Synthetic Lethality and Robustness

Robustness of biological systems

Biological systems tend to be remarkably robust. Not only are they able to withstand changes in their environment but they can also to some extent tolerate random variations in their genome. In systematic genetic studies on the yeast *saccharomyces cerevisiae,* single-gene knock-out mutants were generated for all of its approx. 6000 genes. These studies showed that >80% of these knock-outs grow normally under standard growth conditions. Does this indicate that only 20% of all genes in *saccharomyces cerevisiae* are involved in essential processes? This seems unlikely! Indeed, this number should be understood as an indication of the metabolic and signaling networks in cells being organized in a way that allows the disruption of individual nodes to be buffered. Trying to understand how this metabolic buffer works and figuring out how it results in the robustness of biological networks is the main quest of systems biology.

One of the most important experimental approaches for addressing this question are synthetic lethality screens. These studies are particularly popular in yeast where automated methods are available that help generate the necessary double knockout libraries in a very efficient, high-throughput manner.

Synthetic lethality

Synthetic lethality (Figure 1) occurs when the combination of genetic variations in two different genes leads to cell death, while either variation alone has no or only a very modest effect. In practice the term "synthetic lethality" is used more broadly. It then refers also to cases where a certain combination of harmless mutations generates a pronounced phenotypic effect, without actually causing cell death. Strictly speaking the correct term for such instances would be "synthetic enhancement", "synthetic sickness" or "synthetic fitness".

At first glance the concept of synthetic lethality can be counter-intuitive, but the underlying principle is readily understandable when we consider an analogy drawn from everyday life. Imagine a châlet in the mountains that is equipped with both a gas heater and a wood stove. If either of the two breaks, one can still heat the châlet by using the other heat source. But, if both the stove and the heater break, the situation becomes non-viable.

It is important to understand that synthetic lethal interactions neither require that the two affected genes carry out equivalent molecular functions nor is it necessary that the two gene products physically interact with one another. Although such cases do exist, the vast majority of synthetic lethal interactions occur because the two mutations inactivate key steps in two redundant pathways (see Figure 2 for an example).

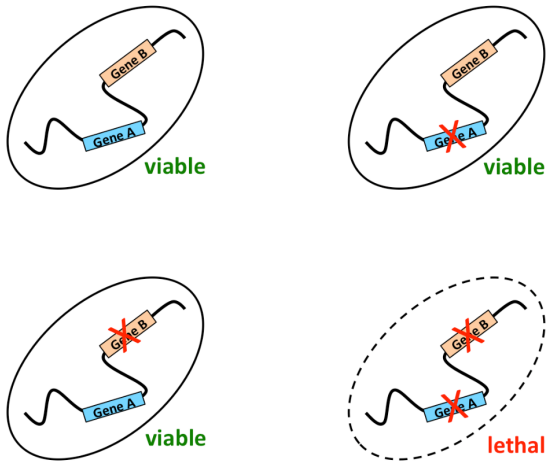


Figure 1 *Schematic representation of the concept of synthetic lethality. Organisms carrying a mutation in either gene A or gene B are viable (i.e. they grow similarly to the wild-type organism, top left) while organisms carrying both mutations (bottom right) can't grow or have severely impaired growth.*

