The role of non-standard translation in Candida albicans pathogenesis

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One sentence summary: Candida albicans uses the fidelity of protein synthesis to spawn novel virulence traits.

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Abstract

Candida albicans typically resides in the human gastrointestinal tract and mucosal membranes as a commensal organism. To adapt and cope with the host immune system, it has evolved a variety of mechanisms of adaptation such as stress-induced mutagenesis and epigenetic regulation. Niche-specific patterns of gene expression also allow the fungus to fine-tune its response to specific microenvironments in the host and switch from harmless commensal to invasive pathogen. Proteome plasticity produced by CUG ambiguity, on the other hand is emerging as a new layer of complexity in pathogenesis and epigenetic regulation. Niche-specific patterns of gene expression also allow the fungus to fine-tune its response to specific microenvironments in the host and switch from harmless commensal to invasive pathogen. Proteome plasticity produced by CUG ambiguity, on the other hand is emerging as a new layer of complexity in pathogenesis and drug resistance. Such proteome plasticity is the result of a genetic code alteration where the leucine CUG codon is translated mainly as serine (97%), but maintains some level of leucine (3%) assignment. In this review, we dissect the link between C. albicans non-standard CUG translation, proteome plasticity, host adaptation and pathogenesis. We discuss published work showing how this pathogen uses the fidelity of protein synthesis to spawn novel virulence traits.

Keywords: Candida albicans, non-standard translation, pathogenesis, drug resistance, genetic diversity, evolution

Introduction

The genetic code establishes the rules that govern the transfer of genetic information from nucleic acids to proteins. By establishing a unambiguous correspondence between codons and amino acids, the genetic code allows for stable inheritance of phenotypic variation produced by proteins upon which natural selection acts (Crick 1968). Despite this, numerous deviations to the standard genetic code have been discovered in both prokaryotes and eukaryotes (Knight, Freeland and Landweber 2001a; Koonin and Novozhilov 2009; Keeling 2016). Most of these alterations occurred in mitochondria due to their small genomes and independent translation machinery—ribosomes and tRNAs (Knight, Landweber and Yarur 2001b; Ling, O’Donoghue and Soll 2015). Although alterations in nuclear genomes are less common, they include sense and nonsense codon reassignments, as well as codon unassignments (Miranda, Silva and Santos 2006; Kollmar and Muhlhausen 2017), with sense-to-sense codon reassignments having the highest potential to boost proteome plasticity.

The only reported cases of sense-to-sense codon reassignment in nuclear genomes invariably involves the leucine CUG codon. The identity of this codon changed three times during the evolution of budding yeasts, with two clones translating the CUG codon as serine (CUG-Ser), one clone translating it as alanine (CUG-Ala) and two clones translating it canonically as leucine (CUG-Leu) (Krasowski et al. 2018). Among the CUG-Ala species are Pachysolen and Nakazawaeae, which are mostly used in biotechnological applications (Muhlhausen et al. 2016; Riley et al. 2016). Most species with biomedical relevance belong to the CTG-clade that comprise the most pathogenic Candida species (Fitzpatrick et al. 2006; Butler et al. 2009). Within this clade, C. cylindracea translates CUGs as serine only, while C. guilliermondii, C. dubliniensis, C. zeylanoides, C. tropicalis and C. albicans ambiguously decode the CUG codon as serine and leucine (Tuine and Santos 1996; M. A. Santos et al. 1997; Suzuki, Ueda and Watanabe 1997; M. A. Santos et al. 2011). Another example of dual codon identity occurs in the Ascoidea-clade species, where Ascoidea asiatica translates CUG stochastically as serine or leucine. In this species, residues encoded by CUG codons in key structural sites are strictly avoided (Muhlhausen et al. 2018).

