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Meiotic Recombination: Genetics' Good Old Scalpel

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In the era of genome engineering, a new study returns to classical genetics to decipher genotype-phenotype relationships in unprecedented throughput and with unprecedented accuracy. Capitalizing on natural variation in yeast strains and frequent meiotic recombination, She and Jarosz (2018) dissect and map to nucleotide resolution, simple and complex determinants of diverse phenotypic traits.

The ultimate aim of genetics is to explain phenotypic traits by their genetic determinants. Since the days of Morgan, genetics is equipped with maps that describe the arrangements of genes on chromosomes and phenotypic properties that they encode. Modern genomewide association studies (GWAS) systemize the mapping of phenotypic traits to loci on genomes. Successes led to identification of genes that encode for particular traits and even to specific nucleotide variations within genes that modify phenotypes, e.g., in diseases (Evangelou and Ioannidis, 2013). Yet, mapping studies face two serious difficulties. First, many traits, such as susceptibility to common heritable disease—not to mention elusive qualities, such as musical talent—involve multiple genes, making it difficult to elucidate the individual contribution of each. Second, limited by a low rate of meiotic recombination that dissects the contribution of nearby loci, association studies often result in too-large genomic regions that often consist of dozens of genes, with a limited ability to find the causative determinants. Recombination is the geneticist's scalpel—the act of recombination can serve as an analytical tool that generates variants that possess alternative genetic combinations. If the recombination rate is high enough relative to the density of sites of variation, it should allow the high resolution that is needed to distill effects of each genetic component. Complementing the pure genetic approach, which observes (i.e., sequences) genomes, is genome engineering that manipulates them. With the advent of genome editing technologies, researchers may not only rely on natural variations; they can design and make them (Barbieri et al., 2017) or randomize genes' sequences (Li et al., 2016) and examine genetic effects on phenotypes. Another research paradigm for genotype-phenotype mapping is experimental evolution. Evolving a strain in the lab toward a particular phenotypic property—e.g., drug resistance—often reveals adaptive genetic determinants (Levin-Reisman et al., 2017). Yet, these paths are limited by human knowledge and by relatively short experimental evolution periods. If we could effectively harness existing variation in wild populations, generate hybrids among them, and scan large enough collections of offspring, then genotype–phenotype mapping could be revolutionized. In this issue of *Cell*, She and Jarosz (2018) achieve this ambitious goal.

Realizing that Baker's yeast features an unusually high recombination rate (Segura et al., 2013), She and Jarosz recognized that this classical model can be used for high-resolution genetic mapping. For a high recombination rate to be helpful, parental strains have to display a sufficient but not too high diversity, and multiple rounds of inbreeding between their offspring must take place. Also, it was clear that large amounts of offspring have to be analyzed so as to realize many of the possible genetic combinations. The authors' mathematical model provides an encouraging result: given the meiotic recombination rate in yeast and a typical density of polymorphism (once every 1,000 nucleotides), an attainable number of individuals (~1,000 clones) could deliver single-nucleotide precision mapping. They start with two natural strains: a wine strain and a clinical isolate. They cross them and inbreed offspring for six generations (F6). They collect more than a thousand F6ers, sequence their genomes, and phenotype their ability to withstand a diverse array of drugs (Figure 1). Sequencing at such depth is the main technology used here that was not available until recently.

By computing association between each site of variation to drug sensitivity, a locus of high association is found, which reassuringly corresponds to a gene from a related metabolic pathway. The gene features several mutations that differentiate between the parental strains. Thanks to the very high recombination rate, the authors are fortunate to get separate yeast clones, each with a unique combination of mutations in the locus. They could thus distil the phenotypic effect of each mutation. One of these mutations, which would have typically been overlooked by most bioinformatics methods (Adzhubei et al., 2010)—as it represents a mutation that does not change the encoded amino acid—turns out to be the most important in improving drug tolerance. But that gene is not the only one related to the trait examined; in fact, a whole network of genes is found to be involved in drug tolerance.

