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Oxylipins as developmental and host–fungal communication signals

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Pathogenic microbes and their hosts have acquired complex signalling mechanisms to appraise themselves of the environmental milieu in the ongoing battle for survival. Several recent studies have implicated oxylipins as a novel class of host–microbe signalling molecules. Oxylipins represent a vast and diverse family of secondary metabolites that originate from the oxidation or further conversion of polyunsaturated fatty acids. Among the microbial oxylipins, the fungal oxylipins are best characterized and function as hormone-like signals that modulate the timing and balance between asexual and sexual spore development in addition to toxin production. Coupled with other studies that implicate a role for fungal oxylipins in pathogenesis by *Aspergillus* and *Candida* spp., these results suggest that host and microbial oxylipins might interfere with the metabolism, perception or signalling processes of each other.

Oxylipin biosynthetic pathways

Lipids are vital constituents of all living cells and provide the structural basis for cell membranes and fuels for metabolism. Recent discoveries also suggest that lipids have major roles as mediators in many eukaryotic multicellular processes including membrane trafficking, cytoskeletal rearrangements and signal transduction cascades [1–4]. Polyunsaturated fatty acids (PUFAs) consist of a major group of lipids that have a crucial role in sustaining membrane fluidity in prokaryotic and eukaryotic organisms. The addition of molecular O_2 to PUFAs leads to the production of a large family of structurally related oxygenated polyenoic fatty acids, collectively known as oxylipins. Oxylipins exhibit crucial biological activities as signals of intra- and inter-cellular communication in plants, vertebrates, invertebrates and fungi. In plants, oxylipins stimulate signals implicated in the mounting of plant defences against pathogens and pests, have antimicrobial effects, provide building units of physical barriers against pathogen invasion, regulate plant cell death and have a major role in the formation of phytohormones and in senescence [5,6]. The oxylipins used by plants to organize defence are similar to signals used in the animal defence arsenal. For instance, eicosanoids in animals (including insects) share high similarity with plant and fungal oxylipins in terms of biosynthesis, structure, function and oxidative modification. Eicosanoids – including prostaglandins (PGs), leukotrienes (LTs) and thromboxanes – are involved in numerous homeostatic biological functions in addition to inflammation and serve as ligands that modulate the expression of genes such as major transcription factors and genes involved in lipid synthesis and secretion [5]. In microbial cells, oxylipins regulate cell growth, differentiation and apoptosis in addition to the development of the infectious process caused by pathogenic microorganisms [6,7]. Oxylipin (eicosanoid) receptors, their genes and their structure–function relationships have been well characterized in vertebrates but not in plants or fungi [5].

The complexity of biosynthetic pathways and diversity of biological activities of plant and fungal oxylipins has only recently started to be unravelled. Phyto-oxylipins are synthesized enzymatically from PUFAs by three major pathways: (i) the lipoxygenase (LOX) biosynthetic pathway; (ii) the endoplasmic reticulum-localized cytochrome P450 enzymes that catalyze the ω- or in-chain hydroxylation of fatty acids; and (iii) an α-dioxygenase that displays similarity with animal cyclooxygenases and catalyzes the α-oxygenation of fatty acids [4]. In fungi, the first biosynthetic enzymes involved in oxylipin biosynthesis were recently identified and show homology to mammalian prostaglandin H synthases or cyclooxygenases (COX) [8–13] (Figure 1). The presence of lipoxygenases and glutathione transferases in fungi known to function in prostaglandin and steroid hormone synthesis illustrates the existence of other potential routes of oxylipin biosynthesis in fungi [12,14]. Additionally, through a non-enzymatic process that is initiated by oxidative stress and free-radical-catalyzed mechanisms, linolenic acid can be converted to an array of prostaglandin-like compounds called phytoprostanes in all aerobic PUFA-containing organisms [15]. In contrast to animals, which use arachidonic acid (C20:4) as the precursor for eicosanoid biosynthesis, plant oxylipins derive mainly from linolenic (C18:3), linoleic (C18:2) and hexadecatrienoic (C16:3) acids whereas fungal oxylipins are primarily derived from oleic (C18:1), linoleic and linolenic acids. These fatty acids are found in phospholipids and acylglycerides but an abundant plant-specific source of PUFAs also resides in chloroplastic galactolipids. In fact, distinct chloroplastic and extrachloroplastic oxylipin biosynthetic pathways exist and produce a complex array of bioactive derivatives [4].

In this review, we highlight the recent advances in the complex biosynthesis and regulation of fungal oxylipins as signalling and developmental modulators, specifically

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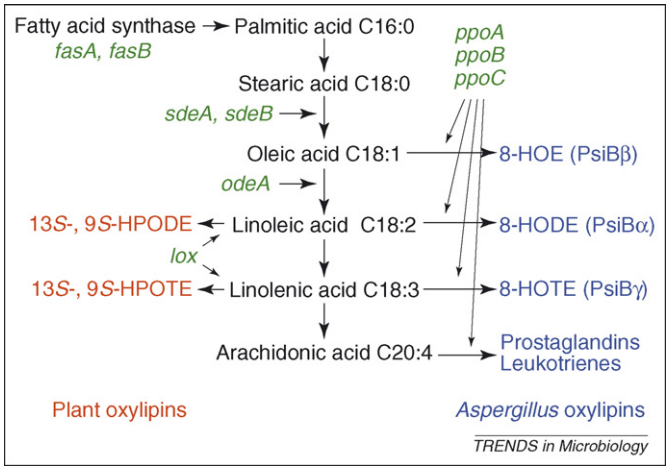

Figure 1. The basic pathway of fatty acid metabolism in *Aspergillus* spp. and plants. Gene name abbreviations: *fasA/B,* fatty acid synthase; *lox,* lipoxygenase; *odeA/B,* oleate delta-12 desaturase; *ppoA/B/C,* psi producing oxygenases; *sdeA/B,* stearic acid desaturase. Oxylipin abbreviations: 8-HODE, 8-hydroxylinoleic acid; 8-HOE, 8-hydroxyoleic acid; 8-HOTE, 8-hydroxylinolenic acid; 9*S*- and 13*S*- HPODE, 9*S*- and 13*S* hydroperoxylinoleic acid; 9*S*- or 13*S*- HPOTE (9*S*- or 13*S*- hydroperoxylinolenic acid).

focusing on progress using the model system *Aspergillus*. The emerging role of oxylipins as communication molecules recognized by both host and microbe and the ensuing consequences of such recognition in host–microbe interactions will be discussed.