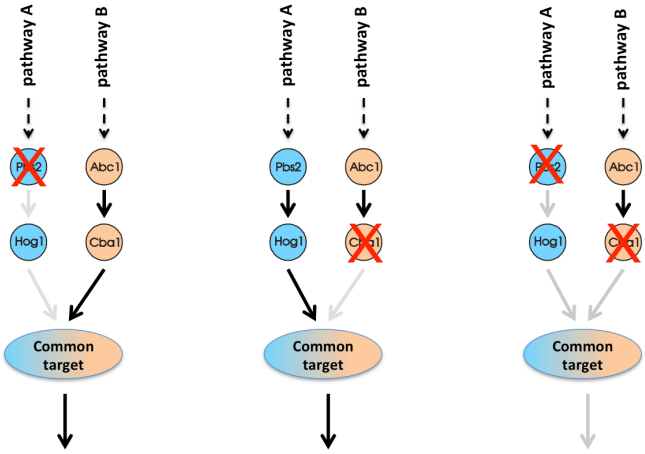


Figure 2 *Example of synthetic lethality resulting from mutations in redundant pathways. Both pathway A and B activate the same target. Activation of this target is essential for the survival of the organism. If one of the genes in pathway A is inactivated (left panel), pathway B can still activate (black arrow) the target and vice versa (middle panel). But if one gene in pathway A is inactivated and a gene in pathway B is inactivated the target is not activated (grey arrow), cannot perform its essential function and the cell dies. Note that Pbs2 and Cba1 may have entirely different molecular functions.*

Of course both pathways in question possibly involve a large number of components. The knockout of any component in one pathway may be synthetically lethal combined with the knockout of any component of the other pathway. Thus, there exist a large number of potentially synthetic lethal interactions (Figure 3). A systematic large-scale study of synthetic lethality in yeast predicted that there could be a total of approx. 100'000 synthetic lethal interactions between pairs of yeast genes.

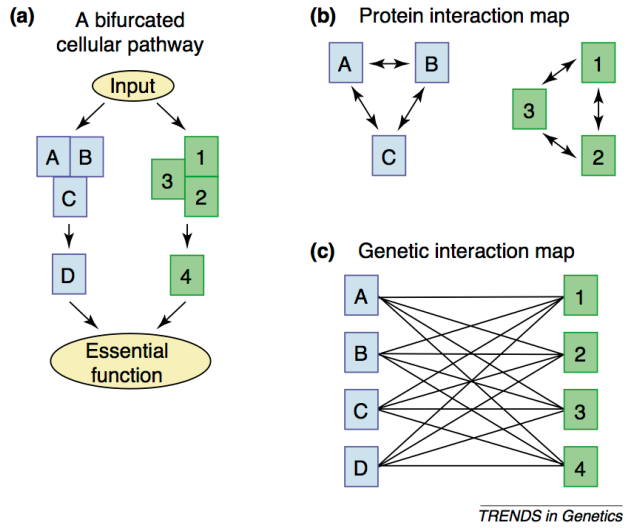
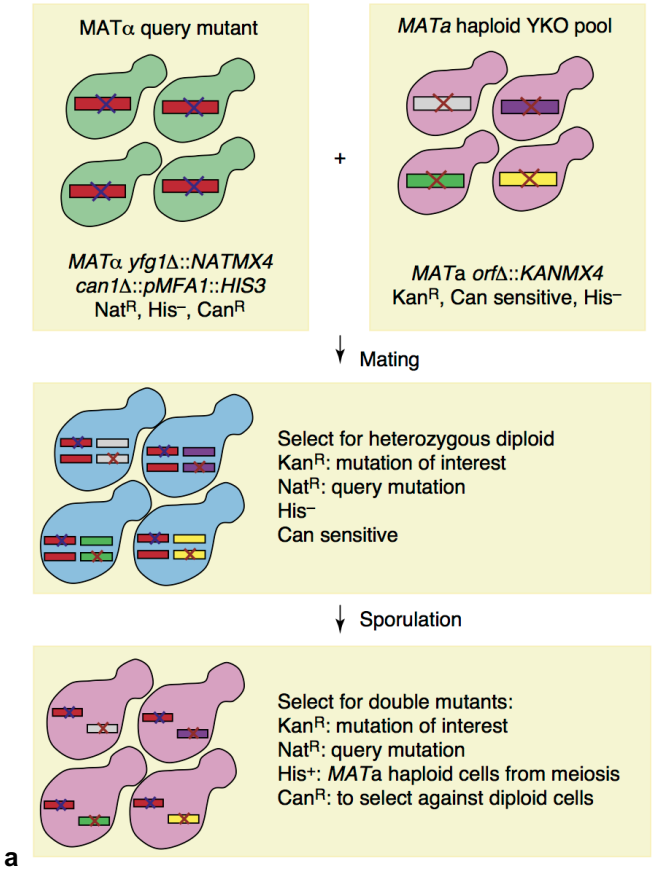


