When nuclear-encoded proteins and mitochondrial RNAs do not get along, species split apart

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The emergence of barriers to reproduction between two populations is one of the most important features of speciation. Among the mechanisms of reproductive isolation are incompatible interactions between gene products of the parental species that reduce the fitness of hybrid individuals. The accumulation of such incompatibilities is described by the Bateson–Dobzhansky–Muller model (BDM) [1] that provides a framework for understanding how genes can coevolve to stay compatible within populations and become incompatible between populations. Only a handful of such loci have been identified and characterized at the molecular level. In this issue of *EMBO Reports*, Jhuang and colleagues [2] show that BDM incompatibilities have accumulated between a nuclear-encoded gene and a mitochondrial ribosomal RNA between two yeast species.

See also: H-Y Jhuang *et al* (January 2017)

Central to the study of speciation is the identification of the barriers that prevent gene exchanges between species and that maintain their genetic independence. Various mechanisms are known to prevent gene flow between species. For instance, pre-zygotic isolation could occur when one species is unable to recognize the opposite sex of the other species as potential mates, or when species are isolated in space. Other mechanisms act after zygote formation, for instance, through the maladaptation of hybrid traits that makes hybrid individuals unable to thrive in the environments available to them. Alternatively, hybrids

may suffer from intrinsic developmental problems and fail to develop normally, leading to poor viability or impaired fertility. Such intrinsic incompatibilities may arise from large chromosomal rearrangements and inversions that prevent proper chromosome segregation [3] or the accumulation of genetic changes between two populations of incipient species, making the alleles of specific genes compatible within species but incompatible when reunited in a hybrid individual (Fig 1A). Because many loci can potentially evolve following this scenario, incompatibilities may accumulate rapidly with time. The nature of these incompatibilities can be diverse, but physical interactions among gene products or gene products and nucleic acids are prime candidates because they are known to coevolve within species and may thus interact in a non-functional manner in hybrids.

Mitochondria-encoded genes have many unique features that make them good candidates to evolve BDM incompatibilities (reviewed in [4]). First, they are under strong coevolutionary constraints because the interactions between mitochondrial and nuclear gene products (mitonuclear interactions) are central to essential cellular functions, most notably aerobic respiration. Second, the rates of evolution of mitochondrial genomes are generally higher than those of nuclear genomes. High mutation pressure, inefficient DNA repair mechanisms, and the mostly asexual reproduction mode of organelle genomes lead to the accumulation of mildly deleterious mutations that are impossible to eradicate, a phenomenon known as Muller's ratchet. These features create a compensatory coevolution dynamics in which

nuclear genes must constantly adapt to the rapidly evolving mitochondrial genomes to preserve the integrity of their shared functions. Finally, mitochondrial DNA (mtDNA) molecules replicate independently from the nuclear genome and are transmitted in a uniparental manner for many species, allowing the possibility for genomic conflicts to arise [5]. In such conflicts, a mitochondrial genome acquires the ability to spread in a population despite its disadvantageous effect on the whole organism's fitness, for instance, in the sex that does not transmit the organelle genome. Some nuclear genes can then evolve to counter the selfish behavior, leading to a coevolution dynamic similar to the compensation model described above.

One powerful model for the study of molecular bases of reproductive isolation and BDM incompatibilities is the yeasts of the genus *Saccharomyces*. Cells of the different species hybridize in the wild and can be crossed in the laboratory, but their progeny is generally sterile, allowing to dissect what molecular functions may go wrong in the hybrids. Importantly, *Saccharomyces* can grow in the absence of functional mitochondria if provided with fermentable sugars such as glucose but relies on functional mitochondrial aerobic respiration if transferred to non-fermentable medium. This unique property allows to screen for defective mitochondrial functions and to construct strains with different combinations of nuclear and mitochondrial genomes.

