1. Introduction

Principal Research Scientist
Science and Technology Associate

In FY2025, I was engaged in finalizing and publishing two manuscripts.

2. Activities and Findings

2.1 Cryptic multicellularity brings selective advantages under stress

Multicellularity has evolved dozens of times across the Tree of Life, each time producing a new kind of individual open to division of labor among its constituent cells. Often, nascent multicellularity remains facultative, manifesting only under conditions that confer a fitness advantage. Here, we investigate the mechanism and selective advantages of cryptic multicellularity in a wild strain of *Saccharomyces cerevisiae*, diploid champagne yeast DB146, which is heterozygous for a nonsense allele at the mating-type locus (Fig 1). Meiotic purification of this trait revealed that DB146 is homozygous for an *AMN1* allele that dysregulates post-mitotic cell separation (Fig 2). Expression of cryptic multicellularity in haploid DB146 derivatives leads to the formation of clonal, multicellular clusters. Systematic analysis of viability in haploid and diploid DB146 derivatives, both unicellular and multicellular, subjected to starvation, desiccation, and low temperature, reveals that multicellular individuals exhibit higher survivorship than their unicellular counterparts under conditions reminiscent of overwintering (Fig 3). Examining other wild strains, we find that ~20% of them exhibit similar ploidy-dependent expression of clonal multicellularity. Altogether, our data suggest that in yeast that sporulate when starved, cryptic multicellularity may be subject to balancing selection, as haploid multicellular progeny enjoy a transient survival advantage that fades once favorable growth conditions resume.

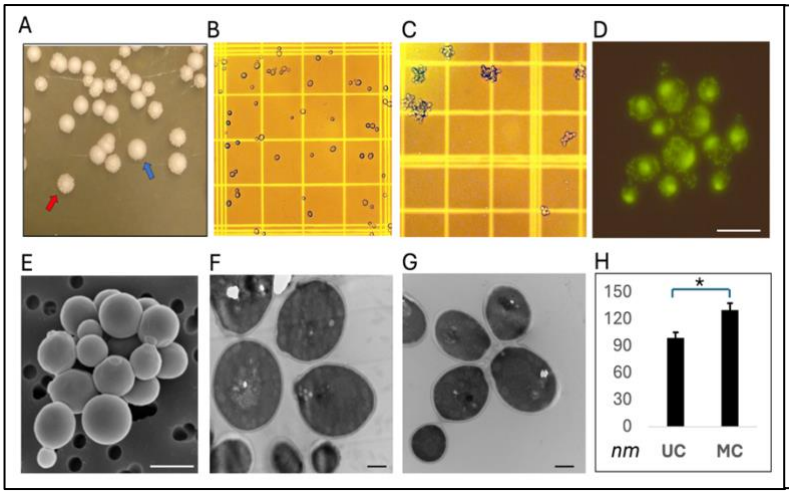

Figure 1. Multicellular phenotype in 42.6, a meiotic derivative of DB146. **(A):** Plating of a mixture of multicellular cultures. Unicellular culture (UC) forms smooth colonies (blue arrow), Multicellular culture (MC) forms colonies with a wrinkled surface (red arrow). (**B):** Single cells in a UC culture (C): Clustering in an MC culture (**D):** A cell cluster with its DNA stained with CyberGreen. The bar is 5µ. (**E):** A cell cluster viewed by SEM. The bar is 5µ. (**E**, **F**): UC (E) and MC (F) viewed by TEM. The bar is 1µ. **(G):** Mean cell wall thickness in a UC and an MC. (**H**) Comparison of cell wall thickness between (UC) and MC). The asterisk denotes significance.

