Published in final edited form as:
Biochim Biophys Acta. 2006 November ; 1761(11): 1391–1399.

Phospholipase A_2 and Phospholipase B Activities in Fungi

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Abstract

As saprophytes or disease causing microorganisms, fungi acquire nutrients from dead organic material or living host organisms. Lipids as structural components of cell membranes and storage compartments play an important role as energy-rich food source. In recent years, it also has become clear that lipids have a wide range of bioactive properties including signal transduction and cell to cell communication. Thus, it is not surprising that fungi possess a broad range of hydrolytic enzymes that attack neutral lipids and phospholipids. Especially during infection of a mammalian host, phospholipase A_2 (PLA_2) enzymes released by fungi could play important roles not only for nutrient acquisition and tissue invasion, but for intricate modulation of the host's immune response. Sequencing of fungal genomes has revealed a wide range of genes encoding PLA_2 activities in fungi. We are just beginning to become aware of the significance these enzymes could have for the fungal cells and their interaction with the host.

INTRODUCTION

Phospholipases (PLs) are ubiquitous enzymes involved in such diverse processes as membrane homeostasis, nutrient acquisition and generation of bioactive molecules. Some phospholipases play a role in microbial pathogenesis and virulence, while other phospholipase-like proteins are found in venoms. Catalytically active phospholipases share a common substrate, the phospholipid, which they preferentially hydrolyze from aggregates as, for example, in micelles or in a bilayer. As eukaroytic microorganisms, fungi have a plasma membrane and multiple intracellular organelles with phospholipid-based membrane layers. Membrane remodeling is an integral part of fungal life, because of many interchangeable morphotypes ranging from unicellular to multicellular and filamentous forms. The outer layer of fungal cells is a rigid cell wall. Thus, fungi are similar to plants, but they lack chlorophyll and the photosynthetic apparatus. The following review on fungal phospholipases will focus mainly on one class of phospholipases, PLA_2, that is likely to be of importance during interaction of fungi with each other, other microorganisms and their mammalian host.

Phospholipases hydrolyze mainly glycerophospholipids, although some also may degrade neutral lipids. Depending on the site of attack, the enzymes are classified as phospholipase A, B, C or D (see Fig. 1). Phospholipase A enzymes hydrolyze the 1-acyl ester (PLA_1) or the 2-acyl ester (PLA_2) of phospholipids. Many fungal species appear to produce phospholipase B

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enzymes that hydrolyze both acyl groups resulting in only minimal accumulation of lysophospholipid product. Hence, these enzymes often also have lysophospholipase activity, removing the remaining acyl moiety on lysophospholipids [1]. Interestingly, some fungal PLBs have been shown to exert transacylase activity since they are able to convert lysopholipids and free fatty acids into phospholipids. Phospholipase C enzymes are phosphodiesterases that cleave the glycerophosphate bond, while phospholipase D enzymes remove the base group of phospholipids (see Fig. 1). For a detailed classification of phospholipase A_2 in humans or other mammalia see the review by Schaloske and Dennis in this issue as well as reviews cited in [2,3].

PHOSPHOLIPASE A_2 FROM THE FUNGAL PERSPECTIVE

Early biochemical and microbiological studies of phospholipid acylhydrolases describe phospholipase A activities in several fungal species including the opportunistic pathogen *Candida albicans* [4–6]. More recent work, however, has demonstrated that many of the characterized fungal phospholipid-specific acylhydrolases are functionally phospholipase B (PLB)-type enzymes with multiple capabilities including those attributed to phospholipase A (i.e. deacylate *sn*-1 and *sn*-2 positions), lysophospholipase and lysophospholipid-transacylase activities [1]. In the following, we will present the few known *senso stricto* PLA_2 enzymes in fungi and then stretch the definition of PLA_2 enzymes to focus on fungal PLBs and patatin-like proteins.

