

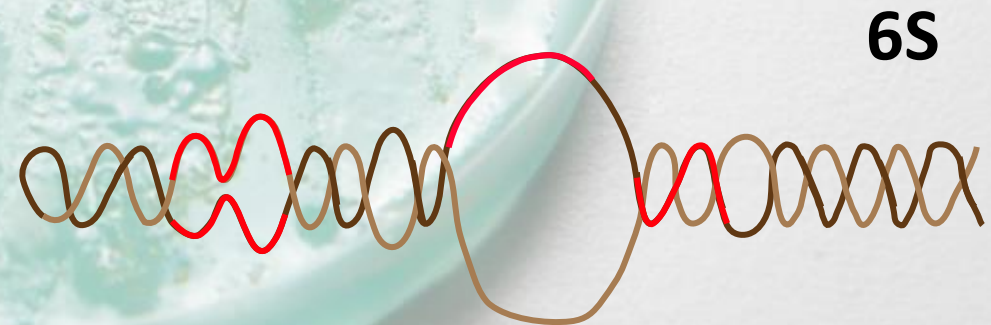
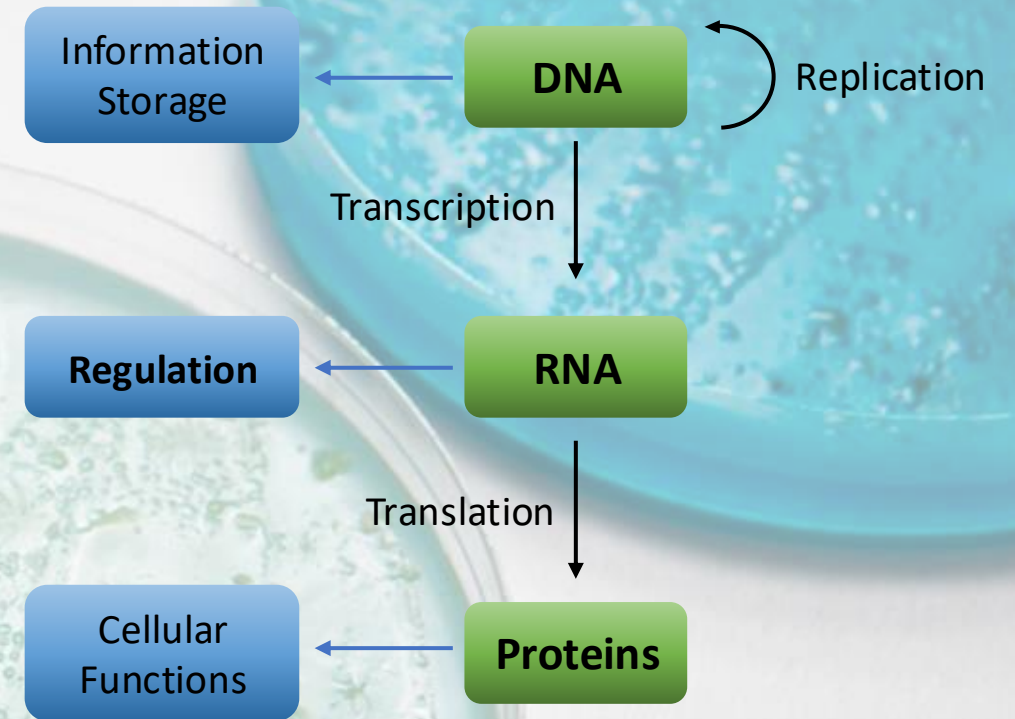
Activity and Inhibition of 6S RNA: A Regulatory Bacterial RNA

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- Due to emerging resistance, the world is running out of new antibiotics¹.
- The function of 6S RNA² makes it a promising target to study the various intracellular functions and antibiotic interactions.



