PNAS COMMENTARY

Transposon load and RNAi loss synergize to drive intraspecies diversity in *Cryptococcus*

Elise Iracane[a] and Alessia Buscaino[a,1]

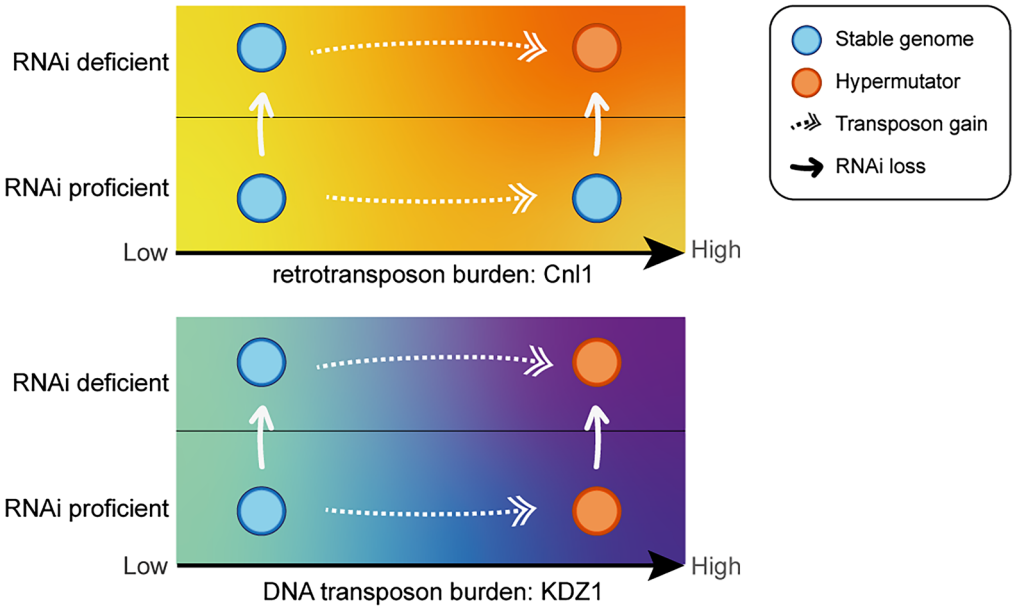

Fig. 1. Pathways enabling hypermutation in *C. neoformans*. The diagram illustrates two distinct pathways to hypermutation based on transposon type. For Cnl1 retrotransposons, only RNAi-deficient strains with high Cnl1 burden develop a hypermutator phenotype. In contrast, hypermutation due to KDZ1 DNA transposons occurs independently of RNAi status, with high KDZ1 burden leading to hypermutation regardless of RNAi proficiency.

Microbial organisms face relentless environmental pressures that demand rapid adaptation for survival (1, 2). This is especially true for pathogenic fungi that must adapt to extreme shifts when transitioning from natural niches to their human host, where they encounter immune defenses and antifungal drugs (3). In PNAS, Huang et al. investigate the evolutionary pathways leading to adaptation in the human fungal pathogen *Cryptococcus* (4).

Cryptococcus neoformans and *Cryptococcus deneoformans* are closely related environmental fungi that can infect humans, causing life-threatening infections in immunocompromised individuals. Treatment options for *Cryptococcus* infections are limited, and resistance to existing antifungal therapies is common, posing serious challenges for clinical management (5). During infection, *Cryptococcus* species must adapt to dramatic environmental changes as they transition from their natural reservoirs in soil and avian habitats to the human host (6). In challenging environments, hypermutator strains—microorganisms with elevated mutation rates—can provide short-term adaptive advantages, despite potential long-term fitness costs (7). The increased genetic variation generated by hypermutation enhances survival under fluctuating conditions and facilitates the acquisition of beneficial traits, including drug resistance (8). Central to this adaptability are transposable elements (TEs), mobile genetic elements that can produce mutations. However, uncontrolled TE activity can disrupt essential genes, imposing significant fitness costs. RNA interference (RNAi) plays a crucial role in suppressing TE mobilization in many organisms by degrading transposon-derived transcripts or seeding repressive chromatin structures (9). Accordingly, a previous study showed that two *C. neoformans* isolates lacking RNAi exhibit hypermutator phenotypes, characterized by elevated mutation rates due to amplification of the retrotransposon Cnl1 (*C. neoformans* LINE-1-like element). While these retrotransposons predominantly accumulate in subtelomeric regions, their integration into certain genes, such as *FRR1*, confers drug resistance (10).

