Immunosensing during colonization by *Candida albicans*: does it take a village to colonize the intestine?

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Abstract

Candida albicans, an opportunistic fungal pathogen and a component of the gastrointestinal tract normal flora, is a frequent colonizer of humans. Is *C. albicans* capable of sensing the immune status of its host, a process we term immunosensing, and, if so, how? *C. albicans* causes serious disease only in immunocompromised hosts and therefore, the ability to immunosense would be advantageous to the organism. We propose a speculative model that, during colonization, *C. albicans* produces phenotypic variants that vary in relative concentration depending on host status. One variant is optimized for persistence as a commensal while the other variant has higher capacity to initiate pathogenic interactions. When the ratio of the two variants changes, the pathogenic potential of the population changes. The critical element of this model is that the *C. albicans* colonizing population is not uniform but is composed of subpopulations of phenotypic variants that are advantageous under different host conditions.

Candida albicans cells colonizing the gastrointestinal tract and causing disease in other niches are not identical

After long-term, peaceful coexistance of *Candida albicans* with its human host, a decline in host immunological function can lead to the development of devastating invasive candidiasis. How does this happen? Unraveling this mystery will be a crucial step in understanding the initiation and progression of disease caused by this opportunistic fungal pathogen.

C. albicans is an important cause of nosocomial infection [1, 2], most likely because hospitalized patients have multiple predisposing factors that favor candidal disease (such as immunosuppression, violation of protective barriers, and use of broad spectrum antibacterial drugs [3, 4]). However, most of the time, the interactions between *C. albicans* and its host are benign. Commensal colonization (see Glossary) by *C. albicans* is common in humans [5]; the organism typically resides in the intestinal tract, genitourinary tract or on the skin as

a component of the normal flora. Colonization is established very early in life and, in one study, was observed in over 50% of one-month old infants [6]. Environmental niches are not thought to be significant in the natural history of *C. albicans*.

In the case of an immunosuppressed patient, *C. albicans* cells that were benignly colonizing can become pathogenic, escape from the intestinal tract and disseminate to distant organs via the bloodstream. Neutropenia and mucosal barrier compromise are important for *C. albicans* escape from the gastrointestinal (GI) tract [7]. When newborn piglets monocolonized in the gut with *C. albicans* are immunosuppressed, *C. albicans* produces invasive lesions in the gut associated lymphoid tissue, showing that *C. albicans* is capable of promoting its own escape from the gut of a compromised host [8].

Mechanistically, what happens when the host becomes compromised and the détente between host and colonizing *C. albicans* cells breaks down? It is possible that during colonization, the organism is always attempting to cause disease. In this scenario, disease is prevented by the constant vigilance of the host and the effects of competition between *C. albicans* and the numerous other microbial colonizers that share the resources within the host.

However, studies show that there are differences in gene expression between cells that are benignly colonizing the GI tract of a host and those that are actively causing disease in other niches [9–12]. For example, the *EFH1* gene, encoding a transcription factor, is relatively highly expressed in cells colonizing the cecum but not in cells occupying a different niche and causing an oral infection (Figure 1) [12]. Since *EFH1* is also not highly expressed during laboratory growth, the intestinal tract is the niche in which this *C. albicans* gene is most highly expressed. Paradoxically, however, expression of *EFH1* is associated with reduced colonization of the intestinal tract. In an animal model, *C. albicans* cells lacking *EFH1* persist in the intestinal tract at higher levels, wild-type cells expressing *EFH1* at normal levels have intermediate persistence and cells that overexpress *EFH1* poorly colonize the GI tract [12]. These findings show that during benign colonization *C. albicans* self-regulates its population size. Perhaps this regulation prevents the organisms from damaging the host or evoking a strong host response. In contrast, Efh1p is less important for pathogenesis from the bloodstream because an *efh1* null mutant is indistinguishable from a wild-type strain in the intravenously inoculated mouse model of fatal systemic candidiasis [12]. These results argue that expression of *EFH1* is part of a genetic program that favors commensalism. Further, organisms must then undergo a transition between the commensal state and the pathogenic state because benignly colonizing organisms in the GI tract and disease-causing organisms in different niches are not identical.

