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[1]Department of Psychology, Yale University, New Haven, CT 06520, USA. [2]Duke Institute for Brain Sciences and Center for Cognitive Neuroscience, [3]Department of Neurobiology, Duke University School of Medicine, Durham, NC 27710, USA. [4]Department of Psychology and Neurosciences, [5]Department of Evolutionary Anthropology, Duke University, Durham, NC 27708, USA.
*E-mail: steve.chang@yale.edu

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Biofilms: Five-Star Accommodations for the Aerobically Challenged

The human microbiome contains a diverse array of microbes that affect human health. A recent study finds that fungal biofilms are capable of supporting growth of anaerobic bacteria, suggesting that these fungi can promote bacterial growth in otherwise toxic environments.

Robert A. Cramer

Environmental oxygen levels have changed significantly over the course of Earth's history and consequently organism oxygen requirements vary throughout the tree of life [1,2]. Oxygen serves as the key electron acceptor in the generation of chemical energy via mitochondrial respiration in most eukaryotes and is essential for biosynthesis of macromolecules such as sterols and porphyrins. Oxygen is also the source of toxic reactive oxygen species that can damage cells and tissue. In fact, many prokaryotic and eukaryotic microbes live anaerobic lifestyles and do not rely on oxygen to grow. For many of these anaerobes, exposure to oxygen is lethal. As a result, oxygen levels are an important factor in determining the composition of a microbial community.

Pioneering research on microbial communities of the human body, collectively termed the microbiome, has revealed the importance of microbes in human health [3,4]. While the majority of human microbiome research has focused on identifying and quantifying bacterial species, it is becoming clear that fungi are also significant components of these microbial communities [5]. As bacteria and fungi are present together in many ecological niches in the human body, a rich opportunity exists for cross-kingdom microbial interactions [6]. Important ongoing research on these cross-kingdom interactions in various compartments of the human body strongly suggests their impact on human health is significant [7]. A particularly clinically relevant question is whether fungal–bacterial interactions can alter the ability of microbes to colonize specific environments of the human body. If so, the spectrum of disease, and subsequent treatments of those diseases, could be directly impacted.

In this issue of *Current Biology*, a report by Fox *et al.* [8] shows that fungal biofilms can support the growth of anaerobic bacteria that would otherwise be too oxygen-rich for these anaerobes (Figure 1). Fox *et al.* focus on *Candida albicans*, a common fungal commensal of mammalian mucosal surfaces including the gastrointestinal (GI) tract [8]. *C. albicans* is well known for its ability to grow as yeast, pseudohyphae or hyphae depending on environmental conditions [9]. In otherwise healthy individuals, *C. albicans* is not often a major agent of infectious disease. However, perturbations to the host immune system and/or microbiome can lead to disease caused by *C. albicans* [10]. As one example, studies in mice reveal that alterations in the microbiome result in increased susceptibility to disease caused by *Candida* [11]. Perhaps more commonly experienced, it is documented that antibiotic usage can increase yeast growth in the female reproductive tract. Consequently, it is clear that bacterial–fungal interactions play a critical role in human health [6,7].

A major feature of *C. albicans* biology that impacts human health resides in its ability to form biofilms [12]. Biofilms are complex, heterogeneous communities of microbes encased in a polysaccharide-rich extracellular matrix that attaches to biotic and abiotic surfaces [13,14]. Their association with human disease is significant as up to 80% of human microbial infections are suspected to result from biofilms according to the National Institutes of Health. Biofilms are often resistant to antimicrobial therapies and provide sources for dissemination of pathogenic microbes throughout the human body. Importantly, biofilms contain complex microenvironments that influence the metabolism and biology of the resident cells, which often consist of multiple species of microbes [15].

While it has previously been shown that bacterial biofilms can promote growth of different bacteria, Fox *et al.* [8] report for the first time that fungal biofilms can also support growth of two obligate anaerobes found in the human GI tract, *Clostridium perfringens* and *Bacteroides fragilis* [16]. As *C. perfringens and B. fragilis* are anaerobes with differing degrees of tolerance to oxygen, the authors' hypothesized that *C. albicans* biofilms contain regions of reduced oxygen levels, or hypoxic microenvironments. Previous studies on bacterial biofilms have shown the presence of hypoxic microenvironments within the biofilm, yet in fungal biofilms this has remained speculative. Gene expression and examination of genetic null mutants in *Candida* species forming biofilms has strongly suggested that fungal biofilms contain regions with low oxygen tension, and moreover it has also been shown that key transcriptional regulators of the fungal hypoxia response contribute to biofilm formation [17,18].

