Compatibility with Killer Explains the Rise of RNAi-Deficient Fungi

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RNA interference (RNAi), lost in a recent ancestor of *Saccharomyces cerevisiae* (fig. S1), can be restored by introducing Argonaute (*AGO1*) and Dicer (*DCR1*) from a close relative, *Saccharomyces castellii* (*1*). The reconstituted pathway silences endogenous transposons, which explains retention of RNAi in some lineages (*1*). But why is it lost in others? To address this question, we determined the consequences of restoring RNAi to *S. cerevisiae*. Restoration of RNAi had little if any effect on growth measured under 50 different conditions, and high-throughput RNA sequencing (RNA-Seq) results indicated minimal changes in nonrepetitive mRNA accumulation (figs. S2 to S6 and tables S1 and S2). However, restoring RNAi to *S. cerevisiae* profoundly affected maintenance of killer, an endemic viral system cytoplasmatically inherited as a double-stranded RNA (dsRNA) virus, L-A, and its satellite dsRNA, M (*2*). M encodes a protein toxin that kills nearby cells while conferring immunity to cells making the toxin, and L-A is required to maintain M (*3*). In the RNAi-competent *S. cerevisiae* strain, endogenous M and L-A dsRNAs were processed into small interfering RNAs and then lost in most cells (Fig. 1A and fig. S7), thereby rendering cells susceptible to killing by toxin from cells that retained killer (fig. S7).

The loss of killer in the RNAi-competent strain illustrated a circumstance in which the viral-defense function of RNAi, known to be beneficial in other contexts (*4*), imparted a net selective disadvantage. This disadvantage, when considered together with the relatively benign effects of losing RNAi in *S. castellii* (fig. S8), which lacks active transposons (*1*), suggested a model to explain why yeasts that lost RNAi have nonetheless succeeded in evolution: Descendents of cells that lose RNAi can acquire and retain killer, which under some circumstances more than offsets the disadvantage of losing RNAi. The killer system is observed in close relatives of *S. cerevisiae* (i.e., yeasts of the sensu stricto clade) (*5*, *6*) (fig. S9), whereas RNAi is absent in all sequenced sensu stricto species yet present in a close outgroup, *S. castellii*. Thus, in our model, loss of RNAi in a recent sensu stricto ancestor enabled one of its descendents to acquire killer, which provided a net selective advantage over its RNAi-containing, nonkiller, and toxin-sensitive neighbors, ultimately giving rise to *S. cerevisiae* and other sensu stricto species.

To test whether our model could apply throughout fungi, we performed whole-genome sequencing to identify RNAi genes in species with killer viruses and RNA analyses to search for dsRNA killer viruses in species with RNAi. RNAi was absent in all species known to possess dsRNA killer, whereas killer was absent in closely related species that retained RNAi (Fig. 1B, fig. S9, and tables S3 and S4). This incompatibility between RNAi and dsRNA killer extended even to *Ustilago*, an evolutionarily distant basidiomycete (fig. S10).

Analyses of synteny and phylogeny of Argonaute and Dicer proteins (fig. S11) indicated that the discontinuous presence of the RNAi pathway in fungi is best explained by its loss in at least nine independent lineages rather than by its acquisition through lateral transfer (Fig. 1B). At least four of these nine lineages included isolates with dsRNA killer viruses (Fig. 1B), which supports our model in which the ability of RNAi-deficient strains to host killer explains their evolutionary success. The other five lineages, for which dsRNA killer has not yet been reported in extant descendants, might have succeeded for other reasons. Alternatively, killer might have been acquired but then lost, just as some descendants of the sensu stricto radiation (including some *S. cerevisiae* strains) have lost killer (Fig. 1B and fig. S9). All nine RNAi losses were relatively recent, suggesting that individuals that lost RNAi earlier left no living descendants. Thus, although compatibility with the killer system can explain the persistence of RNAi-deficient fungal lineages for many millions of years, lineages that lose this elegant transposon defense might be doomed to extinction over the longer term.

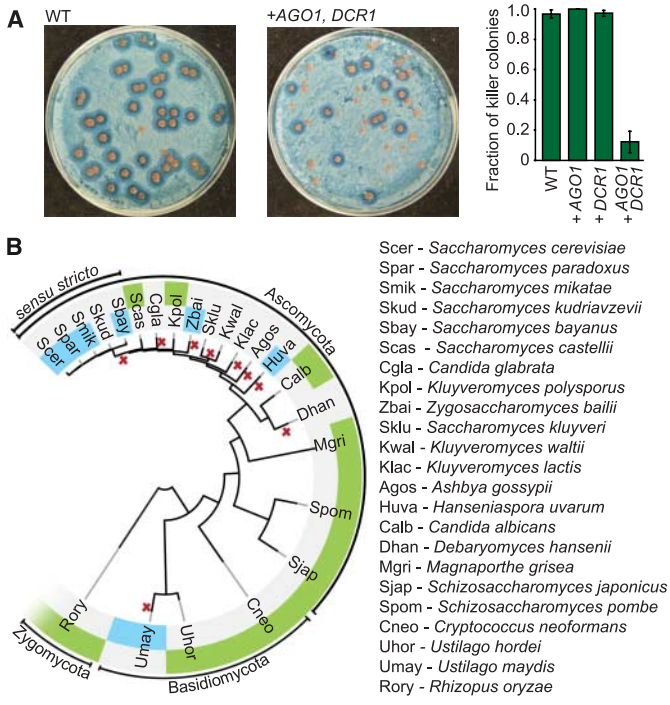

Fig. 1. Incompatibility between RNAi and killer. (**A**) Killer activity of the parental (WT) and RNAi-reconstituted *S. cerevisiae* (*+AGO1, DCR1*) strains. Killing-zone assays (photos) detected killing as clear halos surrounding colonies that had been transferred to plates with a lawn of a sensitive strain (*7*). Graphed (right) are results of four independent experiments (error bars, standard deviation). (**B**) Phylogenetic tree of representative fungal species, indicating the presence of dsRNA killer viruses (blue shading), the presence of RNAi (green shading), and inferred loss of RNAi (red x).

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/333/6049/1592/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 to S6
References (*8*–*46*)

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