Unlike A. asiatica, CUG-encoded residues may exist at functionally relevant positions in C. albicans and other CTG-clade spp. Most residues are located at the protein surface, where both leucine and serine can be incorporated without major impact on protein structure or function, but a few are located in positions where a serine or other polar amino acid is conserved in homologous proteins (Rocha et al. 2011). This is particularly intriguing because inaccurate production of proteins is generally viewed as a nuisance to biological systems (Kapur and Ackerman 2018; M. Santos et al. 2018). Nonetheless, recent data challenged these assumptions and strengthen the idea that protein mistranslation is a double-edged sword with potentially adaptive functions (Ribas de Pouplana et al. 2014; Schwartz and Pan 2017). Several studies have shown that, under stress, increased dual-translation of a codon enhances microbial fitness and modulates host-microbe interactions (Carlson et al. 2009; Netzer et al. 2009; Evans et al. 2018).
The concept of adaptive translation is greatly exemplified in C. albicans, where natural dual-translation of CUGs (which are normally translated as ~3% Leu and ~97% Ser but can change) expands the proteome exponentially (6438 CUG-containing genes can potentially encode 283 billion proteins, under a 50%–50% scenario) (Gomes et al. 2007; Rocha et al. 2011; M. A. Santos et al. 2011). The biological implications of this are profound, as each cell may contain a unique combination of protein molecules, generating an enormous biological complexity in this important human fungal pathogen. In this review, we dissect how protein diversity caused by variable translation of the CUG codon is emerging as a new key player in C. albicans adaptation, pathogenesis and drug resistance.

NON-STANDARD TRANSLATION IN CANDIDA SPP

At least 31 Candida spp. are known to cause disease in humans. Although the incidence of Candida spp. varies geographically, globally it is estimated that ~92% of the infections are caused by only five species: C. albicans, C. glabrata, C. tropicalis, C. parapsilosis and C. krusei (Pichia kudriavzevii) (Pfaller et al. 2010; Guinea 2014; Gabaldón et al. 2016). C. albicans is the most common pathogen isolated, but the prevalence of non-albicans species has been increasing in the past years. Other increasingly important causes of candidiasis include: C. lusitaniae, C. guilliermondii, C. dubliniensis, C. kefyr (Kluyveromyces marxianus), C. famata (Debaryomyces hansenii), C. incospicua, C. rugosa, C. dubliniensis, C. norvegensis (Pichia norvegensis) and the multi-drug resistant C. auris (Pfaller et al. 2010; Papon et al. 2013; Gabaldón et al. 2016, CDC 2019). Apart from C. glabrata, C. krusei, C. kefyr, C. norvegensis and C. incospicua, all Candida spp. listed belong to the CTG clade, a large group of yeasts that reassigned the leucine CUG codon (Butler et al. 2009; M. A. Santos et al. 2011; Papon et al. 2013; Du et al. 2020). Within this clade, C. albicans, C. zeylanoides, C. lusitaniae, C. tropicalis, C. parapsilosis, C. guilliermondii and C. rugosa naturally translate the leucine CUG codons as both serine and leucine (Suzuki, Ueda and Watanabe 1997; Gomes et al. 2007; M. A. Santos et al. 2011). This group of Candida spp. with dual CUG codon translation comprises most of the pathogenic Candida species, whose change of identity of the CUG codon is mediated by a novel tRNA$_{CAG}^{Ser}$ that contains identity elements for both the seryl- and leucyl-tRNA synthetases (SerRS and LeuRS, respectively). SerRS recognizes the 3 GC base pairs in the extra arm and the discriminator base (G73), while LeuRS recognizes A35 and m1G37 in the anticodon loop (red circles). The SerRS recognizes the discriminator base (G73) and three GC base pairs of the extra-arm (blue circles). Notably, the presence of G73, instead of U31, in the anticodon U-turn decreases leucylation efficiency of the tRNA$_{CAG}^{Ser}$ in vitro (Miranda, Silva and Santos 2006) This double recognition by LeuRS and SerRS leads to the synthesis of two aminoacyl-tRNAs (charged with Ser or Leu) that compete for CUG codons. (B) CUG decoding during mRNA translation. Under standard growth conditions, C. albicans translates approximately 97% of CUG codons as Ser and approximately 3% as Leu (Miranda, Silva and Santos 2006; Gomes et al. 2007). Figure created with Biorender.com.
To evaluate the impact of CUG codon ambiguity on the proteome, Rocha and colleagues conducted a deep structural analysis of proteins containing CUG-encoded residues (Rocha et al. 2011). The alignment of 680 protein sequences with CUG-encoded residues in C. albicans with orthologs of six other fungal species revealed that 90% of CUG codons are located at non-conserved positions. The same pattern was observed for the other CTG clade species analyzed, whereas CUGs in S. cerevisiae are evenly distributed in the protein sequences (Rocha et al. 2011). The distribution pattern of CUGs in the CTG clade species suggested that reintroduction of CUGs in the genomes, after the CUG reassignment, was selected to avoid protein misfolding caused by serine substitution for leucine. Proteins with CUG-encoded residues were uniformly distributed across diverse functional categories. However, the 10% of conserved CUG-encoded residues were mainly located in proteins involved in biological processes associated with virulence and pathogenesis, such as biofilm formation, mating, morphogenesis and adhesion. They also correlated with signal transduction, suggesting a pivotal role for CUG decoding ambiguity in pathogen-host interaction (Rocha et al. 2011).