The versatile role of fungal oxylipins

Oxylipin production is widespread among filamentous fungi, yeasts and oomycetes (protists resembling fungi in life style) and, in many cases, these compounds have a role in both organismal development and communication with the host on a cellular basis [7,16] (Table 1).

One of the few extracellular signals known to regulate both asexual and sexual spore development in fungi is a mixture of oxylipins collectively called psi factor (Figure 1) that are proposed to be hormone precursors that repress conidiation and promote precocious sexual development [17] (Figure 2). Extensive chemical studies of psi factor resulted in the identification of linoleic and oleic acid derived oxylipins. Psi factor is a mixture of hydroxylated oleic, linoleic and linolenic acid derivatives (termed psiAα, β and γ, psiBα, β and γ and psiCα, β and γ) produced by *Aspergillus nidulans* [18] and probably other fungi. The proportion of psiA to psiB and psiC is postulated to regulate the ratio of asexual to sexual development in *A. nidulans*. Specifically, PsiBα and PsiCα are reported to stimulate sexual and inhibit asexual spore development whereas PsiAα was antagonistic to the effects of PsiBα and

Table 1. Examples of fungal oxylipins and their putative function[a]

Formal name	Abbreviation or common name	Fungal source	Function
3,7,11-trimethyldodeca-2,6,10-trien-1-ol	Farnesol	*Candida*	Yeast to hyphal transition Quorum sensing
8*R*-hydroxy-octadeca-9,12-dienoic acid	8-HODE psiBα	*Gaeumannomyces* *Aspergillus* *Laetisaria* *Leptomitus*	Stimulation of sexual development
(5*S*, 8*R*)-dihydroxy-octadeca-9,12-dienoic acid	5,8-DiHODE psiCα	*Aspergillus*	Stimulation of sexual development
8*R*-hydroxy-octadeca-9,12-dien-5(S)-olide	psiAα (lactone ring of psiCα)	*Aspergillus*	Inhibition of sexual development
8*R*-hydroxy-octadeca-9-monoenoic acid	8-HOME psiBβ	*Aspergillus* *Gaeumannomyces*	Not known
(5*S*, 8*R*)-dihydroxy-octadeca-9-monoenoic acid	5,8-DiHOME psiCβ	*Aspergillus*	Not known
8*R*-hydroxy-octadecen-9–5(*S*)-olide	PsiAβ (lactone ring of psiCβ)	*Aspergillus*	Not known
(7*S*, 8*S*)-dihydroxy-octadeca-9,12-dienoic acid	7,8-DiHODE	*Gaeumannomyces*	Not known
3-hydroxy-5,8,11,14-eicosatetraenoic acid	3-HETE	*Dipodascopsis* *Candida*	Surface ornamentation Cell aggregation Spore release
3-hydroxy-5,8-tetradecadienoic acid	3-HTDE	*Mucor*	Not known
3,18-dihydroxy-eicosatetraenoic acid	3,18-HETE	*Candida* *Leptomitus*	Not known
15*S*-hydroperoxy-eicosa-5,8,11,13-tetraenoic acid	15*S*-HPETE	*Saprolegnia, Achlya*	Not known
Prostaglandins	$PGF_{2\alpha}$ PGE_2 PGD_2	*Lipomycetaceae* *Aspergillus, Saccharomyces,* several human pathogens [20]	Induce yeast to hyphal transition in *Candida*
Leukotrienes	LTB_4, CysLT	Several human pathogens [20]	Not known
9*S*-hydroperoxy-octadeca-10,12-dienoic acid	9*S*-HPODE	*Saccharomyces* *Fusarium*	Not known
8*R*-hydroperoxy-octadeca-9,12-dienoic acid	8-HPODE	*Gaeumannomyces*	Not known
15,16-dihydroxy-hexadeca-9-enoic acid	Ustilic acid A	*Ustilago maydis*	Antibiotic
2,15,16-dihydroxy-hexadeca-9-enoic acid	Ustilic acid B	*Ustilago maydis*	Antibiotic
13*S*-hydroperoxy-octadeca-9,11-dienoic acid	13*S*-HPODE	*Saccharomyces, Fusarium, Pleurotus*	Not known

[a]The original references that demonstrate the presence and putative function of fungal oxylipins are cited in Refs [7,16,18,79].