Figure 3 *Comparison between the genetic (**c**) and physical (**b**) interaction networks that will be observed for the same biological pathway (**a**). Note that the genetic interactions are much more numerous than the physical interactions between the gene products. Note that the genes A,B,C,D and 1,2,3,4 are not synthetically lethal with one another. (Ooi et al. 2016)*

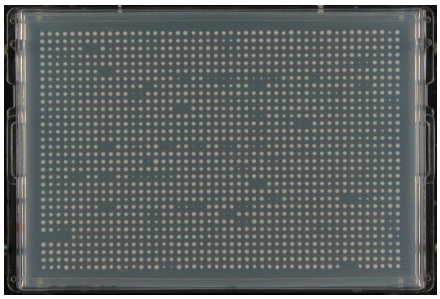
Automated methods allow rapid synthetic lethal screens in yeast

Testing whether a synthetic lethal interaction exists between two genes requires generating organisms in which both those genes are inactivated. In most organisms the process of generating such homozygous double knockouts would be a cumbersome and time-consuming process.

Not so in the yeast *saccharomyces cerevisiae.* For this model organism a number of very efficient methods for generating double knockouts have been developed. The particularly popular SGA (synthetic genetic array) method for generating double knockouts is based on two knockout libraries. The first library consists of yeast cells whose genomes are identical, except for the fact that in every cell a different gene has been replaced by a gene that confers resistance against the antibiotic nourseothricin. Ultimately there is one cell for every gene in the genome, in which that gene has been replaced. Also, the cells have been selected to have mating type **α**. In the second library every gene has been replaced by a gene conferring resistance against kanamycin. Here, cells have been selected to be mating type **a**. In order to generate a double-knockout mutant for genes A and B, one crosses cells missing gene A from the first library and cells missing gene B from the second library. By using induced sporulation to return the crosses to the haploid state and by selecting both for kanamycin and nourseothricin resistance, one obtains the desired double knockout cells.



a



b

Figure 4 *(**a**) Schematic showing the construction of a double knockout library for a query gene with all other knockouts from the yeast knock out library (YKO). (**b**) agar plate growing 1536 yeast colonies from a double knockout screen. Note that most colonies grow normally but some grow significantly slower or don't grow at all. (Ooi et al. 2006 trends in genetics, wikimedia by Masur)*

This process has been highly automated so that it is now possible to routinely perform synthetic lethality screens (Figure 4).

Cells from line A in which one selected gene (called the "query" gene) is knocked out, can very rapidly be crossed with the full library B. The resulting cell lines can then be used to identify synthetic lethal interaction of the query gene with any of the other genes.

Synthetic lethal screens reveal networks of genetic interactions and illuminate the mechanisms underlying the robustness of biological systems

The speed and efficiency of automated synthetic lethal screens on *saccharomyces cerevisiae* has made it possible to conduct these screens on an increasingly large scale. As a result, they are starting to reveal the network of genetic interactions that robustly sustain the functioning of yeast cells in the face of random gene inactivation.

But, despite the tremendous technical progress, a full synthetic lethal screen of all possible double-knockout combinations remains to be a very expensive and time-consuming undertaking. Furthermore, in order to find all possible synthetically lethal interactions, such screens would have to be repeated under a range of different growth conditions. Also, many synthetically lethal interactions may only become evident in the case of a triple or quadruple knockout.