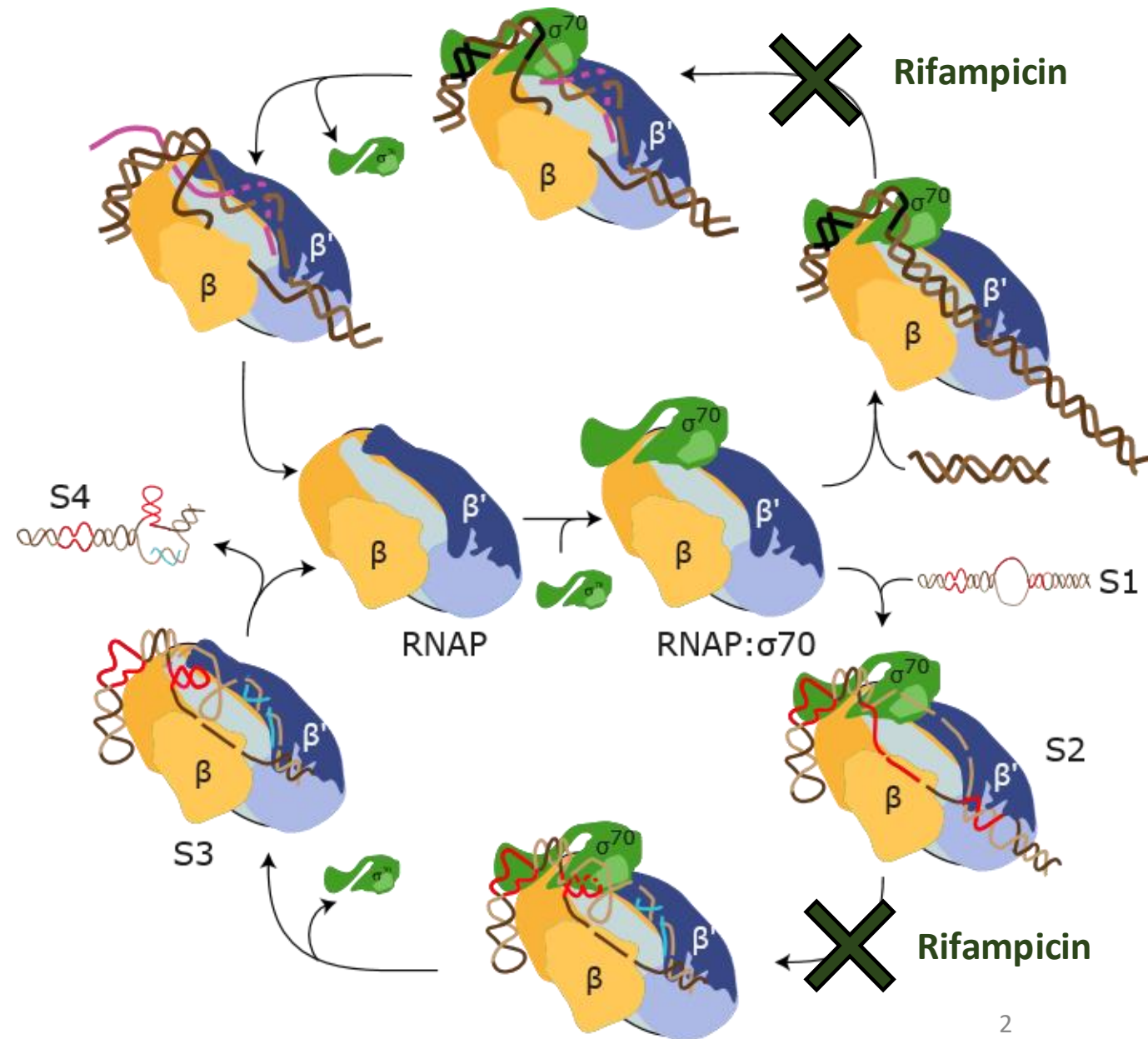
1. <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
2. Bonar et al., RNA, 2022