Fox *et al.* [8], however, provide direct support for the occurrence of zones with low oxygen in *C. albicans* biofilms by quantifying oxygen levels throughout the fungal biofilms with a miniaturized Switch-able Trace Oxygen Sensor capable of detecting oxygen concentrations as low as 10 nM. Within these fungal biofilms oxygen was present in a gradient from 300 μM at the top of the biofilm to less than 50 μM at the bottom. Consequently, *C. perfringens* and *B. fragilis* were able to survive within these fungal biofilms even when the co-cultures were prepared in the presence of oxygen concentrations normally inhibitory to growth of these bacteria.

Perhaps even more intriguingly, *C. perfringens* induced aggregation of *C. albicans*, which Fox *et al.* define as 'mini-biofilms', that allowed *C. perfringens* to survive in an otherwise oxygen-replete environment. The authors propose that these aggregations expand the definition of a fungal biofilm from beyond a surface-attached community to include these suspended aggregates. The strongest support for this conclusion comes from the observation that the master transcriptional regulators of the surface attached biofilm genetic program, including Brg1, Tec1, Rob1, Bcr1, Ndt80, and Efg1 [19], are largely required for aggregate formation.

Regardless of whether these aggregates can be considered true biofilms, they support survival of anaerobic bacteria. While oxygen is almost certainly playing a major role in fungal biofilm-mediated bacterial survival, it is unclear if the fungal biofilm also contributes other critical molecules that allow these anaerobic bacteria to survive and proliferate. This is an important question considering that preventing anaerobic bacterial growth in fungal biofilms may prove to be clinically important, particularly in the context of polymicrobial infections such as those often encountered in chronic wounds [15]. Presumably, if factors in addition to oxygen are enhancing bacterial growth, their depletion from the infection site microenvironment could augment existing treatment strategies.

Intriguingly, while the anaerobic bacteria and *C. albicans* interactions did not significantly alter the structure of the fungal biofilm, bacteria commonly found in the GI tract that are less sensitive to oxygen reduced the overall thickness of the fungal biofilm. These bacteria, including *Klebsiella pneumonia* in particular, induced expression of the master regulator of the *C. albicans* white–opaque phenotypic switch WOR1, suggesting a key role for this important transcription factor in fungal–bacterial interactions. Does altering the thickness of the fungal biofilm represent a strategy by bacteria to increase/decrease oxygen availability to meet their specific metabolic growth requirements? What role does WOR1 specifically play in the interactions between *Candida* and gut-associated bacteria? An answer to this question will require more in-depth investigation into the underlying mechanisms driving these fungal–bacterial interactions. The authors were able to show that cell-free culture supernatants or heat-killed *C. perfringens* were able to induce aggregation of *C. albicans*; however, the signals and receptors involved in the fungal–bacterial interactions under study remain to be elucidated. At least with regard to the *C. perfringens*–*C. albicans* interaction, the reported results strongly suggest that unknown molecules found within or on bacteria can be detected by *C. albicans* and induce the biofilm genetic program. Given the importance of *C. albicans* biofilms in disease, discovering these underlying mechanisms could lead to development of a novel therapeutic targeted at blocking induction of the biofilm-inducing genetic program.

Finally, as alluded to above, while the results presented are from *in vitro* studies, there are significant human health implications for these findings that warrant further investigation. For example, how does growth in a fungal biofilm affect the virulence and drug resistance of these bacteria? How does the presence of specific bacteria alter fungal physiology, virulence, and drug responses? And how do these polymicrobial interactions affect immune responses and tissue healing? Moreover, while these binary interactions lend themselves to experimental approaches to uncover mechanism, it is unclear how fungal biofilm formation and bacterial–fungal interactions would play out *in vivo* when signals from multiple species are being integrated into the response. These interactions, of course, are critical in determining the localized composition of microbial communities in the human body, and it will be fascinating to uncover the underlying mechanisms that ultimately yield the ecology of the microbiome in a given individual that impacts human health and disease.

