Mechanisms of antifungal resistance revealed by quantitative growth curve analysis

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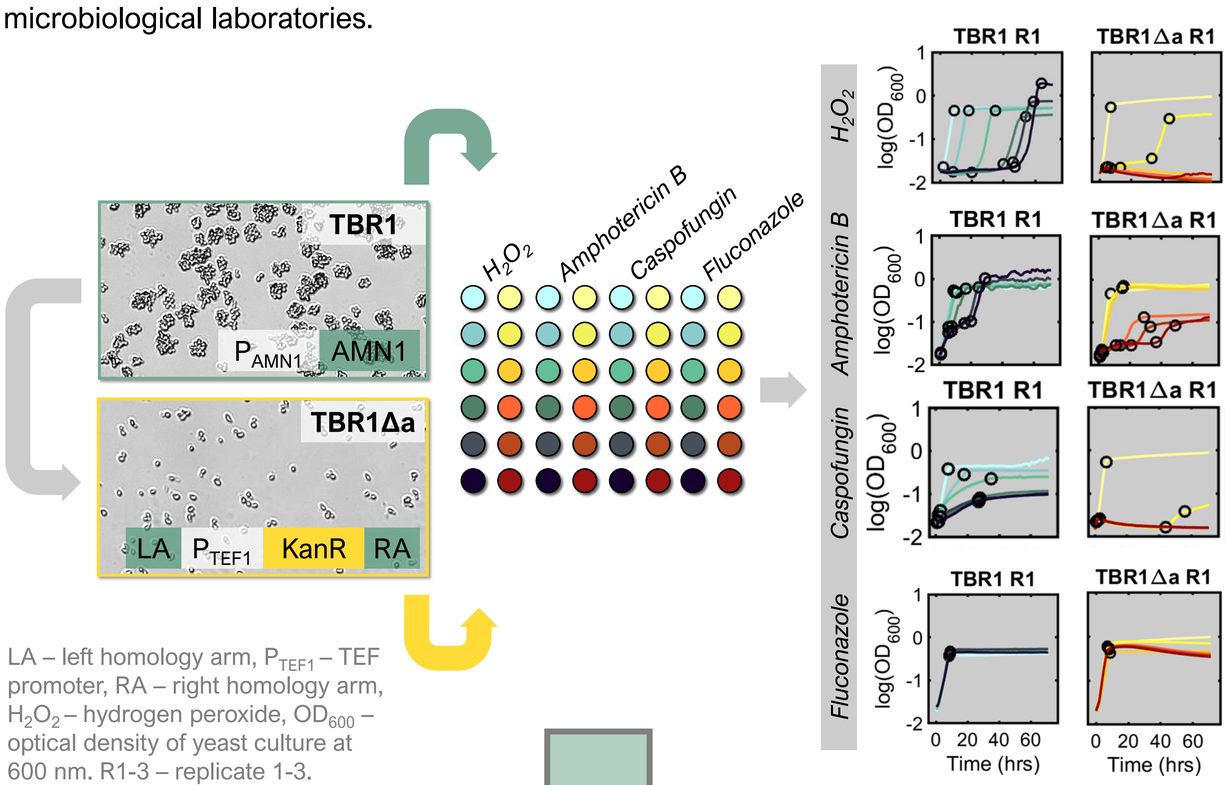
ABSTRACT

Drug resistance is the most clinically relevant adaptive *response* of infectious agents to antimicrobial treatment. How microbial pathogens respond to antibiotics dictates the drug selection, dosage, timing, and combination. These decisions, often made in clinic, are far from being fully algorithmized. The complexity of this decision-making increases when testing a *novel drug formulation* or managing an *emerging pathogen*. Here, we develop the algorithms of drug response analysis and interpretation of the potential mechanisms that shaped the observed response. By testing our methodology on two yeast morphologies (multicellular and unicellular *S. cerevisiae* TBR1[1], different by a single gene – *AMN1*[2]) exposed to H_2O_2 and three antifungal drugs, we demonstrate how such molecular mechanisms as drug influx, metabolism, and toxicity-induced cellular response could contribute to the shape of growth kinetic curve – time-course data widely accessible to microbiological laboratories.



LA – left homology arm, P_{TEF1} – TEF promoter, RA – right homology arm, H_2O_2 – hydrogen peroxide, OD_{600} – optical density of yeast culture at 600 nm. R1-3 – replicate 1-3.

