The sixth sensor: A Candida albicans biofilm master regulator that responds to inter-kingdom interactions

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Invasive candidiasis is the leading cause of mortality from fungal infections and results in at least 50,000 deaths per annum globally. Historically, Candida albicans has been associated with most of invasive candidiasis cases. This picture is changing with the emergence of Candida glabrata as a major pathogen in Northern Europe, the US and Canada, and Candida parapsilosis in southern Europe, Asia and South America. Nevertheless, C. albicans remains a key pathogen and is usually the most abundant Candida species in epidemiological surveys of invasive fungal disease. In addition to invasive infections, Candida spp. also cause localized infections of the oral or vaginal mucosae, the lungs in cystic fibrosis patients or of wounds in burns victims. Further, they can colonise a variety of artificial materials placed in or on the body including catheters, tracheoesophageal speech valves and contact lenses. In these cases, Candida spp are almost always found in polymicrobial biofilms, where they are associated with a variety of bacteria.

Candida spp are well-adapted to cohabit with bacteria, and exist as harmless commensals in polymicrobial biofilms throughout the human gastrointestinal tract. In the oral cavity, these biofilms may contain 100 or more species of bacteria in association with one or more species of Candida. Clearly, there is a vast potential for inter-kingdom interactions between fungi and bacteria in such situations. Within this complex network of interactions, there are some clear associations that stand out. In particular, Candida spp thrive in low-pH environments and are commonly found together with saccharolytic Gram-positive bacteria such as Lactobacillus spp and Streptococcus spp in biofilms throughout the gastrointestinal tract or the vulvovagina and with Staphylococcus spp on skin. Evidence is accumulating that interactions between Candida and bacteria are critical for colonisation, biofilm formation and the yeast-hyphal transition in C. albicans, which is strongly linked to its ability to invade host mucosal surfaces and cause disease. However, the central regulatory pathways in Candida responsible for sensing and responding to interactions with bacteria are not well understood at present.

In the current issue of Virulence, Xu and coworkers describe the identification of a master regulator in C. albicans that is required for sensing interactions with the common oral colonizer Streptococcus oralis. Previous work had identified 6 master transcriptional regulators that are required for biofilm formation by C. albicans: Bcr1, Brg1, Efg1, Tec1, Ndt80 and Rob1. By screening a panel of strains in which the reporter gene encoding mCherry was placed under the control of each regulator, EFG1 alone was upregulated by co-culture with S. oralis in vitro biofilms, organotypic mucosal models and on the tongue of orally infected mice. Xu and colleagues next explored the impact of S. oralis on EFG1-dependent processes in C. albicans using a homozygous efg1 mutant and a genetic complementation strain. EFG1 is a well-characterized transcriptional regulator that is known to be required for C. albicans filamentation. In nutrient-restricted media, S. oralis promoted elongation of hyphae in C. albicans wild-type or the efg1 revertant, but not in the efg1 ΔΔ mutant, indicating that EFG1 is essential for the promotion of hyphal formation by S. oralis. Moreover, C. albicans biofilm formation in an organotypic tissue culture model was promoted by S. oralis and this process was also dependent on EFG1. Interestingly, in the mouse model of tongue colonisation, S. oralis only partially restored biofilm formation in the efg1 ΔΔ mutant. In this more complex environment there may be other pathways in C. albicans that act independently of EFG1 to sense and respond to S. oralis.
To identify potential downstream effectors of EFG1 involved in interactions with S. oralis, Xu et al. assessed the expression of genes encoding 3 cell surface exposed proteins that are known to mediate interactions with oral streptococci: ALS1, ALS3 and HWP1. Of these, only ALS1 was upregulated by S. oralis in the efg1 revertant and this gene was not upregulated in the efg1 Δ/Δ mutant. Further analysis confirmed the described previously finding that an als1 Δ/Δ mutant was deficient in coaggregation with S. oralis, and showed that this mutant was also defective in forming dual-species biofilms. Overexpression of ALS1 in the efg1 Δ/Δ mutant partially restored the ability of the mutant to form thick biofilms with S. oralis, and significantly increased the capacity of C. albicans to recruit S. oralis to the biofilm. Overall, these data demonstrate that ALS1 is an important mediator of C. albicans-S. oralis interactions and works together with EFG1 to promote mucosal biofilm formation in the presence of S. oralis.

There are several key questions that remain unanswered from this work. In particular, what is the upstream signaling and sensing pathway that results in the upregulation of EFG1 in response to S. oralis, and is the signal common to all strains of S. oralis or to other species of oral streptococci? A variety of small signaling molecules released by oral streptococci have been shown to modulate biofilm formation and/or hyphal formation by C. albicans including autoinducer-2, hydrogen peroxide, competence-stimulating peptide and the fatty acid signaling molecule trans-2-decenoic acid.8-11 Could any of these be the trigger for EFG1 gene regulation? It is possible that the signal is also produced by staphylococci since S. aureus-induced mortality in a mouse model of intra-abdominal infection is dependent on EFG1.12 The mechanism by which C. albicans detects the signal and couples it to regulation of EFG1 is also unclear. Expression of EFG1 is dependent on protein kinase A and the cyclic AMP signaling pathway.13 The adenylate cyclase Cyr1 is considered to play a key role in production of cAMP and this in turn is regulated in response to a variety of stimuli including peptidoglycan breakdown products. Therefore it is possible that bacterial cell lysis and peptidoglycan release may be a key stimulus for C. albicans. This would be consistent with observations that expression of EFG1 was not strongly induced until relatively late (24–36 h) after co-inoculating C. albicans with S. oralis.7

It is likely that multiple pathways exist for signaling between C. albicans and oral streptococci. For example, recent work has shown that S. mutans is able to restore the biofilm forming ability of C. albicans mutant lacking the master transcriptional regulator BCR1.14 It is not yet known whether EGF1 is required for this interaction. However, in contrast to the interaction with S. oralis, C. albicans responded to S. mutans by upregulating all 3 genes HWP1, ALS1 and ALS3. In addition, S. mutans glucosyltransferase B (GtfB) was critical for the stimulation of biofilm formation in C. albicans bcr1 Δ/Δ. Although S. oralis produces a glucosyltransferase, it is not a direct homolog of S. mutans GtfB.15

The study by Xu and coworkers provides new insights into the interactions between C. albicans and oral streptococci. Further identification of the signaling pathways involved in Candida-bacteria interactions may ultimately lead to novel approaches for interfering with these interactions and preventing fungal infections. Importantly, the work highlights the need to consider the wider environment when assessing the genetic pathways involved in Candida biology. Phenotypes that are apparent in simple laboratory models may not be so relevant in environments where Candida are naturally found, where there are complex interplays between fungi, bacteria and the host.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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