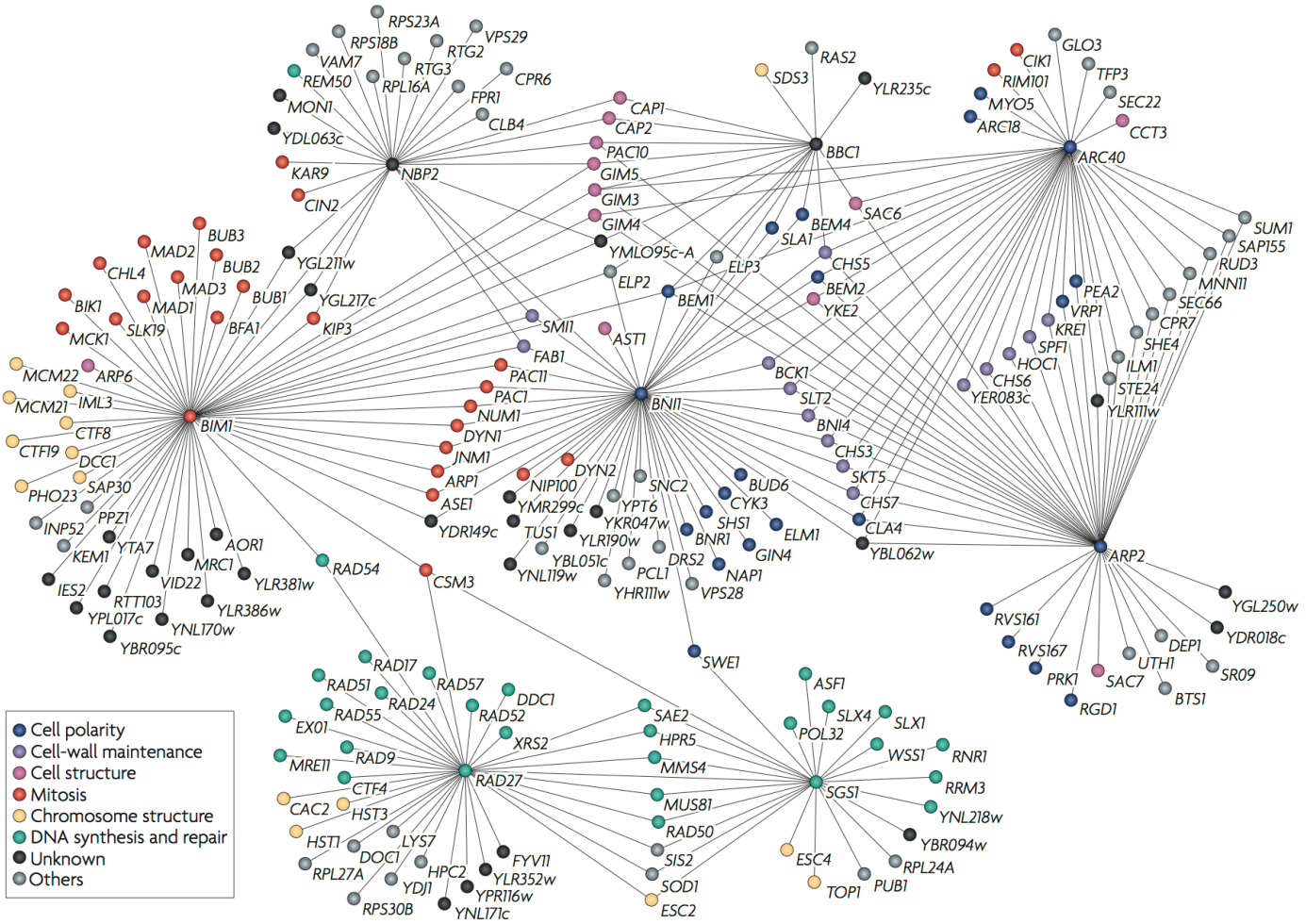


Figure 5 *Network of synthetic lethal interactions generated by performing synthetic genetic array (SGA) analysis in which single gene knockout strains for each of the following eight query genes BNI1, RAD27, SGS1, BBC1, NBP2, BIM1, ARP2 and ARP40 were crossed with each of the 5000 single-gene knockout lines of the yeast knock out library (YKO). Out of the total of 40'000 cell lines 291 showed synthetic lethal interactions. Genes are shown as circles and interactions as lines. As expected the query genes form the hubs of the resulting network, but these hubs are connected via genes that show synthetic lethal interactions with two or more of the query genes.*