CONCLUSIONS

1. *AMN1* deletion causes the transition to unicellularity in clumping yeast phenotypes (such as S. cerevisiae TBR1).

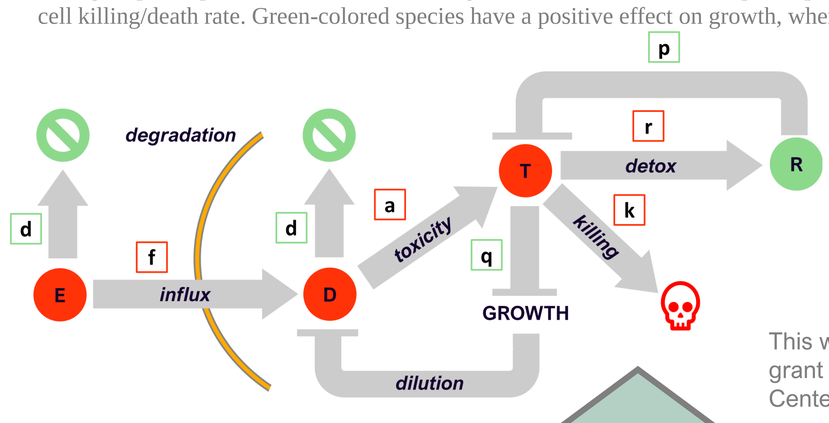
2. H_2O_2, amphotericin B, caspofungin, and fluconazole evoke mechanistically different, yet interpretable responses in yeast.

3. Experimental models of single yeast cells and clumps facilitate estimations of drug penetration and Its effects on cells and the effect of multicellularity on drug resistance.

4. Analyzing and comparing drug-affected growth phase parameters helps narrow down on the mechanisms driving the molecular responses in each phase.

5. Computational deterministic modeling of kinetic growth curves helps *categorize* (sensitivity, tolerance, and resistance) and *mechanistically describe*(drug efflux, metabolic change,) experimentally observed drug response.

Schematic illustration of modeled the variables (molecular species): E (external drug), D (internal drug), T (toxicity), R (detox machinery) and rates: q – the drug threshold to inhibit the growth, f – drug influx rate, a – drug-induced cell toxicity, p – drug response production rate, r – the drug threshold to induce the response production, d – spontaneous drug decay, k – cell killing/death rate. Green-colored species have a positive effect on growth, whereas the red-colored ones suppress growth.