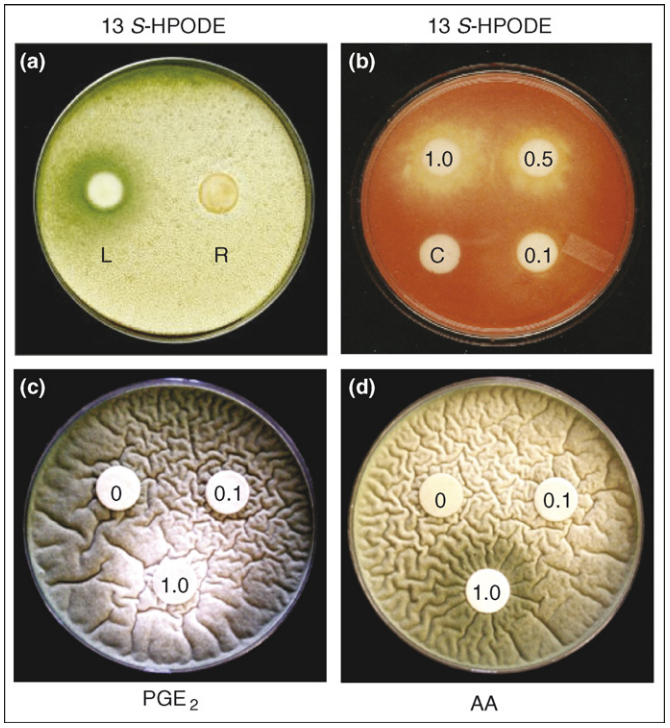

Figure 2. Sporogenic effects of oxylipins and arachidonic acid on *Aspergillus*. **(a)** Induction of asexual development in *Aspergillus flavus* by the plant-derived oxylipin 13*S*-HPODE at 1 mg/disk (left, L). Solvent control treatment on right (R). Photograph reproduced, with permission, from Ref. [80]. **(b)** 13*S*-HPODE inhibits norsolorinic acid (orange pigment precursor of aflatoxin) production in *A. flavus* at 0.5 mg and 1.0 mg/disk. C = solvent control. **(c)** Prostaglandin PGE_2 and **(d)** arachidonic acid (AA) affect *Aspergillus nidulans* development. Wild-type cultures were treated with filter paper disks containing the solvent control, 0.1 mg of AA or PGE_2, and 1.0 mg of AA or PGE_2. The 1.0 mg PGE_2 inhibited asexual sporulation in *A. nidulans* in part (c); by contrast, the 1.0 mg AA induces asexual sporulation in *A. nidulans* in part (d). Parts (c) and (d) reproduced, with permission, from Ref. [11].

PsiCα, inhibiting sexual and enhancing asexual spore development [17]. PsiBα had also been previously isolated from the basidiomycete *Laetisaria arvalis* and was named laetisaric acid (8-hydroxylinoleic acid); it has a biocontrol activity against the soil pathogens *Rhizoctonia solani* and *Pythium ultimum* and several other plant pathogens [19].

The arachidonic acid prostaglandin metabolites PGF_2 and PGF_2-lactone have been detected in several environmental yeasts of the Lipomycetaceae family (*Dipodascopsis, Lipomyces, Myxozyma* and *Zygozyma*) and *Saccharomyces cerevisiae* [7,16]. The pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* and the filamentous fungus *Aspergillus fumigatus* produce both PGs and LTs and their amounts are significantly increased after exogenous application of arachidonic acid [20]. PGE_2 and the primary metabolite of thromboxane A_2 (TXA_2), TXB_2, induce germ-tube formation in *C. albicans*, an activity that could be partially inhibited by antibodies against PGE_2 and TXB_2 [21]. The addition of PGE_2 to *A. nidulans* cultures inhibits conidia formation whereas its non-oxygenated precursor arachidonic acid induces conidiation [11] (Figure 2). Furthermore, COX inhibitors, including aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), inhibit 3-HETE (3-hydroxyeicosatetraenoic acid), PGE_2 and PGD_2 production in several members of the Lipomycetaceae family, supporting the view that a COX-like enzyme was active in these fungi [16,22]. COX or LOX inhibitors (e.g. aspirin, indomethacin and salicylic acid) also impair fungal development [23] in addition to PG production and biofilm formation in *C. albicans* [24].

In the prokaryotic kingdom, several species of lipogenic diffusible molecules regulate a variety of responses such as bioluminescence, virulence and biofilm formation in a density dependent manner through the quorum sensing mechanism [25]. Recent studies in fungi led to the discovery of the oxygenated lipid farnesol, which regulates quorum sensing, yeast-to-hyphal transition and biofilm formation in the human pathogen *C. albicans* [26,27]. A bacterial virulence factor structurally similar to farnesol was shown to inhibit the dimorphic transition in *C. albicans* [28], providing evidence for a cross-kingdom communication through an oxylipin molecule. Farnesol could be an important molecule in shaping fungal communities because it also promotes apoptosis in *A. nidulans* [29].

Aspergillus oxylipin biosynthetic genes

Several studies have demonstrated the presence of oxylipin-generating oxygenases in fungi including *Fusarium* spp., *Gaeumannomyces graminis*, *Laetisaria arvalis*, *Cercospora zeae-maydis* and *Ustilago maydis* [8,30–32] but their role in fungal biology has not been elucidated. Linoleate diol synthase (LDS), which catalyzes the enzymatic conversion of linoleic acid to psiBα and subsequently into (7*S*,8*S*)-dihydroxylinoleate, was the first oxylipin biosynthetic enzyme that was biochemically characterized from the fungus *G. graminis* [33]. The encoding gene, *lds*, has been cloned [8] but has not been inactivated in *G. graminis*. Based on sequence homology studies, Lds led to the discovery of three oxylipin biosynthetic genes (named Ppo: psi-producing oxygenases) in *A. nidulans* [9,10,12] and *A. fumigatus* [11], which were inactivated in both species.

Chemical analysis of the mycelia of the different *Δppo* mutants led to the conclusion that PpoA contributes to the production of the linoleic-acid-derived psiBα oxylipin [9] and PpoC to the formation of the oleic-acid-derived psi factor component psiBβ [10]. Inactivation of the third fatty acid oxygenase, *ppoB*, resulted in a strain with reduced levels of psiBβ suggesting that it might also be involved in the production of oleic-acid-derived psi factor [12]. The catalytic region of the three Ppo proteins are between 40–45% similar to that of human cyclooxygenase-2 and further analyses demonstrated that deletion of *ppoA* and *ppoC* resulted in a decrease of 14% and 36%, respectively, in PG production in *A. nidulans* [11]. Additional characterization of the *ppo* genes in *A. fumigatus* showed that the *ppo* genes also contribute to PG production in this species.