Could colonizing microorganisms detect conditions in the host in order to undergo a change in gene expression and phenotype under appropriate conditions? Here, we propose a speculative hypothesis in which *C. albicans* uses phenotypic variation to test host status, a process we will term 'immunosensing'. In this article, we discuss how a complex colonizing population composed of different phenotypic variants could test host status.

A hypothesis: immunosensing through population dynamics

We hypothesize that during colonization, a population of *C. albicans* cells produces phenotypic variants differing from each other in gene expression patterns. The resulting heterogeneous population, composed of distinct subpopulations, is capable of immunosensing. For the purposes of discussion, we will term the phenotypic variants 'farmers' and 'adventurers' (Figure 2). It should be emphasized that this model is a hypothesis and cells specifically corresponding to farmers and adventurers have not been described.

Most of the colonizing cells are proposed to be farmers, who do not interact aggressively with the host. In fact, *C. albicans* farmers might pacify the host, exerting an immunosuppressive effect by promoting the expansion of regulatory T cell populations [13]. This effect could aid the survival of both farmers and adventurers.

Adventurers could interact more aggressively with the host. For example, they could colonize to higher levels, or express virulence-promoting phenotypes such as increased adhesion and increased invasiveness. In a normal host, adventurers also stimulate an anti-*Candida* host response. Importantly, we also propose that adventurers are more susceptible to the host response than farmers.

In a healthy host, we would envision that most of the *C. albicans* cells are farmers and the subpopulation of adventurers is relatively small because of their susceptibility to the host response. The rate of interconversion between farmers and adventurers could be constant or could change depending on host conditions. Interactions between farmers, adventurers, the bacterial microbiota and the host establish a relatively stable situation, where *C. albicans* colonizes but does not harm the host. Presumably, the ratio of farmers and adventurers in the *C. albicans* colonizing population is maintained at an optimal level that permits long term colonization without damage to the host.

If the host is deficient in mounting a response, due for example to neutropenia, we propose that the adventurers outcompete the farmers because they could be capable of colonizing to higher levels. As a result, adventurers could come to predominate in the population. Thus, when the host becomes compromised, the makeup of the colonizing population changes (Figure 2). The number of adventurers increases in this situation, so the chance that *C. albicans* cells will escape from the intestinal tract and produce disease also increases.

We propose that adventurers are more pathogenic than farmers. Therefore, it could be advantageous to limit their numbers during colonization of a healthy host. The proposed mechanism ensures that large numbers of adventurers only accumulate in a compromised host.

Our speculative model thus proposes that in response to changes in the host, an overall change in the phenotype of the colonizing *C. albicans* population occurs. In this way, the organisms respond to host status, maintaining colonization when the host is healthy but invading tissue and causing disease when the host is compromised. The ability to produce a complex, heterogeneous colonizing population composed of phenotypic variants with different properties is proposed to be crucial for optimal host interaction.

Phenotypic variation is well known in *C. albicans*

The phenotypic variation discussed above is hypothetical but there are several known examples of phenotypic variation in *C. albicans*. A well-studied case involves two cell types, termed 'white' and 'opaque,' that exhibit cooperative behavior. The existence of white cells, opaque cells and switching between them was first observed in a particular clinically isolated strain of *C. albicans* [14]. It was later appreciated that switching between white and opaque cells occurred in cells that were homozygous at the mating type locus, *MTL* [15, 16]. Further, opaque cells are the mating-competent form of *C. albicans* [15–18]. White and opaque cells differ in the expression of hundreds of genes [19] showing that their phenotypes are distinct in many ways.