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1. Smukalla et al. Cell 2008, 135 (4), 726-737.
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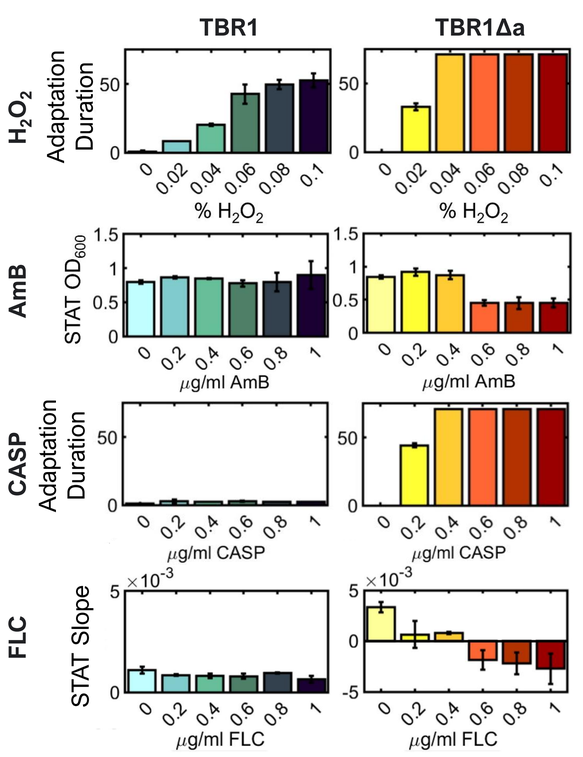
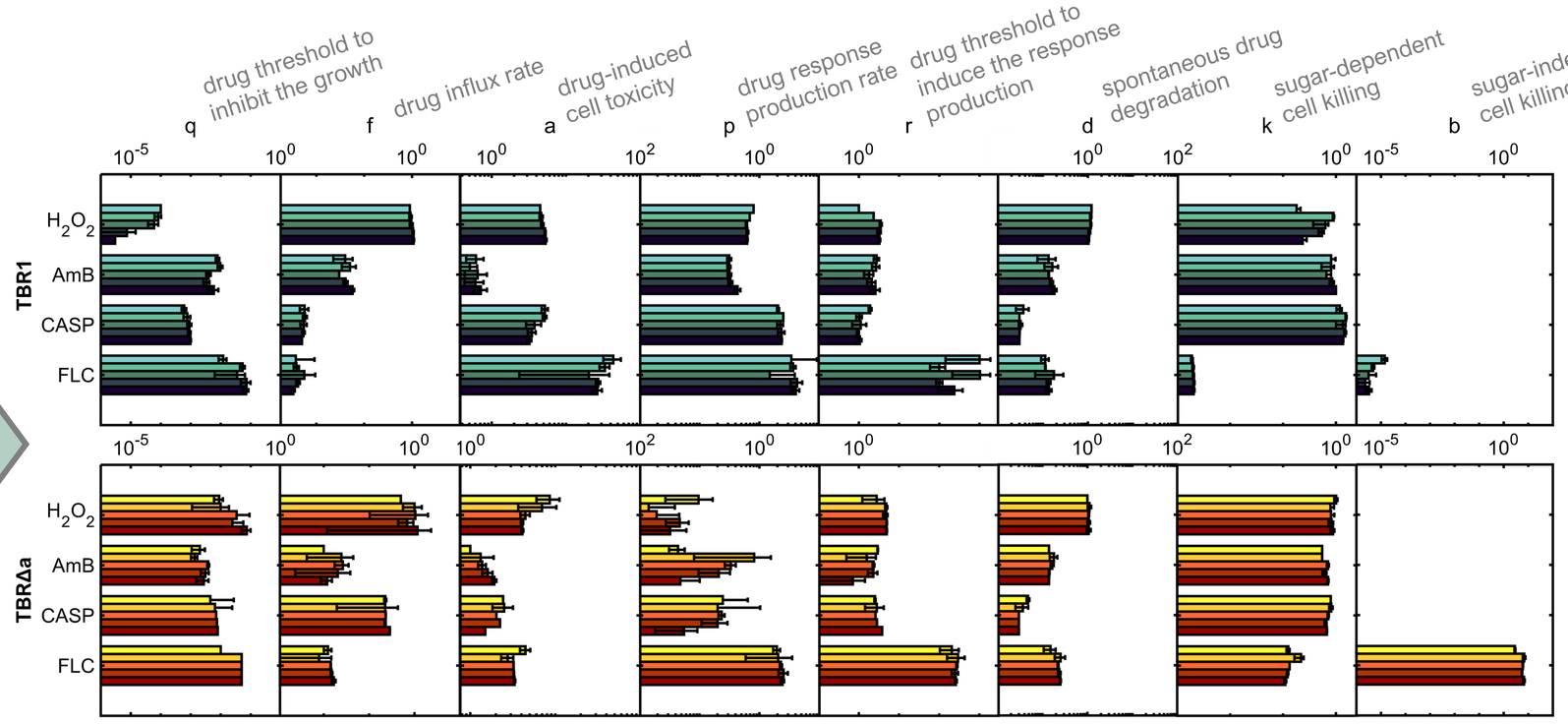
RESULTS

1. Growth curves, broken down into growth phases by our algorithm, showed that every drug produced a *phase-dependent* dynamic response. Hence, H_2O_2 increased the duration of the adaptation phase, amphotericin B created additional (“pregrowth” and “regrowth”) phases and lowered the carrying capacity, caspofungin reduced growth rates at all phases, and fluconazole produced a negatively sloping response at stationary phase.



Legends. H_2O_2 – hydrogen peroxide, AmB – amphotericin B, CASP – caspofungin, FLC – fluconazole.

3. Finally, we applied the parameter values and actualized them to simulate growth curves at each condition. Comparing the parameter values between strains and conditions allowed us to understand the mechanisms of action of each drug on each strain, and subsequently narrow down on the potential causes of drug resistance in the multicellular and/or *AMN1* expressing yeast strains.



2. We next designed a simple model simulating the logistic growth curves and applied least-square non-linear fitting to the experimental curves to obtain numerical values for the parameters in the model. Certain model assumptions aligned with our understanding of the drug action and cellular response and helped us form comprehensive hypotheses of the mechanisms yet undescribed.

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