Difficult-to-treat infections: the role of bacterial biofilm & persisters

S. aureus internalisation and induction of antimicrobial tolerance

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DISCLOSURE

Copenhagen, Denmark
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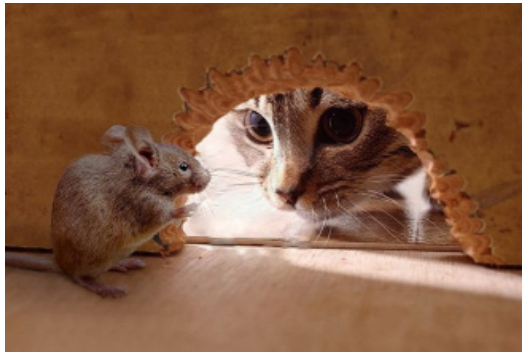
33rd ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

How do bacteria protect themselves against antibiotics ?

"offensive strategy": to develop resistance mechanisms

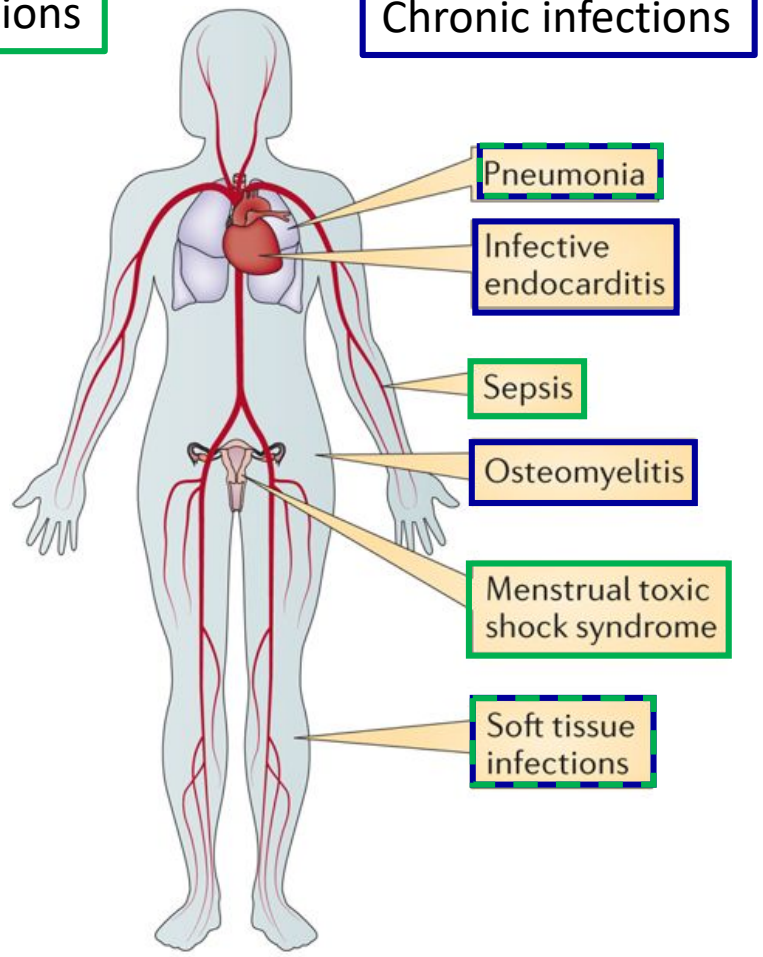
"defensive strategy": to adopt 'hidden' mode of life



Main infections caused by *S. aureus*

Acute infections

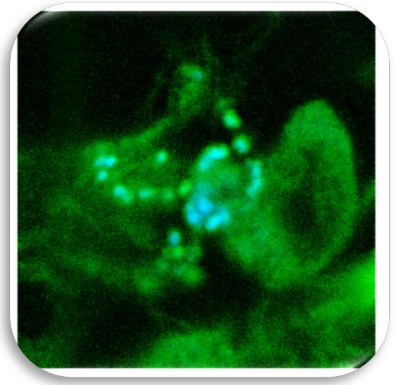
Chronic infections



Nature Reviews | Microbiology

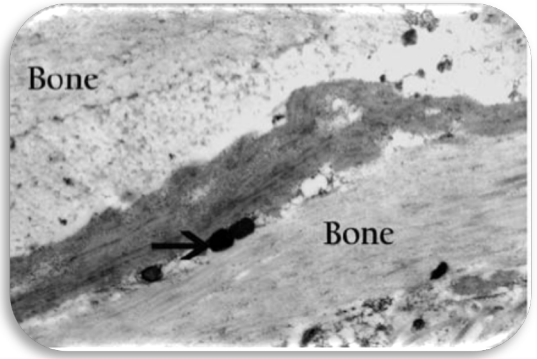
Salgado-Pabón & Schlievert; Nat Rev Microbiol. 2014; 12:585-91

Macrophages – CF lung



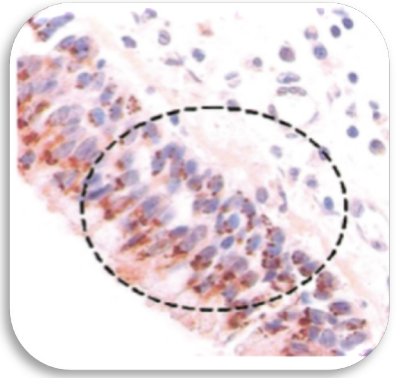
Li et al, Infect Immun. 2017; 85(5). pii: e00883-16

Osteoblasts



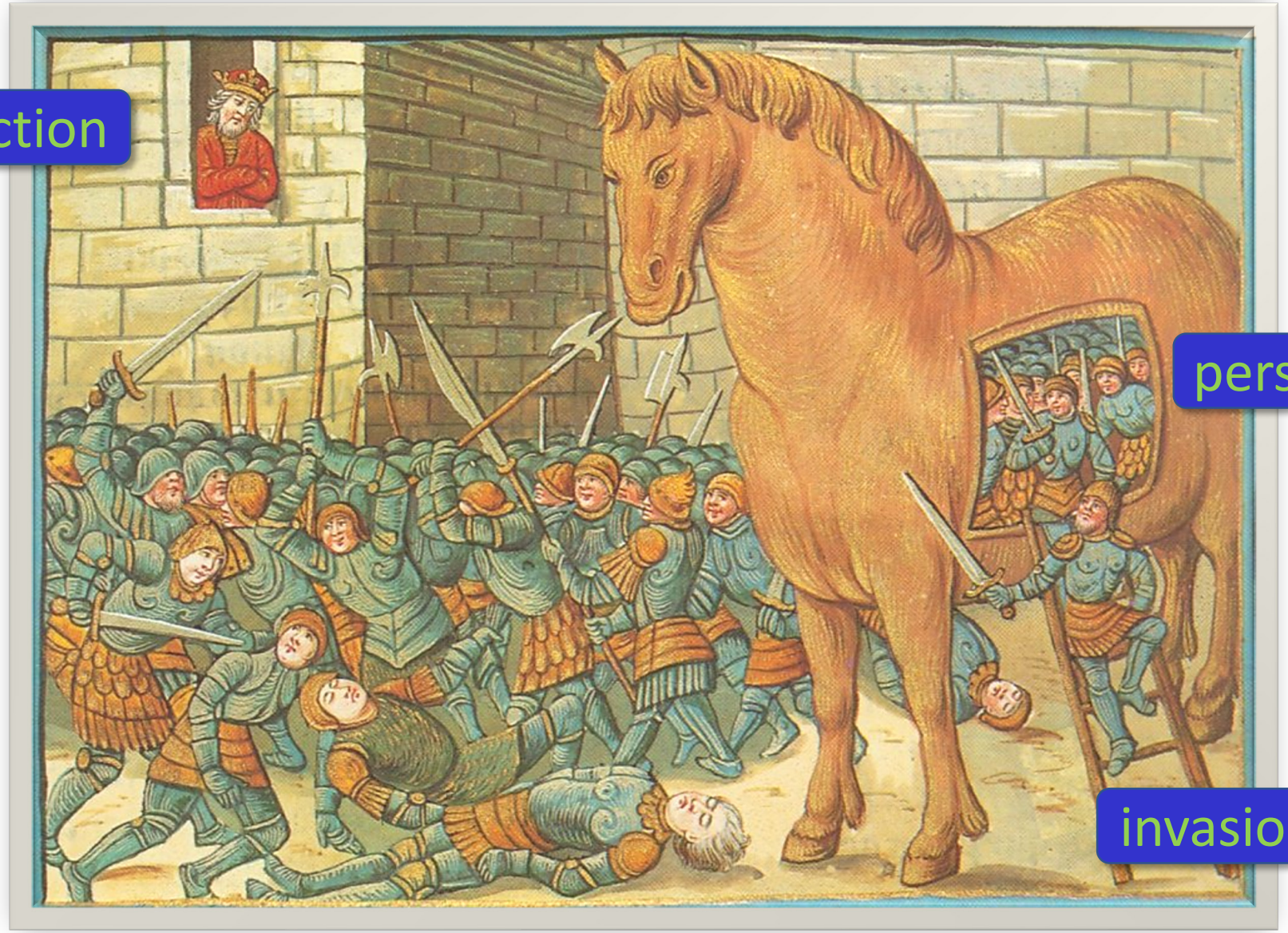
Bosse et al., J Bone Joint Surg Am. 2005; 87: 1343-7

Chronic rhinosinusitis

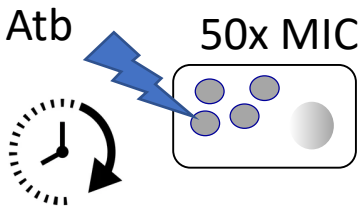


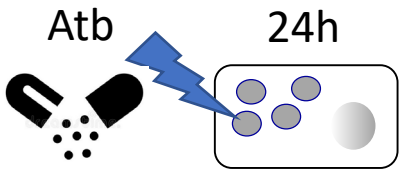
Clement et al, J Infect Dis. 2005;192:1023-8

Benefits of intracellular life

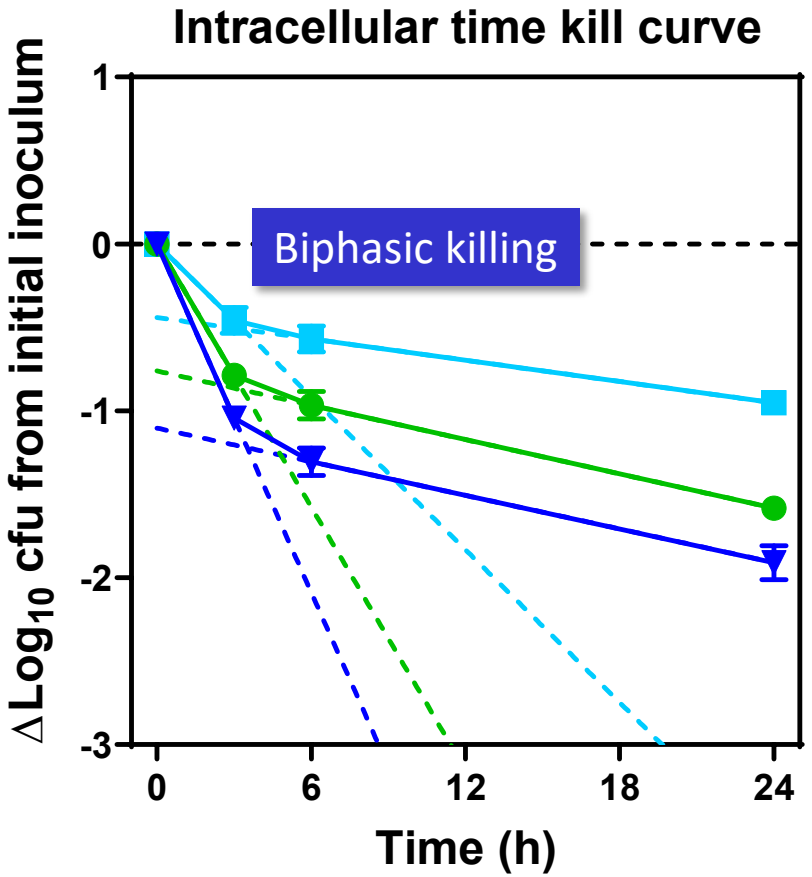


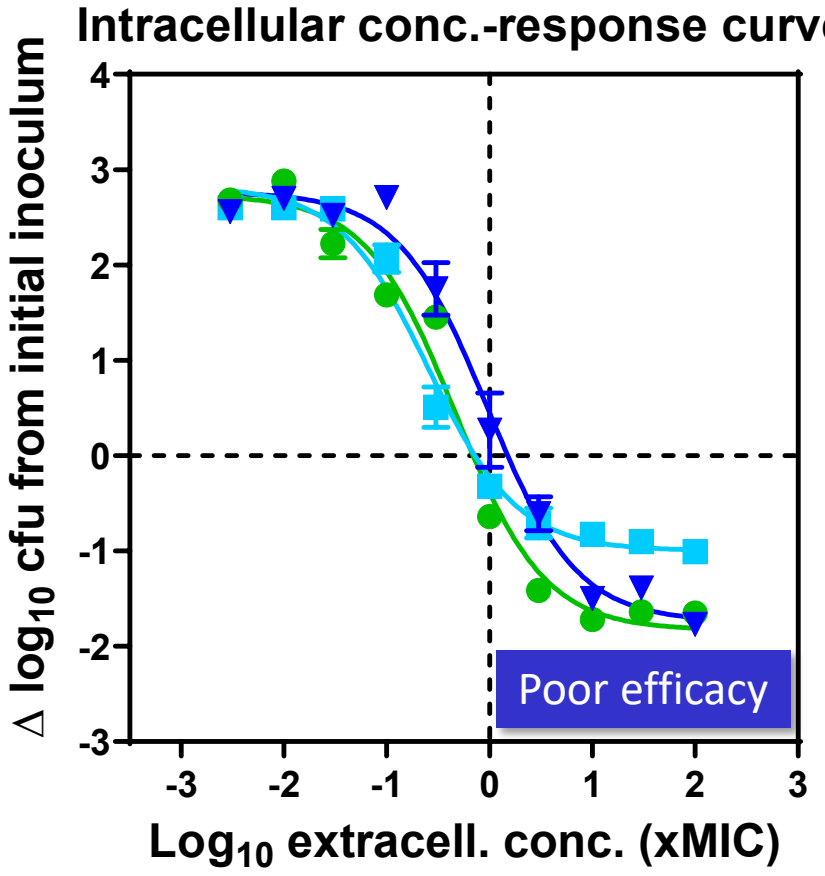
Intracellular activity of antibiotics





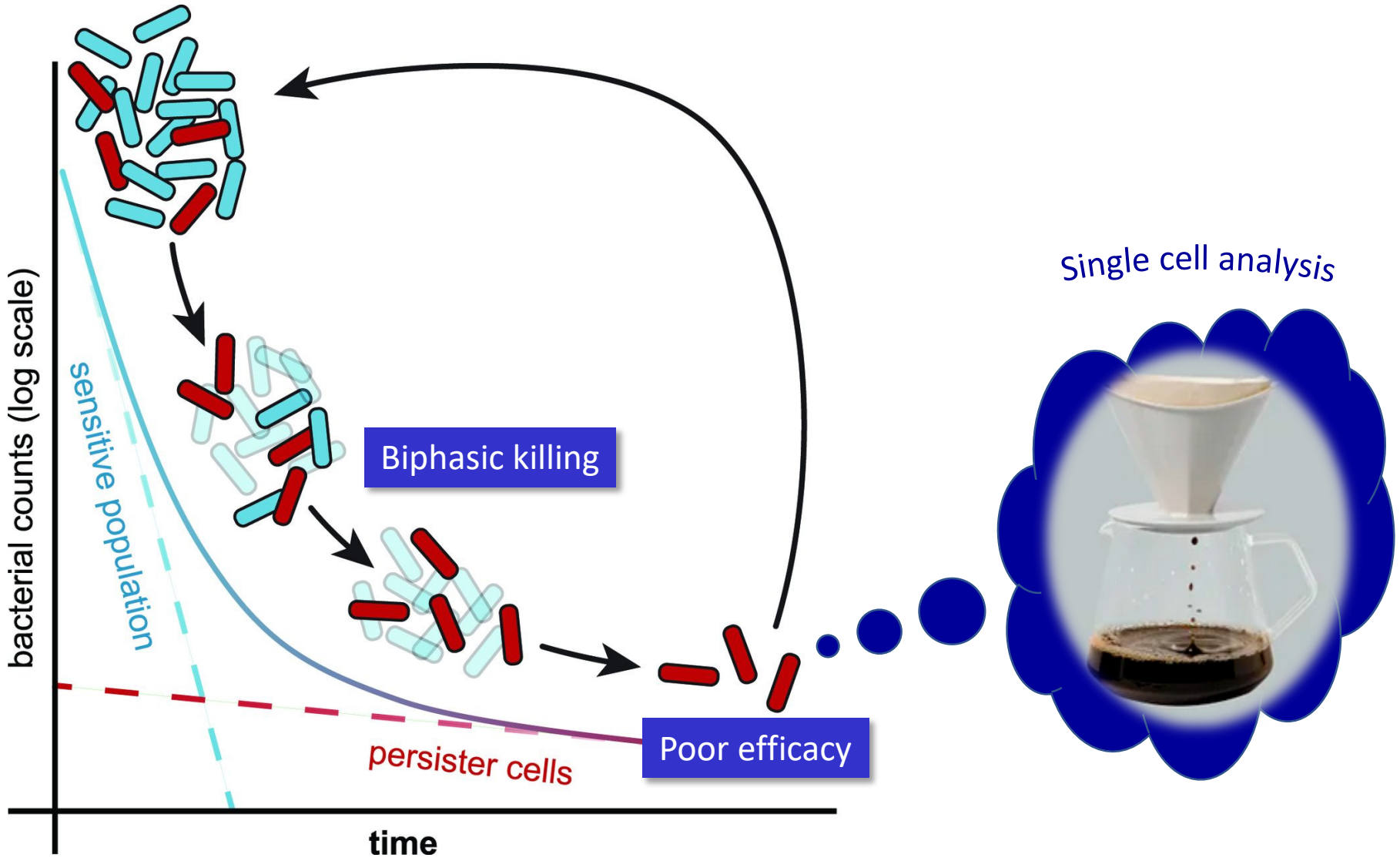
oxacillin clarithromycin moxifloxacin





Peyrusson et al, Methods Mol Biol. 2021; 2357:239-251.

