

Pearls

Regulatory Circuits That Enable Proliferation of the Fungus *Candida albicans* in a Mammalian Host

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A Rich Fungal Microbiome within Us

The human body harbors trillions of microorganisms—a rich and diverse microbiota that plays key roles in human health and disease. All three domains of life—eukaryotes, archaea, and bacteria—are represented in this large assembly of microbes. Among eukaryotes, fungi are particularly prominent residents of the human body. For example, 101 species belonging to 85 fungal genera have been found in the oral cavity of healthy people [1]. Similarly, >200 fungal species (half of them novel or unnamed) representing at least 50 genera have been found in the murine gut [2]. By comparison, there are ~150 bacterial species (or phylotypes) inhabiting the mouths of healthy individuals [3] and ~500–1000 bacterial species in their intestines [4,5]. Although the methodologies differ, as do the classification schemes, it seems that the taxonomical diversity of the fungal component of the microbiota may not be so different from its bacterial counterpart, even though the latter has been much more heavily catalogued. With the exception of a handful of species, however, very little is known about the biology of the members of the human “mycobiome” (*i.e.*, fungal microbiome) and even less about the interactions that they establish with the human host.

In this review we focus on the most widely studied fungus that inhabits the human body, *Candida albicans*. Most, if not all, healthy adults carry this species asymptotically in their gastrointestinal (GI) tract. Despite its status as a commensal microorganism, *C. albicans* is also a leading cause of mucosal disease in healthy hosts and systemic, life-threatening infections in individuals with debilitated immune systems, such as AIDS patients or people receiving chemotherapy. Not surprisingly, it is this “virulent” or “pathogenic” facet of *C. albicans* that has traditionally received the most attention from researchers. More recently, however, several laboratories have begun to explore the tactics that the fungus uses to proliferate in its more common niche, the mammalian gut. These studies have uncovered unexpected connections between the commensal and pathogenic lifestyles. We highlight some of these findings here.

Regulation of Nutrient Acquisition Is Pivotal for Colonizing the Mammalian Gut

The gut is a crowded environment. In the colon alone, *C. albicans* cohabits with ~10¹¹ microbial cells per milliliter of intestinal content [6]. Food resources may be plentiful but competition is obviously high (reviewed in [7]). The ability of *C. albicans* to colonize and proliferate in the GI tract has been studied in mice receiving antibiotics orally; while these animals are not natural hosts of *C. albicans*, they likely serve as reasonable proxies. Because transcription regulators are central elements within the gene network of any organism, the use of them as entry points to the dissection of this trait has proved an effective strategy. A recent screen carried with *C. albicans* in a mouse model of GI tract colonization [8] evaluated 77 transcription regulator mutants for their ability to endure in the intestine for several weeks after oral

inoculation. This group of mutants was chosen because none of them showed overt defects under a wide variety of conditions *in vitro* [9], hence they would be candidates for regulating circuits needed specifically *in vivo*. The screen identified six transcription regulators required for *C. albicans* to colonize the murine gut, four of which (*RTG1*, *RTG3*, *TYE7*, and *LYS144*) controlled the expression of genes responsible for the acquisition and metabolism of nutrients, particularly carbon and nitrogen sources. A significant fraction of the target genes of these regulators (identified by full-genome chromatin immunoprecipitation experiments) are indeed upregulated in *C. albicans* cells growing in the mouse intestine [10]. It is noteworthy that none of the four regulators is required for the species to grow under standard laboratory conditions [9], which reinforces the notion that significant resources are devoted to simply obtain food in the GI tract. It is plausible that these stringent conditions are set, at least in part, by the presence of a competing gut microbiota; consistent with this idea, genes that enable murine gut colonization by intestinal microbes such as *Citrobacter rodentium*—a natural mouse pathogen—are not required to colonize the intestines of germ-free mice [11].

