Group 10

Helena Binková
Emeline Debalme
Simon Lee
Jeff Tamburi

BIO 411: Project 2

1) *Bud23* is one of the previously uncharacterized genes discovered by Ni and Snyder as having a role for proper diploid budding pattern in *S. cerevisiae* (see p. 34 of week 6 lecture). What experiments could you envisage to better understand how the Bud23 protein contributes to this process (mention at least four)?

In order to understand the contribution of Bud23 proteins to diploid budding patterns, we will analyze the phenotype of the cells after modifying the expression of the Bud23 gene. Thus, the 4 experiments we are talking about below are **Functional Genetic Screens** methods. In order to fully understand the function of Bud23, we will consider the cases when the gene is inhibited/deleted, the proliferation of cells involved with the deletion of Bud23, and when the gene is overexpressed.

What is the phenotype of cells with inhibited/deleted Bud23?

- **Knock-out with CRISPR-Cas9:** One of the methods we can use to study the Bud23 protein is by knocking out the Bud23 gene itself using CRISPR-cas9. By deleting the Bud23 gene, we don't allow it to be expressed and there is a lower chance of off target effects. To conduct the actual experiment, we can begin by designing multiple sgRNA-expressing lentiviruses and check. Since we are interested in a specific gene we would design an array in which we can have our sgRNA target specifically our Bud23 gene to look for specific perturbations directly on the proper diploid budding patterns.
- **RNAi:** A very common method like the CRISPR method is to use targeted silencing using RNA interferences (RNAi). These methods are powerful because we identify components of known biochemical and cellular pathways. (And because we are leveraging these known components we are able to study the interaction between genes that reveal the functionality of our genes of interest.) So, we could design small RNAs complementary to a fragment of Bud23, which will allow inhibition of the gene thanks to the RISC complex.

Following these 2 methods, we can observe the phenotypes of Bud23 deficient yeasts by using the Calcofluor White dye.

Is the proliferation rate of cells affected by the deletion of Bud23?

- **Deletion libraries:** Another method to study gene function is through studying how the proliferation rate is impacted through deletion libraries. In brief, deletion libraries are a large set of possible deletion strains that contain unique barcodes that we can use to study the relative abundance of each mutant. So in the context of our experiment design, if the abundance of the

bar-code of our Bud23-deleted yeast strain decreases then it means that Bud23 has a pathway involved in the proliferation of S. cerevisiae

What is the phenotype of an over expression of Bud23?

- **CRISPR/Cas9 overexpression:** CRISPR/cas9 systems can also be used in the context of "activating" specific genes through overexpression. Since we are interested in the role for proper diploid budding pattern in *S. cerevisiae,* an overexpression experiment would be a good way to observe the different pathways of the Bud23 gene which was something we could not do with a loss-of-function type of approach through deletions (Prelich 2012).

Sources:

- Prelich G. Gene overexpression: uses, mechanisms, and interpretation. Genetics. 2012 Mar;190(3):841-54. doi: 10.1534/genetics.111.136911. PMID: 22419077; PMCID: PMC3296252.

2) In anticipation of the upcoming temperature increase on our planet, you want to identify *S. cerevisiae* genes that are dispensable for growth at 32 C but necessary for growth at 37 C. How could you design an efficient screen to identify such genes?

In order to identify the function of a gene, we can analyze the phenotype of the mutant missing this gene. To do that we will perform a **Functional Genetic Screen** by using deletion libraries. We will create a pool of mutant S. Cerevisiae presenting a deletion in one specific gene each. We could then identify thanks to unique bar-codes for each mutant, the genes affected by the different temperature growth conditions.

1. **Generating a complete deletion library**

We want to generate a pool of S. cerevisiae mutants, each deleted for a specific gene. So, we will have a pool of approximately 6000 mutants as S. cerevisiae have ~6000 genes. Moreover, these strains will be bar-coded to uniquely identify each deletion mutant. Each gene is deleted in the Open Reading Frame (ORF), which means from start to stop codon, and replaced by a KanMX deletion cassette by homologous recombination. This cassette is composed of a KanMX gene which is linked to 1 or 2 unique 20-mer tags (DNTAG and UPTAG). These unique tags and their associated primers are named "bar-codes".

2. **Growth of the pools under 32C and 37C**

Once we have our pools of S. cerevisiae mutants, we want to observe their growth under 32C and 37C. We thus need to perform 2 experiments: one at 32C and the other at 37C. In each experiment, cultures containing each deletion mutants are grown on plates, and samples of the cultures are taken at different times.

3. **Analyzing the abundance of each bar-code**

The genomic DNA from the sample is purified and the bar-codes sequence are amplified. By quantifying each bar-code, we can determine the abundance of each mutant strain.