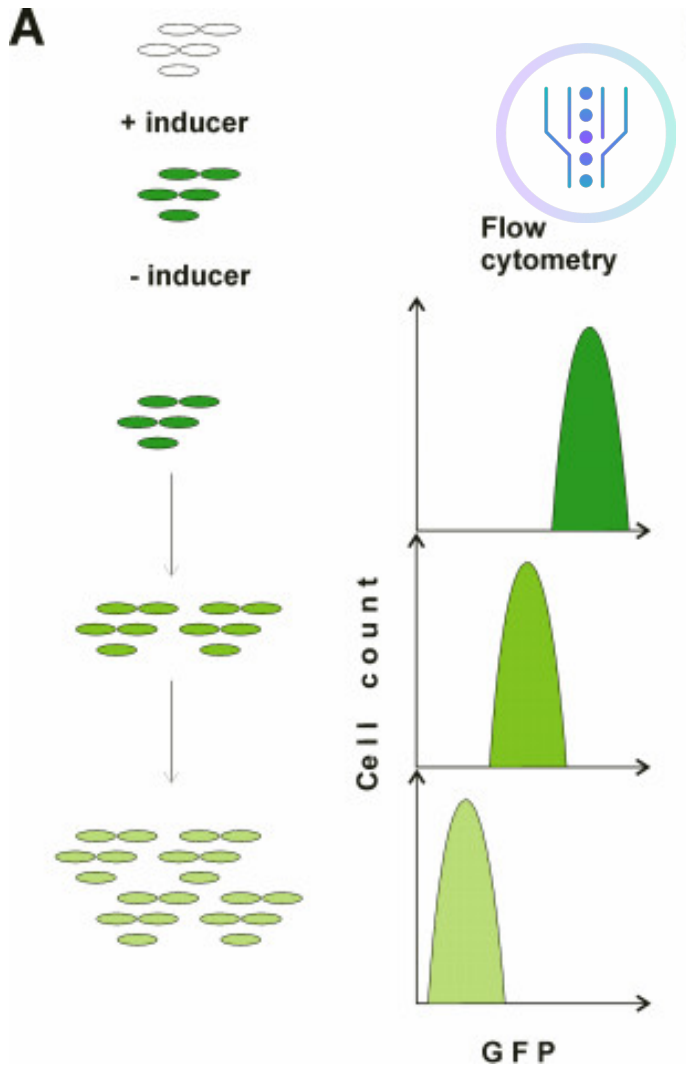
Persisters and antibiotics



Harms et al., Science 2016;354:aaf4268

Following bacterial multiplication at the single cell level and in real time

Multiplying bacteria



Roostalu et al., BMC Microbiol. 2008; 8:68.

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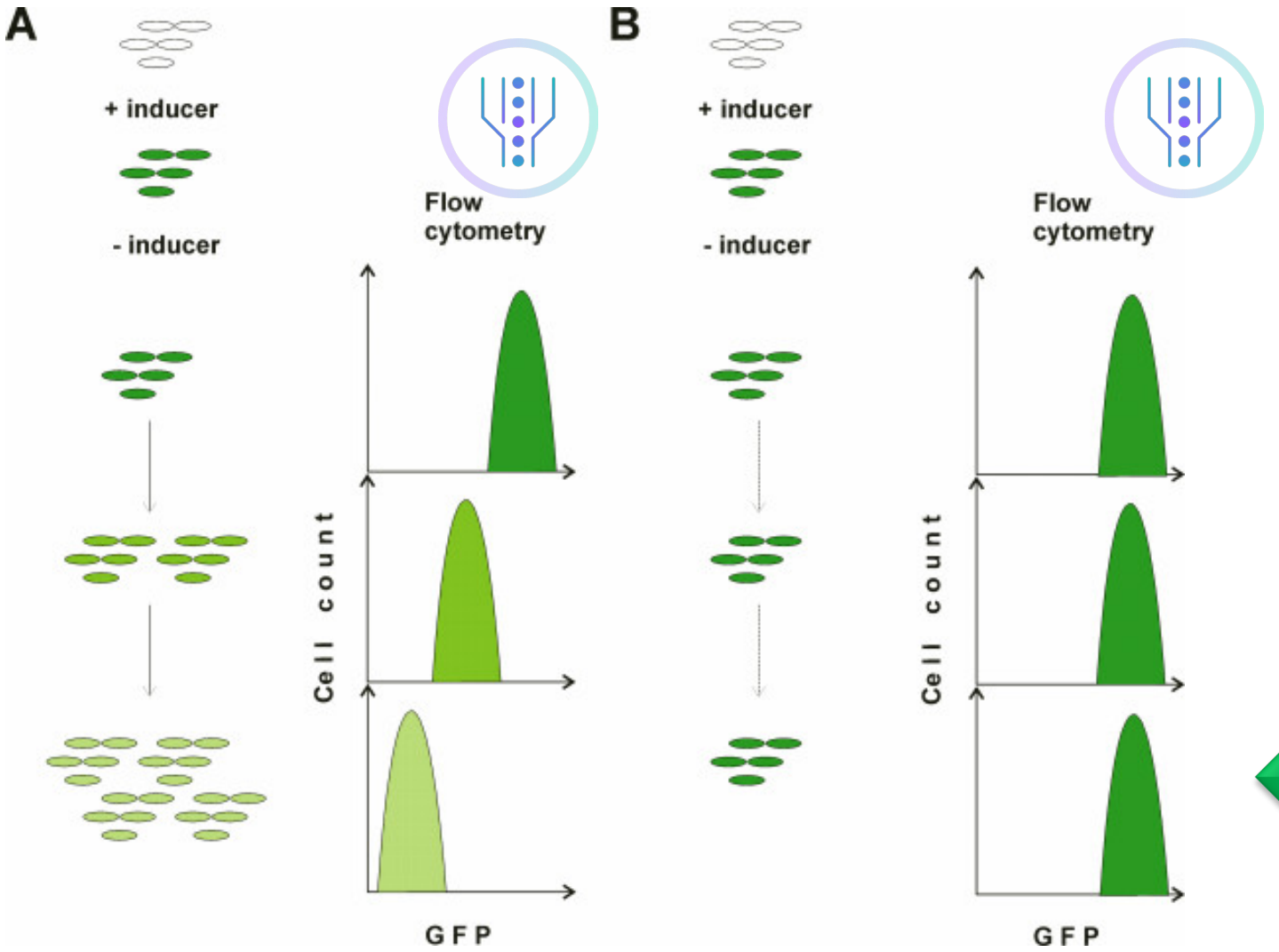


Multiplying bacteria

NON-multiplying bacteria

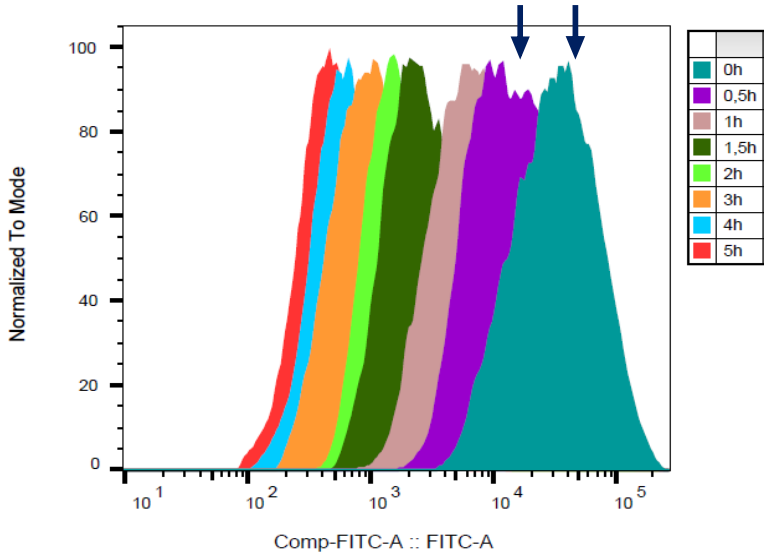
Roostalu et al., BMC Microbiol. 2008; 8:68.

Application to *S. aureus* planktonic cultures

O/N induction
TET 125 ng/mL

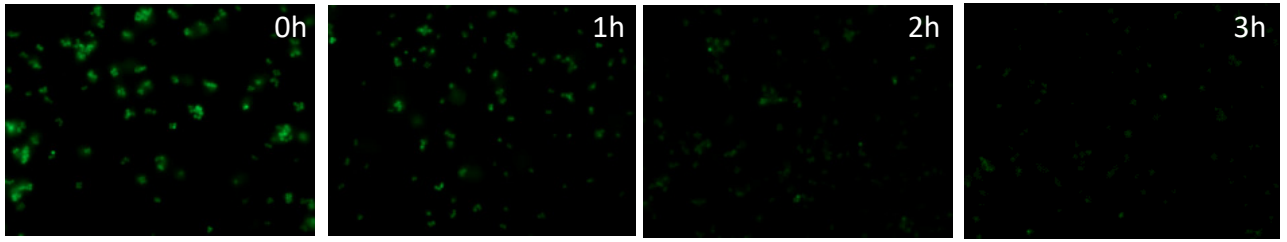
Entry into
exponential phase

Fluorescence : +1h, +2h, +3h…



Growth rate

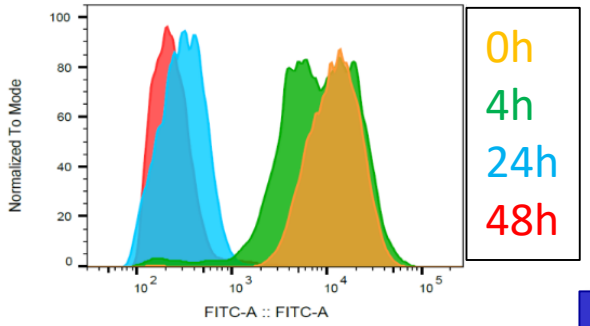
- 28.5 min / generation
- 30.3 min / generation (CFU method)

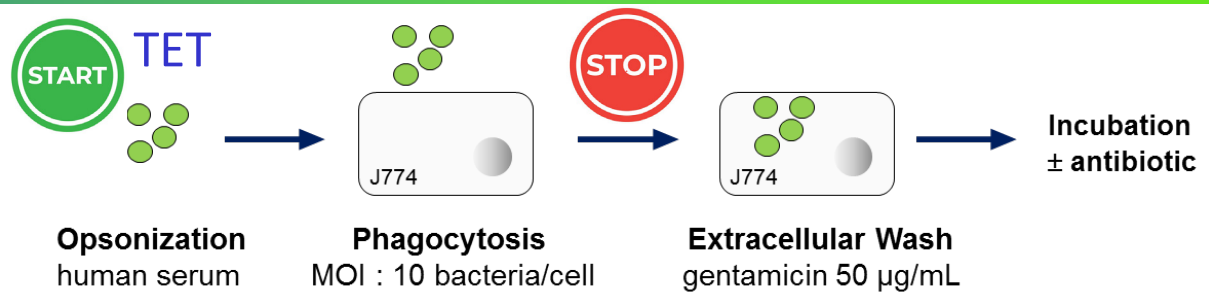


Peyrusson et al, Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o
antibiotic
(gentamicin 5xMIC)





Inoculum actively dividing inside the cell

Peyrusson et al, Nat. Comm. 2020; 11:2200

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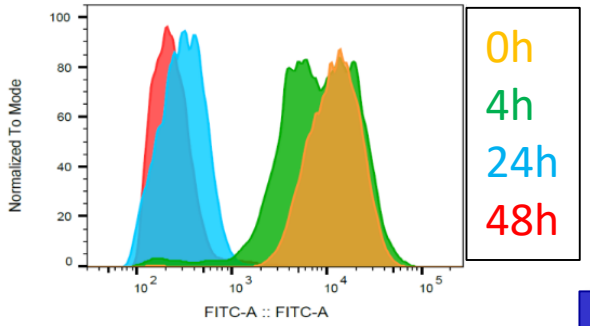
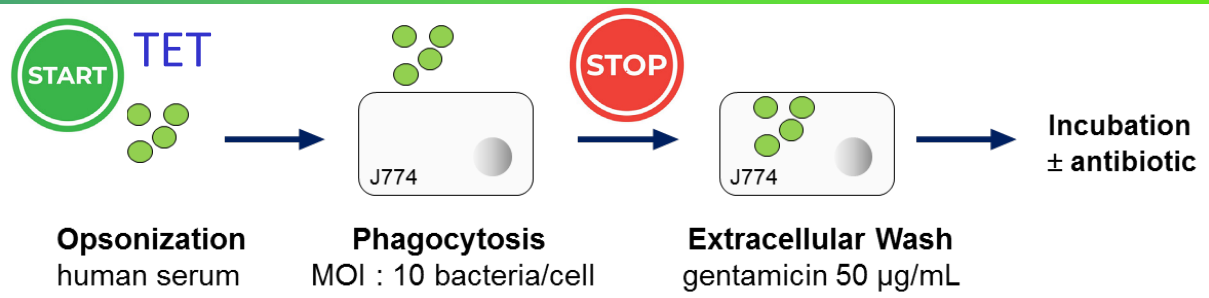




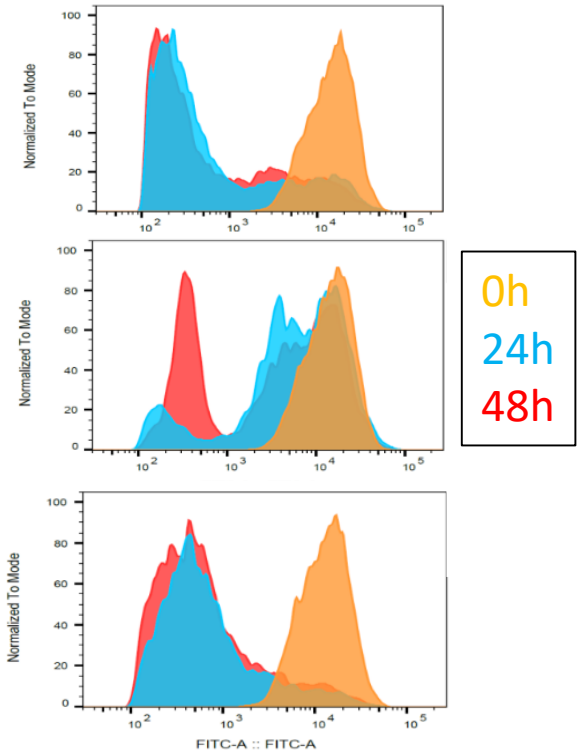
Inoculum actively dividing inside the cell