Secretory PLA_2 in fungi

A ‘genuine’ phospholipase A_2 activity with exclusive specificity for the *sn*-2 position of phospholipids has been described in the ascomycete truffle *Tuber borchii* [7] and the corresponding cDNA sequenced. For example, the TbSp1 encoded enzyme (M_r 19 kDa) has negligible lysophospholipase activity, thus the products of 1,2-dipalmitoyl-phosphatidyl choline hydrolysis are 1-palmitoyl lysophosphatidyl choline and free palmitic acid [7]. Similar to other $sPLA_2$, the TbSp1 phospholipase activity is Ca^{2+}-dependent, and possesses the $sPLA_2$-typical His/Asp dyad and cysteines for disulfide bond formation, albeit the latter in a lower number [4 cysteines versus 10 to 16 in other $sPLA_2$s]. The clear structural and functional similarities of the TbSp1 enzyme to $sPLA_2$ in groups I, II, III, V, IX to XIII have led to the formation of a new group XIV within the PLA_2 family utilizing a catalytic histidine with TbSP1 as a founding member [3]. Further members in this group of microbial PLA_2s are an enzyme from *Streptomyces violaceoruber*, the first $sPLA_2$ identified in a prokaryote [8], and two orthologous enzymes found in *Str. coelicolor* [9]. Structural characterization of the *Str. violaceoruber* $sPLA_2$ protein revealed the surprising fact that the overall structure of this enzyme is completely different from those of eukaryotic $sPLA_2$s, however, similarities between the proteins’ sequences and catalytic mechanisms are clearly detectable [8,10–12]. Another fungal member of group XIV is the secreted protein p15 (see Figure 2) which was recently isolated from the ascomycete *Helicosporium* sp. HN1 [13–15]. The 15 kDa protein was discovered when bacterial and fungal culture supernatants were screened for modulatory effects on neuronal differentiation and survival [16]. Nerve growth factor (NGF)-induced outgrowth of neurites from rat pheochromocytoma PC12 cells was potently stimulated by p15. Further studies identified p15 as a secretory PLA_2 and revealed that its enzymatic activity, rather than receptor binding, is essential for neuritogenesis [17,18]. PLA_2s with a preference for phosphatidylcholine, such as p15 or mammalian group X $sPLA_2$s, release lysophosphatidylcholine which is a crucial second messenger for stimulation of neurite outgrowth in PC12 cells [18]. Our recent BLASTp [19] searches within fungal protein sequences in GenBank reveal the presence of possible TbSP1 orthologues in some filamentous ascomycetes including *Aspergillus oryzae* (BAD01581, BAD01582), *Gibberella zeae* (XP_384087), *Magnaporthe grisea* (XP_363441) and *Neurospora crassa* (CAD21498,

XP_958163) (see Table 1 and Fig. 2). The sequences are similar in size and contain the conserved His/Asp dyads, however, their reliable classification as $sPLA_2$s requires functional characterization.

Studies on the role of TbSP1 in the ectomycorrhizal ascomycete *T. borchii* have shown that the expression of the $sPLA_2$ homologue is increased during C- and N-deprivation [7] and mycorrhiza formation [20]. The enzyme is reportedly both cell surface-associated and secreted [7]. To date it is not clear whether this $sPLA_2$ plays a role in nutrient acquisition and/or in lipid-mediated signaling in the fungi or in the plant-fungus interaction. Released lysophospholipids and unsaturated fatty acids could represent signaling molecules or precursors thereof. For instance, released polyunsaturated linoleic acid could be polyhydroxylated to oxylipins by the action of fungal linoleate diol synthases. Oxylipins are known as hormone-like signals, for example, in *A. nidulans* for regulating the balance of sexual and asexual spore formation as well as host colonization and mycotoxin production [21,22]. Recently, oxylipins have also been detected in *T. indicum* [23], thus lending support to a possible involvement of these molecules in the biology of truffles.

