolerance phenotype, *not* multidrug tolerance.

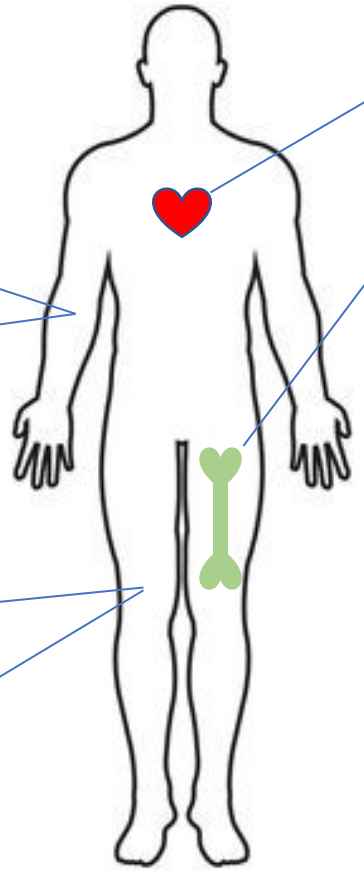
∴ Our clinical *Rel* mutations highlight the diverse roles the stringent response plays in host-pathogen interactions.

Future Questions

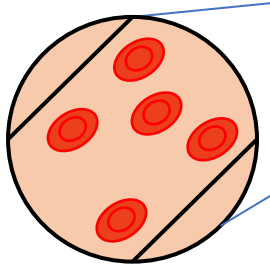
Competitive advantage at metastatic foci?

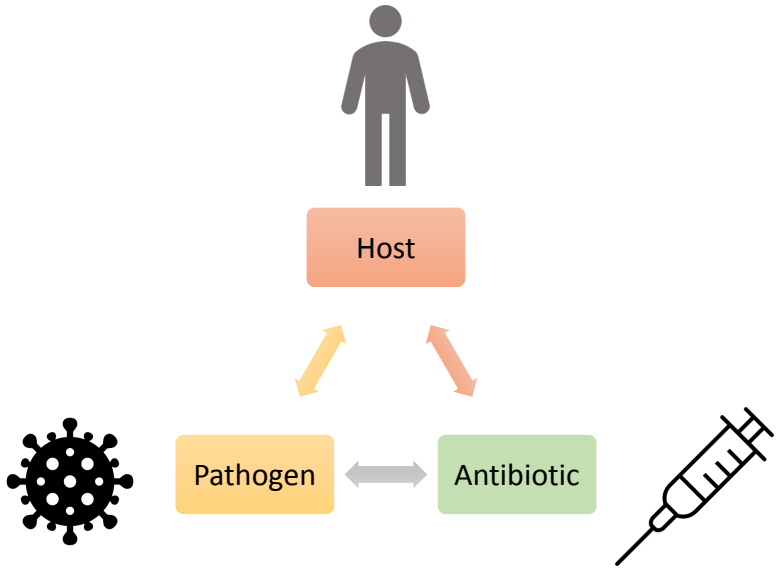
Increased survival within phagocytes?





Competitive advantage within the bloodstream?





Tolerance that emerges only in presence of host factors?