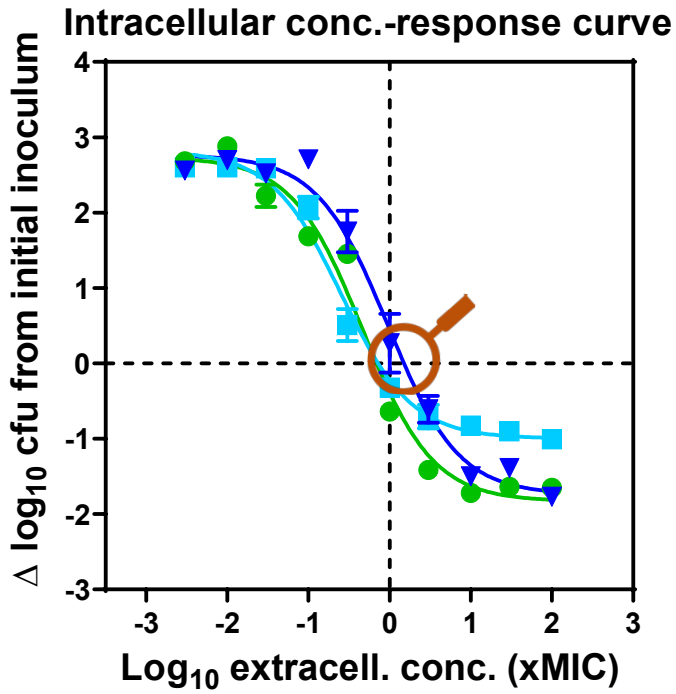
2 x MIC

Oxacillin

Clarithromycin

Moxifloxacin





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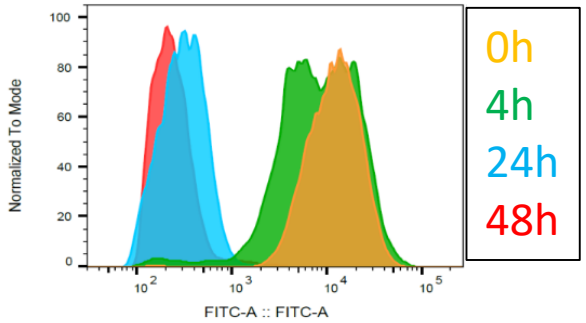
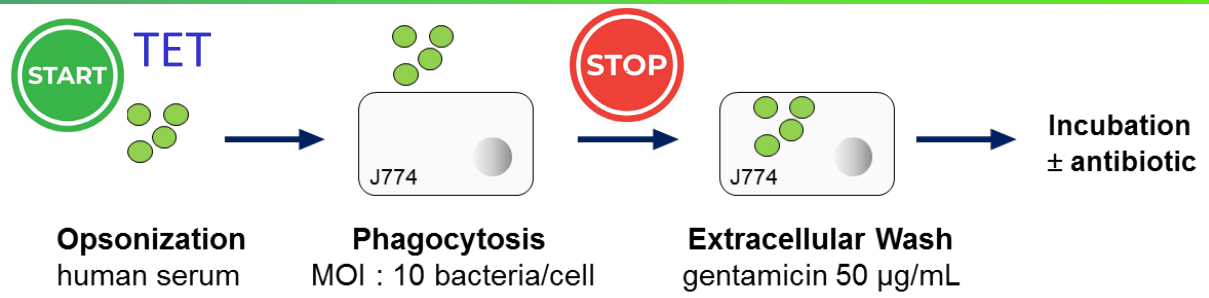




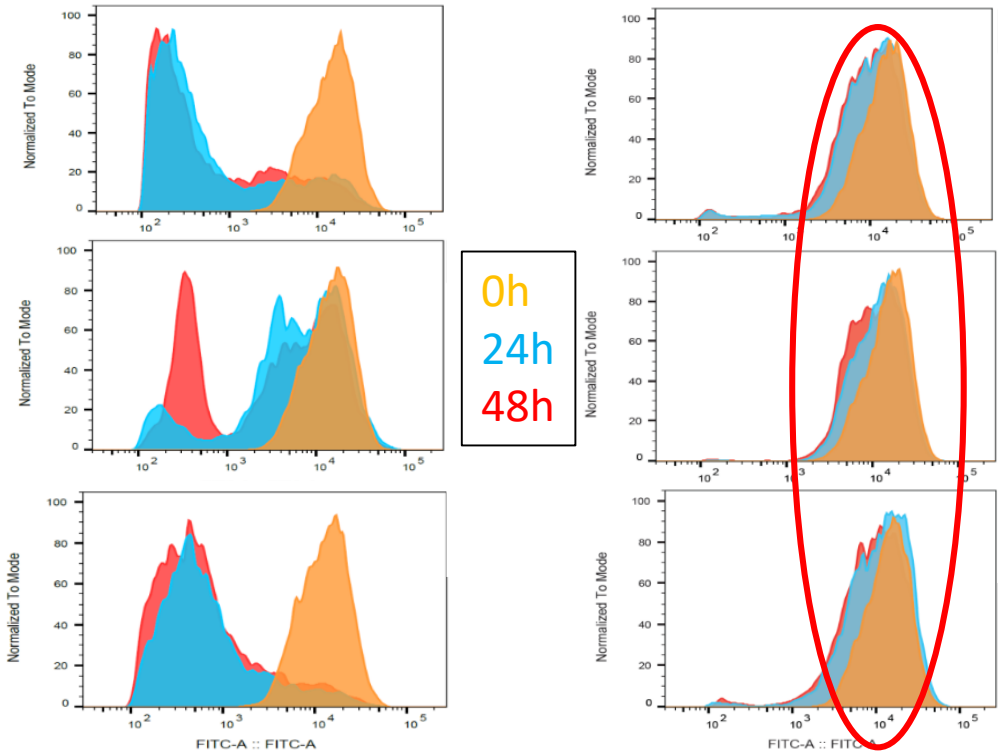
Fully non-dividing population

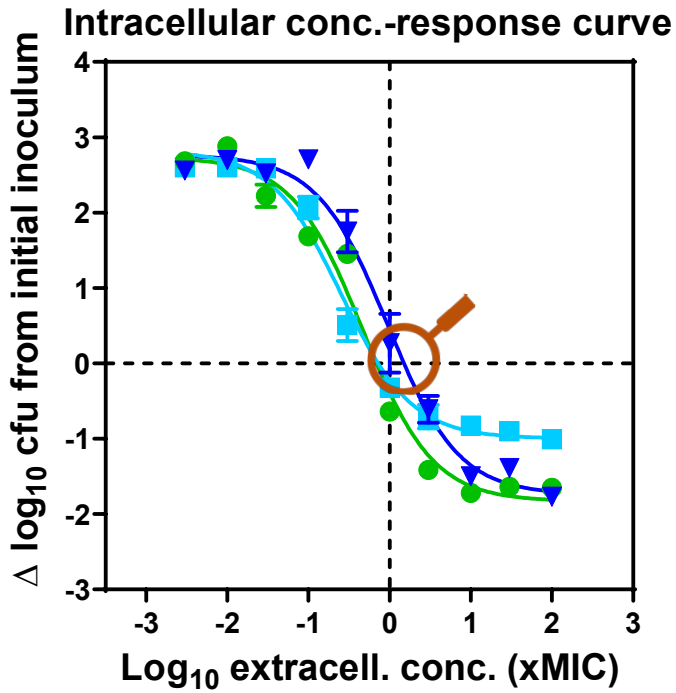
2 x MIC — Antibiotic pressure — 50 x MIC

Oxacillin

Clarithromycin

Moxifloxacin

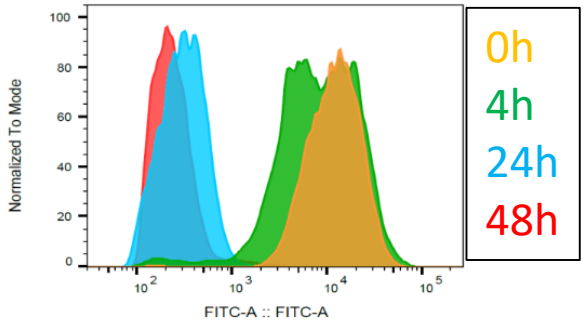


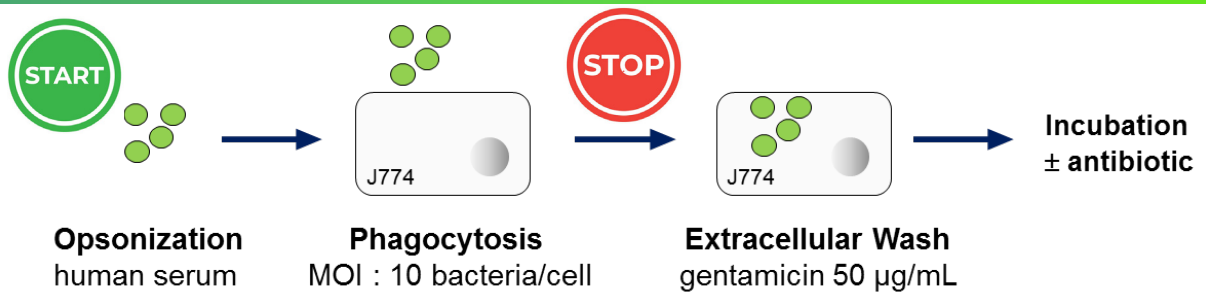


Peyrusson et al, Nat. Comm. 2020; 11:2200

Application to intracellular *S. aureus*

w/o
antibiotic
(gentamicin 5xMIC)