Cytosolic PLA_2 in fungi

A $cPLA_2$ encoding gene (*plaA*) has recently been isolated from *A. nidulans* [24]. PlaA is an 837 amino acid protein (theoretical M_r 92.3 kDa) that in BLASTp analyses displays the highest sequence similarity with potential but so far uncharacterized paralogues and orthologues in other ascomycetes like *A. fumigatus*, *A. oryzae*, *M. grisea*, and *Coccidioides immitis*. Besides these fungal proteins, PlaA appears to be more closely related to mammalian-type $cPLA_2$ proteins than to fungal PLBs. The protein sequence harbors no signal sequence indicating intracellular localization of PlaA. An N-myristoylation site for lipidation in the N-terminal region, a structural feature also found in human GIVC PLA_2 (cPLA2 γ; [25]), might be involved in interaction with membranes and proteins or have regulatory functions. Conserved catalytic residues comprised of the Ser/Asp dyad and the additional arginine in human GIVA PLA_2 [25] is represented in the PlaA protein sequence (Fig. 2). Moreover, a serine residue equivalent to the regulatory serine in GIVA PLA_2 was identified that is phosphorylated by MAPK [26]. However, PlaA lacks the calcium-dependent lipid-binding domain (C2 domain) present in some GIV PLA_2s (Fig. 2), although requires Ca^{2+} for hydrolytic activity towards phosphatidylethanolamine and phosphatidylcholine, the latter being the preferred substrate [24]. It is intriguing to speculate whether PlaA (or a paralogue) is involved in release of oxylipin precursors from phospholipids.

PLBs – are they fungal GIV PLA_2 homologues?

Phospholipase B enzymes harbor three distinct activities: a *sn*-1 and *sn*-2 fatty acid ester hydrolase, a lysophospholipase and a transacylase activity. PLB activities have been described in humans and other mammals [27–34], bacteria [35] and especially in fungi [1]. Morgan *et al.* found a phospholipase B lacking the typical GxSxG lipase motif in the social amoeba *Dictyostelium discoideum* that is therefore not related to the aforementioned PLBs, but has potential protist, invertebrate and mammalian homologues [36]. Many fungal species have more than one PLB gene. For example, the genome of the non-pathogenic yeast *Saccharomyces cerevisiae* encodes three [37–39], while the genome of the opportunistic fungal pathogen *Candida albicans* encodes a PLB family with five members [40,41], (Fig. 2). We will focus our review on PLBs from fungi that are pathogenic to humans because of the potential role of these enzymes in pathogenesis of fungal infections.

Cryptococcus neoformans—The basidiomycete *Cryptococcus neoformans* is an opportunistic pathogen that causes severe infections of the central nervous system in immunocompromised patients [42]. Chen and coworkers isolated a secretory PLB that has

been implicated in virulence [43,43]. The enzyme is a glycoprotein with hydrolytic (PLB, lysophospholipase) and lysophospholipase/transacylase (LPTA) activities at acid pH. The Cn*PLB1* gene was cloned from opportunistic *Cr. neoformans* var. grubii and functionally characterized by generating a gene knock-out strain [45]. The *cnplb1* null mutant was significantly less virulent in a mouse inhalational model and a rat meningitis model. More recent studies have demonstrated that CnPLb1 is crucial for the establishment of interstitial pulmonary infections, possibly by facilitating adhesion to lung epithelial cells, and for hematogenous and lymphatic dissemination from the lung [46,47]. PLB activity, however, is not required for macrophage-facilitated dissemination of intracellular cryptococci to the brain. The same group has also shown that anti-CnPlb1 antibodies can be found in sera of patients for up to two years after cryptococcal infections [48] and that specific PLB inhibitors can exert antifungal activities towards cryptococci [49]. Coe *et al.* isolated another lysophospholipase and LPTA encoded by the Cn*LYSO1* gene that shows functional dependence on CnPlb1, thus suggesting a post-translational interaction of the two unrelated enzymes [50]. A Cn*PLB1* gene homologue (Cb*PLB1*) was also described in the strictly pathogenic *Cr. neoformans* var. *gattii* (serotypes B and C), a variety biochemically and genetically distinct from *Cr. neoformans* var. *grubii* (serotype A) and *Cr. neoformans* var. *neoformans* (serotype D) [51]. The teleomorph (sexual form) of *Cr. neoformans* var. gattii is *Filobasidiella neoformans* var. *bacillispora* while the other varieties correspond to *F. neoformans* var. *neoformans*. Due to mounting molecular evidence the classification of *Cr. neoformans* var. *gattii* in the new species *Cr. bacillisporus* has been proposed [52,53], thus our designation Cb*PLB1* (Table 1 and Fig. 2).