Genetic analysis of the role of Ppos in *A. nidulans* physiology demonstrated that the three deletion mutants exhibited distinct regulation of meiospore and mitospore development, supporting the role that was previously attributed to psi factor as a sporulating agent. Based on the effects of *ΔppoA* and *ΔppoC,* it was speculated that psiBα and psiBβ have opposing functions and the occurrence of both of them is indispensable to balance the sporulation program [10] (Figure 3). Overexpression

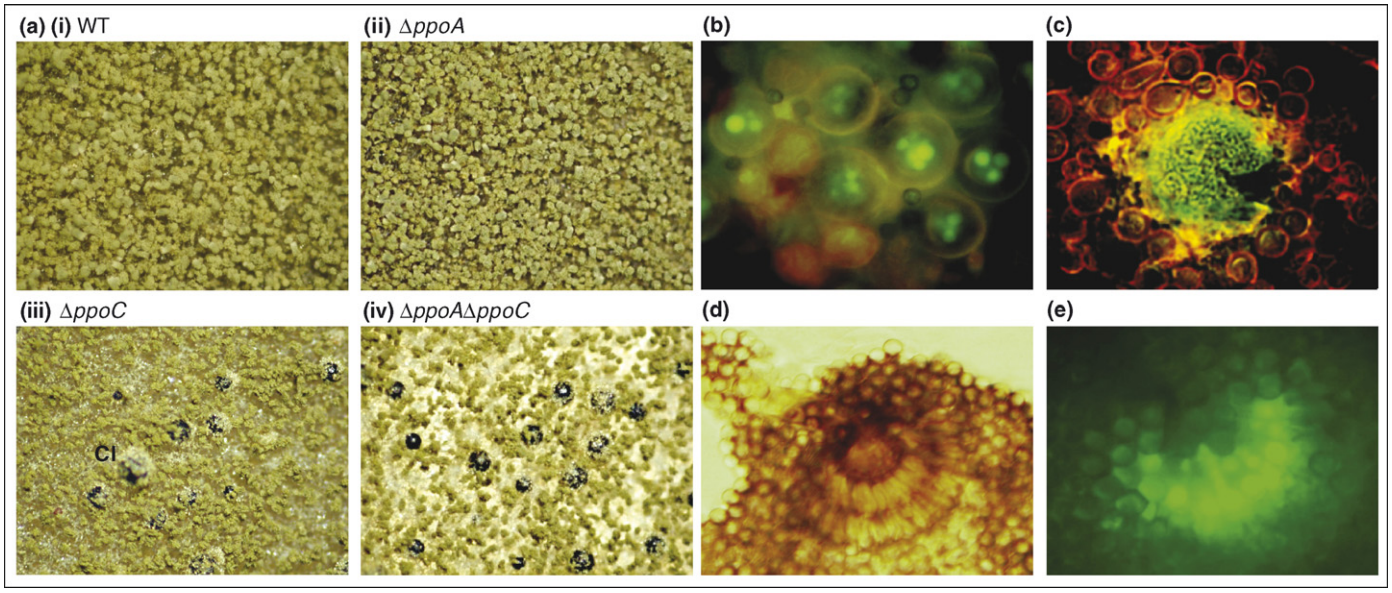

Figure 3. Oxylipins coordinate control of *Aspergillus nidulans* asexual and sexual development. **(a)** *ppoA* and *ppoC* genes are essential for balancing conidiophore and cleistothecia formation [e.g. in the wild type (i)]. (ii) In the *ΔppoA* mutant, sexual sporulation is suppressed and asexual fruiting bodies are induced. The opposite occurs in (iii) *ΔppoC* and (iv) *ΔppoAΔppoC* deletion strains, in which sexual sporulation is induced and asexual fruiting bodies are suppressed. Cleistothecia are observed as black balls [labelled 'Cl' in part (iii)] and smaller green spheres are conidiophore heads. Photographs reproduced, with permission, from Ref. [10]. The remaining parts of the figure show that PpoA localizes in lipid bodies of asexual and sexual fruiting bodies. **(b)** Localization of PpoA in lipid bodies in Hülle cells (primordial cells that surround the cleistothecia in *A. nidulans*) as seen by GFP fluorescence microscopy. **(c)** Localization of PpoA in the young developing cleistothecium surrounded by Hülle cells as seen by GFP fluorescence microscopy. Isolated conidiophore under **(d)** light microscopy and **(e)** fluorescence microscopy depicting the PpoA localization in lipid bodies in asexual fruiting bodies [9].

of *ppoA,* a strain that overproduces psiBα [9], resulted in a decrease of the ratio of asexual to sexual spore development six-fold. The original psi feeding studies showed similar results by adding exogenous psiBα in confluent mycelial cultures. Asexual development was significantly delayed and reduced in the double *ΔppoAΔppoC* and triple *ΔppoAΔppoAΔppoC* mutants concomitant with a precocious increase in production of ascospores. Thus, based on these results, it seems that the bioactive oxylipins produced by the Ppo enzymes collectively inhibit sexual sporulation or induce asexual production. Loss of these signals leads to a mis-scheduled developmental program with a precocious advent of sexual spore production and delay in asexual induction (Figure 3a).

Phylogenetic analyses showed that *ppo* genes are present in both saprophytic and pathogenic Ascomycetes and Basidiomycetes, suggesting a conserved role for Ppo enzymes in the life cycle of fungi [12].

Fungal 'oxylipin signature'

In-depth analyses of plant oxylipin pathways have uncovered complex and tight control of oxylipin production, presumably required for appropriate development in changing environmental milieus [34]. Temporal and spatial activity of different oxylipin biosynthetic enzymes seems to be of fundamental importance for normal growth; this is particularly true for lipoxygenase isoforms [35]. Activities and compartmentalization of the biosynthetic oxylipin enzymes are of paramount importance in determining the oxylipin profiles that will lead to the appropriate developmental pathway. Recent analyses indicate that the phyto-oxylipin pool of a given organelle, tissue, plant or species confers an 'oxylipin signature' to that respective entity [36,37]. It is proposed that the oxylipin signature is predictive of the execution of specific developmental pathways for the organism [4,36].

Biochemical and transcriptional analysis showed a temporal and spatial expression of the different *A. nidulans* oxylipin biosynthetic enzymes that might be of fundamental importance for their activity. The three *A. nidulans ppo* genes are not expressed constitutively in vegetative and developmental cultures, indicating the presence of a complicated but organized network of signalling cascades that synchronize oxylipin production in different tissues over time. *ppoA* expression is associated with formation of the asexual and sexual fruiting bodies and further green fluorescent protein (GFP) fusion studies indicated that the location of PpoA is highly correlated with its target organs, conidiophores and cleistothecia [9] (Figure 3b–e). *ppoC* is expressed at higher levels through a longer time period than *ppoA* whereas *ppoB* showed almost undetectable transcript levels throughout the *A. nidulans* developmental cycle [10,12].