Antibiotic pressure

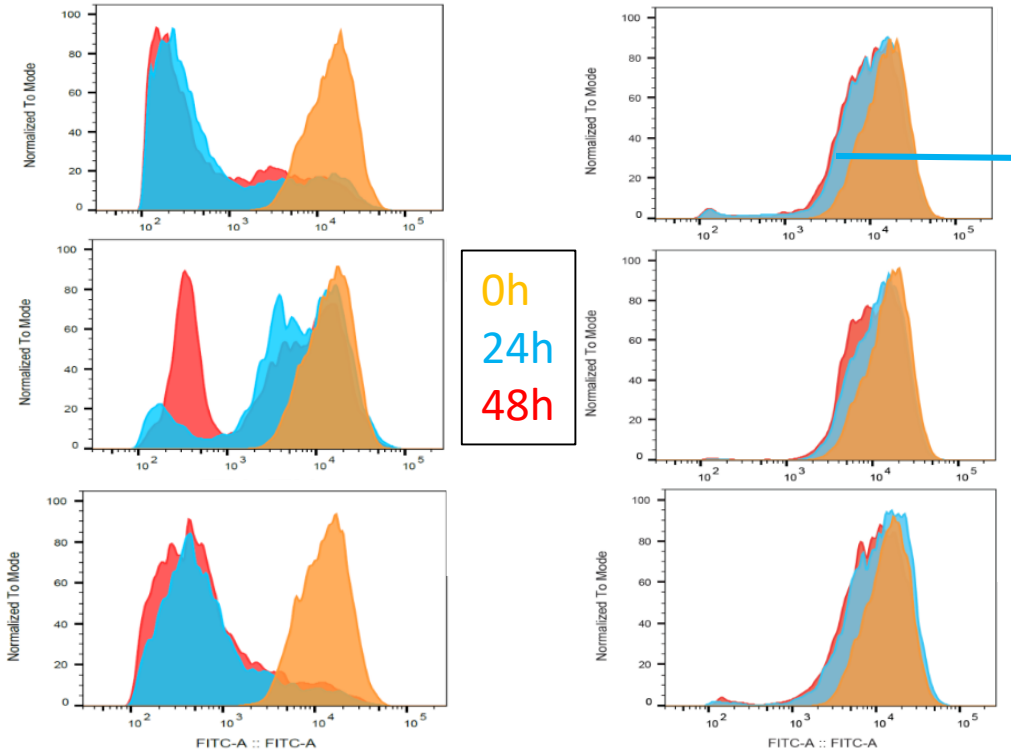
2 x MIC

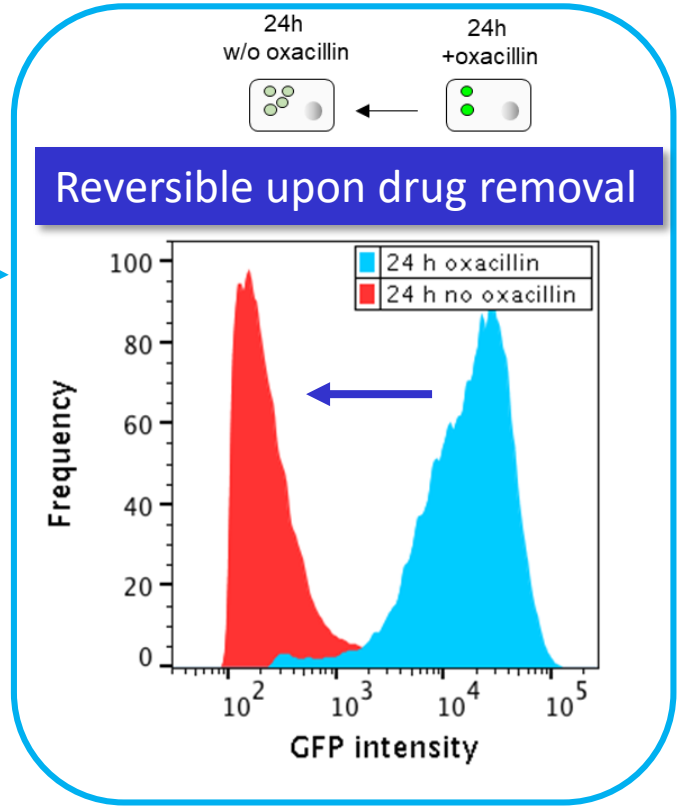
50 x MIC

Oxacillin

Clarithromycin

Moxifloxacin





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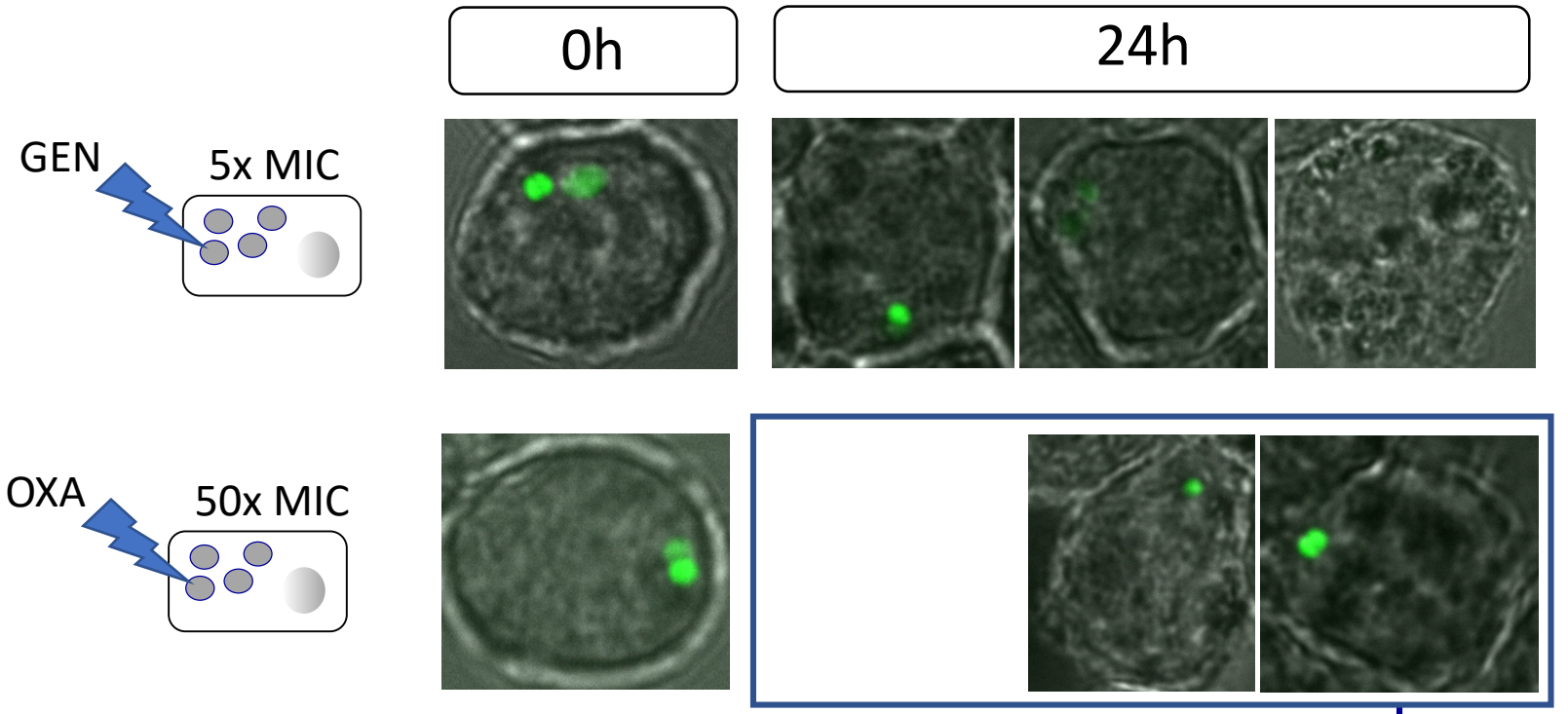
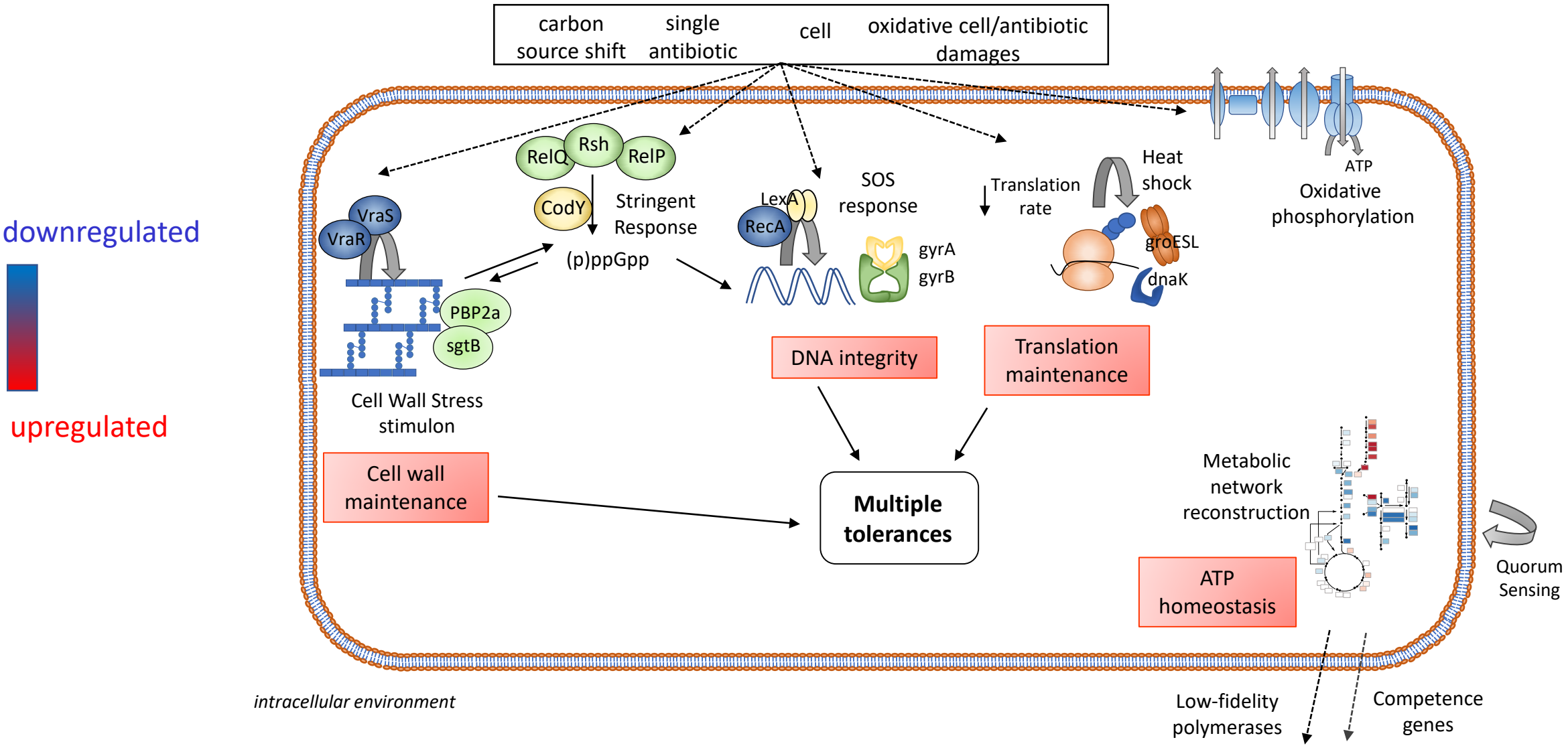
Non dividing bacteria persist inside the cells



Cell sorting and transcriptomic analysis

Peyrusson et al, Nat. Comm. 2020; 11:2200

Intracellular persisters: a global view



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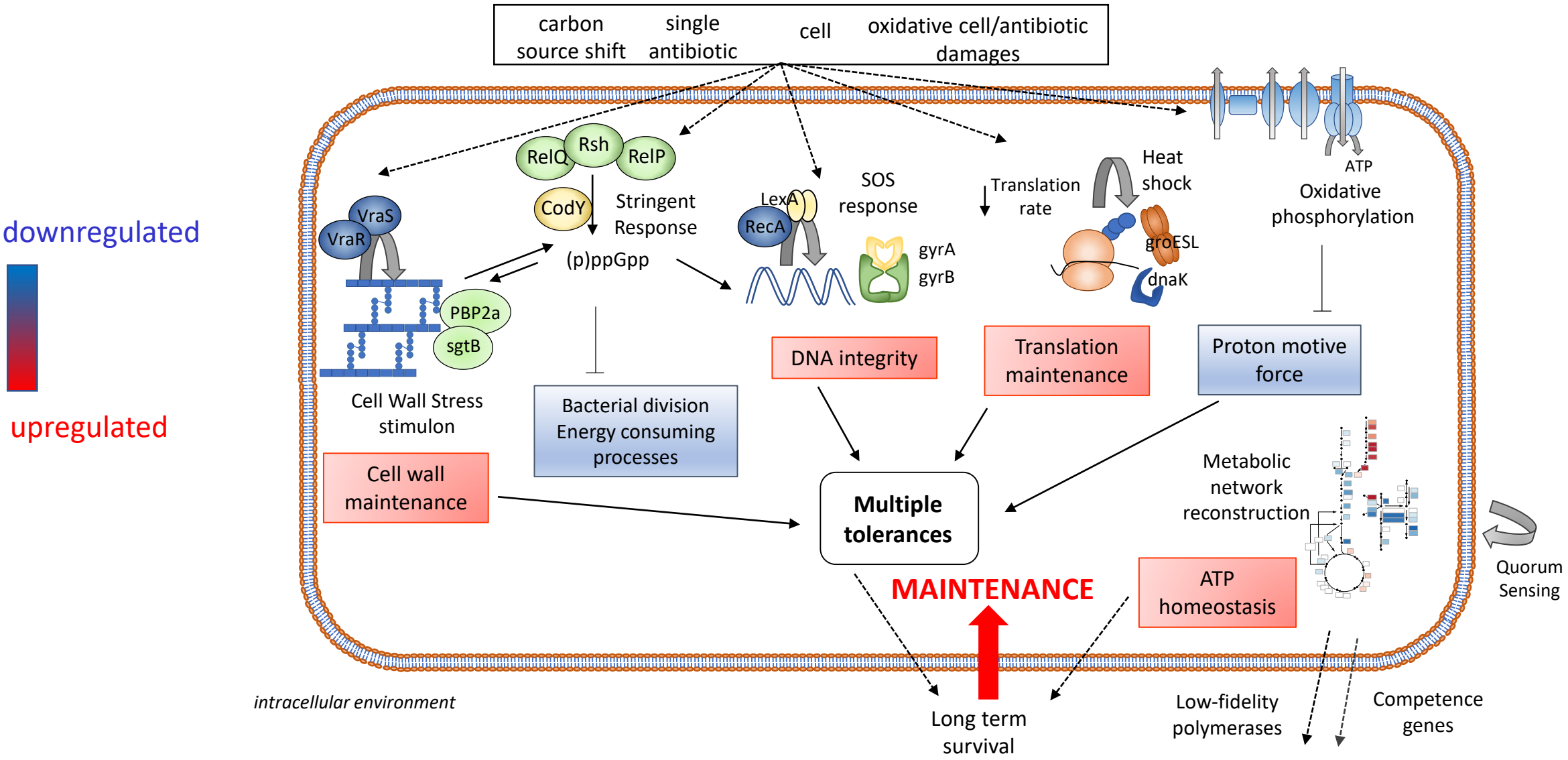
Intracellular persisters: a global view



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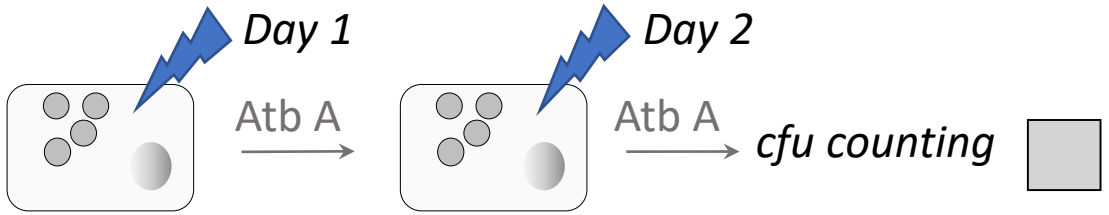
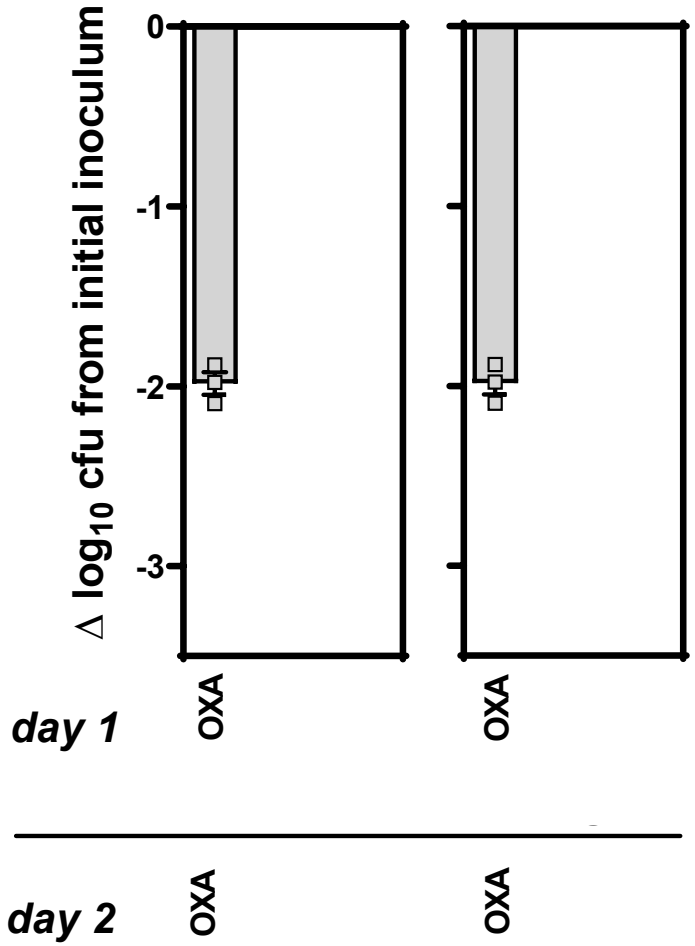
Multidrug tolerance

Multidrug tolerance





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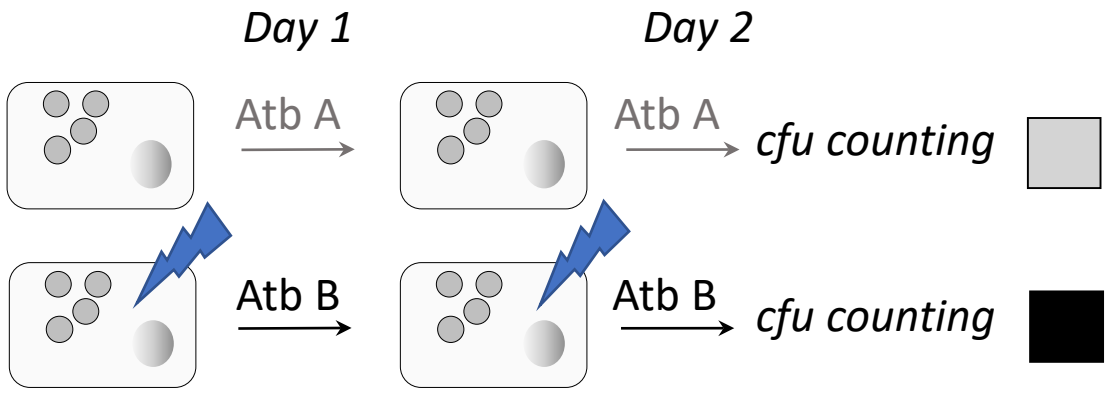
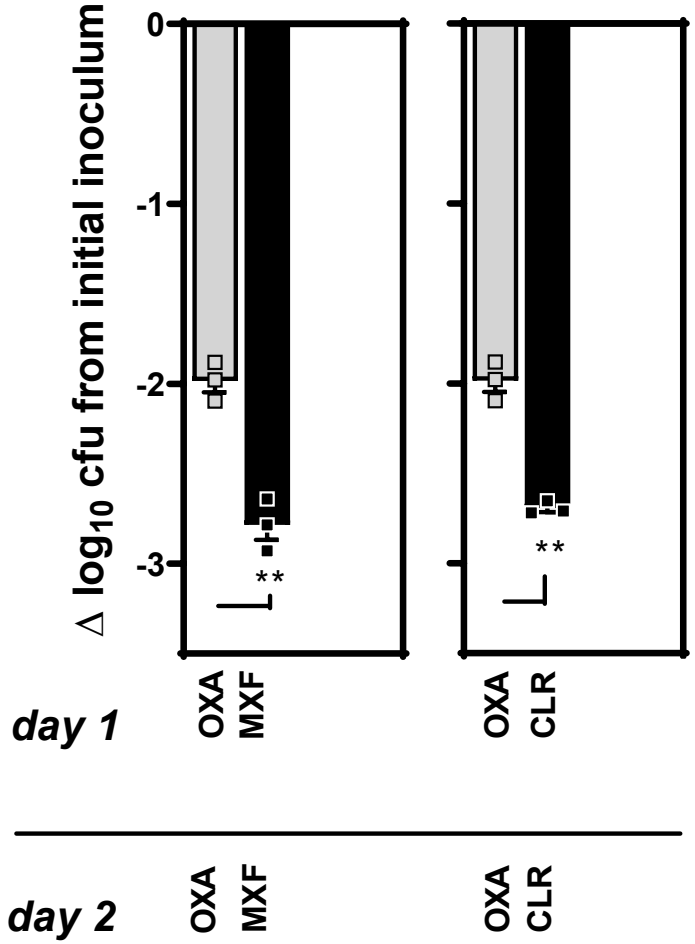
Multidrug tolerance





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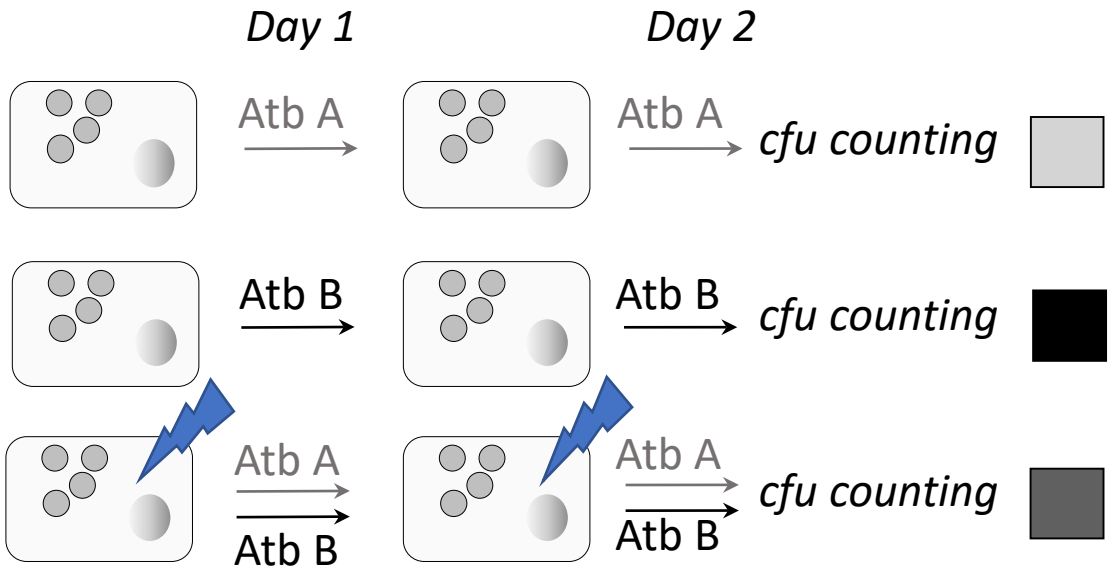

