

SP-05. Royal Society of Chemistry Chemical Biology

SP-05.01 - Probing bacterial survival strategies: inhibitors of (p)ppGpp synthesis

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Persistence is a bacterial bet hedging strategy that allows for temporary tolerance to antibiotic treatment. This phenotypic switch paves the way to the chronicity of certain infections and to the insurgence of genetic resistance. Here we present our work on targeting bacterial persisters via inhibition of the upstream of the stringent response (SR), one of the working hypothesis for their formation. The SR is triggered by the accumulation of the second messenger (p)ppGpp, promoted by a superfamily of enzymes called RSH (RelA/SpoT Homologue). We performed fragment-based virtual screening on the synthetase catalytic site of our model bifunctional protein RelSeq, selecting three main chemotypes. Thermal shift analysis on RelSeq constructs highlighted interesting affinities of some selected fragments, along with the desired selectivity over the hydrolase domain. The most promising scaffold was therefore selected for the development into a higher affinity ligand.

Keywords: Persisters, (p)ppGpp, design

Supported by: ERC-StG-2017 (grant n. 758108)

SP-05.02 - Many birds with one stone: targeting a universal signaling pathway of bacteria to improve antimicrobial therapy

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Winning the war against resistant bacteria will require a change of paradigm in antibiotic discovery. A promising new direction is the targeting of non-essential pathways required for successful infection, such as quorum-sensing, virulence and biofilm formation. Similarly important will be strategies to prevent or revert antibiotic resistance. Here we argue that the ppGpp signaling pathway should be a prime target of this effort, since its inactivation could potentially achieve all these goals simultaneously. The hyperphosphorylated guanine nucleotide ppGpp is an ancient and universal second messenger of bacteria that has pleiotropic effects on the physiology of these organisms and has been implicated in the long term survival and the development of virulence and antibiotic tolerance and persistence in diverse bacteria. The cellular concentration of ppGpp is controlled by enzymes of the RSH (RelA SpoT Homology) family. Long RSH proteins are bifunctional enzymes capable of synthesizing and degrading ppGpp, whereas short RSH, also known as SAS (Small Alarmone Synthetases), are single domain proteins that only synthesize ppGpp. Despite the importance of this pathway, there are remarkably few inhibitors of the RSH enzymes described in the literature. Here we will describe our efforts to develop a pathway-specific whole cell assay capable of identifying inhibitors of both the long RSH and SAS enzymes and preliminary results of the screen of two types of small molecule libraries.

Keywords: ppGpp, Rel, RSH, SAS, persistence

Supported by: FAPESP, CNPq