The precise regulation of each *ppo* gene could be under an oxylipin-driven regulatory feedback circuit. For example, *ppoC* expression is inhibited in a *ppoA* overexpression strain and *ppoA* expression is downregulated in an oleate desaturase mutant (*ΔodeA*) that accumulates high levels of the PpoC product psiBβ [38]. These results led to the hypothesis of the existence of a fungal 'oxylipin signature profile' that functions as a 'master switch' in adjustment to diverse and variable environmental conditions and provides a fitness mechanism to the organism by temporally balancing meiospore to mitospore development. The relative production, activities and compartmentalization of the Ppo enzymes should be of paramount importance in determining oxylipin profiles of different fungal tissues. Microscopic studies using the fused *gfp::ppoA* allele led to the conclusion that PpoA is

localized to lipid bodies in the fruiting bodies [9] (Figure 3b; Figure 3e). It is well known from studies in plants and mammals that the surface proteins of lipid bodies probably have a role in lipid-body biogenesis, trafficking, mobilization and metabolism. Lipid body proteins include the oxylipin-producing enzymes prostaglandin H synthases in mammalian tissues and some lipoxygenases in plants [39].

Regulation of *Aspergillus* oxylipin pathways

Major players participate in the complex regulation of *ppo* genes including the light sensor protein VeA [40], the protein degradation machinery COP9 [41], the asexual transcription factor BrlA [42], the sexual transcription factor NsdD [43], the lipogenic transcription factors SREBP-1 and SREBP-2 [44] and a G protein pathway [45] (D.I. Tsitsigiannis and N.P. Keller, unpublished) (Figure 4). VeA and the COP9 signalosome directly or indirectly regulate the transcription of *ppoA* [9]. In fact, the existence of psi factor was discovered in a COP9 mutant that resulted in the overproduction of psi [46]. Whether directly or indirectly, the COP9 signalosome seems to function in negative regulation of psi production in *Aspergillus*.

Further studies with the *A. nidulans* Δ*ppo* mutants led to the characterization of feedback loops between PpoA and PpoC and the cleistothecial (NsdD)-specific and conidiophore (BrlA)-specific transcription factors, indicating a mechanism by the organism to maintain tight control of the meiospore to mitospore ratio [10]. Additional data suggest that PpoA and PpoC and/or their products serve as antagonistic molecular signals of lipogenic genes through regulatory feedback loops in the cellular machinery of the fungus [10]. Deletion of *ppoC* led to a significant increase in the transcription of genes involved in fatty acid biosynthesis and a concomitant increase in the total amount of fatty acids in the fungal thallus. By contrast, Δ*ppoA* lowered the transcriptional level of the lipogenic genes. Studies in primary rat hepatocytes and cultured 3T3-L1 adipocytes showed that arachidonic-acid-derived oxylipin metabolites (e.g. prostaglandin E_2) suppress FAS expression through a G-protein-coupled-receptor (GPCR) prostanoid signal transduction cascade [47]. Thus, in analogy to these studies, and based on the results that SREBP expression is altered in Δ*ppoC* and Δ*ppoA* mutants, we hypothesize that PpoC and PpoA product(s) modulate SREBP expression indirectly (SREBP factors are known to regulate transcription of lipogenic genes in mammals), perhaps by initiating autocrine/paracrine antagonistic signalling cascades that couple fatty acid anabolism to various developmental programs in *A. nidulans*.

Oxylipins and secondary metabolism

Fungi are among the most prolific producers of secondary metabolites that can be either beneficial (e.g. antibiotics) or detrimental (e.g. mycotoxins) to humankind [48]. Experiments to illustrate the effects of Ppo proteins on secondary metabolism indicated that the *A. nidulans* Δ*ppoA*Δ*ppoC* and Δ*ppoA*Δ*ppoB*Δ*ppoC* mutants were unable to produce the mycotoxin sterigmatocystin (ST) *in vitro* or *in planta* and led to an overproduction of the antibiotic penicillin (PN) [49]. These findings were correlated with decreased

