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EDITORIAL

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

Nicholas S. Jakubovics

Centre for Oral Health Research, School of Dental Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne, UK

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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.[1] 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.[2] 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.[3] 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*.[4] 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.[5] 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.[6] 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*.[7] 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 *in vitro* biofilms, organotypic oral 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* Δ/Δ 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* Δ/Δ 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*.

CONTACT Nicholas S. Jakubovics nick.jakubovics@newcastle.ac.uk Centre for Oral Health Research, School of Dental Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4BW, UK.
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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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