Managing Iron Toxicity in the Mammalian Gut

Iron is an essential nutrient for microorganisms but at high levels it can become extremely toxic. The abundance and availability of iron vary greatly in different locales of the human body: relatively high levels (~10⁻⁴ M) are found in the GI tract as the majority of dietary iron is not absorbed [12], whereas the concentration in the bloodstream is orders of magnitude lower (~10⁻²⁴ M free Fe³⁺) [13]. *C. albicans*, an organism that typically inhabits the gut but that can also cross into the bloodstream, harbors a regulatory circuit composed of three transcription regulators (*SFUI*, *SEFI*, and *HAP43*) that controls the expression of iron uptake genes and iron utilization genes [14]. *SFUI* is required to persist in the GI tract [14] and for resistance to iron

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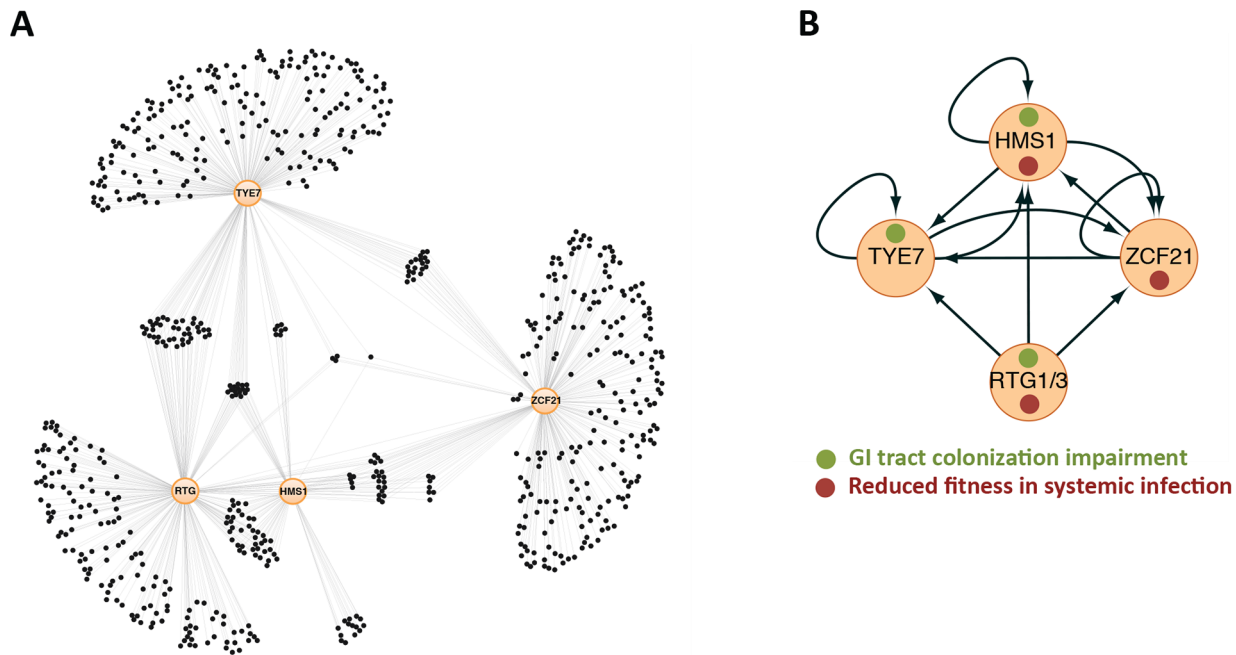


Figure 1. Gene regulatory network directing *C. albicans* proliferation in a mammalian host. (A) Gene network composed of the transcription regulators *RTG1/3*, *HMS1*, *ZCF21*, and *TYE7* (orange circles) and their target genes (black circles) as determined by full-genome chromatin immunoprecipitation. Thin lines indicate binding of the specified regulator to a target gene. About a quarter of the genes in the network (those in the middle) are targets shared by two or more regulators. (B) Relationships among the “master” regulators at the core of the network. Arrows represent protein-DNA interactions. Notice that the circuit displays multiple autoregulatory, feed-forward, and feedback loops. Adapted from [8]. doi:10.1371/journal.ppat.1003780.g001

toxicity *in vitro* [9], suggesting that the primary role of this circuit in the gut is to protect *C. albicans* from iron’s noxious effects. *SEF1*, on the other hand, is required not only for the fungus to endure in the intestine but also for full virulence after bloodstream infection [14]. Thus, *C. albicans* uses a common circuitry to balance its need to uptake iron in one niche (*i.e.*, in the bloodstream) and protect itself from its toxic effects in other locale (*i.e.*, the GI tract).

In addition to circuits that govern carbon, nitrogen, and iron acquisition, gene products that function as adherence molecules [15] and in the detoxification of reactive oxygen species [16] have also been shown to play roles in gut colonization. In fact, *ECE1* and *SOD5*, which influence adhesion and protection against reactive oxygen species, respectively, are among the most highly upregulated genes when *C. albicans* is growing in the GI tract compared to standard laboratory conditions [15,16]. Host cells such as macrophages and neutrophils produce reactive oxygen species, raising the possibility that *SOD5*’s role may be to defend the fungus from these molecules [16] during colonization of the GI tract. Other transcription regulators reported to play roles in gut colonization have been shown to be rather pleiotropic (*e.g.*, *EFG1* [17], *CPH2* [10], and *EFH1* [15]).