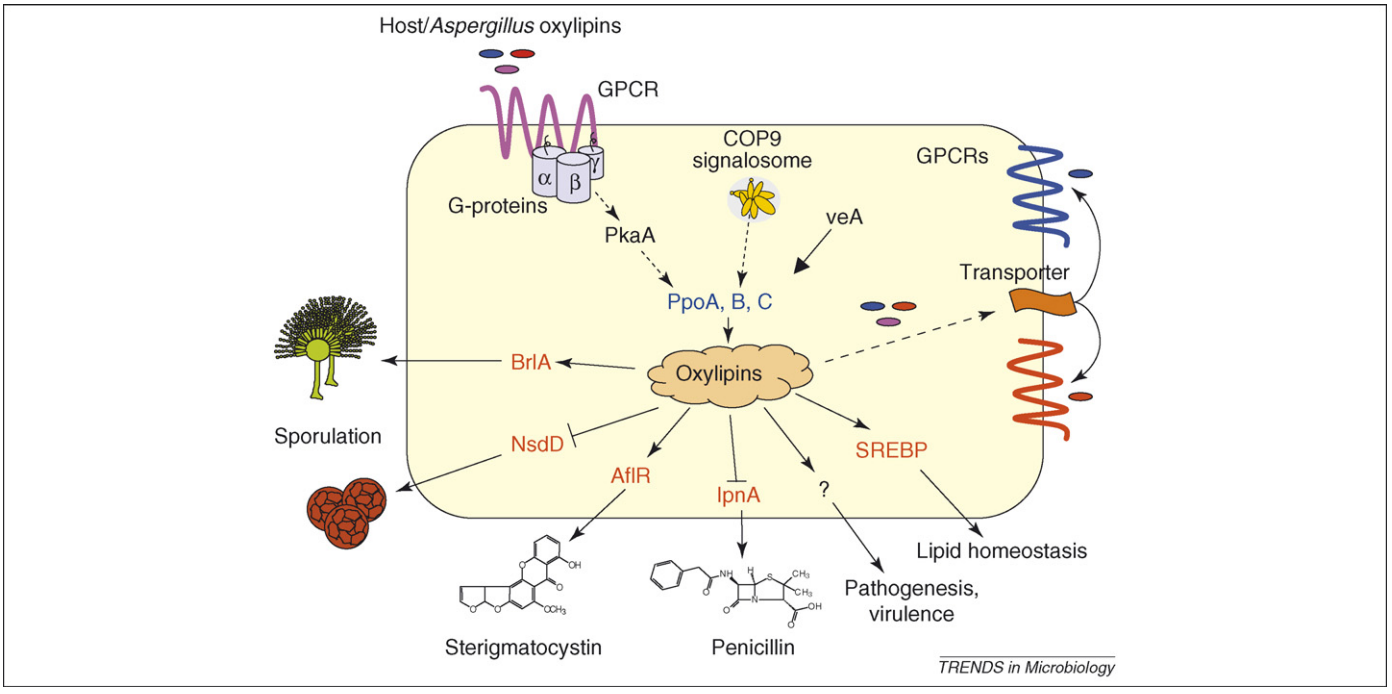

Figure 4. A hypothetical model depicting the *Aspergillus*–host oxylipin signalling in a fungal cell. Ppo enzymes are putative cyclooxygenase-like enzymes generating different oxylipin species, including prostaglandins. Oxylipins regulate the sporulation program of the fungus through the cleistothecial (NsdD) and conidiophore (BrlA) specific transcription factors, the fungal cell lipid homeostasis through the putative lipogenic transcription factors SREBP-1 and SREBP-2, fungal development and secondary metabolism (e.g. sterigmatocystin and penicillin) through a G protein pathway cascade involving protein kinase A (PkaA). The light sensor protein VeA and the protein degradation machinery COP9 have also been implicated in the complex regulation of *ppo* genes. Based on *A. nidulans* and *A. fumigatus* studies, oxylipins are involved in plant and mammalian pathogenesis or virulence. We hypothesize that oxylipins (besides their endogenous cellular role) exit the cell through the mediation of specific transporters and then function as autocrine or paracrine ligands that can bind to and sensitize self or host GPCRs, activating downstream signalling cascades.

expression of genes involved in ST biosynthesis and increased expression of the PN biosynthetic gene *ipnA*, thus suggesting that oxylipin species regulate secondary metabolites at the transcriptional level. The inverse regulation of ST and PN in the double and triple mutants was reminiscent of the opposite regulation of these two metabolites observed in a heterotrimeric G protein mutant ($FadA^{G42R}$) of *A. nidulans* [50]. The constitutively activated Gα-subunit $FadA^{G42R}$ suppresses *aflR* expression (*aflR* is a transcription factor required for ST biosynthetic gene expression [51]) but enhances gene expression levels for *ipnA*. This suppression of *aflR* in $FadA^{G42R}$ is mediated by PkaA [52] and thus it was postulated that oxylipin signalling might be PkaA mediated. The significance of G protein signalling pathways in natural product biosynthesis, sporulation and virulence reveals that ligands such as oxylipins might be important in initiating these cascades, presumably through GPCRs or similar cell surface proteins [45,48] (Figure 4).

Additional studies support a global role for *ppo* genes in natural product biosynthesis. For example, disruption of a *ppo* orthologue in *Fusarium sporotrichioides* impaired T2 toxin production [53] and studies of the plant pathogenic fungus *Cercospora zeae-maydis* showed that the *lds* gene (a *ppo* homologue) is upregulated under conditions that favour the production of the mycotoxin cercosporin in this fungus [32]. Additionally, long chain unsaturated fatty acid mutants with oxylipin defects are altered in ST and aflatoxin (AF) production at the level of gene regulation [54].

The manipulation of the different *ppo* genes in filamentous fungi could enable the increased production of pharmaceuticals or the elimination of fungal toxins by providing improved strains of engineered organisms and designing novel control strategies to reduce the survival and spread of mycotoxigenic aspergilli, respectively. In mammals, NSAIDs (e.g. aspirin, indomethacin, ibuprofen) known to block cyclooxygenase (COX)-derived PG synthesis are commonly used as analgesics and anti-inflammatories [55]. Aspirin inhibits a unique mechanism of action on COX by covalently acetylating a serine residue blocking proper substrate access and orientation at the active site. This opens up the possibility that Ppo enzymes, the counterparts of COX genes, might be good targets for future fungicides.

Oxylipins and pathogenesis

In addition to the role of oxylipins as agents that modulate intrinsic metabolic functions such as growth and maturation in fungi, the effects of fungal oxylipins on the host–pathogen interaction were also examined. Ppo null strains of *Aspergillus* grew and sporulated poorly on peanut seeds; this was coupled with a decrease in the production of the degradative enzyme lipase and the inability to produce the mycotoxin ST *in planta* [49]. These data might help to explain why *A. nidulans* and *A. parasiticus odeA* mutants, altered in oxylipin production, are impaired in the ability to colonize peanut and corn seed [56]. These results suggest that changes in the oxylipin profile, herein achieved through *odeA* or *ppo* mutations, lead to a malfunctional signalling system in the fungal cell that results in an inability to regulate the myriad of processes required for pathogenicity.

Surprisingly, the *A. fumigatus* triple *ppo*-silenced mutant that showed a 12% decrease in PG production was hypervirulent in the invasive pulmonary aspergillosis murine model system and showed increased tolerance to H_2O_2 stress than the wild type [11]. H_2O_2 treatment of fungal propagules is an indirect method of measuring putative resistance of the pathogen to host reactive-oxygen species (ROS), a major host antimicrobial effector system also active against *Aspergillus* conidia. Increased resistance to a ROS-mounted defence could protect a pathogen and render it more virulent. It is possible that Ppo products, PG and/or other oxylipins could serve as activators of mammalian immune responses contributing to enhanced resistance to opportunistic fungi and as factors that modulate fungal development contributing to resistance to host defences.