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Harrison Lab

Urish Lab

Nguyen Lab

Van Tyne Lab

Dimitrov Lab

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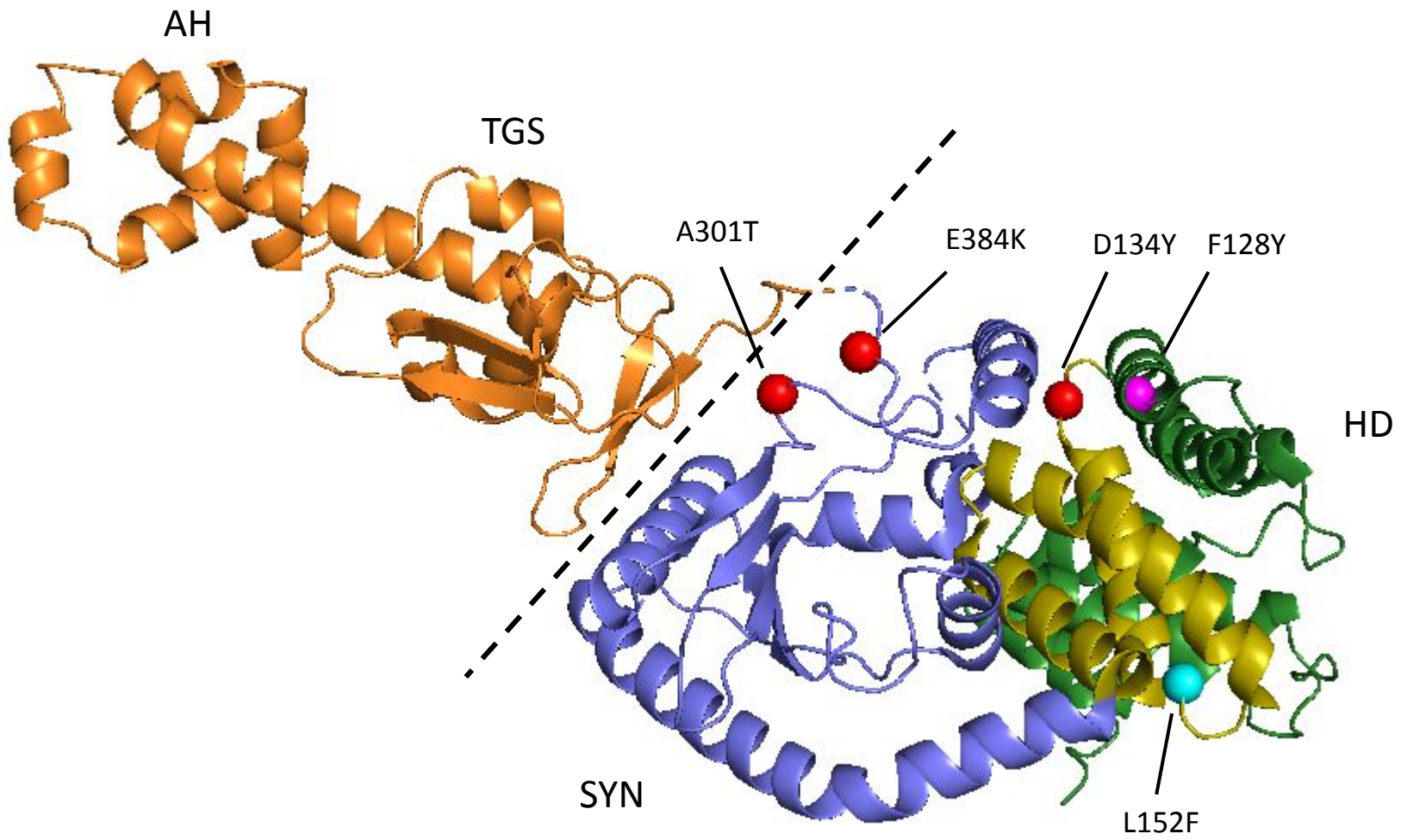
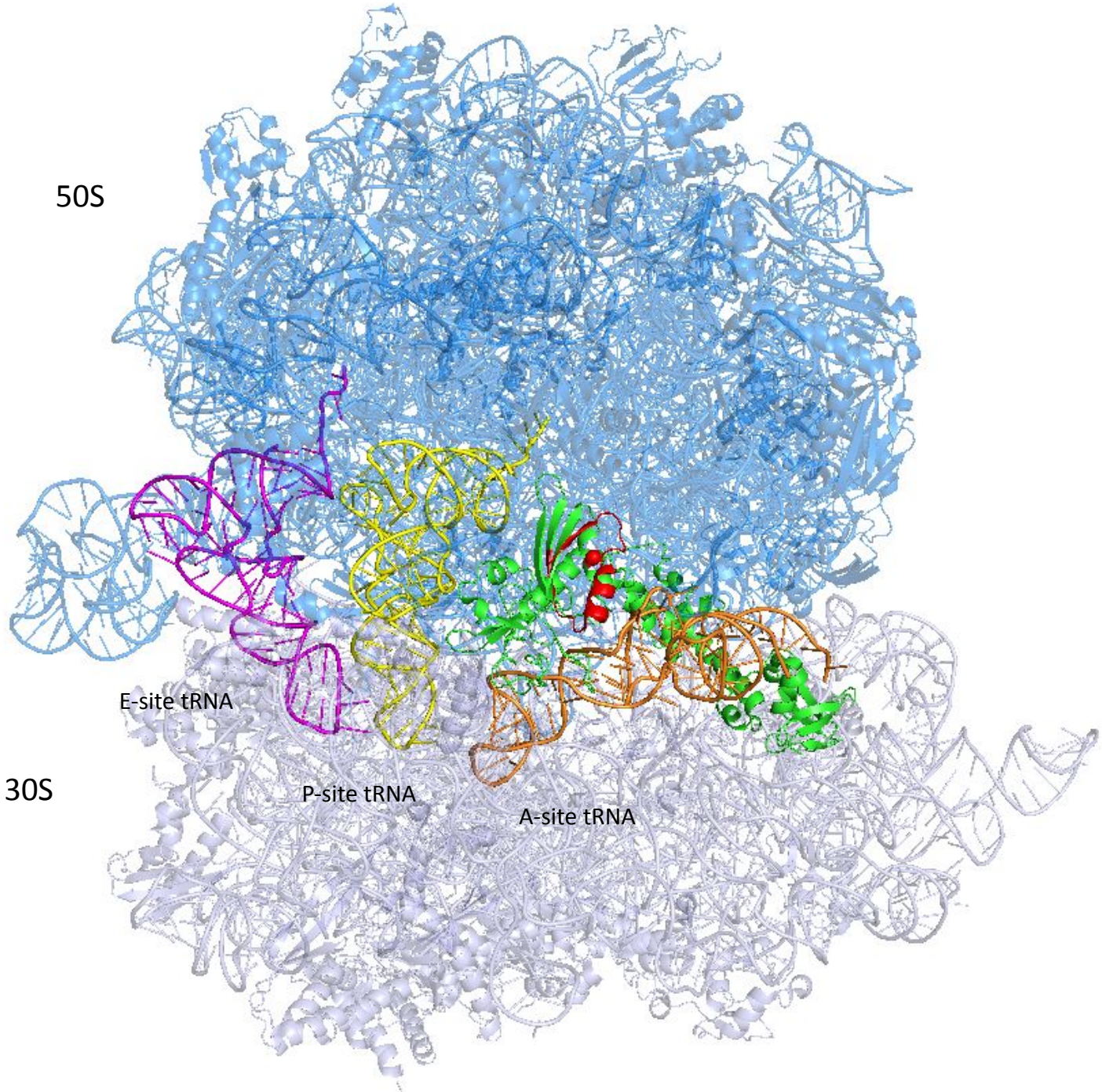


