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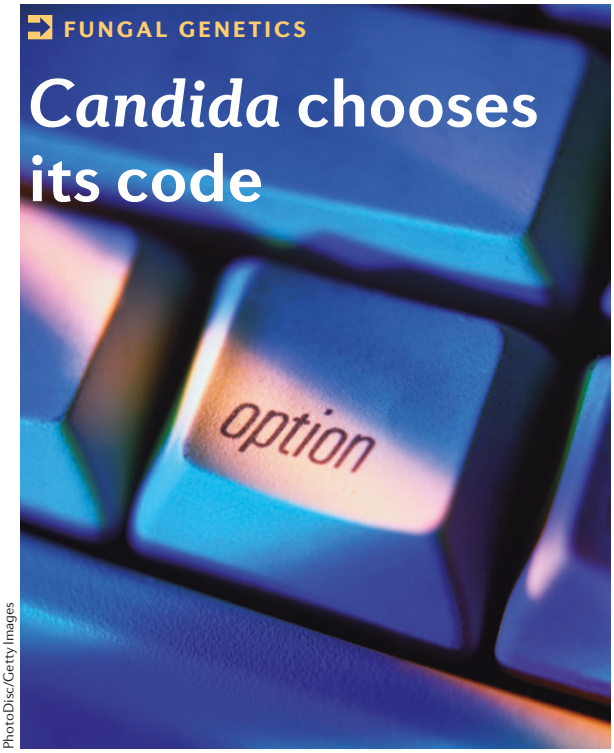
FUNGAL GENETICS

Candida chooses its code



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> genetic and phenotypic diversity … allows the fungus to adapt to its environment.

The theory of a universal genetic code was discarded when it was discovered that several organisms, including many fungi, have reassigned codons to non-standard amino acids. For example, in most organisms, CUG codes for Leu, but in *Candida albicans* it can also be translated to Ser. More than 60% of *C. albicans* genes contain CUG sites that are often decoded as Ser and only rarely as Leu. However, it was unclear whether such codon reassignments provide evolutionary advantages or represent neutral changes. A new study now shows that CUG ambiguity in *C. albicans* leads to more genetic and phenotypic diversity, which allows the fungus to adapt to its environment.

Mutations in tRNAs are a frequent source of codon reassignments; *C. albicans*, for instance, has a gene that encodes a non-standard Ser-incorporating tRNA ($tRNA^{Ser}_{CAG}$). The authors produced a panel of mutants in which one or two copies of a Leu-incorporating tRNA ($tRNA^{Leu}_{CAG}$) were added or replaced $tRNA^{Ser}_{CAG}$. Using a GFP reporter system, they determined that the levels of Leu incorporation varied from 1.5% for a control strain with two $tRNA^{Ser}_{CAG}$ copies to 98% for the mutant in which both $tRNA^{Ser}_{CAG}$ copies were replaced with $tRNA^{Leu}_{CAG}$. Leu levels inversely correlated with growth rates in the mutants, and Leu-incorporating mutants formed irregular colonies and showed altered cell morphology indicative of changes in the cell wall. These results suggest that the CUG^{Ser} reassignment provides a fitness benefit to *C. albicans*.

Why, then, has *C. albicans* retained the ability to incorporate Leu, albeit at low levels, and not switched completely to a CUG^{Ser} code? To detect potential advantages of Leu incorporation, the growth of Leu-incorporating mutants was determined under 30 different conditions, including different temperatures or the presence of antifungal drugs. No clear pattern in changes of growth was detectable, but in some conditions Leu incorporation was beneficial. Two mutant strains with intermediate Leu levels were resistant to fluconazole, a commonly used antifungal drug. The reason for the resistance was unclear, but genetic analysis showed that these two strains had mutations and loss of heterozygosity in genes involved in the calcineurin signalling pathway, which responds to fluconazole-mediated membrane stress. Interestingly, the mutant with almost complete Ser-to-Leu replacement showed the highest genetic diversity of all tested mutants, with many SNPs that probably represent compensatory mutations. This diversity affected genes involved in many processes, including filamentous growth and cell adhesion, both of which are important for virulence.

On the basis of this and the observed cell wall alterations, the authors hypothesized that CUG ambiguity affects *C. albicans* pathogenesis, because cell wall changes might affect the recognition of fungal pathogen-associated molecular patterns by innate immune cells. Consistent with this, Leu-incorporating mutants triggered increased production of pro-inflammatory cytokines by human monocyte-derived dendritic cells *in vitro* and, despite low fungal growth rates, caused severe inflammation in mice.

Thus, genetic ambiguity itself and the genetic changes that occur in response to it allow *C. albicans* to adapt to environmental factors such as host immune responses and antifungal drugs, and might represent a general evolutionary process.

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ORIGINAL RESEARCH PAPER Bezerra, A. R. *et al.* Reversion of fungal genetic code alteration links proteome instability with genomic and phenotypic diversification. *Proc. Natl Acad. Sci USA* http://dx.doi.org/10.1073/pnas.1302094110 (2013)