Studies of *C. albicans* also support a role for oxylipin synthesis in pathogenesis of mammalian mycopathogens. The *C. albicans* yeast to hyphal transition is often associated with progression of infection and, therefore, fungal oxylipin regulation of morphogenesis can be considered a pathogenesis mechanism. Treatment of strains isolated from patients with recurrent vaginal candidiasis with aspirin inhibits not only oxylipin production but also fungal growth [57]. Furthermore, *C. albicans* induces PGE_2 production by host cells, which can also exert immunosuppressive activity. This indicates that both host and fungal lipids have a role in immunomodulation and control of morphogenesis in *C. albicans*. Recent studies demonstrating that sublethal concentrations of several COX inhibitors (aspirin, diclofenac and etodolac) suppressed PG production and biofilm formation in *C. albicans* suggest that PG production could be a significant virulence factor in biofilm-associated infections [24,58].

Oxylipins as communication molecules

Plant oxylipins are produced by distinct plant LOX isozymes that preferentially introduce molecular oxygen into linoleic and linolenic acids either at C-9 (9-LOX) or at C-13 (13-LOX) of the hydrocarbon backbone of the fatty acid. Numerous plant LOX isoforms have been found to accumulate at different developmental stages, such as flowers, mature seeds or mobilization of storage lipids in germinating seedlings [35]. The LOX regiospecific oxygenation leads primarily to two groups of compounds, the 9*S*- and 13*S*- hydroperoxylinoleic acid (9*S*- or 13*S*- HPODE) or 9*S*- or 13*S*- hydroperoxylinolenic acid (9*S*- or 13*S*- HPOTE) (Figure 1). The 13-monohydroperoxides are precursors of biologically active compounds such as traumatin, jasmonic acid and methyl jasmonate, which have hormone-like regulatory and defence-related roles in plants including the induction of the expression of some pathogenesis-related (PR) proteins (e.g. defensins) or enzymes involved in the biosynthesis of phytoalexins [59]. The 9-monohydroperoxides are converted into compounds whose physiological actions are not known, although there is evidence that some of them have antimicrobial properties and induce the hypersensitive reaction (HR), a form of programmed cell death and one of the active defence resistance mechanisms against microbial invasion [60]. A recent

study examined the direct antimicrobial activities of 43 natural oxylipins against a set of 13 plant pathogenic microorganisms including bacteria, oomycetes and fungi based on *in vitro* growth inhibition assays. This study showed that most oxylipins are able to impair growth of some plant microbial pathogens including mycelial growth and spore germination [61].

The structural similarity of plant and fungal oxylipins has given rise to a hypothesis that they are important molecules in cross-kingdom communication based on the *Aspergillus*–seed pathosystem. *In vitro* studies showed that linoleic acid and its two plant oxylipin products 9*S*-HPODE and 13*S*-HPODE have a significant role in differentiation processes in *A. nidulans, A. flavus* and *A. parasiticus* (Figure 2a–b). Whereas all of the 18 C polyunsaturated fatty acids promoted asexual sporulation in all three species, 9*S*-HPODE stimulated and 13*S*-HPODE inhibited mycotoxin production (Figure 2b) [62,63]. Additionally, 13*S*-HPODE inhibited sexual spore production and sclerotial production in *A. nidulans* and *A. flavus*, respectively [64]. Other LOX pathway metabolites (e.g. methyl jasmonate, aldehyde products of 13*S*-HPODE and 13*S*-HPOTE) have also been reported to inhibit or stimulate fungal development and AF production [65–67]. These data led to the proposal that plant oxylipins affected *Aspergillus* developmental processes owing to their mimicry of native *Aspergillus* oxylipins. Because *A. nidulans* psiBα oxylipins are also derived from linoleic acid, seed fatty acids might regulate fungal development by mimicking and/or interfering with signals that regulate fungal sporogenesis. 9*S*-HPODE stimulated sexual spore production in *A. nidulans*, which suggests a similarity in the function of 9*S*-HPODE to the effects observed in *ΔppoC*, *ΔppoAΔppoC* and the triple *ppo* mutant in sexual sporulation. By contrast, the inhibitory effects of 13*S*-HPODE on sexual spore production were similar to the *ΔppoB* and *ΔppoA* phenotypes of decreased ascospore production.

Evidence for a role of oxylipins in plant–bacterial interactions also exists. A recent report indicated that recognition of the *Pseudomonas syringae* avirulence protein AvrRpm1 during the HR induces 9- and 13-lipoxygenase-dependent oxylipin synthesis in *Arabidopsis thaliana*, with the 13-lipoxygenase pathway dominating over that of the 9-lipoxygenase. The major oxylipins accumulated were jasmonic acid, 12-oxo phytodienoic and dinor-oxo phytodienoic acid. The last two were found to be esterified to a novel galactolipid named arabidopside E. This substance accumulated to surprisingly high levels, 7–8% of total lipid content, and inhibited growth of the bacterial pathogen *in vitro* [68].

Fungal infections also induce plant *LOX* gene expression. *Aspergillus* infections of peanut seed results in a concomitant repression of 13-*LOX* and induction of 9-*LOX* that leads to an overall increase in the AF/ST stimulating oxylipin (9*S*) and decrease in the AF/ST repressive oxylipin (13*S*) [69]. In maize seed, *Aspergillus* and *Fusarium* infections induce expression of *cssap92* (*ZmLOX3*: 9-*LOX*) in maize lines susceptible to AF contamination but repress *cssap92* expression in lines resistant to AF production [70]. These studies further support a case for specific 9-*LOX* genes as susceptibility and specific 13-*LOX* as resistance factors in mycotoxin contamination in seed crops.

