The epitranscriptome as an underappreciated regulatory layer in human fungal pathogens

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Genetic mutation and epimutation mechanisms are clear drivers of antifungal drug resistance, yet the role of RNA modifications (i.e., the epitranscriptome) remains relatively untested regarding resistance or even general stress response. Here, deletion of the *Aspergillus fumigatus* tRNA-modifying isopentenyl transferase ortholog, Mod5, led to altered stress response and unexpected resistance against the antifungal drugs 5-fluorocytosine (5-FC) and 5-fluoroorotic acid (5-FOA), but not other common antifungals. Simultaneous profiling of transcriptomes (mRNA-seq and nano-tRNA-seq) and proteomes (LC-MS/MS) revealed no changes to the canonical 5-FC stress response pathways; however, an upregulation of known efflux pumps was observed. The promoters of these efflux pumps harbored transcription factor binding sites for the cross-pathway control (CPC) gene CpcA, a Gcn4 ortholog, which is known to coordinate the response to hypomodified tRNAs. In fact, *cpcA* was prematurely upregulated in the *mod5* knockout compared to the wild type, and deletion of *cpcA* in the *mod5* deletion strain reverted the resistance phenotype as well as some stress defects. Intriguingly, induction of histidine starvation with 3-amino-1,2,4-triazole also facilitated 5-FC resistance, suggesting that conditions where CPC is activated may result in transient 5-FC resistance. Future work to understand how RNA modifications themselves change globally in response to stress and drug treatments in *A. fumigatus* is now underway.