In a mixed population, white and opaque cells cooperate to enhance mating [20]. Through release of pheromone, opaque cells signal white cells. As a result, the white cells aggregate, adhere to a substratum and form a biofilm [21]. Within the biofilm, pheromone gradients

produced by opaque cells are stabilized, promoting mating. Thus, through pheromone secretion, a small number of opaque cells influence the behavior of a large number of white cells and the white cells create an environment that is conducive for mating. This example shows that interaction between phenotypic variants in a mixed population can enhance a biological activity.

Other examples of phenotypic variation in *C. albicans* involve either cellular or colonial morphology. For example, *C. albicans* can grow in either a yeast form or as filamentous hyphae [22]. The transcription profiles of the two forms are quite different, with large numbers of genes that are differentially regulated during laboratory growth [23–25]. Variation in cellular morphology at the population level produces differences in colony morphology [26]. Switching between colony morphologies is regulated and is derepressed in the absence of the histone deacetylase Sir2 [27].

These examples show that phenotypic variation in *C. albicans* is not unusual. During murine intestinal colonization, both yeast and hyphal forms are produced [12, 28]. Thus, at least one type of phenotypic variation occurs during colonization. Further, the cooperation between white and opaque cells shows that a mixed population need not be simply the sum of its parts; rather, a mixed population might have increased capabilities that neither single population possesses.

For other microorganisms, phenotypic variation contributes to colonization and persistence

The notion that a colonizing population of organisms can be composed of distinct subpopulations of phenotypic variants that promote colonization, virulence and persistence during stress is well established for several microorganisms. Organisms that persist in a host for long periods of time and cause chronic infections often exhibit antigenic variation [29], i.e. variation in expression of surface proteins that are antigenic. Antigenic variation is proposed to contribute to colonization or infection in several ways [30]. Antigenic variation enhances persistence by allowing organisms to evade the host immune response. An infection that lasts longer could prolong the period of high infectivity, enhancing transmission of the organism. Antigenic variation can also allow an organism to infect a previously infected host. In addition, different variants of an antigenic protein might differ in activity, altering the course of the disease. All of these effects can be beneficial for the infecting organism.

Antigenic variation has been described in bacterial, fungal and protozoan species [30]. For example, in *Borrelia* species, antigenic variation contributes to persistence in a host during relapsing fever or Lyme disease [31–33]. In *Plasmodium spp.*, the parasites that cause malaria, the products of the *var* genes are proteins that are expressed on the surface of mammalian red blood cells infected with the organism. Different *var* genes encode proteins that differ in antigenicity and ligand binding specificity [34, 35]. Through differential expression and recombinational generation of new sequences [36, 37], the parasites produce extensive antigenic diversity, allowing them to evade the host immune response.

An invasive subpopulation that promotes intestinal colonization by *Salmonella* Typhimurium illustrates how one phenotypic variant can influence colonization by other cells in the population [38, 39]. By invading intestinal tissue, the invasive cells provoke the host inflammatory response. Inflammation promotes luminal colonization by *S.* Typhimurium possibly because *S.* Typhimurium possesses multiple mechanisms for resisting the toxic inflammatory response while competing commensal organisms do not. Although the invasive subpopulation is killed when the bacteria invade intestinal tissue, their

activity ensures that conditions that facilitate colonization by the bulk of the bacterial population are established.

'Persisters,' dormant cells that are highly resistant to antimicrobial compounds, represent another example of phenotypic variants that form a subpopulation of cells within a population [40, 41]. Treatment of a population with an antimicrobial drug kills the vast majority of the cells. However, the persisters survive due to their high drug resistance, enabling the population to recover when the drug is removed. Whether persisters promote host colonization by allowing colonizing populations to resist host-produced antimicrobial compounds or contribute to recurrence of infection following antibiotic treatment remain open questions.