Characterization of *A. nidulans* mutants that harbour the maize 9-LOX *ZmLOX3* under the control of the constitutively expressed promoter of glyceraldehyde-3-phosphate dehydrogenase (*gpdA*) gene led to increased production of conidia and ST (similarly to the *in vitro* action of 9*S*-HPODE), suggesting that *ZmLOX3* can partially substitute for native Ppo dioxygenases in *A. nidulans*. Additional experiments showed that peanut seed 13-LOX (*PnLOX2–3*) expression is decreased when infected by *A. nidulans Δppo* mutants compared with infection by wild type, providing genetic evidence that fungal oxylipins are involved in plant *LOX* gene expression changes, potentially leading to alterations in the fungal–host interaction. The data herein support the hypothesis that oxylipin cross-talk in the seed-*Aspergillus* pathosystem is reciprocal (M. Brodhagen *et al*. unpublished). Other oxylipins postulated to be important in seed–*Aspergillus* interactions include volatiles (e.g. C-6 aldehydes, alcohols and their esters) [71]. These compounds, formed through the hydroperoxide lyase pathway, also seem to be important for signalling within and between plants and for enabling the interacting pathogenic organisms of plants to recognize or compete with each other [72].

Perception model of oxylipin signals

In analogy to the PG perception in mammalian cells [5], the prime mode of oxylipin action might be as ligands through specific GPCRs, many of which have been cloned recently in *A. nidulans*, thus enabling specific receptor agonist and antagonist examination [45,73] (Figure 4). GPCRs are the largest group involved in recognizing diverse external signals and regulating different cellular processes by association with heterotrimeric G proteins. In *S. cerevisiae* three known GPCRs are important for perception of pheromones and carbon source and have crucial roles in mating and filamentous growth. Filamentous fungi contain a larger number of GPCR-like genes [74]. By contrast, the only identified putative GPCR homologues in plants are GCR1 in *Arabidopsis* and RCG1 in rice; however, plants contain a large number of seven transmembrane proteins and receptor-like kinases that might serve as oxylipin receptors [75].

We speculate that fungal oxylipins are not stored but are synthesized *de novo* from oleic and polyunsaturated fatty acids when cells are activated by external or internal stimuli. Oxylipins exit cells by facilitated transport through specific transporters and function locally at low levels (nM concentrations) through autocrine or paracrine processes (i.e. they signal at or immediately adjacent to their site of synthesis) on cell surface receptors linked to G-proteins. Recently, a mammalian GPCR (G2A) was discovered to function as a receptor for 9-hydroxyoctadecadienoic acid (9-HODE) and other oxidized free fatty acids, thus supporting our hypothesis [76]. Activation of G-protein-associated receptor leads to changes in proximal components of receptor-mediated pathways (e.g. G proteins, Ras) that activate intermediate signalling components (e.g. phospholipase C, PkaA and PKCs), and finally cAMP or calcium levels, which serve as second messengers that trigger signalling mechanisms with pronounced effects on various cellular functions. A single

oxylipin might have pleiotrophic effects owing to the existence of multiple receptors for each lipid species. In turn, these receptors might exert different responses on different tissues. Based on studies in mammals, fungi and plants, PUFAs or oxylipins can trigger activation of a wide range of cellular responses such as defence and stress responses, pathogenicity arsenals, cell wall formation, secondary metabolism and oxylipin biosynthesis itself [10,49,77,78]. This suggests oxylipin production and perception is important in the coordination of these responses and might guide adaptable responses of pathogenic fungi upon encounters with the host (Figure 4).

Concluding remarks and future perspectives

Our knowledge of oxylipin-mediated signalling in mycopathogens is still in its infancy. Evidence of an endogenous system balancing meiospore and mitospore production, secondary metabolism and fungal pathogenesis was uncovered with the characterization of the *Aspergillus* oxylipin biosynthetic *ppo* genes encoding fatty acid oxygenases similar in sequence to prostaglandin synthases. The relative levels and timing of differential *ppo* gene expression and the spatial distribution of their products determine fungal development. Apparently, Ppo proteins and/or their enzymatic products are involved in sophisticated interactions between several gene products or other molecules. Orthologues of these genes are found in filamentous fungi and, coupled with the numerous studies linking oxylipin production with fungal sporulation, support a case for conservation of an oxylipin-driven mechanism regulating developmental and pathogenesis-related processes in fungi. Despite these important recent findings, many questions remain to be answered, some of which are listed in Box 1. Lack of the *ppo* genes led to *Aspergillus* strains that were unable to produce the mycotoxin ST and showed a low potential to colonize host plant seeds. However, this was in stark contrast with the finding of a hypervirulence phenotype of *A. fumigatus* strain defective in *ppo* gene expression, as tested in a murine model of invasive aspergillosis. These results suggest a unique role of fungal oxylipins in different hosts and future studies are needed to delineate the different host and fungal factors that determine the disease outcome. The availability of several fungal genome databases in combination with microarrays and ligand–receptor studies will shed light on signal transduction pathways involved in oxylipin perception and function in different hosts.

Implications from these studies are far-reaching and suggest that fungal development in host–fungal interactions could be greatly influenced by lipid content of the host and the set of oxygenases expressed by the pathogen, both of which affecting various virulence and pathogenicity attributes. Potentially, modification of lipid profiles of either the host (generation of transgenic plants with altered lipid content) or the fungus (fungicides targeting the oxylipin biosynthetic enzymes, such as aspirin) could lead to novel control strategies of mycopathogens.

Box 1. Unanswered questions about the signalling and communication role of fungal oxylipins

- What is the full set of oxylipin biosynthetic enzymes and their products in fungi?
- Are fungal GPCRs oxylipin receptors?
- Can fungal oxylipins function as ligands of host receptors, and vice versa, modifying signalling pathways and modulating the infection process?
- Do plant or mammalian oxylipins mimic the endogenous biological activities of fungal oxylipins?
- What are the signal transduction cascades or circuits that enable fungal cells to sense and respond to the oxylipin signals?
- What are the molecular mechanisms that determine the direct or indirect transcriptional role of oxylipins?
- What are the mechanisms that enable oxylipins to regulate fungal secondary metabolism?
- Does the fungal 'oxylipin signature profile' determine the developmental program and fitness mechanism of a fungus in different environments?
- What is the role of oxylipins in inter-organismal ecology?

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