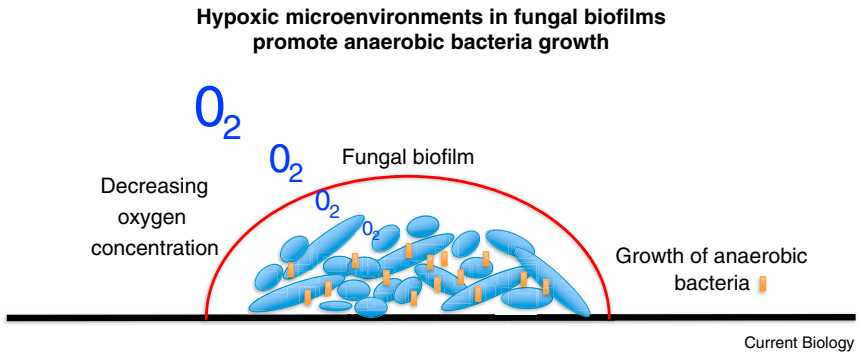

Figure 1. Fungal biofilms contain low oxygen (hypoxic) regions that support anaerobic bacterial growth.

Biofilms formed by the fungal pathogen *Candida albicans* are heterogeneous with multiple morphological forms present including yeast (blue round ovals), pseudohyphae (slightly elongated blue ovals), and hyphae (fully elongated hyphae). An extracellular matrix largely composed of polysaccharides encompasses the biofilm (red outline, white interior). As one moves from the exterior of the biofilm colony to the interior near the surface (black) the oxygen gradient drops dramatically, allowing proliferation and survival of anaerobic bacteria such as *Clostridium perfringens* (orange rods).

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Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, 74 College Street Remsen 213, Hanover, NH 03755, USA.
E-mail: Robert.A.Cramer.Jr@dartmouth.edu

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Cellular Energetics: Actin and Myosin Abstain from ATP during Starvation

Energy is required for all cellular functions and diverse mechanisms allow the cell to overcome acute energy starvation. A new study reveals that starved cells inhibit type V myosins and actin depolymerization, measures that may conserve energy while temporarily retaining cell polarity.

Destiney Buelto[1] and Mara C. Duncan[2,*]

Energy starvation at the cellular level is surprisingly common. In multicellular organisms, energy starvation occurs during heart attack, stroke, hypoglycemia, and in highly proliferative cancers. In microbes, energy starvation occurs whenever the microbe encounters a nutrient-poor environment. Even after decades of research, new aspects of starvation biology are still emerging. In this issue of *Current Biology*, Xu and Bretscher [1] expand the cellular repertoire of starvation responses by identifying two previously unknown responses to glucose starvation — inhibition of myosin V function and inhibition of actin turnover.

Starvation elicits diverse responses, which are frequently ancient, highly conserved and, importantly, dictate whether a cell survives or dies in response to acute starvation [2]. These responses allow the cell to access alternative sources of energy, enter quiescence or engage in programmed cell death, which may provide an advantage for the organism or population [3,4]. Each of these responses requires consumption of energy and other raw material upfront before any benefits are gained. For example, amino acids and ATP are needed for the synthesis and function, respectively, of transporters that allow the cell to access alternative exogenous energy sources. Similarly, quiescence and programmed cell death also require energy [2,5]. Accordingly, energy starvation immediately inhibits processes with high-energy demands, such as translation, which consumes four ATP equivalents per amino acid added, and plasma membrane ATPase activity, which can consume enormous amounts of ATP [6,7]. The prompt shutdown of these processes conserves raw material that the cell needs for other uses. In the absence of exogenous sources, the cell can also produce limited raw material for quiescence by consuming stored carbohydrate reserves, as well as cell-surface and cytosolic components [4,8,9]. Starvation responses thus either provide necessary nutrients or perform activities needed for long-term survival of the organism or population.

The new work from Xu and Bretscher [1] adds to our understanding of the responses to acute energy starvation in eukaryotes. It follows the previous observation that the cortical actin cytoskeleton becomes depolarized upon glucose starvation in the yeast *Saccharomyces cerevisiae* [10]. In yeast, cell polarity depends on actin cables, which are functionally distinct from the endocytic cortical actin cytoskeleton [11]. Actin cables are long bundles of many short actin filaments aligned with the fast-growing end oriented towards the site of growth (Figure 1A) [11]. Actin cables act as tracks for type V myosins, Myo2 and Myo4, which direct polarized delivery of secretory vesicles, organelles, and RNA. This polarized delivery of secretory vesicles is required to maintain cell polarity [12]. Thus, the transient depolarization of the cortical cytoskeleton was thought to reflect the depolarization of the actin cables and the subsequent loss of polarized delivery of secretory cargo.

The current work challenges this model. The authors find that, upon glucose starvation, Myo2 and Myo4, but not type I or II myosins, become stably associated with polarized actin