2017

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FEBS Letters

FEBS PRESS
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COMMENTARY

Quorum sensing in *Candida albicans*: farnesol *versus* farnesoic acid

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Candida albicans is a clinically important dimorphic fungus that exhibits either a budding yeast or a mycelial-hyphal or pseudohyphal growth, depending on environmental conditions. The yeast-to-mycelia morphologic transition, which is generally regarded as an important virulence determinant [1], depends on the inoculum size of liquid cultures. The yeast form is favored when cultures are inoculated at $> 10^6$ cells·mL^{-1}, whereas the mycelial form is favored at inoculum densities $< 10^6$·mL^{-1}. Farnesoic acid (FA) and farnesol (FOH) are two related sesquiterpene quorum-sensing molecules (see Fig 1 for stuctures and abbreviations) that, upon accumulation, prevent the yeast-to-mycelial conversion. Oh *et al*. [2] showed that *C. albicans* strain ATCC 10231 excretes FA, while Hornby *et al*. [3] showed that *C. albicans* A72 and SC5314 excrete FOH. Subsequent work indicated that 10231 was the only isolate of *C. albicans* that fails to produce detectable FOH [4]. Moreover, when tested on *C. albicans* A72, FA had only 3.2% of the hyphal-inhibitory activity relative to FOH [5]. These observations raised two questions: 1., Do FOH and FA block mycelial development via the same molecular mechanism; and 2., What is the biochemical or physiologic difference in strain 10231 that underlies excretion of FA and not FOH?

Do farnesol and farnesoic acid share a common mechanism of action?

This question has now been partially answered by Ahn *et al*. [6], who explored how FA blocks mycelial development of *C. albicans* SC5314. Previously, the same authors had shown that *CaPHO81*, a key component of the phosphate starvation response signal transduction pathway [7], is required for inhibition of hyphal development by FA [8]. The *pho81*Δ mutant cells existed exclusively as filaments and were insensitive to inhibition by FA. In the current study, they have identified a previously unknown transcriptional activator of *PHO81*, *Ca*Hot1p (orf 19.3328), which binds to the *PHO81* promoter region and induces the expression of *PHO81* in response to FA treatment. Similar to the *pho81*Δ strain from their previous work, the *hot1*Δ mutant cells grew exclusively as filaments and were insensitive to FA inhibition of hyphal morphogenesis. Most critically, two lines of evidence indicated that FOH and FA, despite their close chemical similarity (Fig. 1), use separate pathways to block hyphal development. First, the *hot1*Δ mutant cells had lost their sensitivity to FA but remained sensitive to FOH. Second, the mRNA abundance of *HOT1* and *PHO81* increased dramatically between 40 and 240 min following treatment with FA, but remained unchanged following treatment with FOH [6]. Indeed the study of *PH081* and *HOT1* was driven in part by the previous finding that these genes were among those induced in *C. albicans* 10231 following the addition of FA [9]. This dichotomy between FOH and FA responses is analogous to that described by Hall *et al*. [10] who showed that FOH and 3-oxo-C_{12}-homoserine lactone, a quorum-sensing molecule secreted by *Pseudomonas aeruginosa*, block hyphal development by a different pathway from that used by the 12-carbon alcohol dodecanol. Dodecanol exerted its effects through a mechanism involving the *C. albicans* hyphal repressor Sfl1p. Deletion of *SFL1* did not affect the response to FOH but did interfere with the response to dodecanol. Importantly, Sfl1p can also be activated by the bacterial signaling molecules DSF (cis-1-methyl-2-dodecenoic acid) and BDSF (cis-2-dodecenoic acid), which are structurally and functionally related to FA [11]. Our working hypothesis is that farnesoic acid aligns with DSF and BDSF since they are all organic acids with 12-carbon chain lengths.

Abbreviations

BDSF, cis-2-dodecenoic acid; DSF, cis-1-methyl-2-dodecenoic acid; FA, farnesoic acid; FOH, farnesol.