To validate the functional impact of Ser-for-Leu substitutions, Rocha and colleagues determined the crystal structures of the two isoforms of SerRS (SerRS_Ser197 and SerRS_Leu197) (Rocha et al. 2011). SerRS has a CUG-encoded serine residue at position 197 located at the dimer interface. The Ser-for-Leu substitution induces a local rearrangement, which increases slightly (27%) the activity of the SerRS_Leu197 isomorph (Rocha et al. 2011, Robbins, Caplan and Cowen 2017). Similarly, Zhou and co-workers showed that the unique CUG-encoded residue present at position 919 in LeuRS is not conserved among eukaryotes, but is involved in tRNA binding and aminocylation. Ser-for-Leu substitution at the unique CUG site increased ~30% the LeuRS activity without causing major structural alterations (Zhou et al. 2013). These studies suggest that leucine incorporation at CUG sites could be regulated by a fine balance of the SerRS and LeuRS isoforms activity. However, further investigation is needed to clarify this issue as we are still far from understanding the molecular mechanisms that regulate CUG codon ambiguity.

Another example of the impact of CUG codon ambiguity on protein function is the C. albicans eukaryotic translation initiation factor (eIF4E). This cap-binding protein mediates mRNA binding to the ribosome and to translation initiation factors (Hinnebusch and Lorsch 2012). Feketová and colleagues showed that eIF4E has two variants due to CUG ambiguous decoding: eIF4E_Leu116 and eIF4E_Ser116 (Feketova et al. 2010). This residue is located in the surface of the protein, adjacent to the eIF4G binding region. Ser-for-Leu did not impair activity, but the eIF4E_Leu116 was temperature sensitive (Feketová et al. 2010), suggesting that C. albicans may reprogramme translation from CAP-dependent to CAP-independent mRNAs at high temperatures.

In another study, Leu-CUG translation decreased the stability and activity of the protein kinase Cek1 without major structural alterations. Cek1 contains a single CUG-encoded residue at a conserved position and is a key kinase of the MAPK cascade directly linked to morphogenesis in C. albicans. Incorporation of Ser at this CUG site induced the autophosphorylation of the conserved tyrosine residue of the Cek1 231TEY233 motif and increased its intrinsic kinase activity in vitro. Unlike the Ser-Cek1 variant, the Leu-Cek1 variant is not autophosphorylated at the 231TEY233 motif within the kinase activation loop (Fraga et al. 2019). Therefore, these in vitro studies demonstrate that Leu/Ser-CUG isoforms of C. albicans proteins can be functional despite some differences in activity and/or specificity. It is also possible that CUG ambiguous decoding could affect protein-protein interactions, disrupting existing networks or producing new ones.

As the CTG clade shows some variability in reassignments and C. albicans tolerates high level of ambiguity at CUG sites (Gomes et al. 2007), the question if C. albicans tolerates ambiguity at other codons arose. To answer this, Simões and colleagues tested if C. albicans tolerates codon ambiguity at other codons (Simões et al. 2016). For this, C. albicans strains were engineered to express tRNA_{CUG,ser} genes with mutated anticodons. Several mutant tRNA_{CUG} that were able to read codons encoding amino acids with different chemical properties, namely Leu, Ala, Gly, Lys, Thr and Tyr were engineered. This approach relied on the fact that eukaryotic seryl-tRNA synthetase (SerRS) does not recognize the tRNA Ser anticodon loop (Lenhard et al. 1999), so alterations in the anticodon do not affect tRNA seryl. In other words, each targeted codon was decoded by the mutant tRNA_{CUG,ser} and by its cognate tRNA. It is important to note that the codons targeted in this study follow a similar codon usage as CUG. Interestingly, expression of the recombinant tRNAs had little impact on fitness (Simões et al. 2016).

The high tolerance of C. albicans to codon ambiguity, alongside the functional consequences of Ser/Leu decoding mentioned above, raise the question of the overall implications of such ambiguity to Candida spp. biology. Recent studies provide evidence that CUG ambiguity may indeed be relevant for adaptation. The double identity of the CUG codon creates statistical proteins whose implications in the many facets of C. albicans virulence will be dissected in the following sections.