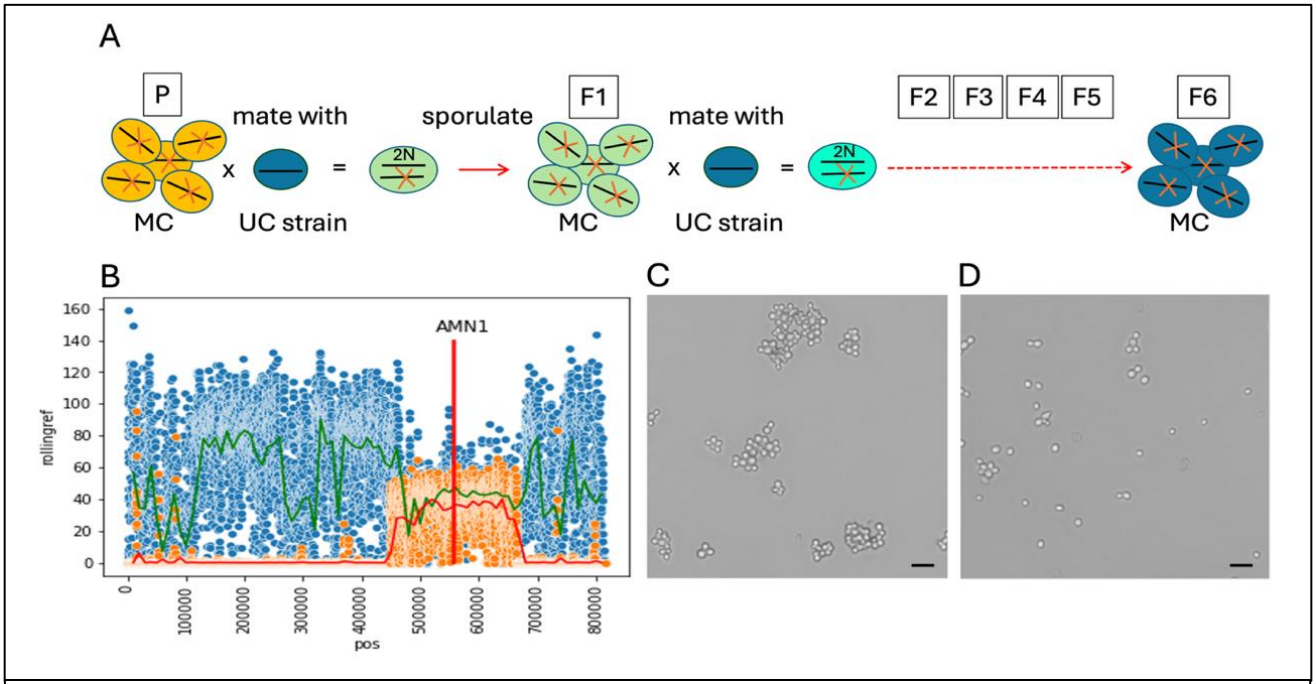

Figure 2. **Finding the genetic determinant of multicellularity in haploid derivatives of DB146.** (**A**): Schematic of meiotic purification to isolate the genomic region carrying the determinant for the multicellular phenotype. P is an MC derivative; F1-F6 are products of successive backcrosses with unicellular lab strain BY4742. (**B**): The introgressed region on Chromosome II in the F6 progeny carries the locus for *AMN1*. (**C**)**:** Photomicrograph of an MC isolate *before* and (**D**)**:** *after* the native *AMN1*^{DB146} was replaced with the orthologous sequence from a laboratory strain BY4742. Scale bar length = 20µm.