6S RNA Regulates Expression of Housekeeping Genes in Bacteria

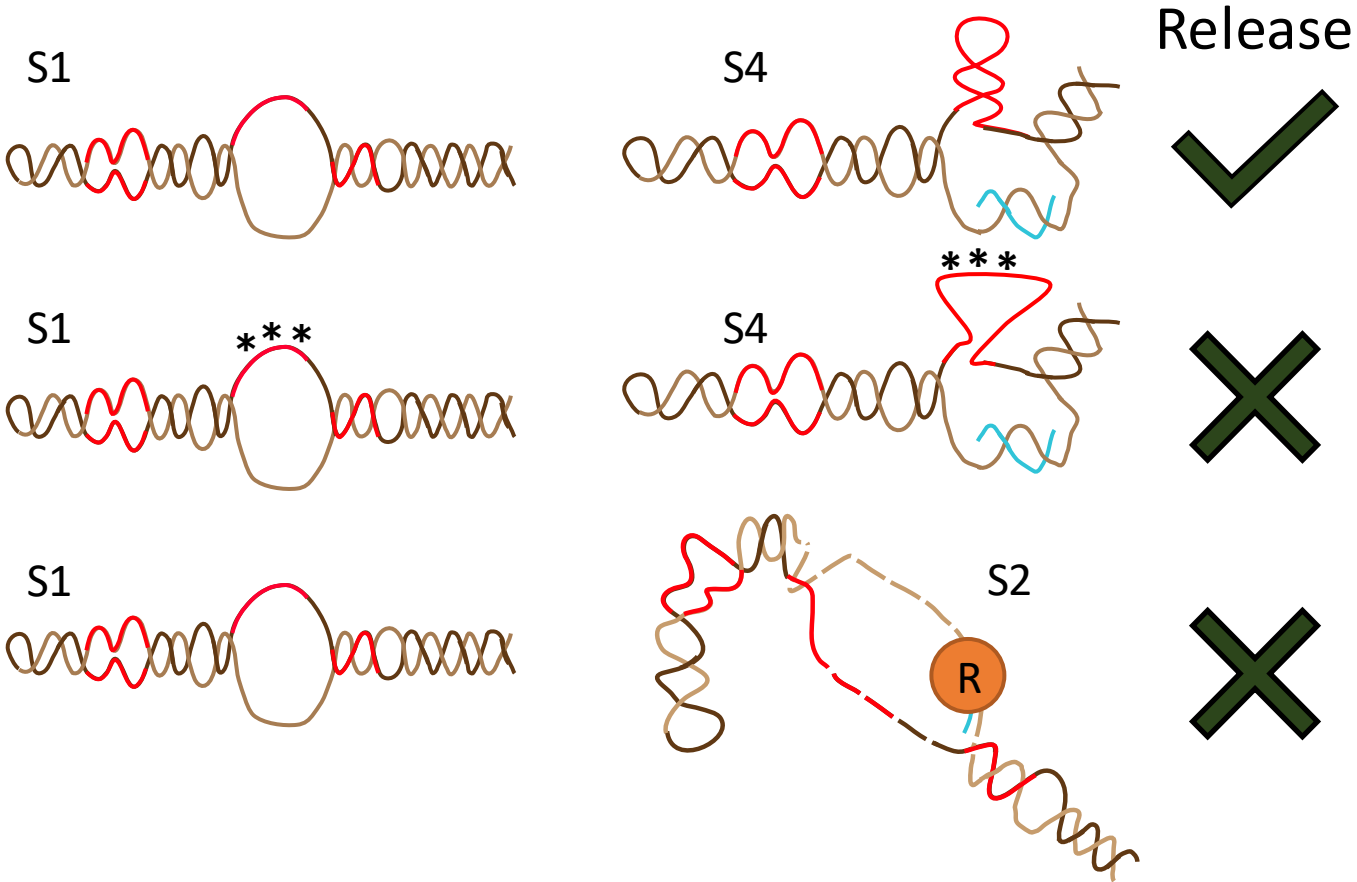
6S RNA can compete with DNA promoters to bind to RNAP (RNA Polymerase) and serves as a template for short product RNA (pRNA) synthesis, which allows it to:

- Release from RNAP in high nutrient conditions or with other release factors³.
- Stay bound to RNAP in low nutrient or **stress** conditions^{4,5}.

Rifampicin (Rif) blocks both pathways: RNA synthesis, pRNA synthesis.



