FUNGAL GENETICS

Telomere transposon takeover in *Cryptococcus*

Identification and analysis of mutator strains in the human fungal pathogen *Cryptococcus neoformans* show that natural loss of RNA interference triggers massive accumulation of Cnl1 retroelements at subtelomeric regions.

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Evolution requires genetic change, yet as many mutations are deleterious to the fitness of an organism, there is not a simple relationship between mutation rate and adaptation. Furthermore, while mutation rates are known to vary between organisms, the causes and consequences of this are poorly understood in many systems, including medically important fungal pathogens. Such mechanisms may be germane to how key traits such as virulence and drug resistance evolve. It has been theorized that selection minimizes the mutation rate, while genetic drift, a non-adaptive mechanism, enables high mutation rates (produced by so-called mutator alleles) to become fixed in small populations[1]. Such a non-adaptive view does not rule out special cases in which the evolution of mutators can be adaptive in the short run (for example, under conditions of varying and strong selection) despite their long-term fitness costs[2]. In either scenario, one anticipates the presence of natural mutators in many populations.

In the microbial world, numerous examples of natural 'mutator' isolates have been found, most often due to loss of mismatch repair functions or via mutations in DNA polymerases. Within the fungal kingdom, the topic of this piece, a salient example comes from the yeast *Saccharomyces cerevisiae*, in which natural diploid isolates have been identified that display a defect in mismatch repair due to an incompatibility between alleles of a mismatch-repair protein complex rather than its wholesale loss[3]. This example is intriguing as it enables the defect in mismatch repair to be readily reversed through the loss of heterozygosity, a high-frequency event, suggesting that increasing mutation frequency is not always a one-way street. Despite the importance of mismatch repair and DNA polymerases in limiting mutations, there are other important anti-mutagenic mechanisms.

When they mobilize, transposable elements can produce mutations and have a major role in evolution[4]. In eukaryotes, mechanisms based on the Argonaute family of small RNA–binding proteins (referred to as RNA interference (RNAi)-related mechanisms) play a key role in suppressing transposon mobilization via the recognition of transposon RNA by a bound small RNA[5]. It is thus surprising that, despite the clear role of RNAi in suppressing mutagenesis by mobile elements, natural mutator alleles defective in RNAi have not been described in any microbe.

Cryptococcus neoformans is a basidiomycete yeast that is more closely related to mushrooms than the model ascomycete yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*. It is of high medical interest as an opportunistic pathogen that preys on highly immunocompromised individuals: *C. neoformans* is the most common cause of fungal meningitis and is responsible for a large fraction of deaths in HIV/AIDS[6]. Writing in *Nature Microbiology*, Priest and colleagues report the identification of natural isolates of *C. neoformans* that are defective in RNAi and harbour a remarkably high load of one specific non-long terminal repeat (LTR) retrotransposon[7]. This work demonstrates that the natural loss of RNAi can indeed lead to a mutator phenotype.

To identify strains with increased mutation rates, the authors surveyed a diverse collection of 387 isolates of *C. neoformans*. The authors plated these

