GENOME-WIDE ANALYSIS OF ELECTRICAL PHENOTYPE USING ISODIELECTRIC SEPARATION

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ABSTRACT

We present the first genome-wide analysis of electrical phenotype, focusing on the budding yeast *Saccharomyces cerevisiae*. Using isodielectric separation (IDS), a microfluidic platform recently developed by our lab, we have screened the haploid yeast knock-out collection to identify genes whose deletion confers an increase or decrease in effective conductivity relative to wildtype cells. Pathway analysis and subsequent characterization of these deletion strains suggest biological processes and genetic pathways associated with distinct electrical phenotype. These results demonstrate the feasibility of IDS as a platform for genetic screens and establish a new class of phenotypic analysis - electrogenomic profiling.

KEYWORDS: Cell Separation, Electrical Properties, Genetic Screen, Dielectrophoresis

INTRODUCTION

Over the past several decades, electrical cell separation methods have had a number of successes, sorting cells according to biologically relevant metrics including differentiation [1] and oncogenesis [2]. However, despite their achievements, these methods remain a niche application. This is largely due to a poor understanding of how a cell's genotype translates to an electrical phenotype. As a consequence, even the most successful applications of electrical separation methods have been *ad hoc*, based upon little or no understanding of the underlying biological mechanism through which differences in the cells' electrical properties arise. The development of more systematic, hypothesis-driven electrical separations requires a comprehensive mapping of genotype to electrical phenotype, as well as technologies capable of delivering this depth of characterization. Here we present a microfluidic platform capable of high-throughput mapping of genotype to electrical phenotype, and the results of a genome-wide screen using this platform to understand the genetic basis for the intrinsic electrical properties of cells.

THEORY

In working towards a comprehensive mapping of genotype to electrical phenotype, we have recently developed a novel microfluidic screening platform, called isodielectric separation (IDS; Figure 1) [3,4]. IDS separates cells using dielectrophoresis in a liquid with spatially varying electrical conductivity; cells within the device are driven to the point where their polarization charge vanishes and they are in dielectrophoretic equilibrium. The device consists of a microfluidic channel across which the electrical conductivity of the liquid varies, as well as electrodes used to deflect cells to their equilibrium positions. Since cells with different electrical properties converge to different equilibrium positions, they are spatially resolved in a continuous manner, independent of cell size, thus providing high throughput and specificity [4]. This allows us to sort and characterize millions of genetically distinct cells in parallel, generating the first "electrogenomic" profile.

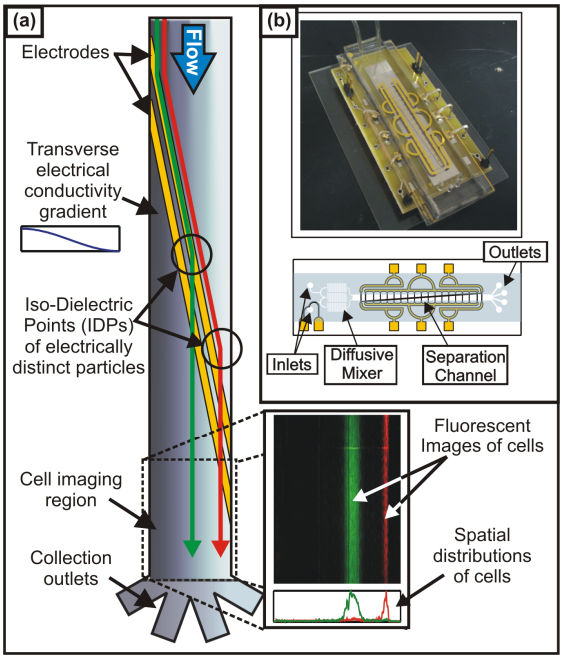

Figure 1: Overview of the IDS device. (a) Cells are carried into a separation channel, across which the electrical conductivity decreases. Electrodes across the diagonal of this channel deflect cells using dielectrophoresis to equilibrium positions (IDPs), where they pass through the electrodes and flow downstream for collection or imaging. (b) Photograph of the device along with a schematic.