Using crosses between two closely related *Saccharomyces* species, *S. cerevisiae*, also known as the baker's yeast, and *S. bayanus*, Jhuang *et al* [2] find that when

Figure 1. Genetic incompatibilities between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

(A) General Bateson–Dobzhansky–Muller model of incompatibilities between pairs of interacting loci. The case where inter-locus incompatibilities (bottom) are dominant can lead to reproductive isolation in F1 hybrids. Recessive interactions will contribute to incompatibilities in F2, recombinant hybrids. (B) Symmetric incompatibility between the nuclear gene *CCM1* and the 15S mitochondrial ribosomal RNA (rRNA). Defective 15S rRNA stabilization reduces hybrid fitness on non-fermentable media, where mitochondrial function is essential.

the chromosome 7 of *S. cerevisiae* is replaced by its homologue from *S. bayanus*, cells are unable to grow on a non-fermentable medium. The authors demonstrate that this phenotype is caused by an incompatibility between *CCM1*, a gene located on chromosome 7, and the mtDNA of the other species (Fig 1B). *CCM1* encodes the mitochondria-targeted Ccm1 protein that contains a pentatricopeptide repeats (PPR) domain. PPR proteins bind RNA and are involved in many processes that regulate RNA, from maturation to translation, and they regulate organelle gene expression in many eukaryotes, including slime molds, plants, fungi, and animals [6]. Ccm1 in budding yeast is required for respiratory growth and maintenance of mitochondrial functions and is known to interact physically with many mitochondrial transcripts, including the 15S rRNA that it stabilizes [7]. Jhuang *et al* find lower levels of Ccm1-bound 15S rRNA in *S. cerevisiae* harboring

the *S. bayanus* *CCM1* allele, suggesting that the incompatibility arises because of impaired 15S rRNA stabilization. By swapping *CCM1* between species, they also demonstrated that *CCM1* incompatibility is symmetric, further enhancing the role of these genes in the isolation between the two species. The *CCM1*–15S rRNA pair adds to two other mitonuclear incompatibility pairs among *Saccharomyces* yeasts that were discovered in previous studies from the same team [8].

If the incompatibility observed was the result of the physical interaction between the RNA molecule and Ccm1, one would predict that mutations in the gene encoding the protein or the RNA itself could restore the incompatible phenotype. Using an experimental evolution approach where they let strains evolve to regain fitness and followed by sequencing of the candidate genes, the authors showed that many mutations in mitochondrial and nuclear genes can restore

the compatibility between the *S. bayanus* nuclear genome and the *S. cerevisiae* mtDNA. Mutations that were found in the *CCM1* gene itself were all within the predicted RNA-binding PPR motifs, confirming their role in the faulty interaction and demonstrating at the same time that these motifs can rapidly evolve to re-establish the proper relationship between nuclear and mitochondrial genes. Some of the mutations identified converged toward the molecular differences already existing between the two species, corroborating their role in the incompatibility. This experiment also shows that mitonuclear coevolution can proceed through cycles of deleterious mutations followed by compensatory ones.

One important finding here is that some of the proteins involved are fast-evolving and could thus represent a hot spot for the accumulation of BDM incompatibilities. To support this observation, Jhuang *et al* provide evidence that PPR proteins in

S. cerevisiae evolve at a faster rate than protein families harboring similar structural or functional characteristics and that many PPR genes underlie incompatibilities among closely related *Saccharomyces* species. Among the many experiments performed, the authors tested the interactions of a dozen of PPR genes from many closely related *Saccharomyces* species with the *S. cerevisiae* mitochondrial genome and found many cases of growth disadvantage on non-fermentable media, suggesting a common role for this gene family in generating mitonuclear incompatibilities. The results suggest that nuclear genes with rapidly evolving regions involved in RNA binding may be particularly prone to accumulating incompatibilities, potentially driven in the case of mitonuclear incompatibilities by the rapid evolution of mitochondrial rRNA [9]. Another example also involving a nucleic acid binding protein has been reported recently between mice

species, in this case involving a DNA binding protein [10] playing a role in DNA double-strand break with a rapidly evolving DNA binding domain that fails to interact properly in hybrid mice.

The results presented by Jhuang *et al* provide insights into an important class of mechanisms that cause hybrid incompatibilities between fungal species. Identifying the preferred paths leading to reproductive isolation is an essential step toward elucidating the mechanisms of speciation itself. Divergence in the interactions between PPR proteins and mitochondrial transcripts could be one general driver in setting the very first steps of isolation between species. This hypothesis is difficult to test for *S. cerevisiae* and *S. bayanus* because they are non-sister species that diverged several million years ago, leaving the possibility that other mechanisms could have arisen earlier than the *CCM1*–mtDNA incompatibility pair. The recent discovery of very closely related

species of yeast [3] will offer a powerful study system to examine what happens during the early steps of species emergence and whether mitonuclear interactions occupy a driving position.

References

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