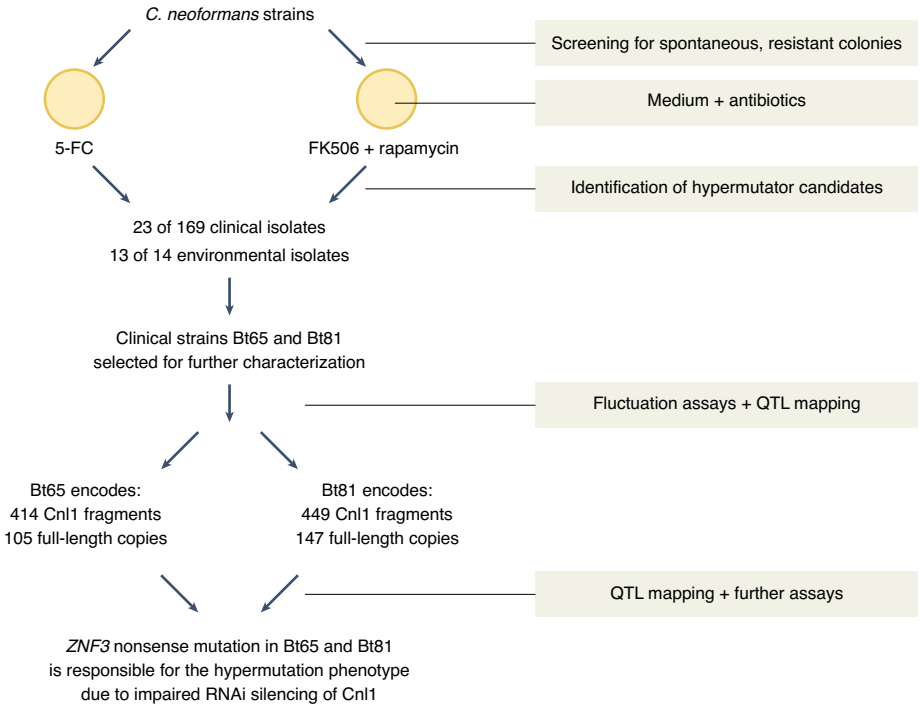

Fig. 1 | Flow diagram of screening approach and key findings. *C. neoformans* strains were screened for resistant colonies on agar plates containing either 5-flucytosine (5-FC), or rapamycin + FK506. The two clinical strains Bt65 and Bt81 were identified and selected for further characterization. Fluctuation assays and QTL mapping revealed that *ZNF3* nonsense mutation in both strains is responsible for the hypermutation phenotype owing to impaired RNAi silencing of Cnl1, a non-LTR retrotransposon.

isolates on media containing toxic levels of the antifungal drug flucytosine or the inhibitors rapamycin and FK506, which inhibit the TOR and calcineurin pathways, respectively, via the protein FKBP12 (Fig. 1). The authors identified dozens of mutator candidates and focused on two clinical *C. neoformans* isolates with the highest apparent mutation rate, Bt65 and Bt81.

Mutation rates in these isolates were significantly greater than in the reference strain, and only slightly less than in a mutant deficient in DNA mismatch repair. Importantly, resistance mutations in the hypermutator strains were largely due to insertions by the non-LTR Cnl1 retrotransposon, which can be found in multiple natural isolates but is largely non-functional in the *C. neoformans* reference strain H99.

Subsequently, by using mutation rate determined by Luria–Delbruck fluctuation analysis as a quantitative trait, Priest and colleagues compared segregants from crosses between the stable reference strain and mutator strains, identifying a significant quantitative trait locus (QTL) associated with a high mutation rate. Analysis of single nucleotide polymorphisms within this QTL revealed a promising candidate gene, *ZNF3*, encoding a C2H2 zinc finger protein of unknown molecular function identified previously to be required for RNAi[8]. Both hypermutator strains contained nonsense mutations in *ZNF3*, and complementation of the *znf3* mutant alleles by introduction of functional alleles from non-hypermutator strains decreased mutation rates. Concordantly, disruption of the RNAi pathway by deletion of the canonical components *AGO1* (encoding the sole Argonaute in *C. neoformans*) or *RDP1* (encoding the sole RNA-dependent RNA polymerase) were sufficient to drive increased mutation rates in the complemented strains. This finding is important because it indicates that RNAi, and not some other function of *ZNF3*, is responsible for the mutator phenotype.

Finally, long-read DNA sequencing revealed a striking feature of the two hypermutator strains: they harbour large Cnl1 arrays of up to 80 kb in length at nearly every subtelomeric region. Furthermore, among *znf3* segregants from hypermutator and reference strain crosses, Cnl1 appeared to rapidly invade naive subtelomeric regions. This finding suggests a plausible evolutionary order of events: loss of RNAi followed by mobilization. Like many transposable elements, Cnl1 appears to have a predilection for subtelomeric regions. This may provide the transposon with the ability to propagate itself without placing an undue mutational burden on its fungal host genome.

These findings by Priest and colleagues demonstrate the existence of a natural variation in RNAi that triggers massive accumulation of a specific transposable element, providing a mechanism by which genomes can quickly grow in size and, potentially, capabilities. More generally, the work illustrates the utility of investigating natural variation in a well-sampled species. Why a specific element is mobilized, how a large copy number is tolerated and whether this mobilization has an adaptive value in specific natural environments remain interesting questions for future studies. The powerful genetic toolbox available in *C. neoformans* makes it an outstanding system for addressing these questions. ❐

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Competing interests

The authors declare no competing interests.