**PHENOTYPIC AND GENOMIC IMPACT OF CUG AMBIGUITY**

Quantification of Leu incorporation at CUG sites using an MS-based reporter system revealed that wild-type C. albicans cells can increase CUG codon ambiguity up to ~5%, when grown in low pH conditions (Gomes et al. 2007). Although small, an increase of incorporation of Leu at CUG sites from the basal 3% to 5% could generate an additional 4.2 × 10⁵ statistical polypeptides (Gomes et al. 2007). Remarkably, recombinant C. albicans strains encoding a yeast Leu tRNA_{CAG,Leu} and incorporating 28% Leu at CUG sites displayed very high morphologic diversity, including ovoid opaque cells, pseudo-hyphal and hyphal morphologies. The phenotypes observed in highly ambiguous cells were similar to the phenotypes that are induced by environmental signals, such as high temperature, low pH and serum. Expression of a mutant S. cerevisiae Leu-tRNA_{CAG,Leu} in C. albicans strains increased Leu incorporation at CUG sites and exposed hidden phenotypic variability, including the same morphological changes reported by Gomes and colleagues, but also enhanced activity of extracellular hydrolases (aspartic proteinases and phospholipases). High-level CUG codon ambiguity also induced mating by upregulating the key regulator of white-opaque switching, which was reflected on the increase of mating competent opaque cells. Furthermore, it revealed up-regulated expression of genes involved in cell adhesion and hyphal development (Miranda et al. 2007). This is in line with other data showing that CUG-encoded residues are enriched at conserved positions in proteins associated with biofilm formation, morphological switching, and adhesion (Rocha et al. 2011).

Miranda and co-workers also showed that C. albicans cells that incorporated 28% Leu at CUG sites had higher adherence to fibronectin and gelatin (Miranda et al. 2013). This is relevant because C. albicans is a successful commensal of mammalian hosts due to its capacity to adhere to host constituents and to avoid im-
CUG ambiguity accelerates acquisition of drug resistance

The increasing impact of *Candida* species on human health is mostly due to the existence of a limited number of approved antifungal drugs and the emergence of drug resistance. Currently, there are only five classes of antifungal agents: polyenes (amphotericin B), azoles (fluconazole, itraconazole, posaconazole, voriconazole and isavuconazole), echinocandins (caspofungin, micafungin and anidulafungin), allylamines (terbinafine) and antimetabolites (flucytosine). The scarcity of antifungals is partially explained by the conservation of eukaryotic gene functions between fungal pathogens and the human host (Perlin, Rautema-Richardson and Alastruey-Izquierdo 2017; Robbins, Caplan and Cowen 2017; Fisher et al. 2018), leading to a reduced number of viable safe targets. Of all classes, the most used against *Candida* infections belong to the azoles, mainly fluconazole, due to its great efficacy, low toxicity, bioavailability and low cost. However, the extensive use of fluconazole has resulted in increased resistance to azoles, turning therapies increasingly ineffective (Castanheira et al. 2017; Robbins, Caplan and Cowen 2017). Therefore, understanding the molecular mechanisms of drug resistance is of crucial importance for the medical community.

Several studies have identified two main molecular mechanisms of acquisition of azole resistance in *C. albicans*. First, overexpression of the drug target gene *ERG11* (14a-lanosterol demethylase) reduces the direct impact of the drug (Oliver et al. 2007). Second, increased efflux of the drug from cells by ABC transporters (encoded by *CDR1* and *CDR2*) (Coste et al. 2006) or by the major facilitator superfamily efflux pump (encoded by *MDR1*) (Dunkel et al. 2008) reduces the effective intracellular drug concentration. In both cases, such alterations can result from point mutations in genes encoding those proteins, in transcription factors regulating mRNA expression levels (Coste et al. 2006; Dunkel et al. 2008), or from increased copy number of the relevant genes, via genome rearrangements (Selmecki, Forche and Berman 2006).