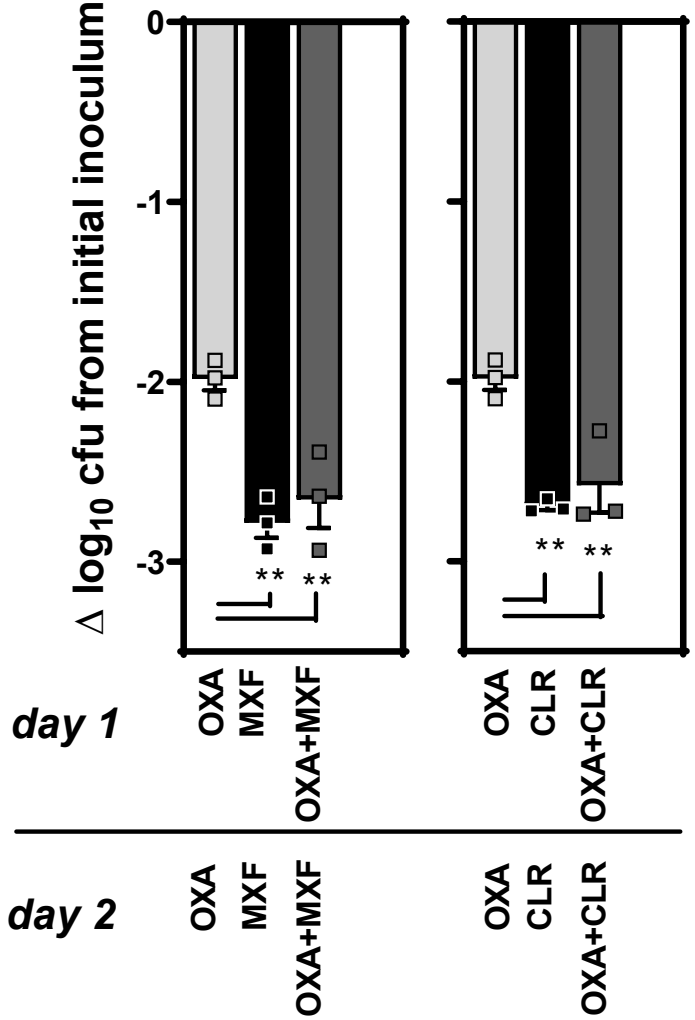

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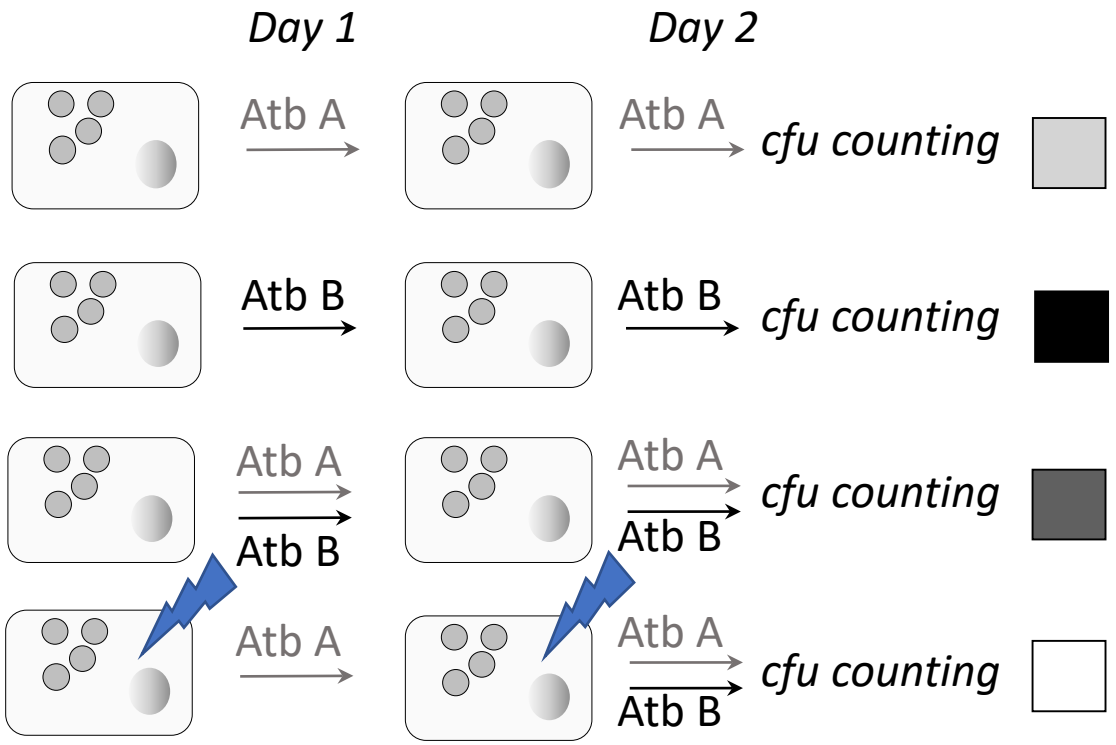
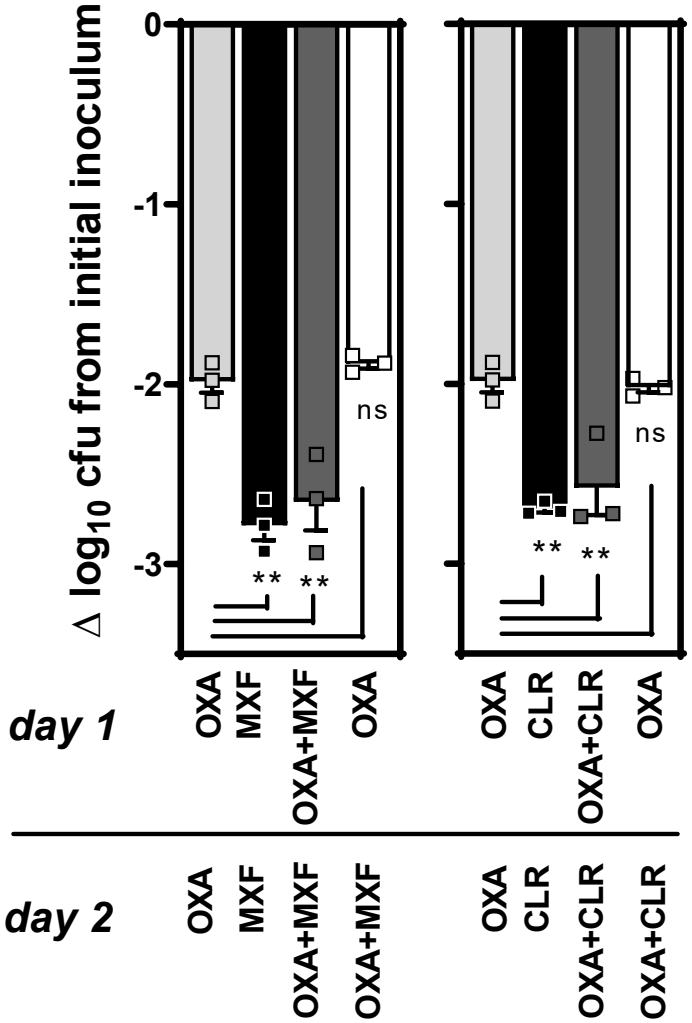


Persister level is defined by exposure to the first antibiotic



Peyrusson et al, Nat. Comm. 2020; 11:2200

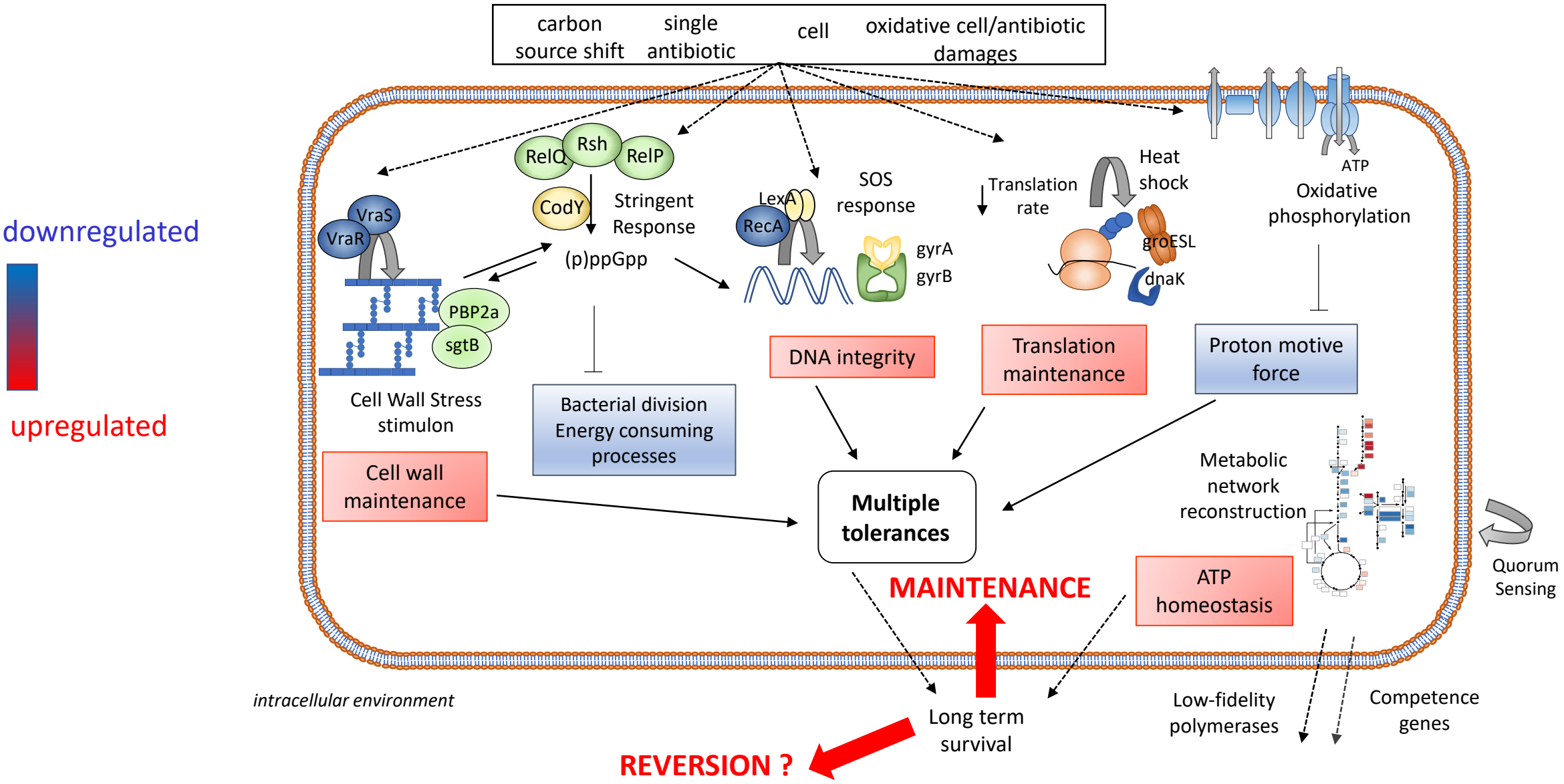
Intracellular persisters: clinical implications

- Intracellular bacteria can remain ‘dormant’ inside eukaryotic cells
- Dormancy is favored by stressful conditions (antibiotic pressure, e.g.)

- Dormancy is associated to the activation of a global stress response
  → Dormant bacteria are ‘multidrug tolerant’
  → Exposure to one drug makes bacteria non-responsive to other classes of drugs

D. Parkins

Intracellular persisters: a global view



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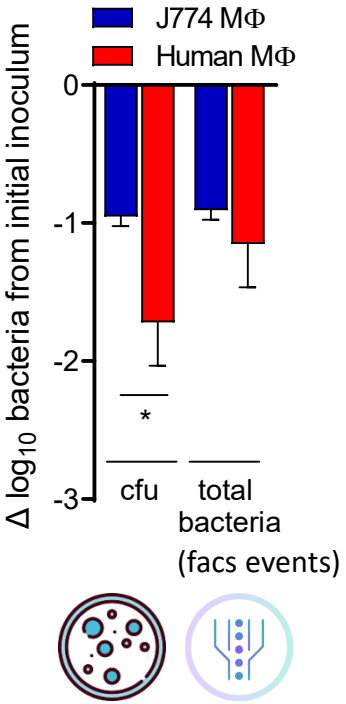
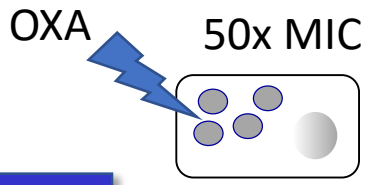
Deepness of dormancy…. Kinetics of awakening

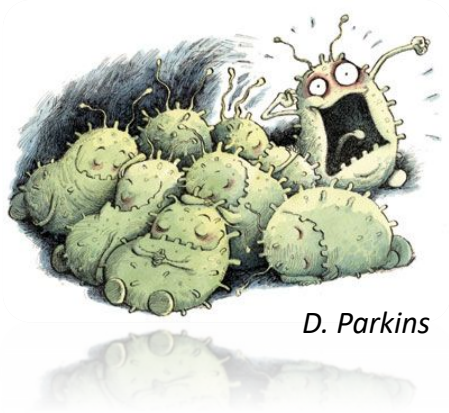
Deepness of dormancy

FACS counts vs colony forming units





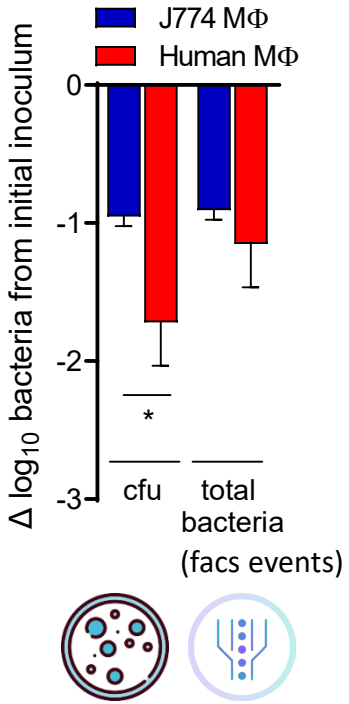
All bacteria do not form colonies when isolated from activated human macrophages

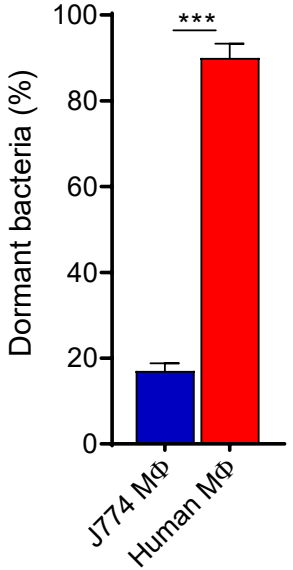


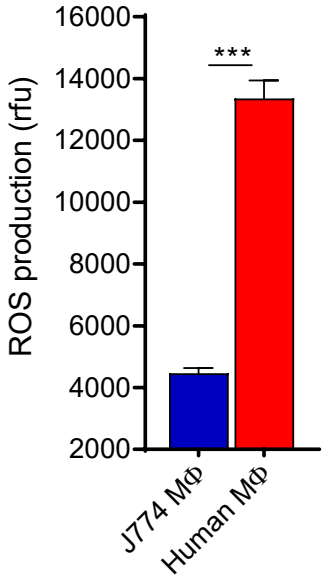
Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