EXPERIMENTAL

Figure 2 summarizes our screening methodology. We have chosen to focus on the budding yeast *Saccharomyces cerevisiae* due to the continued importance of yeast as a model of eukaryotic biology, as well as for the wide variety of genetic libraries available in *S. cerevisiae*. These include the haploid yeast deletion library [5], a collection of ~5000 yeast strains, each lacking a single gene not essential for growth and identifiable by a molecular barcode. We construct a pool of the deletion library, followed by the parallel analysis of the ~5000 strains in parallel. To achieve this, we sort the pooled deletion strains in the IDS device, fractionating cells into different outlets according to their effective conductivities. By collecting cells from these outlets, extracting and amplifying the DNA barcodes encoding the genetic identity of each cell and then sequencing these barcodes, we are able to quantify the abun-

dance of each strain across the four outlets. Normalizing the strain distributions in each outlet to that from the original unsorted pool reveals genes whose deletions increases or decreases the effective conductivity of a cell under the conditions of the screen (i.e. conductivity range and electric field frequency). For our initial profile, we have screened cells under conditions sensitive to the characteristics of the cell membrane and cell wall, using a conductivity gradient ranging from 0.075 S/m to 0.015 S/m, and an electric field frequency of 300 kHz. To assure robust sampling of all strains in each of the sorted fractions, we collect ~10^7 cells in total (>10^6 in each outlet) by operating continuously for ~3 hours at a throughput of ~5×10^4 cells/min.

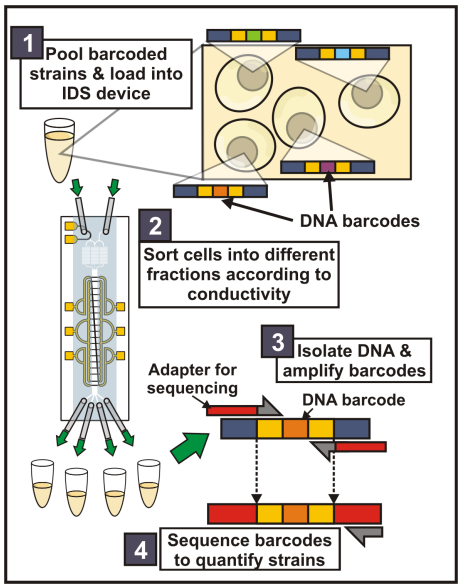

Figure 2: Screening methodology. Starting from a vial of cells containing each strain in the barcoded deletion collection (1), we use IDS to separate cells into four different fractions corresponding to different electrical conductivities (2). After the separation, we use PCR to amplify the strain barcodes (3), and sequence them to determine the abundance of each strain in the different fractions and original pool.

RESULTS AND DISCUSSION

Deep barcode sequencing [6] allowed us to identify deletion strains that were significantly enriched or depleted under a range of medium conductivities. Figure 3*a* shows the comprehensive deletion strain enrichment across each of the four outlets, spanning a range in conductivities from 0.075 to 0.015 S/m. Here, the strains have been arranged in ascending order according to their enrichment at higher medium conductivities. Selecting strains associated with substantial enrichment across the four outlets has identified ~50 genes whose deletion results in a "high conductivity" phenotype (*i.e.* distribution to the *left* of the wildtype strain in Figure 3*b*) and ~15 gene deletions conferring a "low conductivity" phenotype (*i.e.* distribution to the *right* of the wildtype strain in Figure 3*b*).

Pathway analysis on the "high conductivity" and "low conductivity" sets of genes identified in this screen suggests several biological processes and genetic pathways closely associated with distinct electrical phenotype (Figure 4*a*). Statistically significant correlations ($P < 10^{-6}$) among genes associated with the Rim101 pathway (known to play a role in cell wall maintenance [7]), the dynein-dynactin pathway (involved in nuclear transport during mitosis), and the Swr1 complex (involved in transcription regulation through chromatin remodeling) emerge among the high conductivity strains, while the low conductivity strains include several genes associated with the SWI-SNF complex.

In addition to identifying genetic pathways associated with altered electrical phenotype, we have investigated the role of biological processes (through Gene Ontology annotations) and morphology (through previously established datasets [5]) in determining electrical properties. Interestingly, ~43% of the strains significantly enriched at lower conductivities (Figure 4*b*, *left*) bear deletions to genes involved in transcription regulation (representing 9.6% of all genes; $P = 1.4\times10^{-4}$), suggesting that single, loss-of-function mutations may predominantly result in increased effective conductivity, whereas larger-scale changes in gene expression may be necessary to decrease the effective conductivity of a yeast cell under the conditions of our screen. Morphologically (Figure 4*b*, *right*), we find that ~40% of the strains exhibiting distinct electrical phenotypes have been associated with altered morphology, compared with an incidence of ~15% across the entire library. This enrichment of strains with different shapes is generally consistent with changes in dielectric properties predicted for cells more or less elongated than wildtype; however, morphology alone neither fully predicts, nor is fully predicted by, changes in electrical properties.