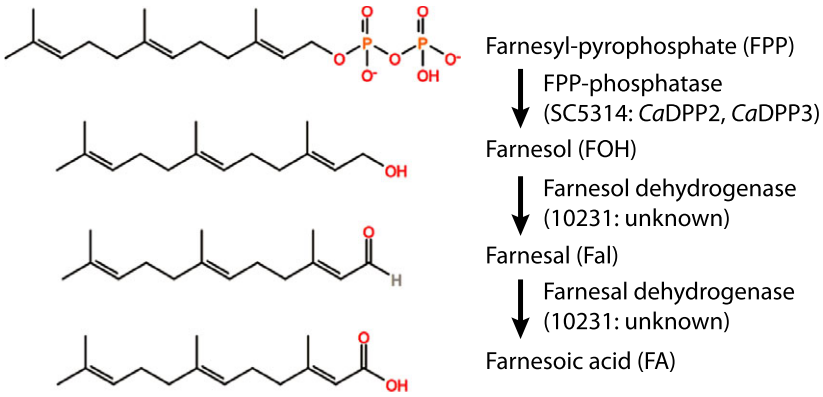

Fig. 1. Structures and biosynthesis of sequiterpene quorum-sensing molecules in *C. albicans*. The most likely explanation for FA production by strain 10231 is the presence of an active farnesol and farnesal dehydrogenase pathway in this strain, and its absence from other clinical isolates. The identities of these dehydrogenases are currently not defined.

An apparent paradox in the regulation of hyphal morphogenesis by CaPho81p

Phosphate starvation in fungi results in an induction of high-affinity phosphate transporters, the secretion of acid and alkaline phosphatases, and the reorganization of cellular metabolism. This allows cells to acquire more of, and to cope with the limitation of, this critical macronutrient. Regulation of genes that are induced by phosphate starvation is well characterized and essentially conserved in ascomycetes such as *C. albicans* and *S. cerevisiae* [7], and the core regulatory elements are detailed in Fig. 2. Briefly, phosphate starvation-responsive genes are under the control of the transcriptional activator Pho4p, which is maintained in a hyperphosphorylated, inactive state by the action of the cyclin/cyclin-dependent kinase complex Pho85p/Pho80p. Pho81p was identified as a cyclin-dependent kinase inhibitor, and its activation by phosphate-limiting conditions leads to a decrease in Pho80/Pho85 activity, leading to dephosphorylation and activation of Pho4. Recent work by Ikeh *et al.* [12] has identified *Ca*Pho4p as an component of the fungal phosphate starvation regulatory pathway that, like *Ca*Pho81p, also plays a role in regulating hyphal morphogenesis. Pho4p is a basic helix-loop-helix transcription factor that binds to and activates genes involved with phosphate acquisition and polyphosphate mobilization, and the work of Ikeh *et al.* [12] showed that a *pho4*Δ mutant strain fails to form hyphal germ tubes under hyphae-inducing conditions. As noted above, in the context of phosphate starvation, the activity of Pho4p is regulated by its phosphorylation status [7] as represented in Fig. 2. A *pho81*Δ mutant strain would be expected to accumulate hyperphosphorylated Pho4p, which is actively excluded from the nucleus and thus inactive as a transcriptional regulator. If we assume that it is the hypophosphorylated, nuclear-localized form of Pho4p that is active in the regulation of filamentation, this raises a conflict with the data presented by Chung *et al.* [8] and Ahn *et al.* [6]. Specifically, a *pho81*Δ mutant strain would be expected to favor the inactive form of Pho4p. This assumption, by analogy to the filamentation defect exhibited by the *pho4*Δ mutant presented in Ikeh *et al.* [12], would lead us to expect that the *pho81*Δ mutant (and by extension, the *hot1*Δ mutant) would fail to form hyphae under favorable conditions. However, the opposite outcome is demonstrated for these mutant strains, with ectopic hyphal formation in conditions that normally favor the yeast form.

This apparent paradox can be explained by at least two different models. First, it could be that the two observations are not actually at odds if one assumes that the hyperphosphorylated form of Pho4p is responsible for hyphal induction via a mechanism that is independent of its activity as a transcription factor. This possibility seems unlikely, as it was shown recently that phosphate starvation itself is a powerful inducer of hyphal morphogenesis in a number of highly virulent clinical isolates [13], suggesting that the *C. albicans* Pho regulon is functionally linked to hyphal induction, most likely via the Pho4p-mediated mechanism presented in Fig. 2. An alternative and perhaps more likely scenario is that Pho81p indeed acts to regulate the phosphorylation status of Pho4p by inhibiting the activity of the Pho80/Pho85 kinase complex, but that it also acts as a negative regulator of the hyphal induction pathway mediated by the Ras/cAMP pathway. This seems more likely, as it has been shown [8] that the *pho81*Δ strain accumulates an abnormally high level of Ras-GTP, and that FA-