A Molecular Basis for the Interplay of Commensalism and Pathogenicity

Many systemic, life-threatening infections in humans are caused by the very same bacteria or fungi that compose their microbiota. That is, these opportunistic pathogens reside in the host as harmless commensals (typically on mucosal surfaces) but can cross the host’s protective barriers and colonize internal organs causing serious disease. The GI tract, for instance, appears to be the ultimate source of the majority of deep-seated *C. albicans*

infections [18,19]. Do entirely different gene sets account for the seemingly disparate behaviors of *C. albicans* as commensal versus pathogen?

Observing phenotypes on a gene-by-gene basis provides a mixed answer. On the one hand, transcription regulators such as *EFG1* [16,17], *SEF1* [14], *RTG1*, *RTG3*, and *HMS1* [8] are necessary for full virulence in mouse models of systemic infection (*i.e.*, as a pathogen) as well as for GI tract colonization (*i.e.*, as a commensal). On the other hand, mutant strains lacking the regulators *TEC1* or *LYS14* show reduced virulence in models of systemic infection but display no obvious defect in colonizing the murine GI tract [8,10,20]. The converse is true for *C. albicans* strains lacking *TYE7* or *SFUI* or ectopically expressing *EFH1* [8,14,15]. However, genes do not function in isolation, and a clearer picture emerges by considering the existing links among some of these regulators.

Five transcription regulators that displayed a significant phenotype “exclusively” in association with the host [8] form a tightly knit circuit (Figure 1). In this circuit, the regulators are connected with one another irrespective of whether they are required for GI-tract colonization, systemic infection, or both. This observation implies that significant portions of the “pathogenic” and “commensal” lifestyles are coupled and controlled by the same core circuitry. Thus, from a gene network perspective, commensalism and pathogenicity do not appear to represent fully independent traits but rather are intertwined. The close links between these two traits may reflect the natural history of *C. albicans*: its association with mammals is ancient [21] and the selection pressure on the fungus has likely been as a commensal organism. Thus, it is plausible that the functions employed by *C. albicans* to spread from the human gut into systemic infections rely on the same regulatory circuitry that evolved to enable growth in the host as a commensal organism. Consistent with this idea, some

impairment in the host immune system is typically required for *C. albicans* to be fully pathogenic.

Commensalism/Pathogenicity Transcriptional Circuit in *C. albicans* Has Structure in Common with Circuits Underlying Cell Differentiation

It has been shown that at the heart of the ability of *C. albicans* to proliferate in the mammalian host lies a highly interconnected transcriptional circuit composed of five transcription regulators (Figure 1). The distinctive feature of this circuit is the existence of multiple connections among all its components; that is the “master” regulators control one another (in addition to their target genes), and the target genes are typically bound by multiple master regulators. Thus, the circuit has many feed-forward and feedback loops. By contrast, many “textbook” genetic circuits—those that most scientists have grown used to—consist of simple, unidirectional relationships among their components, making their behavior more predictable. Because of the distinctly interwoven appearance of the *C. albicans* circuit, it is difficult to predict its behavior without extensive knowledge of the parameters (*e.g.*, concentration of proteins, affinity constants, and the like). It is noteworthy that the overall topology resembles other circuits known to direct well-established cell differentiation processes such as white-opaque switching [22] and biofilm development [23] in *C. albicans*, filamentation in *S. cerevisiae* [24], and embryonic

development in metazoans (*e.g.*, see [25]). The fact that disparate functions in divergent organisms rely on a similar network architecture suggests that these transcriptional circuits may share common, underlying features. It is possible, for example, that the network structure enables the integration of multiple internal and external cues to then specify the precise pattern of target gene expression.

Conclusion

The study of opportunistic pathogens such as the fungus *C. albicans*, which can proliferate in disparate niches of the host either as a commensal or a pathogenic organism, allows us to genetically dissect the features of these two ways of life as well as to reveal the links between them. The results thus far suggest that there may be no clear distinction between the genetic circuitries employed during “harmless” proliferation in the gut or “disease-causing” growth after bloodstream infection. Rather, a single highly interconnected transcriptional circuit (one whose structure resembles others that orchestrate cell differentiation) may govern proliferation in all niches of the mammalian host.

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