In addition, organisms adapt to the host environment upon introduction into a host. For example, *Vibrio cholerae* organisms shed by an infected host suffering from cholera differ phenotypically from *V. cholerae* organisms that are present in the environment [42]. Therefore, within a host, the organisms undergo a transition from one phenotypic state to another. At certain times or in certain locations within the host, the *V. cholerae* population will likely be composed of mixtures of cells in different phenotypic states. Whether the mixed population exhibits properties distinct from those of the individual subpopulations is not known.

As these examples show, populations of microorganisms are often composed of distinct subpopulations. Such complex populations promote colonization, persistence and transmission.

Concluding remarks

Many different organisms generate phenotypic variants during growth within a host. The production of variants affects colonization or pathogenesis in several important ways, for example, allowing immune evasion and long term persistence in the host. For *C. albicans*, we propose that production of a particular type of phenotypic variant allows the organism to monitor the immune status of its host. This immunosensing function represents a new proposed function for phenotypic variation. Future experimental tests of this model will include identification of phenotypic variants produced during colonization by *C. albicans* and analysis of the contribution of variants to pathogenicity (Box 1). A prediction of this model is that mutants that are unable to generate the appropriate phenotypic variants will be attenuated in producing endogenous candidiasis.

Immunosensing through phenotypic variation might not be restricted to fungi. The ability of bacteria to sense the host's immune response and respond to the host by undergoing phenotypic change has been recently discussed [43]. Therefore, the strategy of using a complex colonizing population to monitor and respond to the host could represent a general microbial mechanism for optimizing host-microbe interactions.

Acknowledgments

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Glossary

Colonize	to establish a population in a particular niche, e.g. in a host.
Commensal	an organism that benefits from an interaction with another organism without causing damage to the second organism.
Disease	an alteration of the body that results in the inability of the body or a part of the body to perform its normal function, e.g. due to the effects of a microorganism.
Endogenous candidiasis	disease caused by *Candida* organisms that are already colonizing a host.
Immunosensing	the process by which colonizing microorganisms detect a change in the immune status of the host.

|	
Opportunistic pathogen	a microorganism that can be pathogenic if the host is compromised but is not harmful to a healthy host.
Pathogenic	able to cause disease.

Box 1. Outstanding questions

- Can a subpopulation of cells corresponding to adventurers be identified and isolated? The speculation that subpopulations exist would be supported by the demonstration that such cells occur during intestinal colonization.

- How do adventurers contribute to pathogenesis in animal models of candidiasis? The speculation that farmers and adventurers differ in pathogenic potential could be established experimentally.

- In addition to the properties described here, in what other ways do adventurers and farmers differ from each other? Transcription profiling could demonstrate the full spectrum of activities that are co-regulated with the farmer or adventurer phenotype.

- How commonly in nature do heterogeneous colonizing populations promote colonization and host exploitation? The strategy of producing subpopulations that differ in their behavior in the host might be shared by many microorganisms.