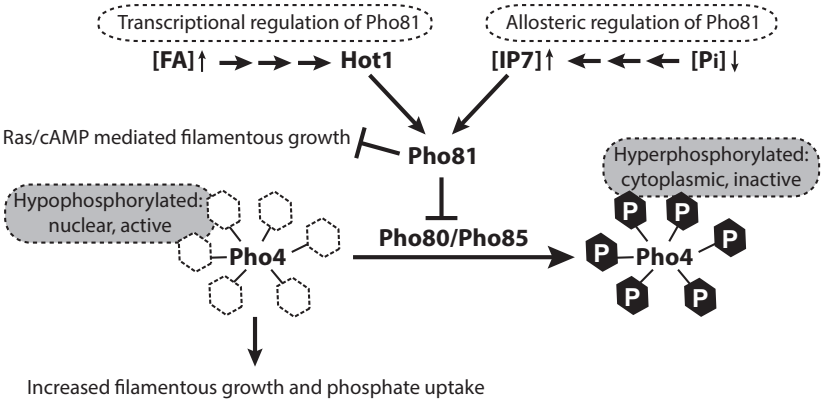

Fig. 2. A model for the dual roles of Pho81 in inducing or suppressing pathways of filamentation. As described in the text, Pho81 negatively regulates the Ras/cAMP-mediated pathway of hyphal morphogenesis, but likely plays a positive regulatory role in promoting hyphal morphogenesis under phosphate-limiting conditions.

mediated inhibition of filamentation in a strain bearing a hyperactive allele of Ras is dependent on the presence of Pho81p. Taken together, the evidence suggests that Pho81p plays a positive role in regulating filamentation via Pho4p activation, while playing a negative role in filamentation by inhibition of the Ras/cAMP pathway. This also suggests that under the conditions operative in these studies, the Ras/cAMP pathway plays a dominant role in the regulation of filamentation, and that the Pho4p-dependent filamentation pathway plays a secondary role.

Why does *C. albicans* strain 10231 secrete farnesoic acid, while other *C. albicans* strains secrete farnesol?

To the best of our knowledge and assessment of the relevant literature, 10231 is the only strain of *C. albicans* reported to secrete FA instead of FOH. Presumably it makes FA from FOH through the pathway that is also employed by insects and other invertebrates for the synthesis of juvenile hormone III, converting FOH to farnesal via an alcohol dehydrogenase, and then to FA via an aldehyde dehydrogenase (Fig. 1). Strain 10231 was isolated in 1943 and has been in the ATCC collection since 1965, although it was used as a test organism for antifungal antibiotics as early as 1956 [14]. It has at least nine alternate strain designations and it is recommended for use in the ASTM Standard Test Method E979-91. This recommendation is fitting because 10231 produces abundant germ tubes [15] and chlamydospores [16] along with forming especially black colonies on BiGGY agar. Thus, it fulfills the three hallmarks for *in vitro* diagnostic characterization of *C. albicans*.

However, in *in vivo* the virulence of strain 10231 may differ from that of other strains in a manner that depends on FOH production [17, 18]. For instance, Navarathna *et al.* [17] showed that FOH acted as a virulence factor in a mouse model for candidiasis; a mutant which produced six times less FOH was ca. 4.2 times less pathogenic than its parent. Similarly, the LD_{50} dose for strain 10231 in a mouse model was 1.2×10^7 cells per mouse [17], a value six times higher than the LD_{50} values for their FOH excreting counterparts [17]. Subsequently, Ghosh *et al.* [18] showed that mouse macrophage cells produced ca. 1000 × less IL-6 mRNA when challenged with strain 10231 than with a series of FOH secreting of *C. albicans*. Strain 10231 elicited only background levels of IL-6 expression. The RAW264.7 mouse macrophage cells produced more IL-6, TLR2, and dectin1 mRNA by 100-, 2-, and 3-fold, respectively, when exposed to a combination of FOH and zymosan than a combination of FA and zymosan [18]. More recently [19] we showed that FOH stimulated the migration of mouse macrophages, induced the rapid depletion of B cells from the mouse peritoneal cavity, and recruited neutrophils, granulocytes, and small peritoneal macrophages to the mouse peritoneal cavity. Unfortunately, this study did not include direct comparisons of FOH *versus* FA or of *C. albicans* 10231 *versus* A72 or SC5314. Taken together, these results strongly suggest that the altered response of the innate immune system to strain 10231 relative to other isolates is due to differences in their response to FOH versus FA. The genetic and biochemical factors which contributed to the selection for FA production versus FOH production are currently undefined. However, their elucidation is likely to provide new details regarding the differences in virulence

and immune responses between 10231 and other clinical isolates of *C. albicans*.