Huang et al. investigate how the loss of RNAi and subsequent accumulation of TEs drive hypermutation and emergence of drug resistance in *Cryptococcus* species (4). Through screening of 387 *C. neoformans* isolates, the authors identified five additional RNAi-deficient strains, revealing that RNAi loss

Author affiliations: [a]Kent Fungal Group, School of Biosciences, Division of Natural Sciences, University of Kent Canterbury, Kent CT2 7NZ, United Kingdom

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[1]To whom correspondence may be addressed. Email: a.buscaino@kent.ac.uk.

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in this fungal pathogen is more prevalent than previously recognized. RNAi, an ancient mechanism conserved throughout eukaryotes, has been independently lost in several organisms (11). Examples include the model yeast *Saccharomyces cerevisiae*, the corn smut pathogen *Ustilago maydis*, and the parasitic protozoan *Trypanosoma cruzi*. RNAi has been lost in the reference strain of the human fungal pathogen *Candida albicans*, although most clinical isolates maintain an active RNAi pathway (12). The evolutionary significance of RNAi loss in certain *C. neoformans* isolates warrants further investigation. One hypothesis suggests that RNAi loss may have allowed several mycoviruses to persist in these species, which can provide benefits by producing toxins (13). While RNA viruses have not been observed in *Cryptococcus*, the presence of mycoviruses in RNAi-deficient *C. neoformans* isolates remains an open question with potential evolutionary implications.

In PNAS, Huang et al. investigate the evolutionary pathways leading to adaptation in the human fungal pathogen *Cryptococcus*

With access to additional RNAi-deficient *C. neoformans* isolates, Huang et al. analyzed how RNAi loss and TE burden contribute to hypermutation (4). Surprisingly, they found that RNAi deficiency alone is insufficient to drive a hypermutator phenotype—only RNAi-deficient strains with high Cnl1 TE burden displayed significantly elevated mutation rates. When RNAi-deficient strains were serially passaged under laboratory conditions, strains with low Cnl1 TE levels did not undergo transposon amplification and consequently did not exhibit hypermutation. However, strains combining RNAi loss with high Cnl1 transposon loads displayed marked increases in mutation rate, demonstrating that RNAi deficiency and TE accumulation have synergistic effects on hypermutation. These findings raise questions about why RNAi-dependent hypermutation only occurs in strains with high Cnl1 burden. The chromatin status associated with Cnl1 may play an additional role in regulating Cnl1 mobilization independently of RNAi. Cnl1 is located in subtelomeric regions that are assembled into heterochromatin and marked by DNA methylation, a specialized chromatin structure known to repress TE mobilization (9, 14, 15). It remains unknown whether heterochromatin controls Cnl1 mobilization in *C. neoformans* and whether this chromatin state differs between strains with high versus low TE burden.

One of the most impactful findings from Huang et al. is the remarkable diversity in mechanisms regulating TE mobilization, hypermutation, and drug resistance among isolates of the same species Fig. 1 (4). Not only is RNAi regulation of hypermutation dependent on Cnl1 copy number, but certain *C. neoformans* isolates also contain a DNA transposon, KDZ1, that appears to evade RNAi control and induce hypermutation even in RNAi-proficient strains. This illustrates the complexity of TE regulation in *Cryptococcus*, where control mechanisms vary widely between different transposable elements. The regulatory landscape of TEs is further diversified across *Cryptococcus* species. In agreement with previous results (16, 17), Huang et al. demonstrate that Cnl1 does not contribute to hypermutation and emergence of drug resistance in *C. deneoformans*, a close relative of *C. neoformans*. In *C. deneoformans*, while some TEs remain under RNAi control, others are active independently of RNAi pathways. Previous studies have shown that host-relevant stresses, such as elevated temperature, drive TE mobilization in *C. deneoformans* through RNAi-independent mechanisms (16, 17). Future studies should examine how the cross talk between different TE regulatory mechanisms varies across host microenvironments.

The findings from Huang et al. underscore two potential evolutionary trajectories for RNAi-deficient *Cryptococcus* strains (4). One pathway leads to hypermutation and potential drug resistance driven by high TE loads, while the other maintains genetic stability through low TE levels, avoiding hypermutation. This diversity has significant clinical implications, as TE-driven hypermutation poses a challenge in managing *Cryptococcus* infections in immunocompromised patients. Understanding TE regulatory mechanisms in *Cryptococcus* could inform future therapeutic strategies.

In conclusion, Huang et al. work highlights the remarkable diversity within and between *Cryptococcus* species, showing how multiple parallel mechanisms control TE mobilization to maintain genome stability while allowing for hypermutation and the selection of novel traits that drive evolution (4). Increasingly, it is clear that intraspecies diversity is a hallmark of fungal species (18–20) which we must consider in order to truly understand how fungi adapt to their environments, interact with hosts, and engage with microbial communities.

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