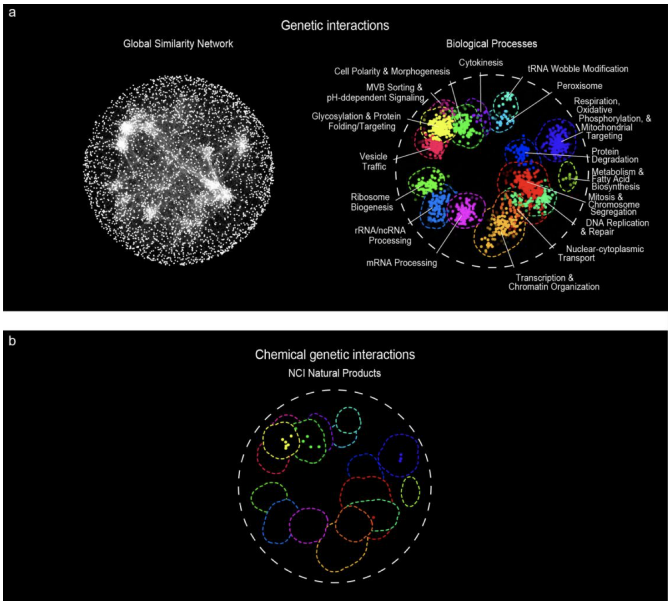
4. **Identifying the genes**

The genes associated with a bar-code amount increasing (=proliferation/growth of cells) at 32C but decreasing (=depletion of cells) at 37C can be identified as genes dispensable for growth at 32C but necessary for growth at 37C.

Sources:

- EASON, Robert G., POURMAND, Nader, TONGPRASIT, Waraporn, et al. Characterization of synthetic DNA bar codes in Saccharomyces cerevisiae gene-deletion strains. Proceedings of the National Academy of Sciences, 2004, vol. 101, no 30, p. 11046-11051.
- GIAEVER, Guri, CHU, Angela M., NI, Li, et al. Functional profiling of the Saccharomyces cerevisiae genome. nature, 2002, vol. 418, no 6896, p. 387-391.

3) The image below stems from a chemical genomic screen in *S. cerevisiae*. Explain how such experiments are conducted in general and enable scientists to predict which biological process is affected by a given chemical compound. Moreover, propose which specific commercially available chemical compounds the yellow and green discs might correspond to (provide one name for each).



(Piotrowski, Li, Deshpande et al., 2017)

Chemical genetic screens are done using libraries of small molecules that have known activities or simply diverse chemical structures. The small compounds can usually allow for temporal control and reversibility on protein inactivation.

It takes several steps to understand which biological process is affected by a given chemical compound. First, a genetic interaction network is established, in which the majority of all possible double mutants is judged. In the example given in lecture 7

(slide 29), synthetic lethality is given for the interaction between two different mutated genes. These interactions can also be more nuanced:

- Negative genetic interaction: when two mutations produce a double-mutant fitness defect that is more severe than expected
- Positive genetic interaction: when two mutations produce a double-mutant fitness defect that is less severe than expected

The set of interactions composes the genetic information profile. Then, genes having similar roles are grouped together and they form clusters representing major biological processes. Furthermore, a chemical-genetic profile is created, that is the negative or positive interactions between the selected compound and all the different mutations. Finally, the main thesis around which it is possible to understand which biological process is affected by a given chemical compound is the following: “If a bioactive compound inhibits a specific target protein, then loss-of-function mutations in the corresponding target gene should mimic the bioactivity of the compound” (Piotrowski, Li, Deshpande et al., 2017). Therefore, by analyzing the similarities between the chemical-genetic interaction profile and the genetic information profile that is clustered based on the different biological processes, it is possible to predict which biological process is affected by a particular drug.

Yellow disc: glycosylation and protein folding/targeting cell wall biogenesis

Example of chemical compound targeting glycosylation and protein folding/targeting cell wall biogenesis: Meclizine (Piotrowski, Li, Deshpande et al., 2017, Figure 2 iii)

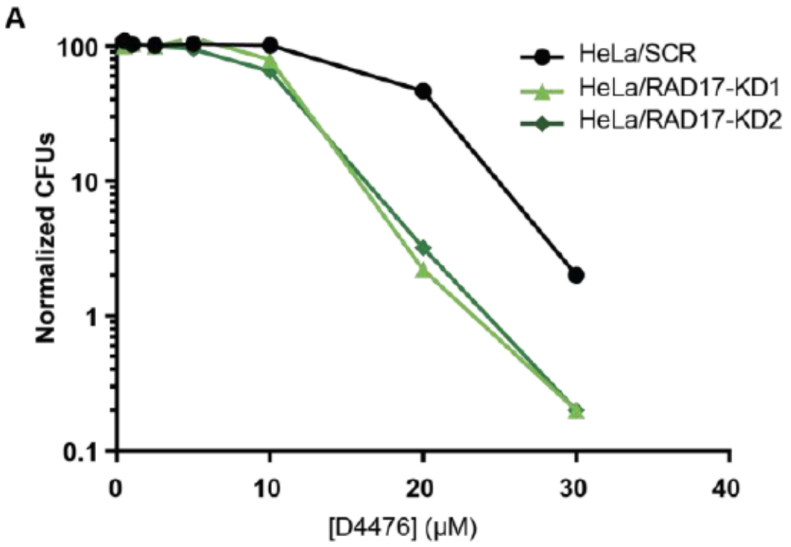
Green disc: cell polarity and morphogenesis