Made a 6S Mutant That is Release Defective

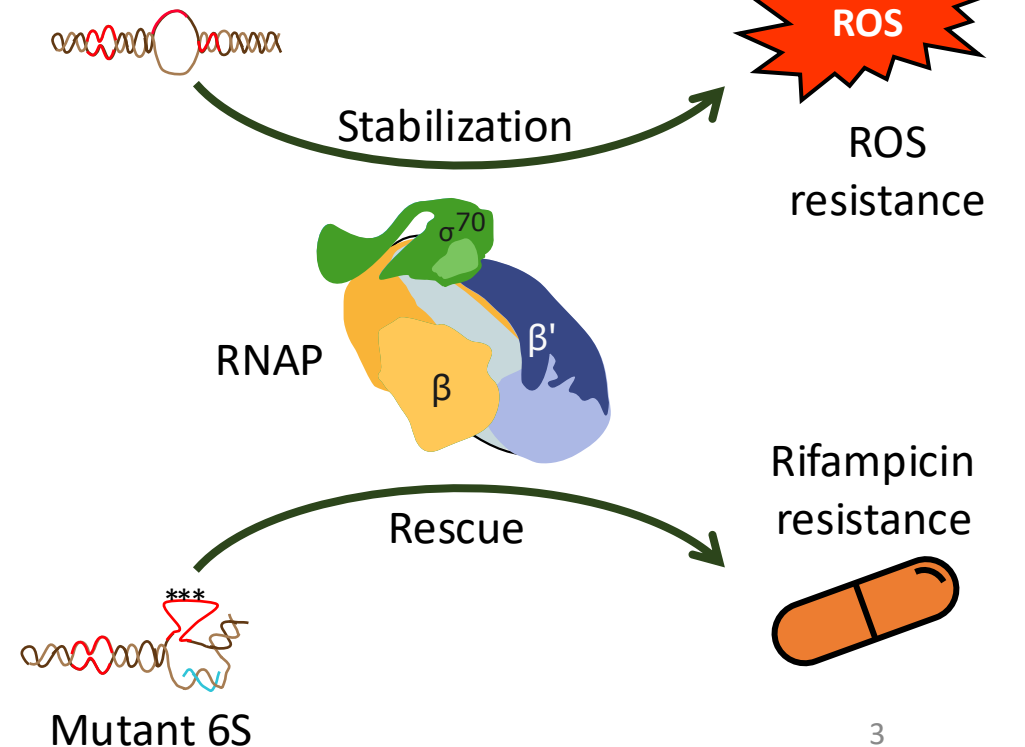


- Point mutations (Sr) prevent/slow down the 6S RNA release from RNAP.
- When Rifampicin is added, the release rate decreases as well.

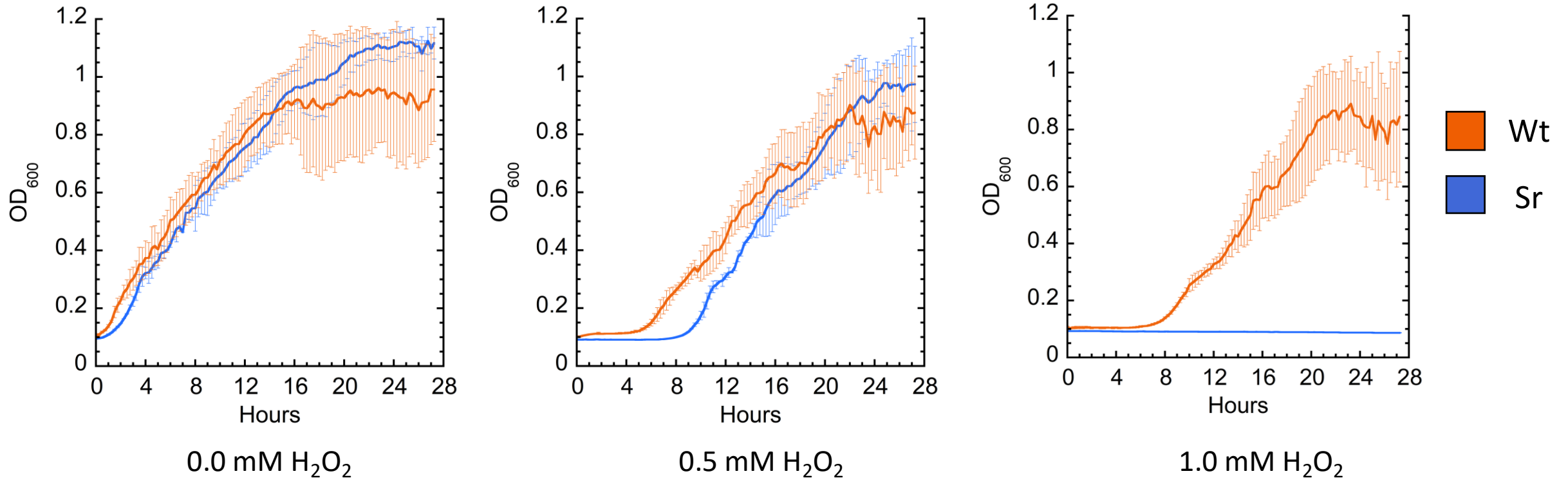
Sequencing results showing 3 point mutations

Reference	TGTAACCCCTTGAACCCCTTGGTTCAAGGTGAAT
Wt_6S	TGTAACCCCTTGAACCCCTTGGTTCAAGGTGAAT
Sr_6S	TGTAACCCCTTGAACCCCTGTGTTCTGAGGTGAAT

