Science and Technology Group
Annual Report FY2022

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Introduction, Activities and Findings

1. Genome restructuring in yeast *Saccharomyces cerevisiae*

About 80% of all cancer genomes are restructured – the genomes evolve fast by recombination, deletion and expansion. Genomic restructuring allows cancer cells to obtain new traits, speed their growth, and evade conventional therapies. However, the molecular and biochemical mechanisms of genome restructuring are still unknown, primarily due to the technical difficulties of studying cancer cells directly. Similarly, yeast cells restructure their genomes under stress, which we discovered in a model deleoped in my lab. Because of multiple similarities between yeast and cancer cells, the molecular mechanisms controlling genome restructuring may also be similar. In this project, we work on discovering the molecular mechanisms of genome restructuring by using yeast *Saccharomyces cerevisiae* as model. To discover rearrangements we are using GenomeMiner, a bioinformatics platform designed to analyze genomic data obtained via long-molecule sequencing on Oxford Nanopore and PacBio systems. In particular, we work on (1) detecting multiple genomic restructuring events in single cells; (2) uncovering genetic regulation of genomic restructuring. (3) using entrapped yeast cells as a model for somatic cells to link molecular mechanisms of genome restructuring between yeast and humans. Understanding genomic restructuring in yeast will allow us to find genes that control genomic restructuring in cancer cells, which can be new cancer drug targets (Fig. 1).

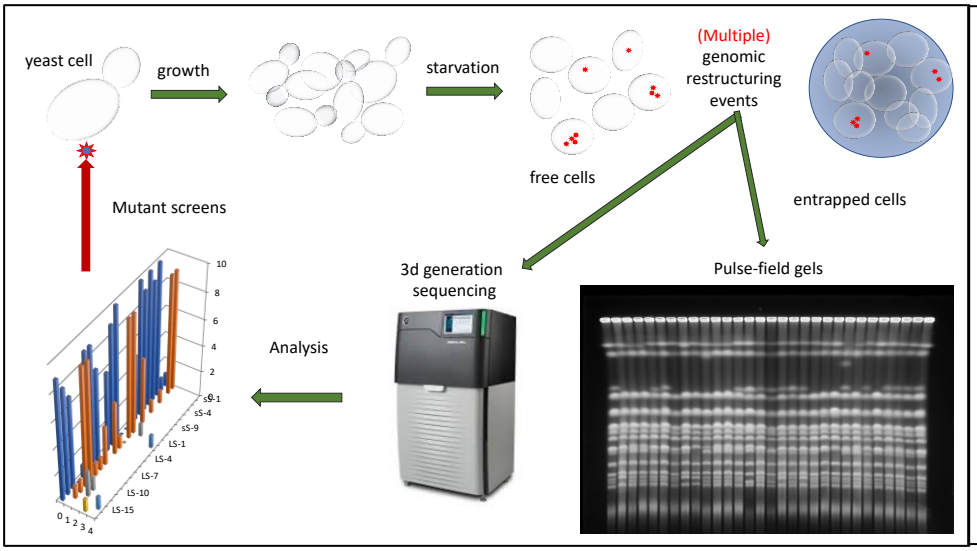

Figure 1. Main flow of genome restructuring analysis. After growth and subsequent starvation/stress, some (rare) cells will develop multiple genomic restructuring events (multiple dots that mark DNA double strand breaks in same cell/red (grey) arrows). These cells will be isolated using screening, analyzed by sequencing and pulsed-field gels to determine the molecular mechanisms of genomic rearrangements.

2. Entrapped microbial cell factories for prodiuction of valuable biologics

Over the past 20 years, bioengineering has made significant advancements in making new microorganisms for various bioproducts, including fuel, pharmaceuticals, textiles, and food ingredients. Approximately 60% of physical inputs to the global economy could be produced biologically, with an estimated annual global impact of $2-4 trillion by 2040. Biotechnology is capable of employing sustainable and environmentally friendly production methods that are increasingly demanded by consumers. Although fermentation-based bioprocesses have the potential to deliver a sustainable supply of products for various industries, there are significant challenges of low yields and a high genetic instability of engineered microorganisms. These challenges limit the efficiency of microbial cell factories, leading to high costs and the lack of competitiveness on the market. To address these challenges we developed a platform that employs cell entrapment in specialized matrices which prevent cell division and protects cells from physical damage. Entrapment stabilizes their genomes because the cell seize cell division, enhances secondary metabolism, increases yields and rates of production, and removes the need for batch processes, thereby decreasing overall production costs. In our Proof-of-concept project we developed a platform for the high-yield bioproduction at a lab scale of an antioxidant dye astaxanthin, as well as a range of biopeptides (an umami taste heptapeptide, oxytocin, hemopressin and two enkephalins) and a microbial natural antibiotic bikaverin in production hosts *Pichia stipitis* and *Saccharomyces cerevisiae*. We showed that several of these high-value biologics are produced from 4-fold to 10-fold higher in entrapped cells compared to free cell production (Fig. 2). The next stage will be to scale up bioproduction to the 5L fermenter scale.