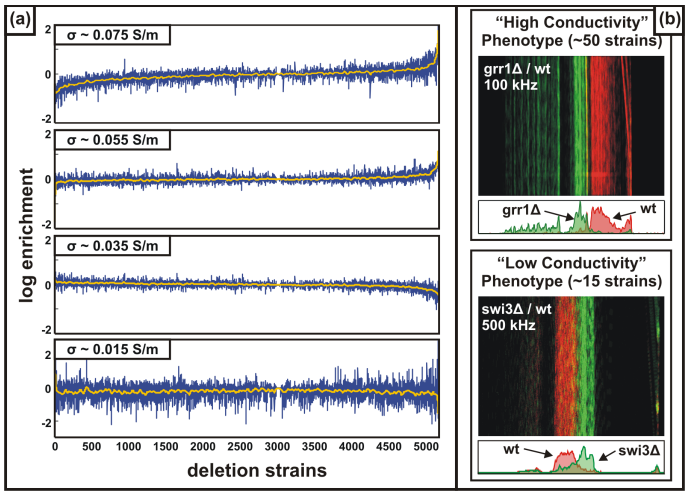

Figure 3: Results from an electrogenomic screen probing cell membrane and wall properties. (a) Plots showing the fold-enrichment across ~5000 yeast strains with individual gene deletions (consensus from two independent separations). Sorting the pooled deletion library at a frequency of 300 kHz into bins spanning a fivefold change in conductivity allows us to identify a number of gene deletions conferring distinct electrical properties. Under the conditions used for these separations, wildtype cells (and strains exhibiting similar electrical phenotype) localize at ~0.035 S/m. (b) Representative distributions for strains identified exhibiting a high conductivity phenotype (grr1Δ) and a low conductivity phenotype (swi3Δ).

To supplement the large scale survey of electrical phenotype, we have performed high-resolution experiments to confirm the phenotypes observed in the pooled screen. Pairwise comparisons between deletion and wildtype strains has yielded a false positive rate of ~10% across 20 strains tested to date, supporting the robustness of the large-scale data. Distributions of deletion strains associated with the Rim101 pathway relative to wildtype (green and red respectively) are shown in the inset of Figure 4*a*. Similarities between these distributions suggests that a universal electrical phenotype may be established from a variety of perturbations to a single genetic pathway.

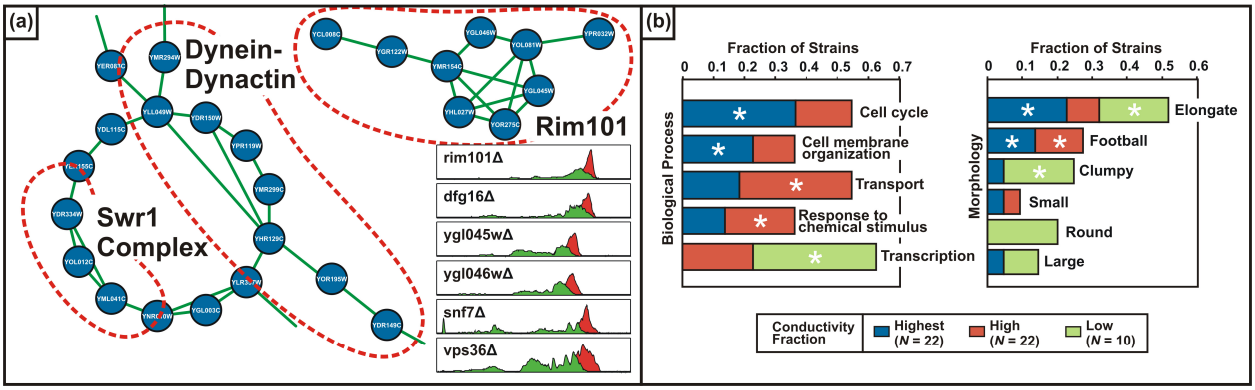

Figure 4: Genetic interactions and common features for deletion strains exhibiting distinct electrical phenotype. (a) Networks of positive genetic interactions (as determined in [8]) among strains with increased effective conductivity suggest the roles of perturbations to the Rim101 pathway, the dynein-dynactin pathway, and the Swr1-complex. The inset shows distributions of deletion strains associated with the Rim101 pathway (green) relative to wildtype (red). (b) Different biological processes (GO annotation, left) and morphologies (right) correlate strongly with different electrical phenotypes. Under the screening conditions we use, we find distinct clustering of biological function with different conductivity fractions (: $P < 0.05$).*

CONCLUSION

This work demonstrates the utility of IDS for performing genome-wide screens of electrical phenotype, and presents results from the first genome-wide analysis of electrical properties in an organism. Collectively, these results establish a new type a genetic screen and a new screening platform, as well as providing a more comprehensive understanding of the genetic basis of electrical properties that could prove valuable in guiding the future development of electrical separations.

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