Wild-type 6S



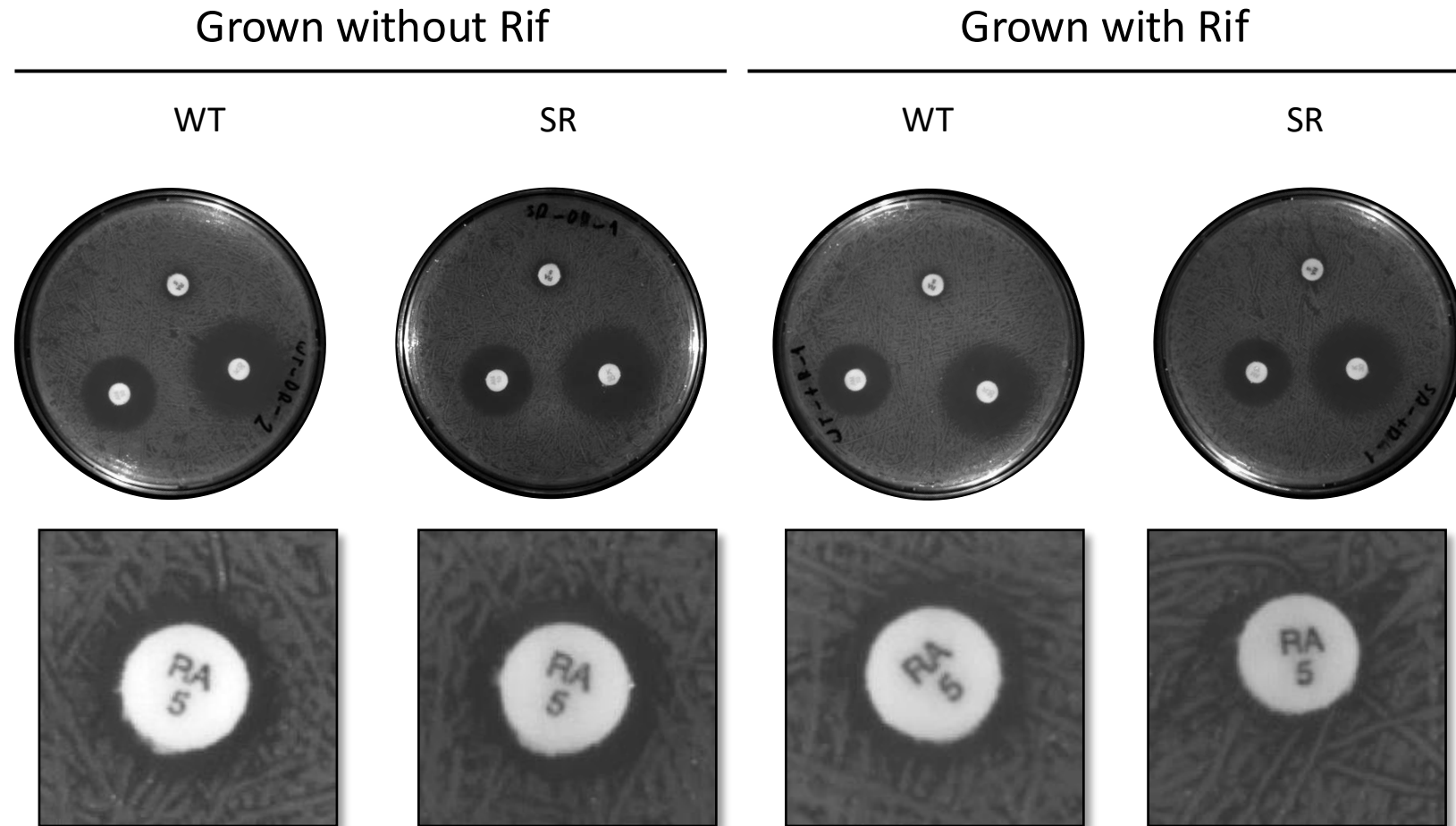
6S RNA is Critical for Oxidative Stress Response



- *E. coli* lacking 6S gene are sensitive to H₂O₂⁶.
- Here we show *E. coli* with release defective (Sr) 6S grow poorly compared to wild-type when H₂O₂ is added to the media.

Mutants Develop Rif Resistance Faster

- *E. coli* were grown in media either with or without Rif.
- Antibiotic disks used to assess whether resistance has occurred: the **closer** bacteria are to disk, the **more resistance** they have.
- Without Rif, both WT and SR have are inhibited by Rif.
- When grown in Rif, **SR develops resistance faster**.



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