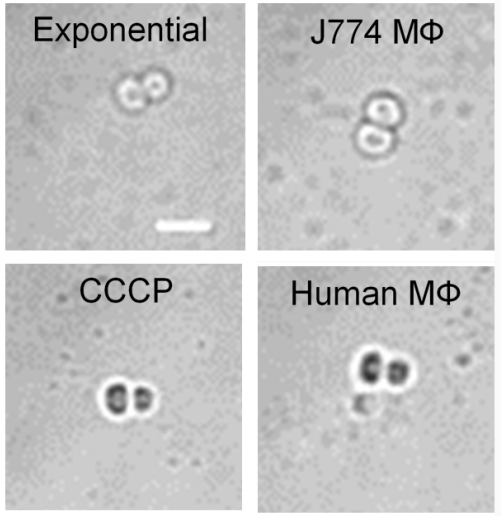
Deepness of dormancy

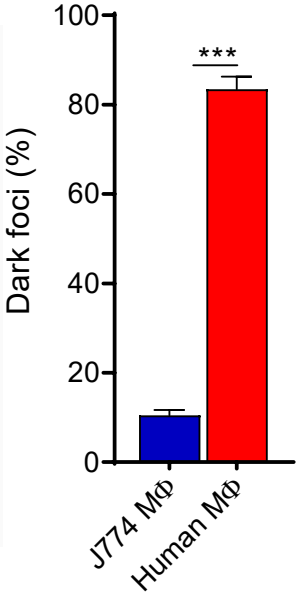
FACS counts vs colony forming units

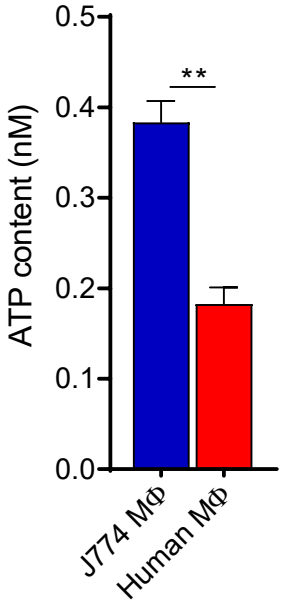










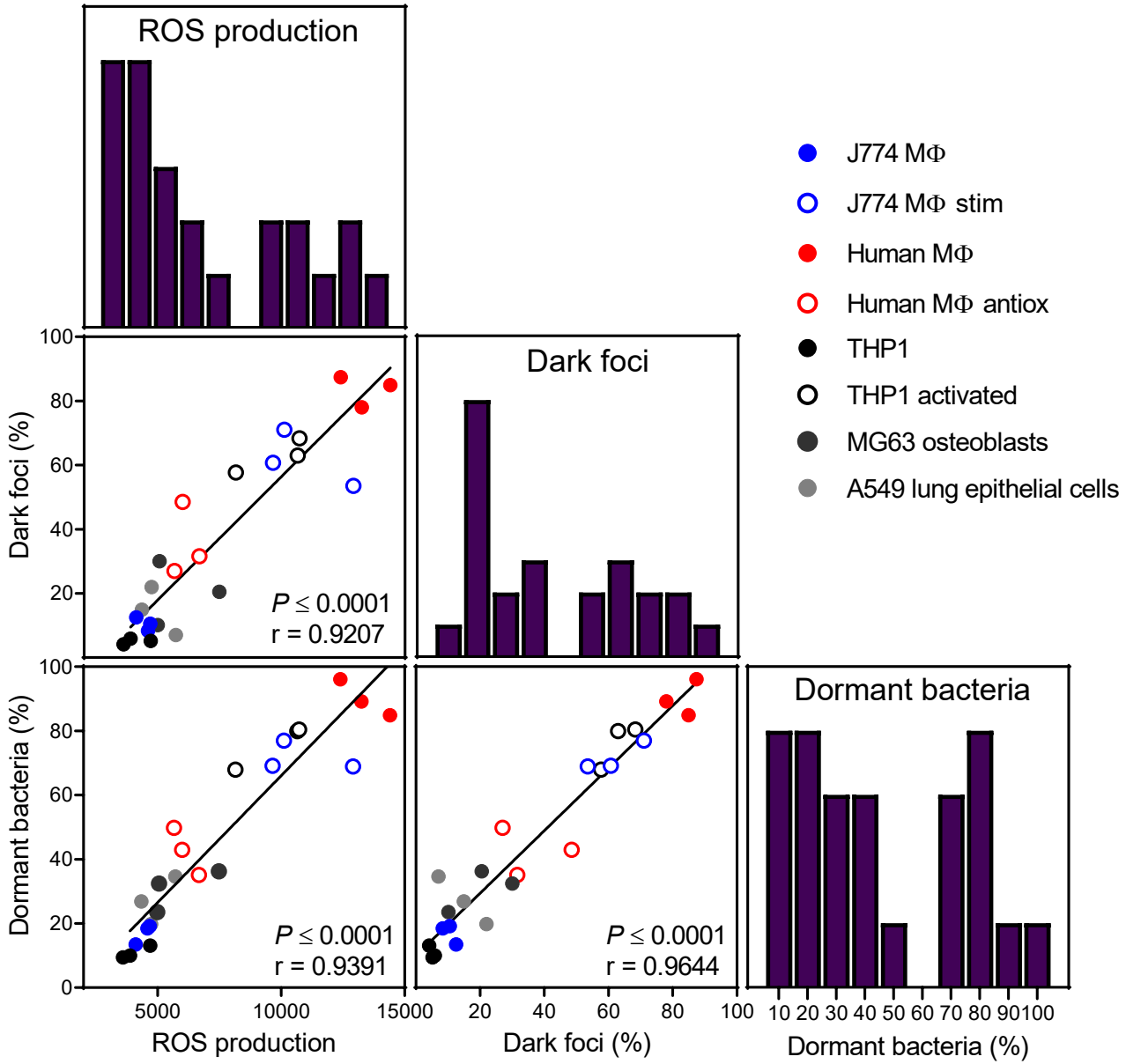


Dormancy level depends on the cell type (oxidative stress level)

Dormant bacteria show protein aggregates and lower ATP content

Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

Deepness of dormancy: impact of the cell type



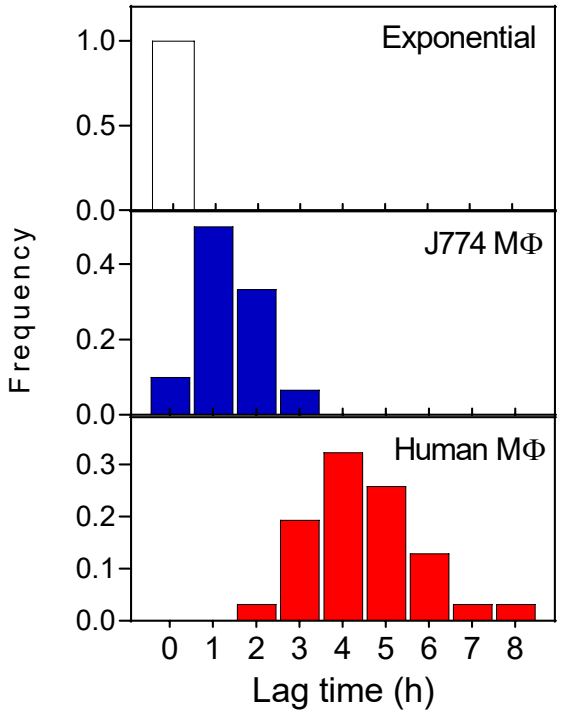
Cell ROS production highly correlated with % dormant bacteria % dark foci

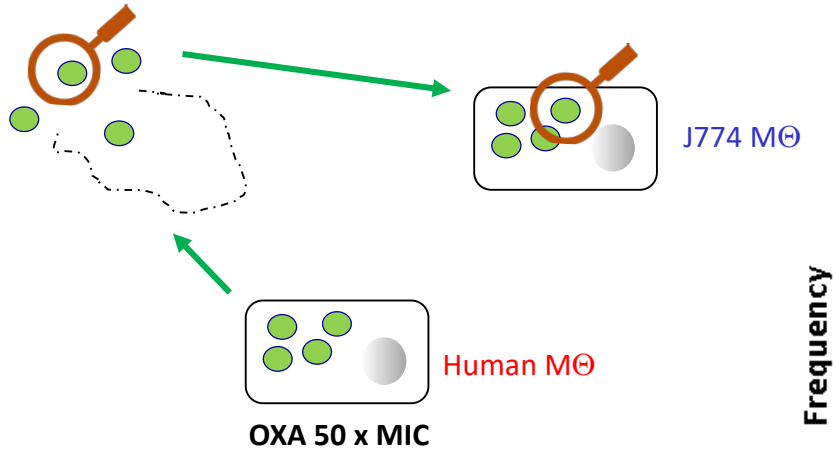
Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

Awakening of dormant forms

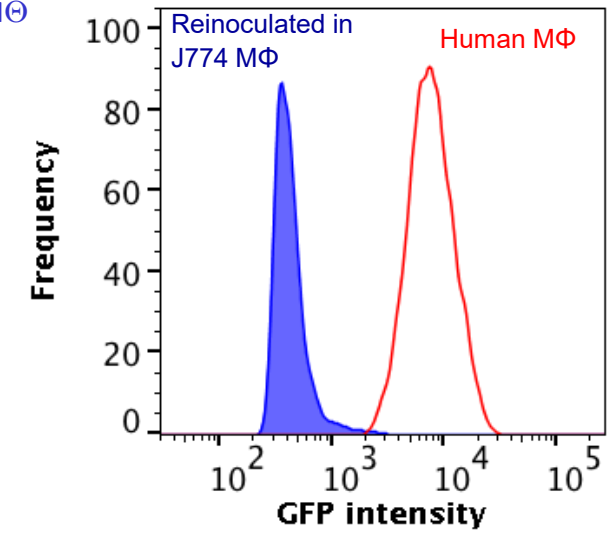
Awakening of intracellular bacteria upon drug removal

In liquid culture





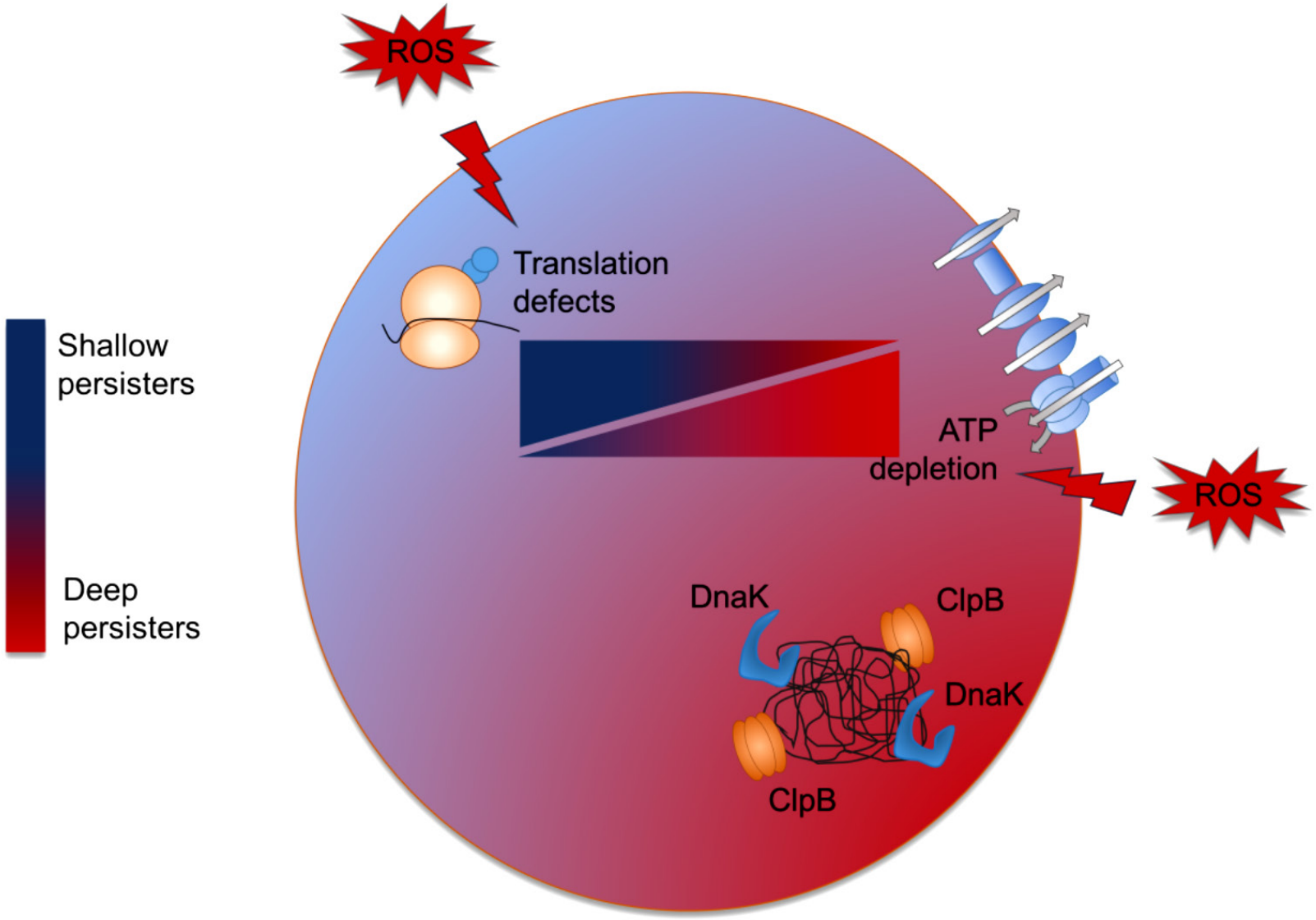
Intracellularly



Awakening time is longer for persisters from oxidative cells

Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

Deepness of dormancy : a balance between translation defects and ROS



Peyrusson et al, Microbiology Spectrum 2022; 10(1):e0231321

Intracellular persisters: clinical implications

- Intracellular bacteria can remain ‘dormant’ inside eukaryotic cells
- Dormancy is favored by stressful conditions (antibiotic pressure, e.g.)

- Dormancy is associated to the activation of a global stress response
  → Dormant bacteria are ‘multidrug tolerant’
  → Exposure to one drug makes bacteria non-responsive to other classes of drugs

- Dormancy is reversible when the stress is relieved
  → A possible reason for recurrence of the infection ?

- If deeply dormant, persisters do no grow on agar plates
  → How to detect them in biological samples ?

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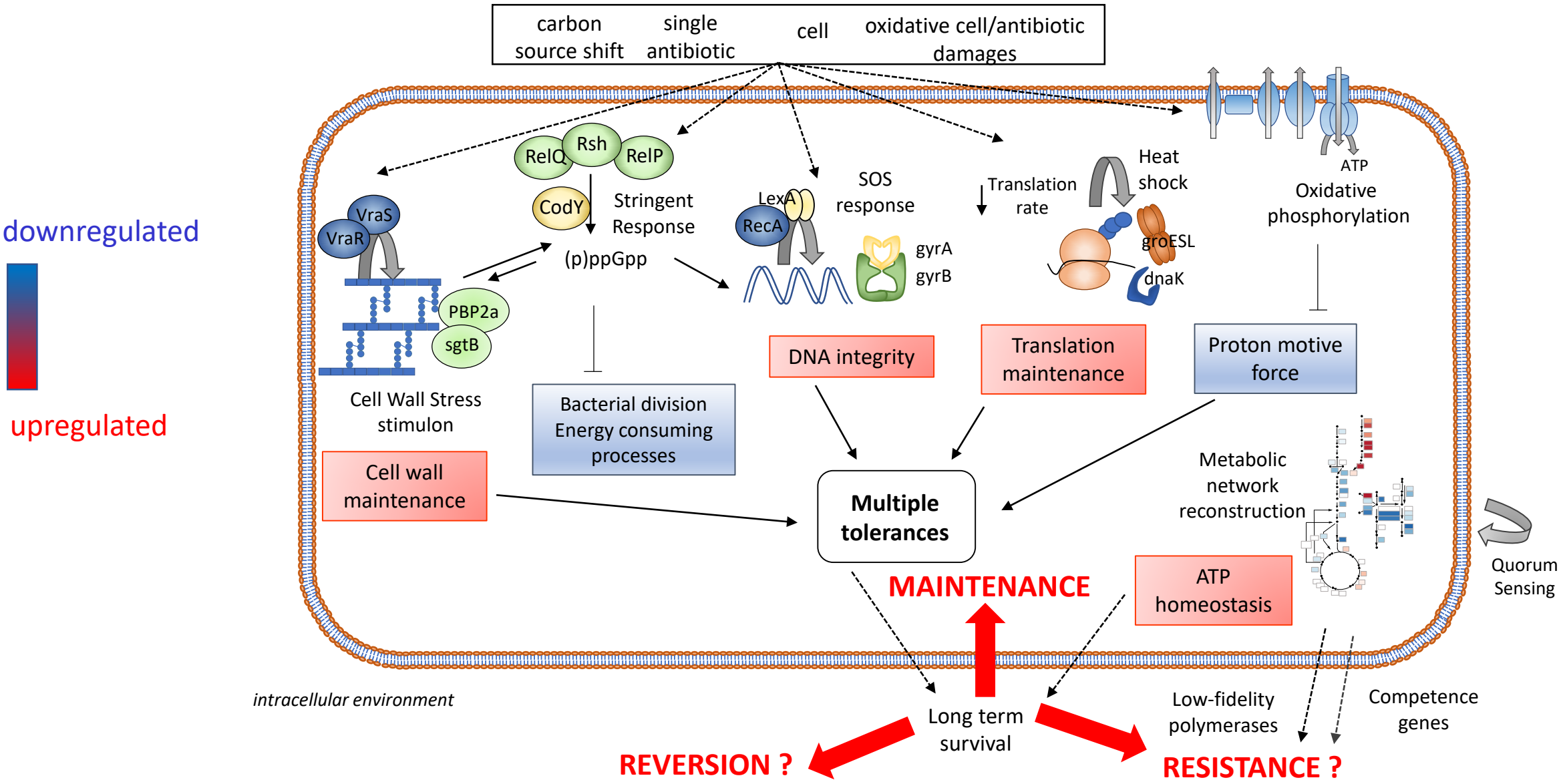
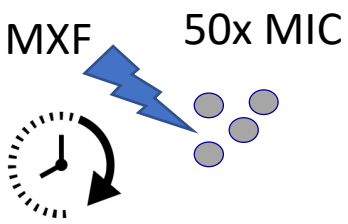
Intracellular persisters: a global view