Interestingly, in some of the examined traits, pairs of causal variants are found within the same gene. Yet, more striking is the observation that in most cases, those pairs of mutations that affect the same trait tend to occur in neighboring genes along a chromosome. While in bacterial operons this could be expected, this thought-provoking finding suggests that the eukaryotic chromosomes consist of "inheritance blocks" consisting of multiple genes each. Such blocks, present already at the parental strains, often

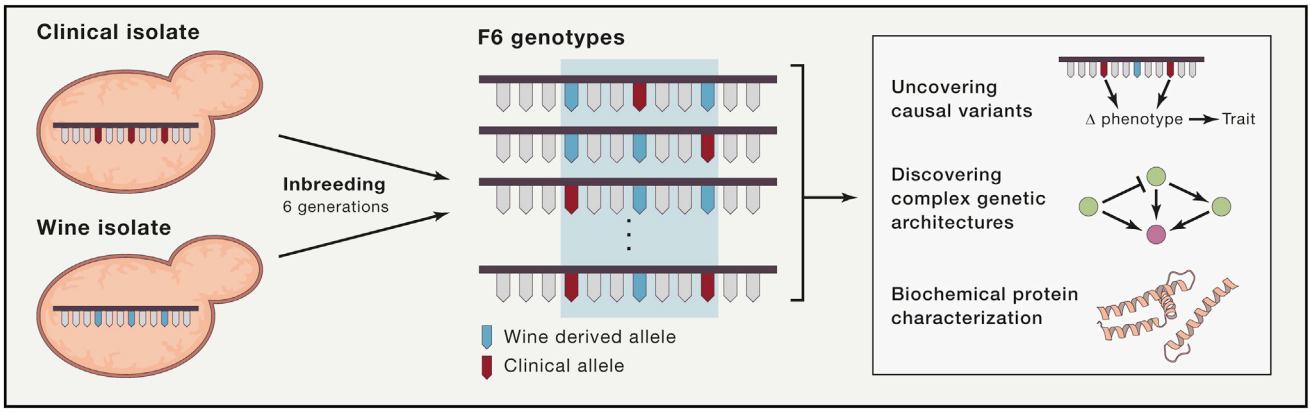

Figure 1. A Pipeline for the Discovery, Mapping, and Characterization of Genetic Determinants of Phenotypic Traits
Two wild yeast strains are inbred for six generations. More than a thousand offspring are collected, which feature recombination between multiple sites of variation. Recombined progenies are phenotyped, genotype-phenotype associations are established for single-nucleotide variants, and causative mutations are assessed at single-gene and -protein levels and at the genetic network level.

contain combinations of mutations that neutralize each other's potential deleterious effects. This arrangement appears to be particularly important as many of the challenges imposed on the cells require dozens of contributing variants. This is a remarkable notion that could further imply that such inheritance blocks may facilitate coordinated horizontal transfer of genetic material in eukaryotes.

The large dataset of 370 causal mutations allows for checking, and often refuting, of some prevailing intuitive beliefs about phenotype-changing mutations. For example, though causative mutations often change protein secondary structures, others act without changing the amino acid. Further, causative variation is seen in promoters, in untranslated regions of genes, and in intergenic regions. Causative synonymous mutations (Sauna and Kimchi-Sarfaty, 2011) are often predicted to show increased translation efficiency, as they here too shift from low- to high-optimality codons. The fact that single codons may exert such effects is remarkable and suggests more convoluted effects, e.g., in optimizing ribosome flow (Frumkin et al., 2017). Further, the genetic code is found to be arranged such that the effect of mutating between synonymous codons may appreciably change translation efficiency, while non-synonymous mutations often show more mild translation effects.

She and Jarosz (2018) bring excellent and exciting results, unprecedented in scope and accuracy and with a refreshing approach. Would this approach help solve complex mapping problems in other organisms? A lot seems to depend on the meiotic recombination rate, the number of offspring, and the depth of inbreeding. Manipulation of recombination rate in yeast has already been done (Sadhu et al., 2016). Would that be attainable in metazoans?

Interestingly, the genes found here to affect a phenotype are largely different from the genes found in genetic perturbation experiments for the same phenotypes. Is there a "right" approach? Scientific research comes at two main flavors. A classical paradigm is to merely observe nature. The tinkerer's approach is to manipulate systems, either randomly or by engineering. The present study brings back to the stage the observer's approach with its full power and beauty. Synergy between the approaches is almost guaranteed.

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