Correspondence

Breaking a species barrier by enabling hybrid recombination

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Hybrid sterility maintains reproductive isolation between species by preventing them from exchanging genetic material1. Anti-recombination can contribute to hybrid sterility when different species’ chromosome sequences are too diverged to cross over efficiently during hybrid meiosis, resulting in chromosome mis-segregation and aneuploidy. The genome sequences of the yeasts Saccharomyces cerevisiae and Saccharomyces paradoxus have diverged by about 12% and their hybrids are sexually sterile: nearly all of their gametes are aneuploid and inviable. Previous methods to increase hybrid yeast fertility have targeted the anti-recombination machinery by enhancing meiotic crossing over. However, these methods also have counteracting detrimental effects on gamete viability due to increased mutagenesis2 and ectopic recombination1. Therefore, the role of anti-recombination has not been fully revealed, and it is often dismissed as a minor player in speciation1. By repressing two genes, SGS1 and MSH2, specifically during meiosis whilst maintaining their mitotic expression, we were able to increase hybrid fertility 70-fold, to the level of non-hybrid crosses, confirming that anti-recombination is the principal cause of hybrid sterility. Breaking this species barrier allows us to generate, for the first time, viable euploid gametes containing recombiant hybrid genomes from these two highly diverged parent species.

Species are formed and maintained by the restriction of gene flow between diverging populations. Barriers to gene flow can be physical, such as geographic distance, or they can be properties of the species themselves. Here, we focus on one such barrier to gene flow, hybrid sterility. Hybrid sterility can be caused by a variety of mechanisms that can generally be classified into two categories: incompatibilities between diverged chromosomes (such as large-scale chromosomal rearrangements and anti-recombination) and incompatibilities between individual genes from the diverging populations1. Here we show that repressing anti-recombination dissolves the reproductive barrier between two yeast species, S. cerevisiae and S. paradoxus, increasing their production of viable hybrid gametes by 70-fold (Figure 1A). We did this by repressing the meiotic expression of just two highly conserved genes, SGS1 and MSH2. Msh2 is a component of the mismatch repair system that removes base-pair mismatches in duplex DNA, both to repair misincorporations in newly synthesized DNA and to inhibit recombination between diverged sequences (anti-recombination). The former activity reduces mutations, and the latter can help maintain genome integrity by limiting ectopic recombination between non-homologous chromosomes and dispersed repeats3. Sgs1 is a DNA helicase that is assumed to act downstream of mismatch recognition by Msh2 to unwind nascent recombination intermediates containing a high density of mismatches3, but also plays a more general function in recombination to disassemble joint-molecule intermediates that could lead to crossovers3. Thus, although completely deleting MSH2 enhances meiotic recombination between the diverged chromosomes of S. cerevisiae x S. paradoxus hybrids, increasing proper chromosome segregation and therefore hybrid spore viability, this benefit is countered by elevated mutagenesis and genome instability in mitotically dividing cells, reducing viability. We therefore replaced the native promoters of MSH2 and SGS1 with the CLB2 promoter, which represses gene expression during meiosis but not mitosis4.

Meiotic repression of either gene alone significantly increased hybrid spore viability (Figure 1A, MSH2 p = 7.99 x 10^-6; SGS1 p = 2.2 x 10^-19). Overall spore viability rose from 0.46%
in the wild-type hybrid to 3.18% in the pCLB2-MSH2 strain and to 20.08% in the pCLB2-SGS1 strain. Spore viability was further improved to 32.65% when both genes were repressed ($p < 2.2 \times 10^{-5}$). Although hybrid fertility was not increased to the level of the parents — the S. cerevisiae and S. paradoxus parent fertilities were 83.75% and 92.25%, respectively — it was well within the range of fertilities of non-hybrid crosses formed from diverged populations of one species or the other. For example, 32–87% for S. paradoxus or S. cerevisiae crosses with collinear genomes, 14–86% for wild S. paradoxus crosses. These results show that anti-recombination determines most of the hybrid sterility barrier between our S. cerevisiae and S. paradoxus strains.

This remarkable restoration of hybrid fertility allowed us to produce a large sample of perfectly euploid hybrid gametes. Any viable gametes produced by a hybrid are usually aneuploid, and this remains the case even when MSH2 is knocked out. By dramatically improving hybrid fertility, we significantly increased the production of hybrid tetrads in which all four spores were viable from 0% in the wild-type hybrid to 5.3% in the double mutant hybrid (Figure 1B; 0 out of 269 versus 108 out of 2,037, respectively, $p = 2.04 \times 10^{-5}$). Because all chromosomes are essential in yeast, we can infer that these full tetrads contain only euploid hybrid gametes. Generation of these hybrids enables the unambiguous analysis of recombination and trait mapping, both of which were previously confounded by aneuploidy in sampled hybrid spores.

Finally, in order to map the genome-wide distribution of crossovers in our pCLB2-MSH2 pCLB2-SGS1 double mutant hybrid, we sequenced the genomes of the 336 hybrid spores from hybrid followed the normal, non-hybrid genomes of the 336 hybrid spores from the hybrid from the wild-type hybrid to 3.18% in the pCLB2-MSH2 strain and to 20.08% in the pCLB2-SGS1 strain. Spore viability was further improved to 32.65% when both genes were repressed ($p < 2.2 \times 10^{-5}$). Although hybrid fertility was not increased to the level of the parents — the S. cerevisiae and S. paradoxus parent fertilities were 83.75% and 92.25%, respectively — it was well within the range of fertilities of non-hybrid crosses formed from diverged populations of one species or the other. For example, 32–87% for S. paradoxus or S. cerevisiae crosses with collinear genomes, 14–86% for wild S. paradoxus crosses. These results show that anti-recombination determines most of the hybrid sterility barrier between our S. cerevisiae and S. paradoxus strains.

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