Pausch 2020
PDB 6YXA



Pausch 2020
PDB 6HTQ


10D 20L 30K 40K 50Y 60I 70T 80I 90V 100R 110K 120Q 130A 140V
SaRel 1 MNNEYPYSADEVLHKAKSYLSADEYEYVLKSYHIAYEAHKGQFRKNGLPYIMHPIQVAGILTEMRLDGPTIVAGFLHDVIEDTPYTFEDVKEMFNEEVARIVDGVTKLKKVKYRSKEEQQAENHRKLFIAIAKDVRVILVKLADRLHNMR 150
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160Q 170I 180I 190T 200Y 210K 220T 230D 240G 250R 260D 270V 280A 290K
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310S 320G 330F 340V 350K 360Q 370A 380Q 390L 400P 410G 420I 430G 440I
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460G 470K 480S 490N 500A 510E 520V 530E 540G 550K 557- 566E 576I 586V
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606P 616K 626D 634- 642W 650Q 660A 670V 680N 690I 700V 710R 720D
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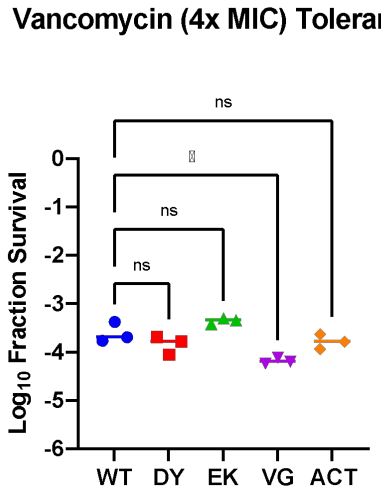
Vancomycin (4x MIC) Tolerance
ns
ns
ns
Log10 Fraction Survival
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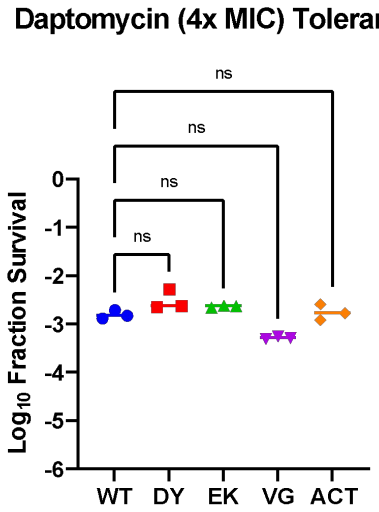
-1
-2
-3
-4
-5
-6
WT
DY
EK
VG
ACT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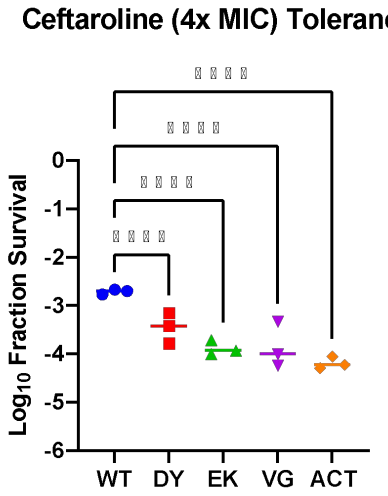
Ceftaroline (4x MIC) Tolerance
Log10 Fraction Survival
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-2
-3
-4
-5
-6
WT
DY
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VG
ACT