The importance of epigenetic pathways in mediating drug resistance in fungi is also well established and involve tuning of a pre-programmed response without permanent genetic changes (Chang et al. 2019). Regulation of translational fidelity may act...
Similarly, as it affords the cell the opportunity of producing an entirely novel set of proteins from the same genetic background. Although most of these proteins will likely be neutral or deleterious in function, a small subset may acquire novel functions entirely. This is the case in bacteria, where Javid et al. showed that sequence variation of antibiotic targets results in resistant phenotypes (Javid et al. 2014). In this study, wild-type mycobacteria and strains with high and low translational fidelity were compared to investigate a direct role of mistranslation in antibiotic tolerance. Low translational fidelity strains had increased antibiotic tolerance and gene deregulation variations were distinctively different and broader in strains that incorporate Leu at high level. While the expression of genes of the ergosterol biosynthesis pathway, encoding the molecular target of fluconazole, was upregulated in both wild-type and high mistranslating resistant strains, the drug efflux pathway was affected differently (Weil et al. 2017). The wild-type strain acquired a known gain-of-function mutation in the transcription factor MRR1, a positive transcriptional regulator of the multidrug efflux pump gene MDR1 (Morschhauser et al. 2007; Dunkel et al. 2008). On the other hand, the high-level Leu-CUG translating strain acquired a previously described A736V gain-of-function mutation in TAC1, the transcriptional activator of the CDR1 and CDR2 genes, which encode the ABC transporters (Coste et al. 2006; Lobberger, Coste and Sanglard 2014). Taken together, and although the stochastic nature of mutations should be stressed here, these results indicated that both strains activated the traditional mechanisms of azole resistance, nonetheless through different drug efflux pathways. Also, the evolution of the hypermistranslating strain, in both the absence and presence of fluconazole, resulted in the fast accumulation of LOH events. A large LOH region in chromosome 5 (Chr5) appeared exclusively in the presence of fluconazole, which is consistent with previous studies of fluconazole-resistant C. albicans isolates (Selmecki, Forche and Berman 2006; Ford et al. 2015). Interestingly, analysis of the relative synonymous codon usage in genes affected by LOH events during evolution revealed three key aspects: (i) the number of CUG codons differed between alleles (a and b) of genes that lost heterozygosity; (ii) genes with a higher number of CUG codons in allele a than allele b (a > b) underwent a reduction in CUGs by changing them to other codons; (iii) genes with a lower number of CUGs in allele a than allele b (a < b) had an increase in the number of CUG codons by changing other codons to CUG. The same pattern was observed in copy number variation analysis of the evolved high-level Leu-CUG translating strain. This resistant strain showed loss of chromosomal repeat regions in Chr2, Chr4 and Chr6, and increased copies of Chr1, Chr4, Chr5 and ChrR. Within these amplified chromosomal regions were a large proportion of genes involved in both translation and drug resistance. Therefore allele-specific gene functions may contribute to rapid adaptation to fluconazole and CUG codon usage and translation seems to have a balancing effect on such genomic events (Bezerra et al. 2013; Kalapis et al. 2015; Weil et al. 2017).

CONCLUSION

The effect of codon ambiguity on phenotypic and genetic diversity of Candida spp. adds a new dimension to the study of genome evolution, phenotypic variation, ecological adaptation, drug resistance and virulence in humans. The direct impact of CUG ambiguity in C. albicans signaling pathways is still largely unexplored, but the accumulating evidence on phenotypes uncovered support the hypothesis that CUG ambiguity modulates host-pathogen interaction and immune responses and accelerates the evolution of...
drug resistance (Fig. 2). The implications are wide and should be further explored at the molecular level.

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**REFERENCES**

Amar A, Al Mamun M, Humayun MZ. Spontaneous mutagenesis is elevated in protease-defective cells. Mol Microbiol 2009; 71:629–39.

Balashov S, Humayun MZ. Mistranslation induced by streptomycin provokes a RecABC/RuvABC-dependent mutator phenotype in Escherichia coli cells. J Mol Biol 2002; 315:513–27.

Bezerra AR et al. Reversion of a fungal genetic code alteration links proteome instability with genomic and phenotypic diversification. Proc Natl Acad Sci 2013; 110:11079–84.

Brown AJ et al. Codon utilisation in the pathogenic yeast, Candida albicans. Nucleic Acids Res 1991; 19:4298.

Butler G et al. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature 2009; 459:657–62.

Carlson BA et al. Selenoproteins regulate macrophage invasiveness and extracellular matrix-related gene expression. BMC Immunol 2009; 10:57.

Castanheira M et al. Monitoring Antifungal Resistance in a Global Collection of Invasive Yeasts and Molds: application of CLSI Epidemiological Cutoff Values and Whole-Genome Sequencing Analysis for Detection of Azole Resistance in Candida albicans. Antimicrob Agents Chemother 2017; 61.

CDC. Antibiotic Resistance Threats in the United States. Department of Health and Human Services. Atlanta, GA, United States: U.S. Department of Health and Human Sources, Centers for Disease Control and Prevention. 2019.