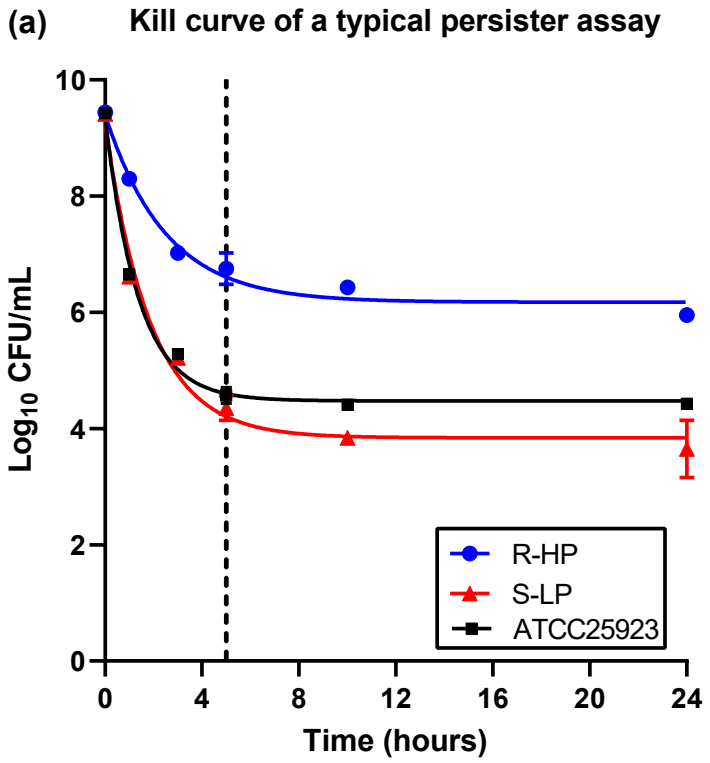


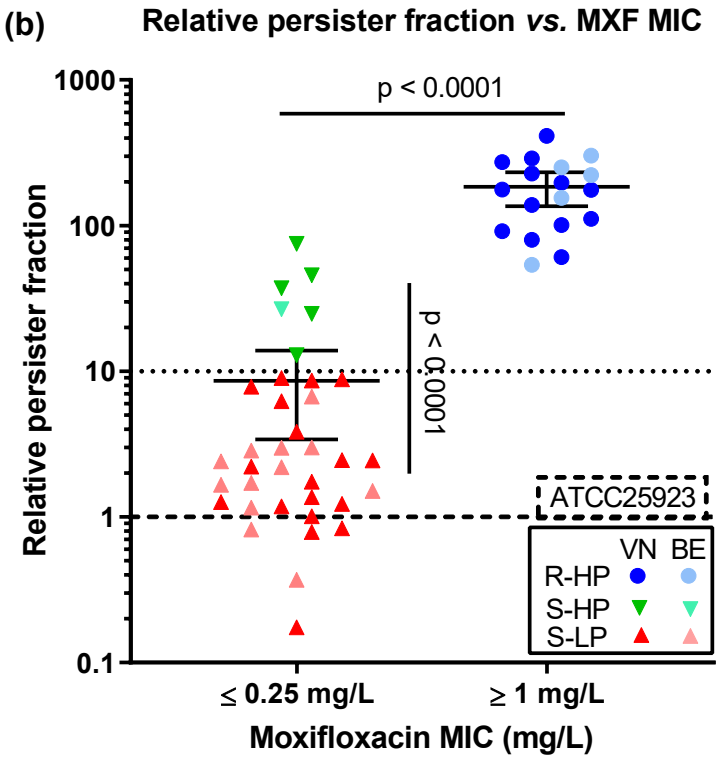
Peyrusson et al, Nat. Comm. 2020; 11:2200

What about clinical isolates ?

Relative persister fraction to the fluoroquinolone moxifloxacin in a collection of clinical isolates







Resistant; High persister

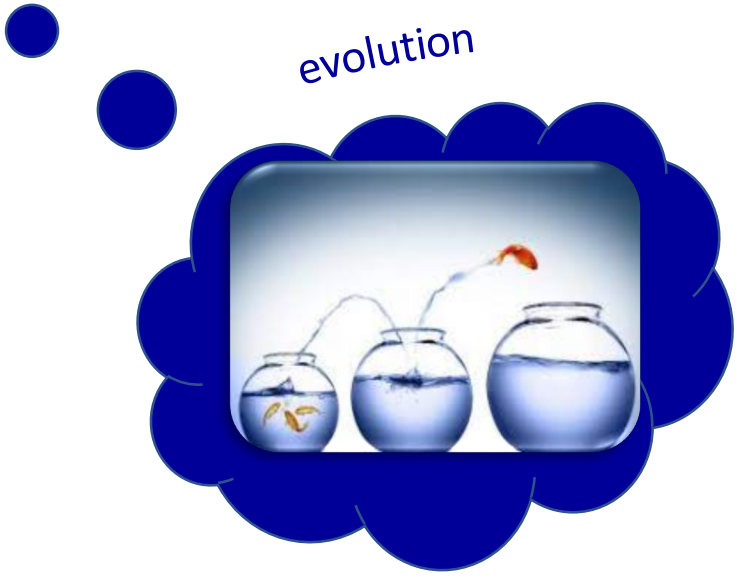
Susceptible; High persister

Susceptible; Low persister

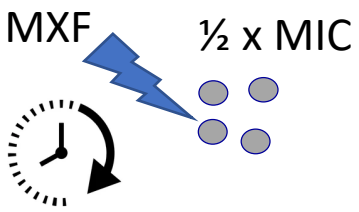
- All resistant isolates have a high relative persister fraction
- Most susceptible isolates have a low relative persister fraction

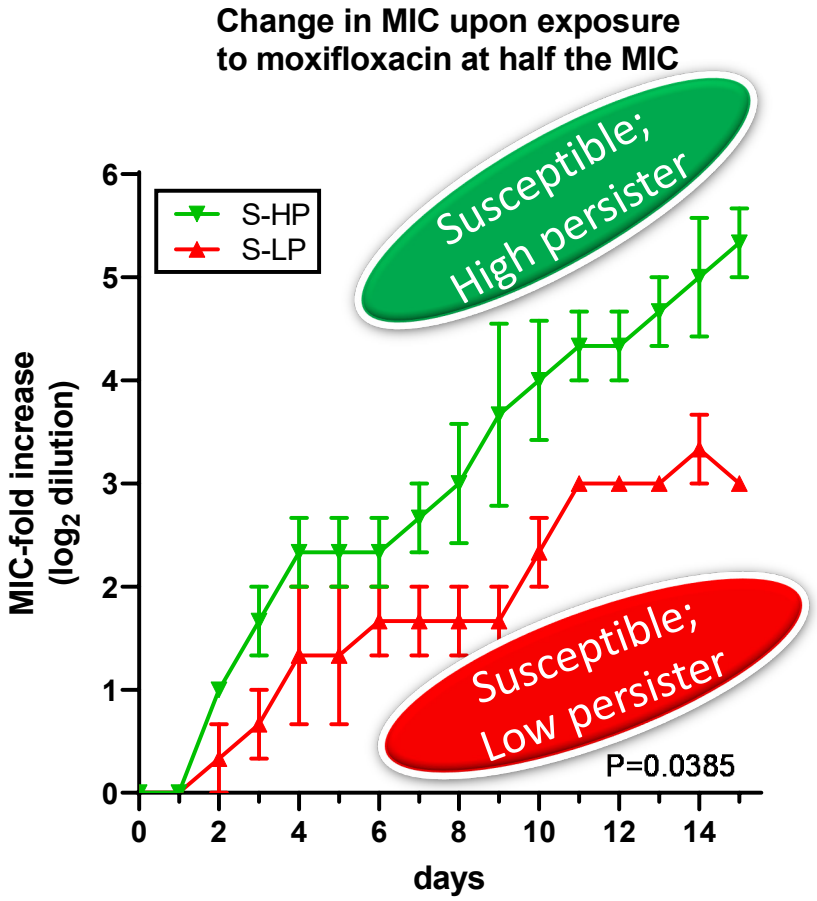
Nguyen et al, Front. Microbiol 2020; 11:587364

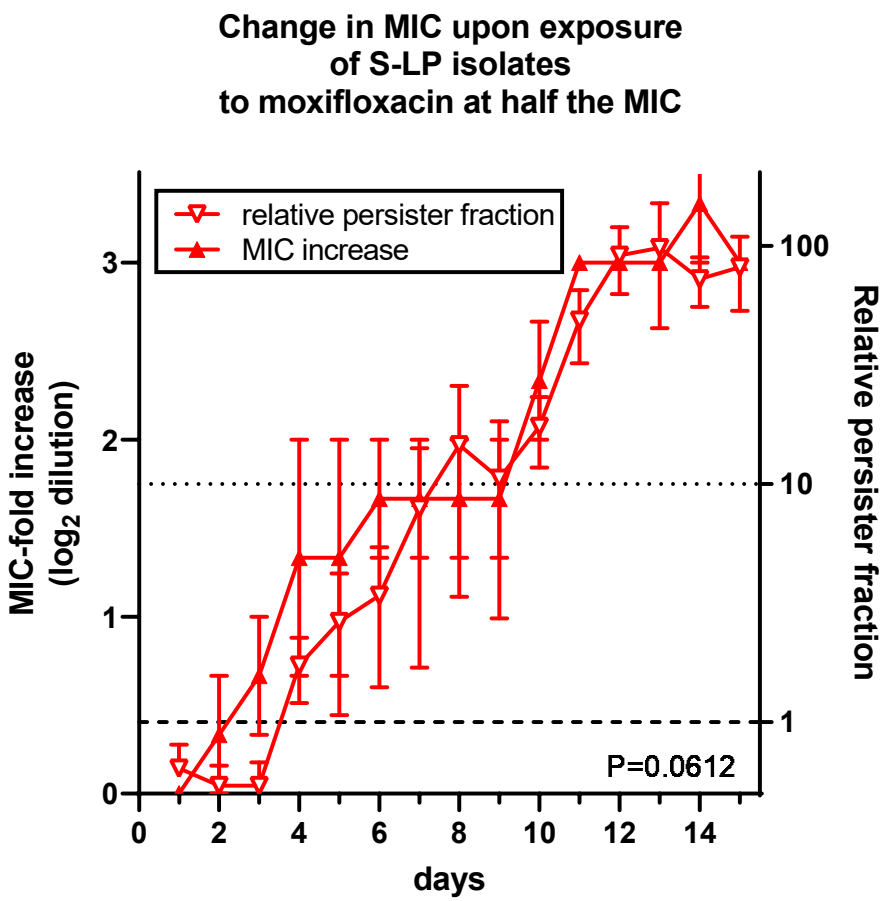
Does persistence prepare for resistance?



Does persistence prepare for resistance?







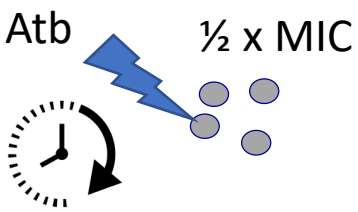
- Persistence accelerates the selection of resistance
- Persistence and resistance are acquired together

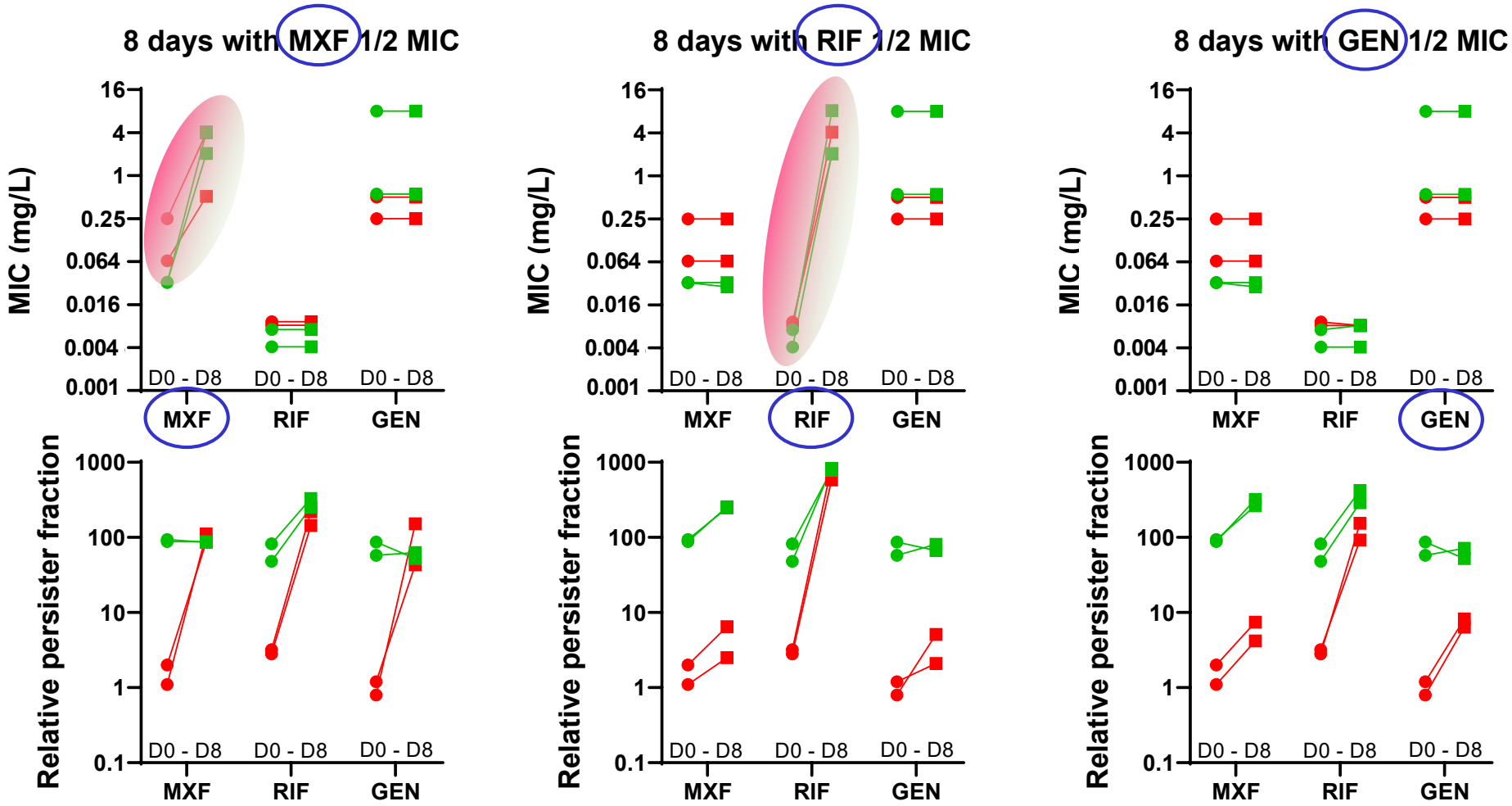
Nguyen et al, Front. Microbiol 2020; 11:587364

Cross-persistence without cross-resistance

Susceptible; High persister to MXF

Susceptible; Low persister To MXF





Exposure to sub-MIC

- selects for resistance to the selecting drug (when possible)

Balcaen et al., unpublished

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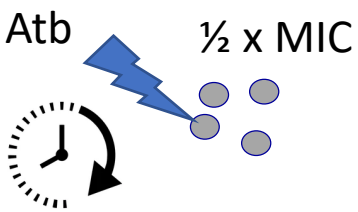