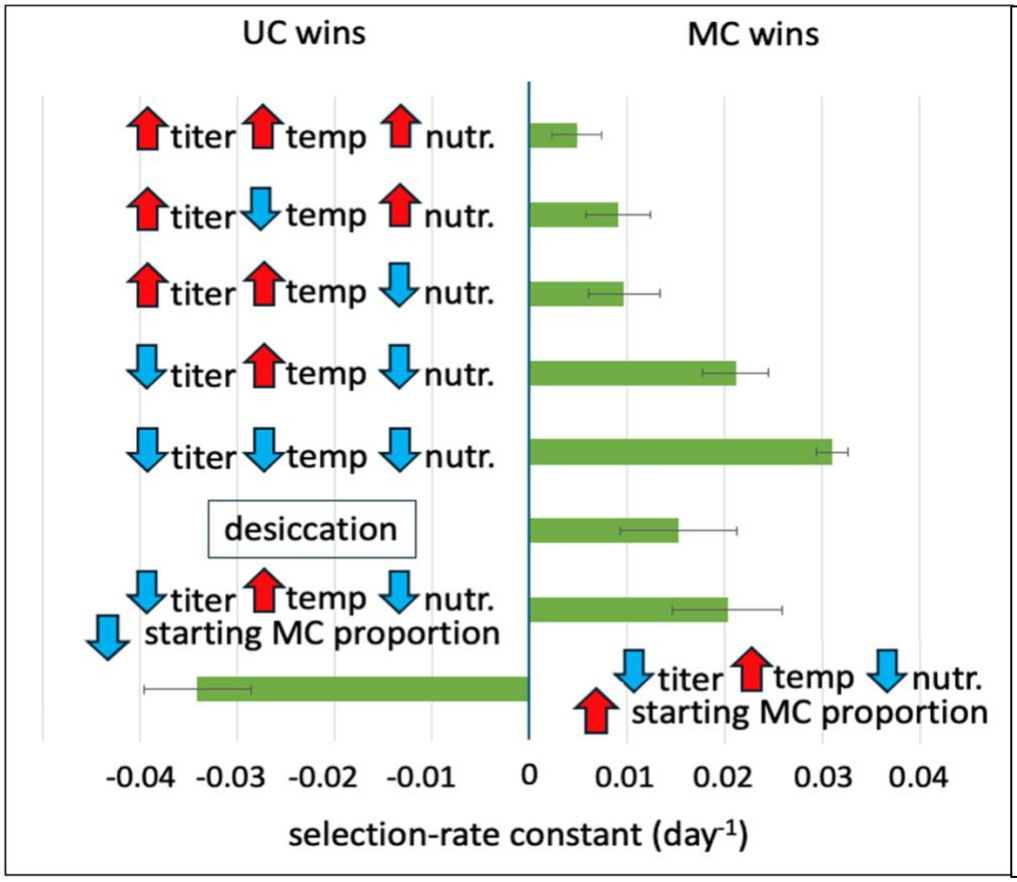

Figure 3. MC isolates have a survival advantage under adverse conditions. Bars represent mean selection-rate constants (1/day) for cultures in which MC were competed head-to-head against UC. Error bars are SEM of selection rates obtained between 0 to 15, 15 to 30 and 30 to 45-day periods. **Red** and **blue** arrows, respectively, denote **high** and **low** values for initial cell density, temperature, or nutrient level (see text for details). Except where noted, MC and UC cultures were mixed in 1:1 proportion. Low and high starting proportions were 1:10 or 10:1 MC:UC, respectively.

2.2 Novel methods of treating tumors based on their metabolism (review/perspectives)

Many aggressively growing tumors rely heavily on rapid glycolysis to produce energy and generate anabolic intermediates needed for growth. This metabolic rigidity in glycolytic tumors, readily detectable by FDG-PET scans, constitutes a targetable vulnerability that can be addressed by several approaches, effectively reducing glucose availability to tumors. For example, a combination of ketogenic diets that restrict dietary carbohydrates and metformin, which suppresses endogenous glucose production via gluconeogenesis, achieves a sustained systemic glucose deficit resulting in significantly slower tumor growth and extended overall survival in preclinical breast cancer models. Furthermore, epidemiological data from diabetic cohorts corroborate these findings, as a combination of metformin with carbohydrate management shows markedly reduced cancer incidence. To prevent glycolytic tumors from escaping glucose restriction through compensatory glutaminolysis, autophagy and neoangiogenesis, the glucose-lowering regimen can be combined with the inhibitors of these pathways. Lastly, normoxic tumor microenvironments that are chemotherapy- and immunotherapy-responsive can be targeted concurrently, creating a spatially complementary, organism-centric mutimodal treatment strategy.

3. Collaborations

Prof. Bill Holben, University of Montana
Prof. Frank Rosenzweig, Georgia Institute of Technology
Prof. Leonid Kalachev, University of Montana
Dr.Roland Degenkolbe, Umirai
Prof. Dorothy Sears, Arizona State University
Prof. Lesley Ellis, University of California at San Diego
Eli Lyons, TupacBio

4. Mentoring

A member of the graduate review committee: successful oral defense for Dr. Taehwan Yung, Georgia Institute of Technology.

5. Publications

1. *Novel methods of treating tumors based on their metabolism*, Dorothy Sears and Eugene Kroll. Cancers, *in preparation.*
2. *Cryptic multicellularity in wild yeast brings selective advantages under stress*. WK Sexton, K Schmidt, Q Dickinson, J Childress, F Rosenzweig, and E Kroll. Proceedings of the Royal Society B. *in revision.*