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Integrated mathematical modeling, experimental and bioinformatics study of Type-II antitoxin-toxin system's response to antibiotic exposure

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Motivation and Aim: Antibiotic persistence refers to a phenomenon in which a small subpopulation of bacterial cells (dormant cells) survives antibiotic treatment despite being genetically identical to the vast majority of cells that are susceptible to antibiotics. Bacterial toxin-antitoxin (TA) modules of Type-II are involved in response to various stressful conditions, such as antibiotic exposure. Elevated toxin levels may induce dormancy. Our goal is to explore how the kacAT Type-II TA system (a member of the GNAT-ribbon-helix-helix family) participates in the antibiotic persistence of *Klebsiella pneumoniae.*

Methods and Algorithms: We tackle this problem by joint theoretical and experimental study of kacAT gene expression regulation [1], as well as with a bioinformatics and statistical analysis of GNAT-RHH loci in completely sequenced genomes of *K. pneumoniae* [2]. To this end, and based on the fact that KacAT complex formation is cooperative, we developed a mechanistic model of system dynamics, which is characterized by highly non-linear feedback loops and a single stationary state (Fig. 1A).

Results: Analysis of our mathematical model [1] enables us to reproduce the following experimental observations: (i) Reduction of [KacA]:[KacT] protein ratio upon antibiotic application (antibiotic levels increase, i.e., antitoxin degradation increases going from left to right along each curve in Fig. 1B). (ii) Large kacAT transcript increase induced by antibiotics, which based on the model can be achieved under strong promoter autorepression by KacAT complex (Fig. 1C). (iii) KacAT overexpression induces tolerance to antibiotic stress, whereas kacAT deletion does not affect this tolerance (predicted large binding affinity Kb in Fig. 1C).

The finding that kacAT deletion does not lead to spontaneous persister formation is consistent with growing experimental evidence in different Type-II TA systems but opposes earlier theoretical models (see, e.g. [3]), which predicted the appearance of bistability. However, these models assumed the cooperative (joint) action of multiple TA systems located at several instances in the genome. So, we performed a bioinformatics analysis of GNAT-RHH family loci in completely sequenced genomes of *K. pneumoniae* [2]. Statistical analysis of thus predicted loci indicates that only one system in the same family as kacAT is strongly preferred (Fig. 2A–E). Additionally, we obtain statistically significant negative correlations between different clades for which experiments indicate their cross-talk so that such interactions are disfavored (Fig. 2F).

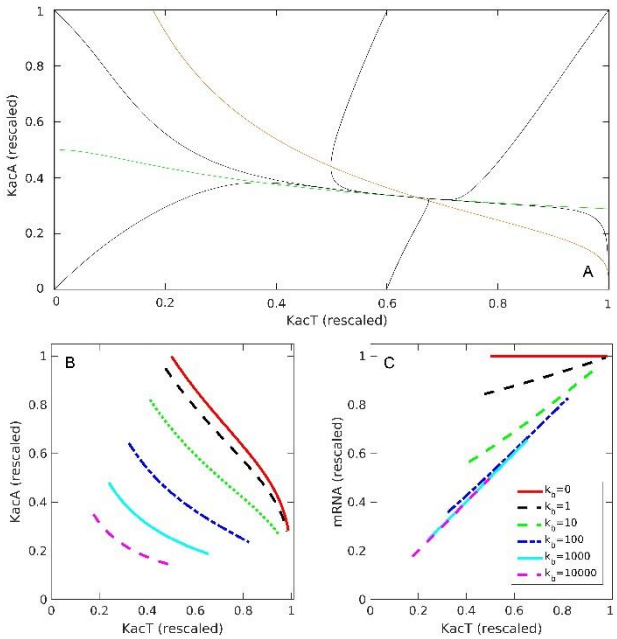

Fig. 1. Model of kacAT expression dynamics. (A) Phase plane analysis. (B) Equilibrium values of proteins KacA *vs* KacT. (C) Transcript kacAT mRNA *vs* KacT. Figure adapted from [1]

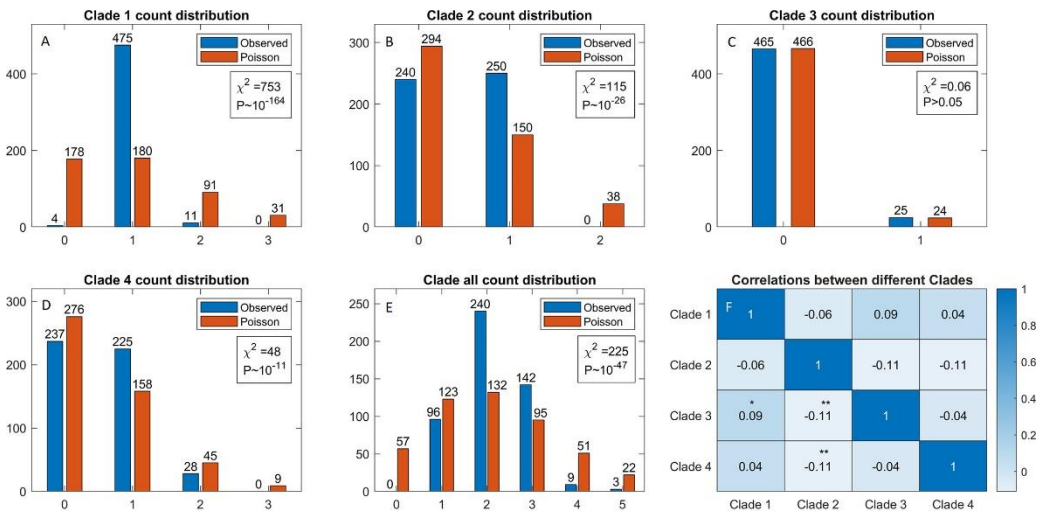

Fig. 2. Statistical analysis of GNAT-RHH TA pairs in *K. pneumoniae* strains. (A–D) Observed *vs* the Poisson distribution counts for clades 1–4 and (E) for all classes together. (F) Spearman's correlations between the counts of different GNAT-RHH clades in *K. pneumoniae* strains. P values of 0.05 and 0.1 are denoted by ** and *, respectively. Figure adapted from [2]

Conclusion: Consequently, based on our quantitative model we obtain that kacAT regulation results in monostable dynamics, and are able to explain all experimental data Moreover, we obtain that kacAT deletion does not lead to spontaneous persister formation, despite previous theoretical predictions, but in accordance with recent experimental evidence. This and our bioinformatics/statistical study challenges the assumption of cooperativity in TA action, possibly explaining the absence of spontaneous persister generation in kacAT. However, TA systems could be involved in antibiotic-induced persister formation, which provides an important future research direction.

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