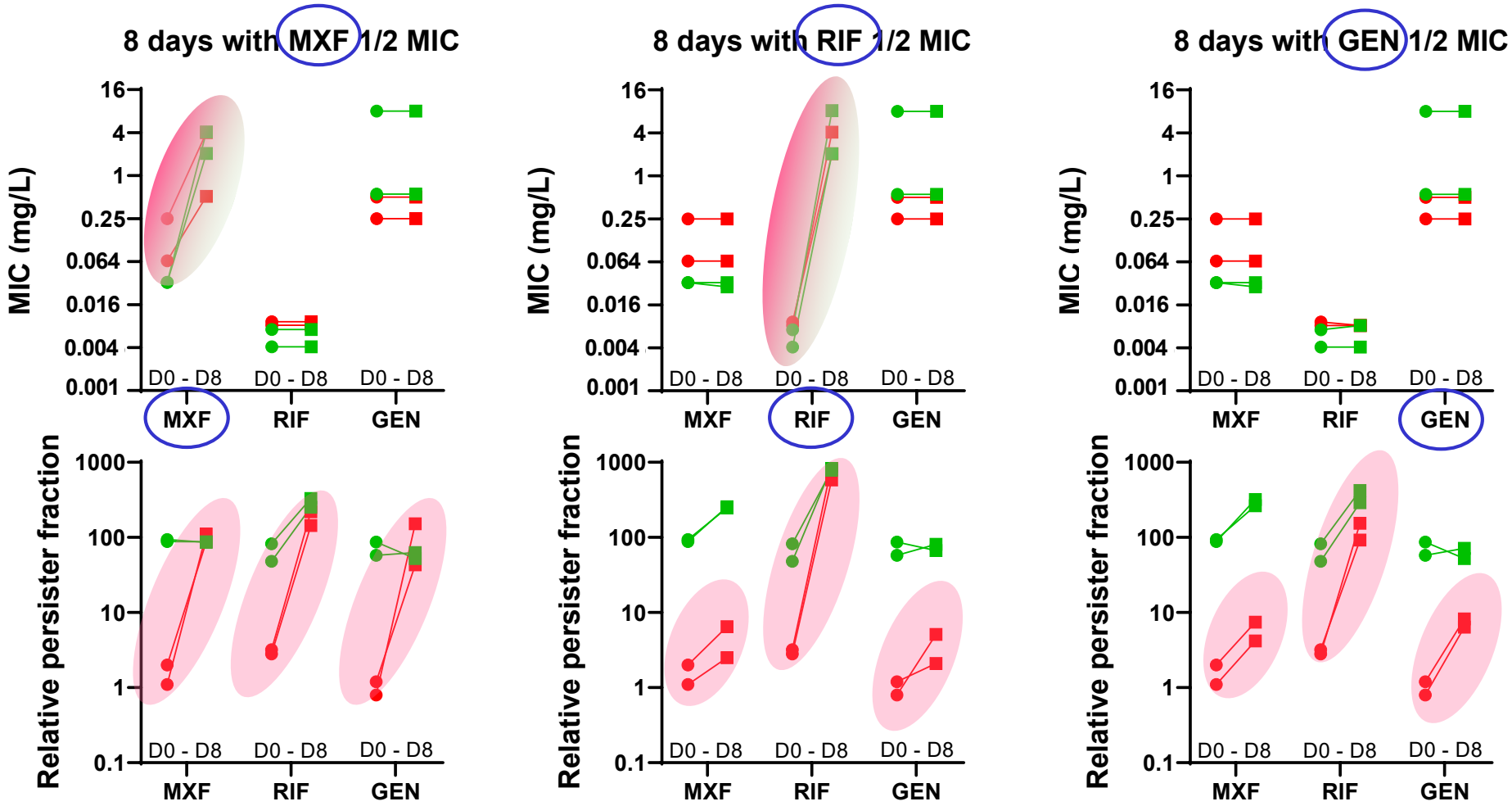

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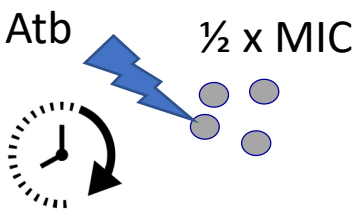
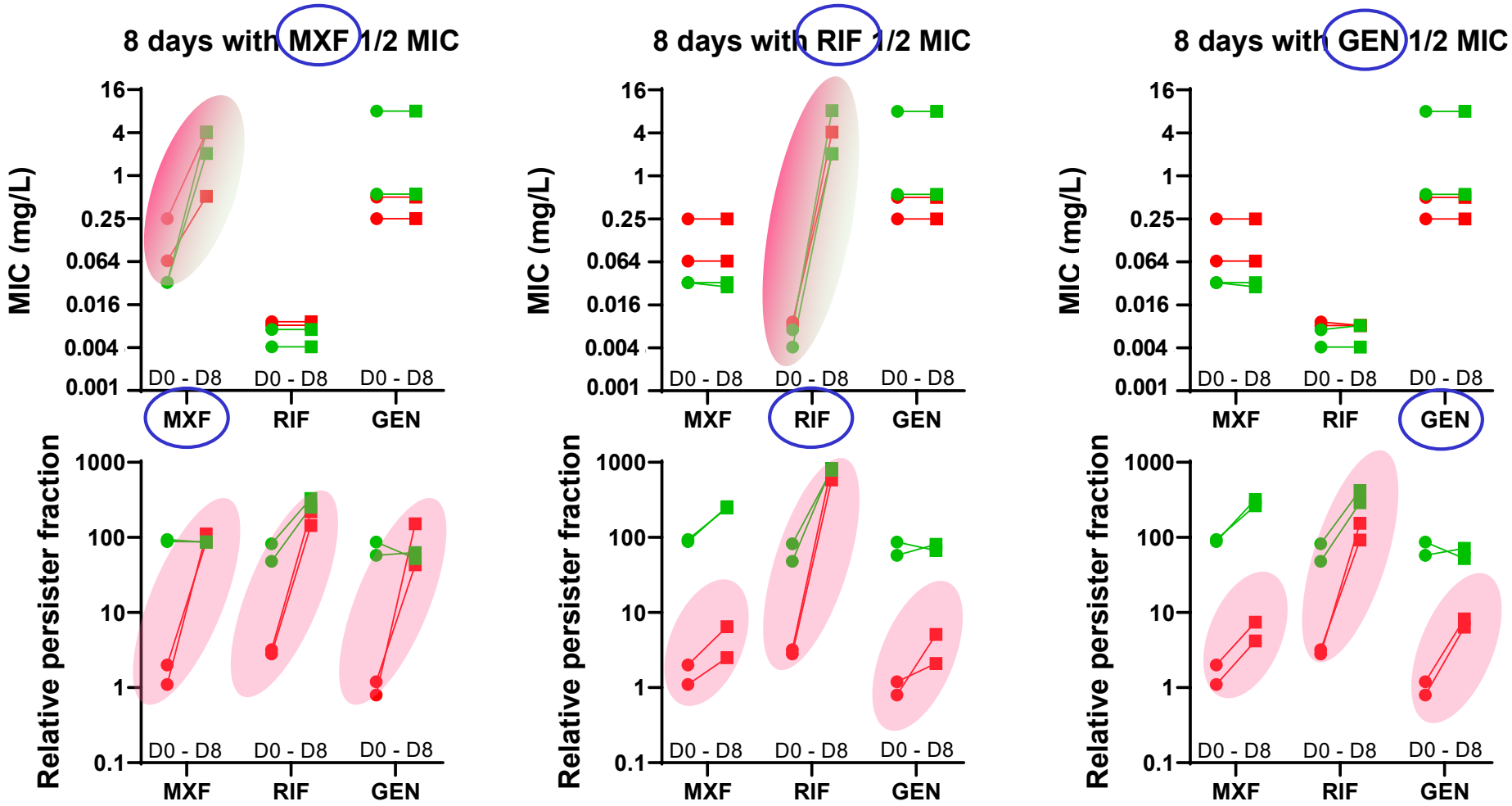




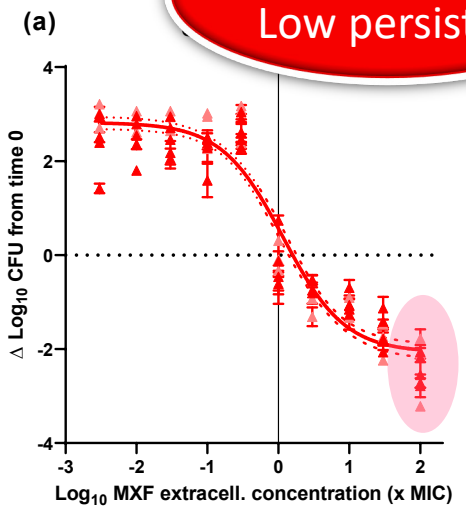
Exposure to sub-MIC

- selects for resistance to the selecting drug (when possible)
- increases persistence to different drug classes

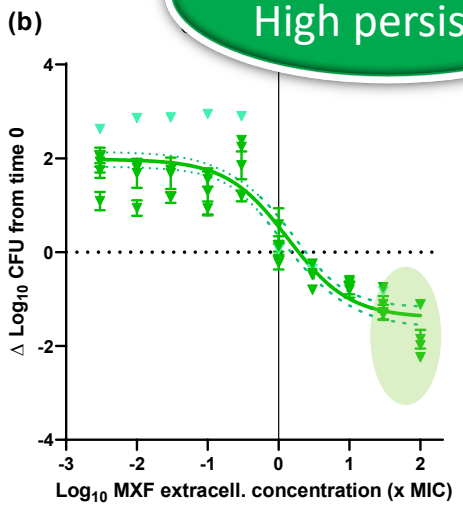
Balcaen et al., unpublished

Persister character to predict intracellular tolerance

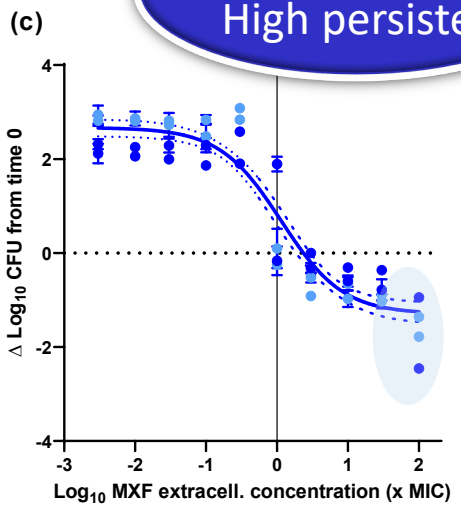
Susceptible; Low persister

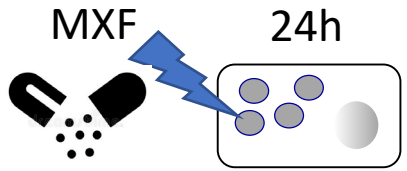


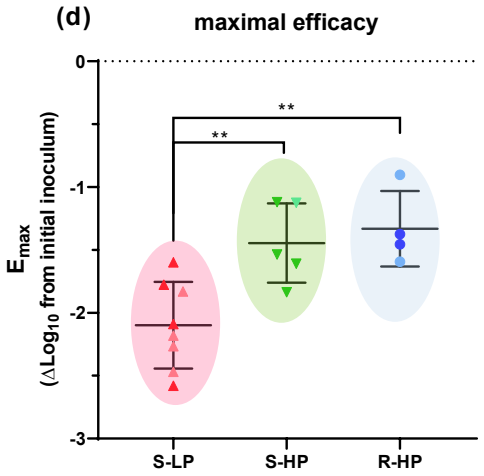
Susceptible; High persister

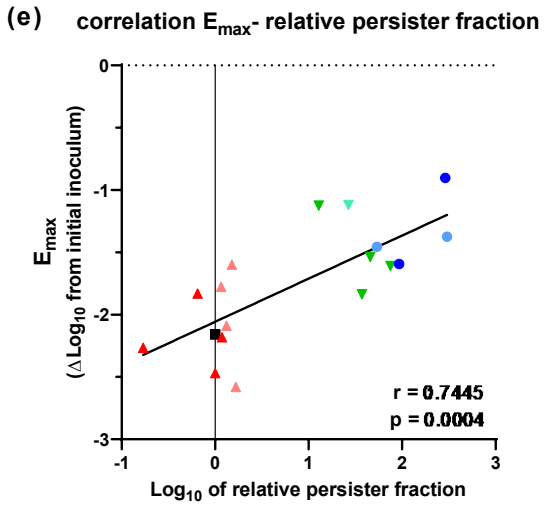


Resistant; High persister









Isolates with higher relative persister fraction are less responsive to moxifloxacin intracellularly

Nguyen et al, Front. Microbiol 2020; 11:587364

Intracellular persisters: clinical implications

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- Dormancy is favored by stressful conditions (antibiotic pressure, e.g.)

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- Dormancy is associated to the activation of a global stress response
  → Dormant bacteria are ‘multidrug tolerant’
  → Exposure to one drug makes bacteria non-responsive to other classes of drugs

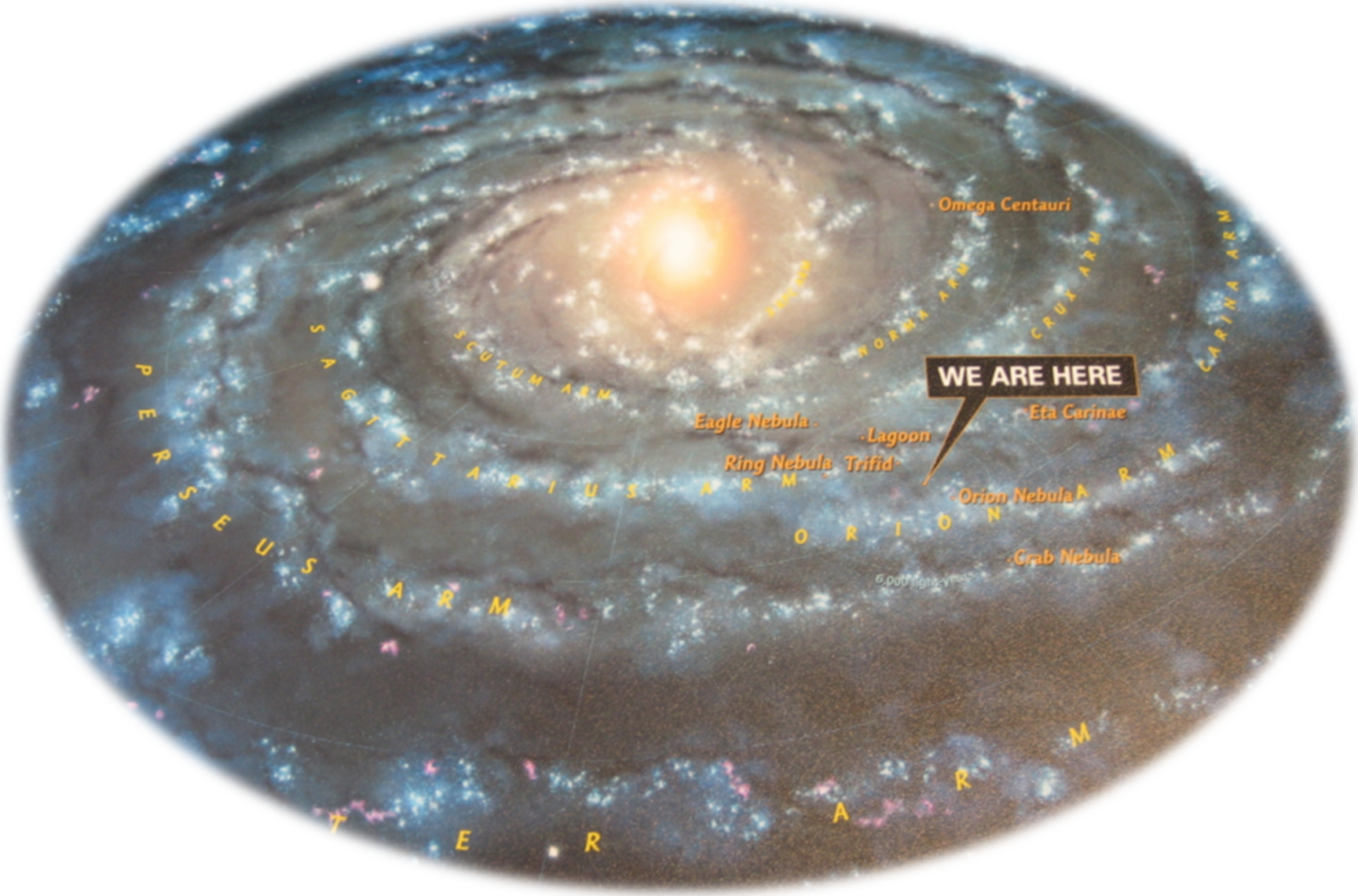
- Dormancy is reversible when the stress is relieved
  → A possible reason for recurrence of the infection ?

- If deeply dormant, persisters do no grow on agar plates
  → How to detect them in biological samples ?

- Clinical isolates differ by the fraction of persisters in their populations
  → Is there a link with the risk of clinical failure ?

- Activation of stress in persisters favors selection of resistance
  → Another reason to see resistance increase when dealing with chronic infections ?

Still more questions than answers ….



Acknowledgments

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Cartoon: https://kids.frontiersin.org/articles/10.3389/frym.2019.00045