Sources of Non-genetic Variability and Heritability in Pheromone Signaling Observed on a Microfluidic Device

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***Short Abstract* — Single cell tracking, fluorescent measurements, and dynamic environmental control produce experimental data highly suitable for systems level analysis of both natural and synthetic biological networks. Here we will describe a microfluidic device capable of high-throughput single cell fluorescent data acquisition and fully automated lineage tracking on the budding Yeast. We provide evidence for non-genetic heritability of signaling capacity in Yeast and highlight the role cell cycle and pheromone pathway components play in modulating the degree of this heritability. Finally a simple model will be used with lineage data to evaluate the contribution of this heritability to the overall heterogeneity observed at the population level. Our results directly address recent theoretical explorations into the potential role of division processes in giving rise to experimentally observed heterogeneity.**

***Keywords* — microfluidics, fluorescent microscopy, lineage tracking, single cell analysis, MAPK signaling, Yeast, non-genetic heterogeneity, noise.**

I. EXPERIMENTAL SETUP

MICROFLUIDIC devices allow for a simultaneous control of cellular environment and high-resolution, single cell measurement of expression [1-3]. We have made a custom 2-layer device [3] that allows for the acquisition of fluorescent data from single cells in 128 chambers for over 10 hours at time intervals that are less than a tenth of a cell cycle. The device allows for dynamic control of the chamber environment allowing for 16 different conditions to be tested simultaneously. Furthermore, 8 different strains can be concurrently assayed in a single experimental run. Each experimental run can therefore provide single cell measurements on fluorescent reporters, cell size, and period. Furthermore by implementing a technique introduced in [4] and an in-house image analysis pipeline we can extract lineage information for thousands of single cells in a fully automated fashion using this device. A fluorescent reporter (mCherry) was used as a read-out of the pheromone signaling pathway and was measured for every cell at every time point in the experiment.

II. RESULTS

Using the expression data in conjunction with the lineage information we calculated correlations between the responses of related cells to quantify the heritability of the capacity to respond to pheromone in 8 different strains. All strains showed significant correlations compared to randomly picked cells indicating presence of a non-genetic inheritance of capacity in pheromone signaling. Interestingly different knockout strains in the pathway elicited different degrees of inheritance; in particular, *fus3* and *ste50* showed extreme and opposite phenotypes in relation to WT. We provide evidence that this variability in inheritance is related to regulatory interactions between pheromone components and the cell cycle that couple the two networks to each other. Furthermore we quantify the drop in response correlation as a function of genealogical distance between cell pairs. Through comparison of this decay rate with the predictions from a basic model we address issues surrounding the source of heterogeneity in clonal populations. We discuss the implications of these measurements for recent suggestions that division noise – and not stochasticity in expression - is what accounts for non-genetic heterogeneity [5].

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