Dynamics of antibiotic stress response by a Type II toxin-antitoxin system

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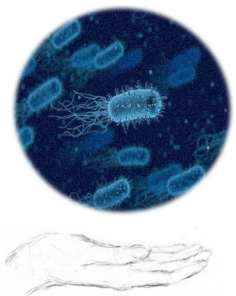

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Outline

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Regulation of Type II TA systems

- Toxin ("T") and antitoxin ("A") are expressed together.
- T generally inhibits translation and leads to cell growth arrest.
- T forms complex with A which prevents T toxicity.
- Complex formation is cooperative.
- Complex of A with T binds to their promoter.
- There is non-linear negative feedback.
- Details may vary for different TA systems.

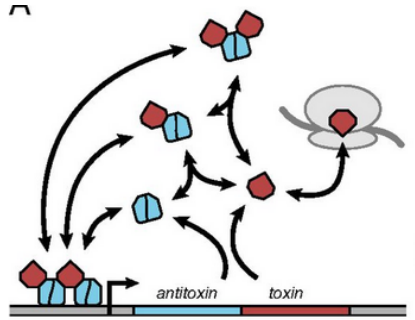

Figure: PMID 23781105: Regulation and complex formation in Type II TA

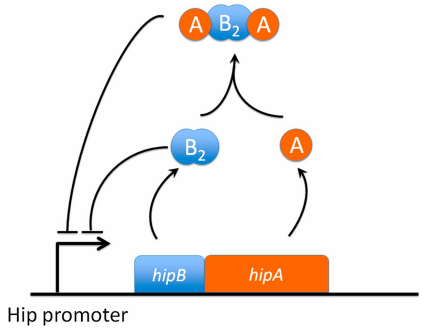

Figure: PMID 24344277: A variation

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Positive feedback due to toxin inhibition of cell growth

- T inhibits cell growth. Slower cell growth leads to lower protein dilution. This leads to smaller effective T degradation.
- It is assumed that several TA systems in the genome may act cooperatively! Therefore, in addition to positive, there may also be a nonlinear positive feedback in the system.

Bistability may emerge!

High toxin state may correspond to persisters.

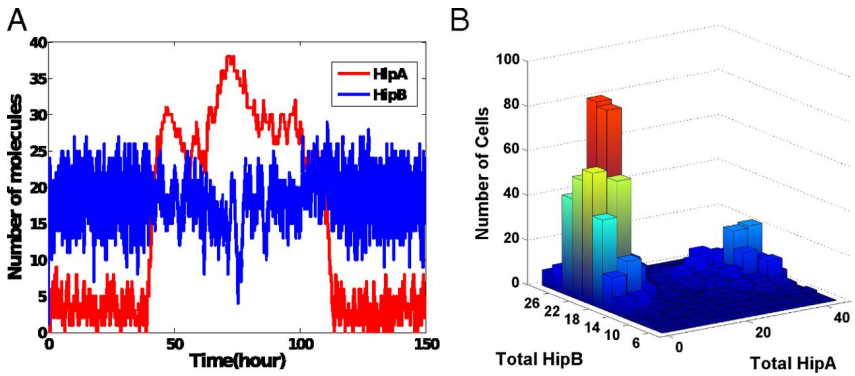

Figure: PMID 24344277: Modelling predicts spontaneous persister formation

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kacAT configuration and experimental observations

kacAT properties

- kacAT architecture generally similar to other Type II systems
- Large binding cooperativity where complex is formed by 2T and 4A
- Strong system response to stress
- However, no induction of spontaneous persistance!
- Antitoxin is degraded in response to stress

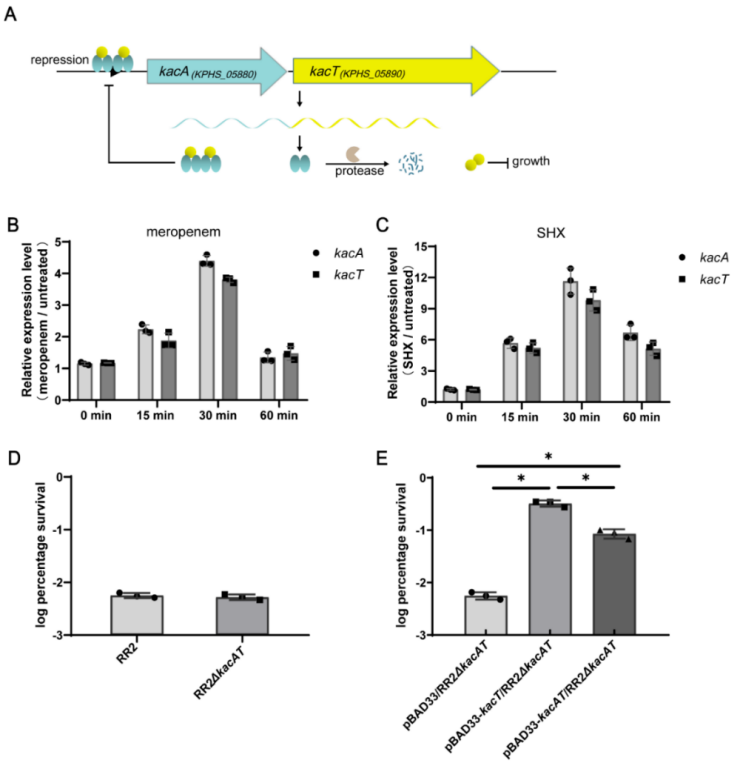

Figure: kacAT regulation and experimental results [1]