References

1 Sudbery PE (2011) Growth of *Candida albicans* hyphae. *Nat Rev Microbiol* **9**, 737–748.

2 Oh KB, Miyazawa H, Naito T and Matsuoka H (2001) Purification and characterization of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. *Proc Natl Acad Sci USA* **98**, 4664–4668.

3 Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P and Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl Environ Microbiol* **67**, 2982–2992.

4 Hornby JM and Nickerson KW (2004) Enhanced production of farnesol by *Candida albicans* treated with four azoles. *Antimicrob Agents Chemother* **48**, 2305–2307.

5 Shchepin R, Hornby JM, Burger E, Niessen T, Dussault P and Nickerson KW (2003) Quorum sensing in *Candida albicans*: probing farnesol's mode of action with 40 natural and synthetic farnesol analogs. *Chem Biol* **10**, 743–750.

6 Ahn CH, Lee S, Cho E, Kim H, Park W and Oh KB (2017) A farnesoic acid-responsive transcription factor, Hot1, that regulates yeast-hypha morphogenesis in *Candida albicans*. *FEBS Lett* **591**, 1225–1235.

7 Tomar P and Sinha H (2014) Conservation of PHO pathway in ascomycetes and the role of Pho84. *J Biosci* **39**, 1–12.

8 Chung SC, Kim TI, Ahn CH, Shin J and Oh KB (2010) *Candida albicans PHO81* is required for the inhibition of hyphal development by farnesoic acid. *FEBS Lett* **584**, 4639–4645.

9 Chung SC, Lee JY and Oh KB (2005) cDNA cloning of farnesoic acid-induced genes in *Candida albicans* by differential display analysis. *J Microbiol Biotechnol* **15**, 1146–1151.

10 Hall RA, Turner KJ, Chaloupka J, Cottier F, DeSordi L, Sanglard D, Levin LR, Buck J and Mühlschlegel FA (2011) The quorum sensing molecules farnesol/homoserine lactone and dodecanol operate via distinct modes of action in *Candida albicans*. *Eukaryot Cell* **10**, 1034–1042.

11 Wang LH, He Y, Gao Y, Wu JE, Dong YH, He C, Wang SX, Weng LX, Xu JL, Tay L *et al*. (2004) A bacterial cell-cell communication signal with cross-kingdom structural analogues. *Mol Microbiol* **51**, 903–912.

12 Ikeh MAC, Kastora SL, Day AM, Herrero-de-Dios CM, Tarrant E, Waldron KJ, Banks AP, Bain JM, Lydall D, Veal EA *et al*. (2016) Pho4 mediates phosphate acquisition in *Candida albicans* and is vital for stress resistance and metal homeostasis. *Mol Biol Cell* **27**, 2784–2801.

13 Romanowski K, Zaborin A, Valuckaite V, Rolfes RJ, Babrowski T, Bethel C, Olivas A, Zaborina O and Alverdy JC (2012) *Candida albicans* isolates from the gut of critically ill patients respond to phosphate limitation by expressing filaments and a lethal phenotype. *PLoS ONE* **7**, e30119.

14 Taber WA (1956) An agar diffusion assay method for the antifungal polyene, candidin. *Can J Microbiol* **2**, 45–55.

15 Kim D, Shin WS, Lee KH, Kim K, Park JY and Koh CM (2002) Rapid differentiation of *Candida albicans* from other *Candida* species using its unique germ tube formation at 39°C. *Yeast* **19**, 957–962.

16 Montazeri M and Hedrick HG (1984) Factors affecting spore formation of a *Candida albicans* strain. *Appl Environ Microbiol* **47**, 1341–1342.

17 Navarathna DHMLP, Hornby JM, Krishnan N, Parkhurst A, Duhamel GE and Nickerson KW (2007) Effect of farnesol on a mouse model of systemic candidiasis, determined by use of a *DPP3* knockout mutant of *Candida albicans*. *Infect Immun* **75**, 1609–1618.

18 Ghosh S, Howe N, Volk K, Tati S, Nickerson KW and Petro TM (2010) *Candida albicans* cell wall components and farnesol stimulate the expression of both inflammatory and regulatory cytokines in the murine RAW264.7 macrophage cell line. *FEMS Immunol Med Microbiol* **60**, 63–73.

19 Hargarten JC, Moore TC, Petro TM, Nickerson KW and Atkin AL (2015) *Candida albicans* quorum sensing molecules stimulate mouse macrophage migration. *Infect Immun* **83**, 3857–3864.