Example of chemical compound targeting cell polarity and morphogenesis: Micafungin (Piotrowski, Li, Deshpande et al., 2017, Figure 2 vi)

Source:

- Piotrowski, J., Li, S., Deshpande, R. et al. Functional annotation of chemical libraries across diverse biological processes. Nat Chem Biol 13, 982–993 (2017). https://doi.org/10.1038/nchembio.2436

4) Below are the results of a clonogenic assay conducted in HeLa cells targeted with two shRNAs (KD1, KD2) against the cell cycle checkpoint protein RAD17, or a negative control shRNA (SCR). Cells were also treated with increasing concentrations of the casein kinase inhibitor D4476. Based on this data, how effective was the RNAi-mediated depletion of RAD17 in your opinion? And how do you think the data would look if you combined RNAi-mediated depletion of RAD17 with CRISPR/Cas9-mediated targeting of casein kinase? Moreover, how does this synthetic interaction compare to that of cells lacking BRAC2 and treated with PARP inhibitors? And, finally, do you think that D4476 is a promising candidate to target cancer cells with hyperactive RAD17?



The RNAi-mediated depletion of RAD17 seems effective. The proliferative potential of the cells was visibly affected by these shRNAs, as we can see when applying the casein kinase inhibitor D4476: at 20 mM of this drug, shRNA-treated cells decreased their colony forming units to approximately 2-3% (compared to without the drug), while non-treated cells were at 50%. We can conclude that RNAi-treated cells are a lot more susceptible to the drug.

CRISPR/Cas9 targeting changes the DNA sequence of cells. Therefore, if cells undergo a CRISPR/Cas9-mediated RAD17 treatment, their casein kinases will be fully non-functional. The data will be a single point as there is no progression in the casein kinase inhibition, and the CFU decrease (compared to before the CRISPR/Cas9 treatment) will probably correspond to the last values of the given plot (or lower), where casein kinase is the most inhibited.

BRCA2 and PARP are involved in two different DNA repair mechanisms, homologous recombination and non-homologous end joining respectively. Inhibiting both proteins in a cell causes synthetic lethality, as the two repair mechanisms cannot compensate for each other anymore. The synthetic interaction between casein kinase and RAD17 inhibition seems to be similar: inhibiting casein kinase in RAD17-depleted cells is toxic and stops proliferation compared to cells with normal RAD17 levels.

D4476 does not seem to be a promising candidate to target cells with hyperactive RAD17. Cells with normal RAD17 function are relatively unaffected by this drug, and only cells with depleted RAD17 are lethal. The pathway involving RAD17 seems to compensate for casein kinase depletion, so hyperactive RAD17 cells could be even more potent than normal cells in presence of a D4476 treatment, which would be counterproductive.

5) Walking on the shores of Lac Léman, you discover a new species of butterflies in which one wing is blue and the other yellow. Fascinated by this discovery, you decide to develop this species as a new model system, and bring back a few males and females to the laboratory. Describe the steps you

would take in the months and years ahead to discover genes that are necessary for making this unusual color dichotomy.

After bringing the butterflies to the laboratory, we develop an environment conducive to their development and mating in order to obtain a high number of individuals. As we work with an unknown animal, we first have to perform a whole genome sequencing using Next Generation Sequencing to obtain the genome sequence.

An option would be to do a CRISPR/Cas9 screen using a gRNA library targeting the whole sequenced genome. We could discover which deletions impact the dichotomic phenotype and deduce which genes are involved. However, this may be too time-consuming and expensive.

Instead, we want to reduce the coverage of the gRNA library, in order not to have to go through the whole genome. For that, we could look into previously published literature, namely literature already studying this particular butterfly, or, if unavailable, other related lineages and species of butterflies. This allows us to align and compare sequences of other butterflies with our sequenced butterfly genome. The majority of sequences should be conserved across species, but identifying differing genes is a good way to find the genes that could be at the origin of the color dichotomy. We then create a gRNA library targeting these differing genes and do a CRISPR/Cas9-mediated deletion using this library in embryonic stem cells (by microinjection or electroporation). We inject the modified embryonic stem cells into early butterfly embryos, producing chimeric butterflies that we breed until obtaining homozygous animals. Thereby, we generate knock-out animals for all the wanted gene sequences. After the butterflies grow, we screen them for an alteration in the dichotomic phenotype. The butterflies with altered phenotypes are retained for genetic analysis, and we identify the involved genes in color dichotomy.

For each discovered gene, some additional analysis can be performed. For example, the relations between the different discovered genes can be analyzed. We could create transgenic animals with double knock-outs to study the synthetic reactions of these genes. From that, we could also deduce some epistatic relations or the order of action of genes, and hypothesize on the function of each gene on the phenotype.