A. *Candida albicans* benign colonization in the murine GI tract
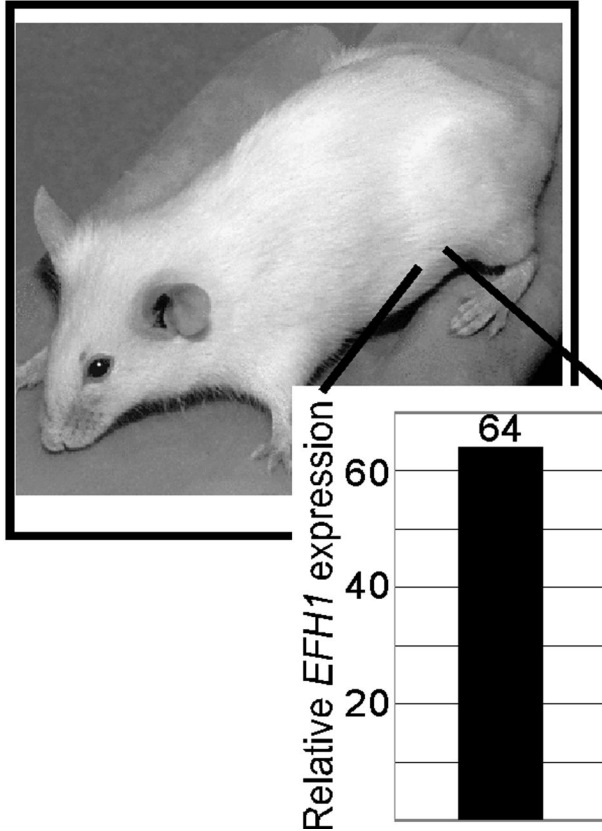
B. *Candida albicans* pathogenic growth in tongue tissue (oral thrush)
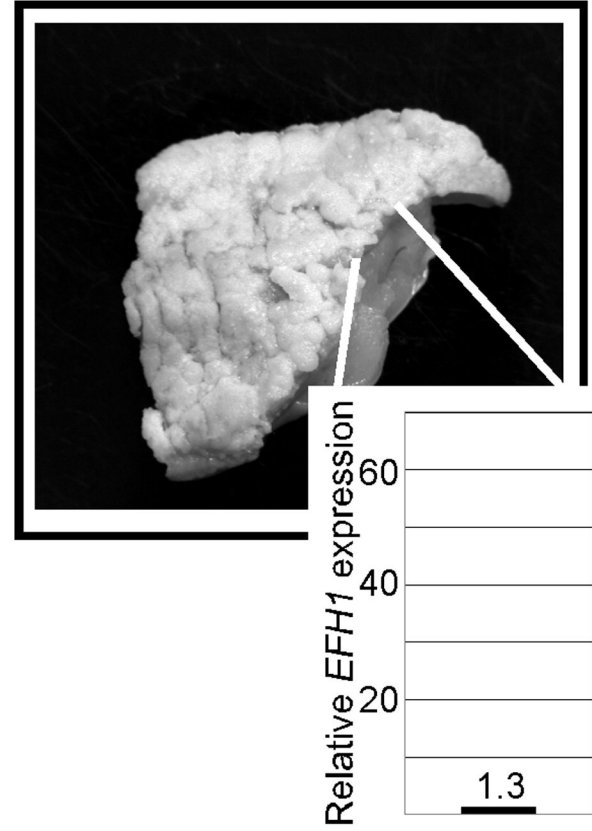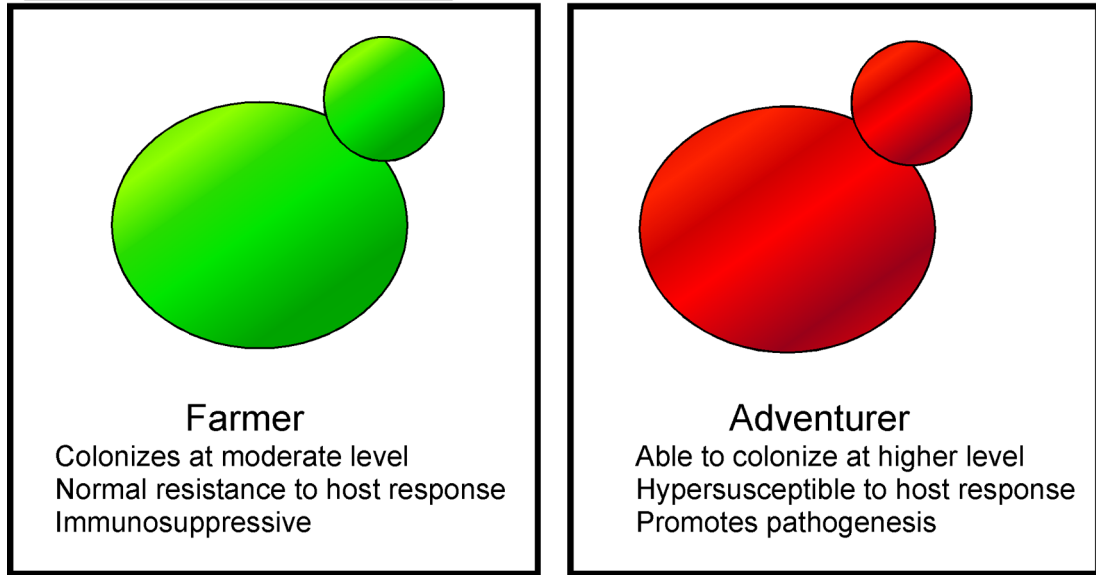
Figure 1.
Differential gene expression in C. albicans cells colonizing the gastrointestinal tract or causing oral thrush. **(a)** Colonization of the gastrointestinal (GI) tract without disease. Wild-type *C. albicans* cells established colonization of the murine GI tract as described previously [9]. RNA was extracted from colonizing *C. albicans* cells recovered from the murine cecum and expression of *EFH1* was measured by real time quantitative reverse transcriptase PCR [12]. **(b)** Oral thrush in an immunosuppressed gnotobiotic newborn piglet. A germ-free piglet monocolonized in the GI tract with *C. albicans* and immunosuppressed developed oral thrush [8]; monocolonized piglets not treated with immunosuppressing drugs do not develop the extensive thrush shown here. (Image copyright © 2000, American Society for Microbiology. Reprinted with permission from [8]). RNA was extracted from pathogenic *C. albicans* cells recovered from the piglet tongue and *EFH1* expression was analyzed as described above [12].