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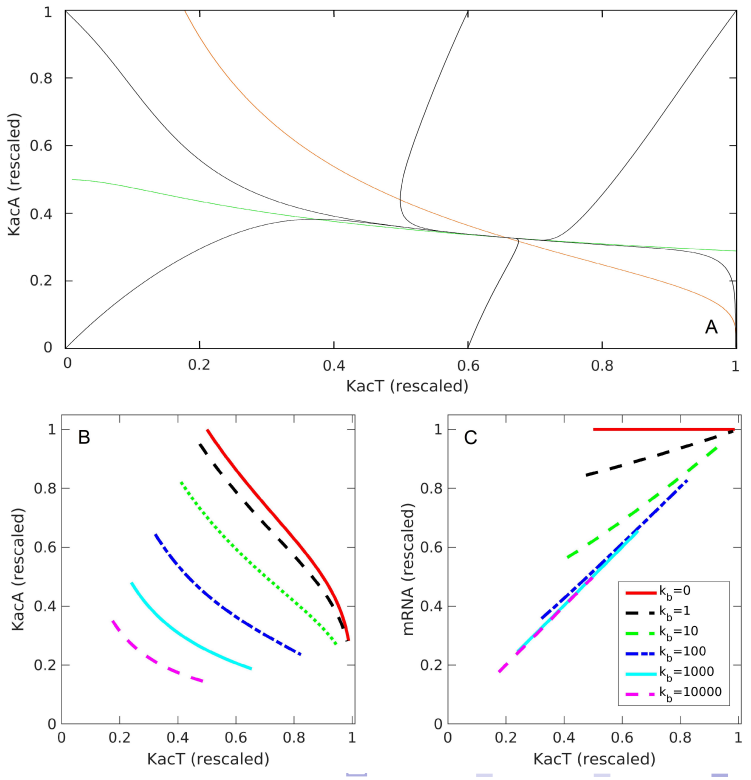
Mathematical model of the system dynamics [1]:

$$\frac{dA}{d\tau} = \frac{2k\phi}{1+K_bA^4B^2} - (1+\delta\lambda)A - 4A^4B^2$$

$$\frac{dB}{d\tau} = \frac{k\phi}{1+K_bA^4B^2} - B - 2A^4B^2$$

Model explains:

- Reduction of the [KacA]:[KacT] ratio upon antibiotic application.
- Large increase in kacAT transcripts induced by antibiotics.
- KacAT overexpression induces antibiotic stress tolerance, whereas kacAT deletion does not affect this tolerance.



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Persister emergence model assumption

- Why kacAT deletion does not lead to spontaneous persister formation despite previous theoretical predictions?
- Theoretical studies assume cooperative (joint) action of several TAs.

Only one system in the same family as kacAT strongly prefered!

Also, statistically significant negative correlations between different clades for which experiments indicate their cross-talk [2]

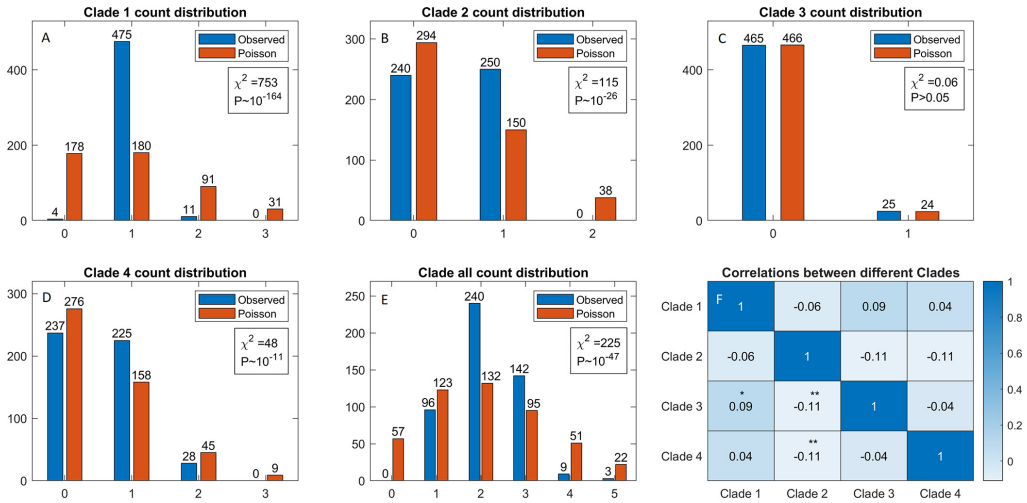

Figure: Statistical analysis of GNAT-RHH TA pairs in *K. pneumoniae* strains.

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Conclusions

Developed mechanistic model of kacAT system dynamics

- kacAT regulation leads to monostable dynamics.
- Can explain all experimental results.
- Explains that kacAT delation does not affect cell growth.

Predicted and analyzed all GNAT-RHH TA pairs in *K. pneumoniae*

- Multiple loci instances (or absence of loci) are strongly disfavoured.
- Crosstalk between loci also appears disfavoured.
- This may contribute to the observed absence of spontaneous persister formation in kacAT.

Outlook: Can kacAT lead to antibiotic induced persister formation?

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References

[1] Peifei Li, Ying-Xian Goh, Bojana Ilic, Cui Tai, Zixin Deng, Zhaoyan Chen, Marko Djordjevic, and Hong-Yu Ou. Antibiotic-induced degradation of antitoxin enhances the transcription of acetyltransferase-type toxin-antitoxin operon. *The Journal of Antimicrobial Chemotherapy*, 78(4).

[2] Ying-Xian Goh, Peifei Li, Meng Wang, Marko Djordjevic, Cui Tai, Hui Wang, Zixin Deng, Zhaoyan Chen, and Hong-Yu Ou. Comparative Analysis of Diverse Acetyltransferase-Type Toxin-Antitoxin Loci in Klebsiella pneumoniae. *Microbiology Spectrum*, 10(4).

Acknowledgments

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