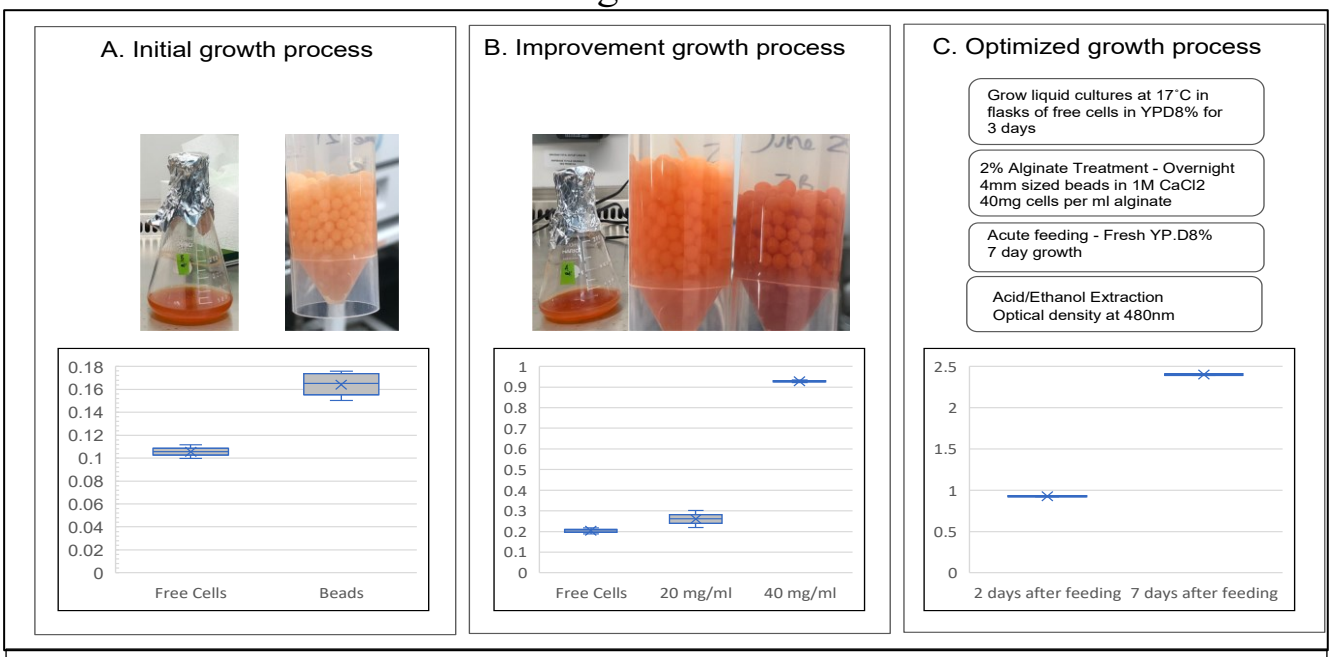

Figure 2. Comparison of the initial growth process (A), improved process (B) and the final process. (C). Please note that the graphs are not to same scale.

3. Combination metabolic therapy for aggressive tumors

The current front-line therapies of surgery, radiation, and chemotherapy have limited success in advanced cancer treatment due to genomic redundancy and cellular heterogeneity of advanced tumors. However, leveraging 100 years of research on cancer biology and the latest tools in molecular analysis, researchers have developed a radically new approach to treating advanced cancer. Instead of targeting cancer genes, they are targeting cancer metabolism, one of the hallmarks of cancer. Aggressively growing tumors often experience intratumoral hypoxia due to insufficient blood supply. Tumor cells adapt to hypoxia by reprogramming their metabolism to rely on glucose fermentation as the primary energetic pathway and drastically increase their glucose intake. Researchers aim to exploit this dependency of hypoxic tumor cells on glucose by developing an evidence-based protocol to limit the total glucose available to a tumor. They aim to starve cancer to slow its growth and integrate multiomic analysis for precision targeting. To investigate whether glucose limitation arrests the proliferation of hypoxic, glycolytic tumors, the researchers propose to test the efficacy of a specialized, high-fat, low-carbohydrate diet, in combination with the glucose-lowering drug metformin, on the growth and metastatic outcome of aggressively growing tumors in an established murine model of triple-negative breast cancer (TNBC). They also aim to address the heterogeneity of cancer by introducing a “multiomics” approach to analyze cancer biomarkers in blood and tissue samples to identify those that positively and negatively associate with therapeutic success. If successful, this combination treatment can be adapted to other solid tumors, using a similar multiomics approach and eventually translated to clinical trials. Our results so far support the expectation that hypoxic tumor tissues are susceptible to even mild glucose limitation in breast cancer tissue culture and in two murine models of breast cancer. First, this study confirmed that cancer cells rely on an abnormally high glucose level to survive in a hypoxic environment, supporting the notion that hypoxic tissues must drastically increase their glucose intake to survive. Second, after limiting the total glucose with the combination of a low carbohydrate (ketogenic) diet and Metformin in a triple-negative breast cancer model, the breast tumor burden was half of that in control groups. Third, the mean latency of tumors in the KM group increased by 36% compared to the mean latency of all other groups, resulting in an additional 31 days of overall survival in the KM group compared to other groups. This can be equated to more than three additional human years of life. This is a significant increase in the TNBC overall survival (15 months post-detection).

Collaborations

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