A. Hypothetical phenotypic variants produced during host colonization

Farmer
Colonizes at moderate level
Normal resistance to host response
Immunosuppressive

Adventurer
Able to colonize at higher level
Hypersusceptible to host response
Promotes pathogenesis

B. Differences in populations of organisms colonizing hosts differing in immune status

Normal Host   Compromised Host

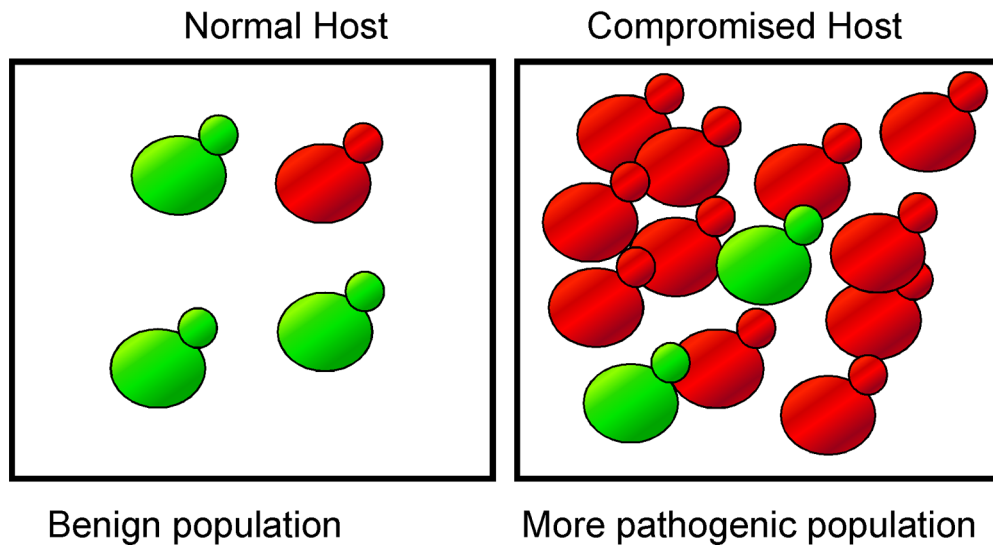
Benign population   More pathogenic population

Figure 2.
Hypothetical *C. albicans* phenotypic variants produced during colonization of a host. **(a)** Depicts two phenotypic variants, farmers and adventurers, that are proposed to differ in interaction with the host and susceptibility to the host response. **(b)** Depicts populations produced in a normal host or a compromised host.