Chang Z et al. Epigenetic mechanisms of drug resistance in fungi. Fungal Genet Biol 2019; 132:103253.

Coste A et al. A mutation in Tac1p, a transcription factor regulating CDR1 and CDR2, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in Candida albicans. Genetics 2006; 172:2139–56.

Crick FH. The origin of the genetic code. J Mol Biol 1966; 38:367–79.

Douglas LM, Konopka JB. Plasma membrane architecture protects Candida albicans from killing by copper. PLoS Genet 2019; 15:e1007911.

Du H et al. Candida auris: epidemiology, biology, antifungal resistance, and virulence. PLoS Pathog 2020; 16:e1008921.

Dunkel N et al. Mutations in the multi-drug resistance regulator MDR1, followed by loss of heterozygosity, are the main cause of MDR1 overexpression in fluconazole-resistant Candida albicans strains. Mol Microbiol 2008; 69:827–40.

Evans CR et al. Errors during Gene Expression: single-Cell Heterogeneity, Stress Resistance, and Microbe-Host Interactions. mBio 2018; 9.

Feketova Z et al. Ambiguous decoding of the CUG codon alters the functionality of the Candida albicans translation initiation factor 4E. FEMS Yeast Res 2010; 10:558–69.

Fisher MC et al. Worldwide emergence of resistance to antifungal drugs challenges human health and food security Science 2018; 360:739–42.

Fitzpatrick David A et al. A fungal phylogeny based on 42 complete genomes derived from superptree and combined gene analysis. BMC Evol Biol 2006; 6.

Ford CB et al. The evolution of drug resistance in clinical isolates of Candida albicans. Elife 2015; 4:e00662.

Fraga JS et al. Genetic code ambiguity modulates the activity of a C. albicans MAP kinase linked to cell wall remodeling. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 2019; 1867:654–61.

Gabaldon T, Naranjo-Ortiz MA, Marcet-Houben M. Evolutionary genomics of yeast pathogens in the Saccharomyces. FEMS Yeast Res 2016; 16.

Gomes AC et al. A genetic code alteration generates a proteome of high diversity in the human pathogen Candida albicans. Genome Biol 2007; 8:R206.

Guinea J. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect 2014; 20:5–10.

Heilmann CJ et al. Surface stress induces a conserved cell wall stress response in the pathogenic fungus Candida albicans. Eukaryotic Cell 2013; 12:254–64.

Hinnebusch AG, Lorsch JR. The mechanism of eukaryotic translation initiation: new insights and challenges. Cold Spring Harb Perspect Biol 2012; 4.

Javid B et al. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. Proc Natl Acad Sci 2014; 111:1132–7.

Kalapis D et al. Evolution of Robustness to Protein Mistranslation by Accelerated Protein Turnover. PLoS Biol 2015; 13:e1002291.

Kapur M, Ackerman SL. mRNA Translation Gone Awry: translation efficiency for rifampicin phenotypic resistance. Proc Natl Acad Sci 2014; 111:1132–7.

Keeling PJ. Genomics: evolution of the Genetic Code. Curr Biol 2016; 26:R851–R3.

Knight RD, Freeland SJ, Landweber LF. Rewiring the keyboard: evolvability of the genetic code. Nat Rev Genet 2001a; 2:49–58.

Knight RD, Landweber LF, Yarus M. How mitochondria redefine the code. J Mol Evol 2001b; 53:299–313.

Kollmar M, Muhlhäuser S. Nuclear codon reassignments in the genomics era and mechanisms behind their evolution. Bioessays 2017; 39.

Koonin EV, Novozhilov AS. Origin and evolution of the genetic code: the universal enigma. IUBMB Life 2009; 61:99–111.

Krassowski T et al. Evolutionary instability of CUG-Leu in the genetic code of budding yeasts. Nat Commun 2018; 9:1887.

Lenhard B et al. tRNA recognition and evolution of determinants in seryl-tRNA synthesis. Nucleic Acids Res 1999; 27:721–9.

Ling J, O’Donoghue P, Soll D. Genetic code flexibility in microorganisms: novel mechanisms and impact on physiology. Nat Rev Microbiol 2015; 13:707–21.

Lohberger A, Coste A, Sanglard D. Distinct roles of Candida albicans drug resistance transcription factors TAC1, MRR1, and UPC2 in virulence. Eukaryotic Cell 2014; 13:127–42.

Massey SE et al. Comparative evolutionary genomics unveils the molecular mechanism of reassignment of the CTG codon in Candida spp. Genome Res 2003; 13:544–57.