Analysis of the amino acid sequences of CnPlb1 and CbPlb1 revealed that both proteins contain putative N-terminal signal peptides for secretion and C-terminal hydrophobic regions with glycosylphosphatidylinositol (GPI) anchor attachment motifs (Fig. 2). Djordjevic et al. using a heterologous expression system in *Saccharomyces cerevisiae* provided experimental evidence that the GPI motif directs CnPlb1 to the plasma membrane and, presumably after further processing, predominantly to the cell wall [54]. Cell wall-bound enzyme might be ready for rapid release into the supernatant by GPI-cleaving enzymes under appropriate environmental conditions [54]. In the plasma membrane, CnPLB1 appears to be specifically associated with lipid rafts, rigid microdomains in the membrane formed by aggregation of sterols and sphingolipids [55]. Surprisingly, PLB activity is suppressed in the lipid raft environment while lysophospholipase und LPTA activities are unaffected.

Pathogenic fungi produce eicosanoids like prostaglandins or leukotrienes from arachidonic acid [56–58]. Noverr and coworkers investigated the role of CnPLB in eicosanoid production in *C. neoformans* [59] and demonstrated that the enzyme is necessary for release of the precursor arachidonic acid from arachidonoyl-phosphatidylcholine.

Aspergillus fumigatus—Recently Shen *et al.* [60] reported three putative PLB genes (af*plb1*, af*plb2*, and af*plb3*) in the filamentous fungus *A. fumigatus*, a major opportunistic pathogen in humans. *A. fumigatus* causes a wide range of disease including allergic bronchopulmonary aspergillosis and invasive aspergillosis [61]. Gene transcripts of af*plb1* and af*plb3* were induced by lecithin (dipalmitoyl phosphatidylcholine), a major component of lung surfactant. This could be an indication that the PLBs are indeed involved in virulence because the lung is the main portal of entry for *A. fumigatus*. Proteins encoded by af*plb1* and af*plb3* bear the structural hallmarks for secreted and possibly GPI anchored enzymes, a signal sequence and a hydrophobic C-terminus. AfPlb2, on the other hand, lacks a signal peptide according to sequence analysis. All three enzymes harbor the typical catalytic triad comprised of Arg, Ser, and Asp. This triad can be found in other fungal PLBs and human cytosolic PLA_2 [25]. The functional role of the AfPLBs in cell biology and virulence remains to be tested. The extracellular PLBs, AfPlb1 and AfPlb3, as well as the secreted phospholipase C (PLC)

activity may be important in the interaction of *A. fumigatus* with host cell membranes [62, 63].

Candida albicans—With the advent of the AIDS pandemic and the increased number of patients with impaired immunity due to cancer or iatrogenic immunosuppression in transplant medicine, the incidence of fungal infections has risen dramatically. For example, *Candida* species, and predominantly *C. albicans* are now the fourth most commonly isolated pathogen from nosocomial blood stream infections in the US [64] and oral candidiasis is one of the first indicators of HIV disease progression.

Early studies on extracellular phospholipases in *C. albicans* described phospholipase A and lysophospholipase activities [4,6,65]. A putative role of extracellular phospholipase was proposed later by Pugh and Cawson [66] who found the highest phospholipase production at the tip of hyphae invading chicken chorioallantoic membranes which they used as a tissue invasion model. The first unequivocal characterization of an extracellular phospholipase activity required the purification to homogeneity as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The isolated 84 kDa glycoprotein retained Ca^{2+}-independent PLB activity as well as lysophospholipase and LPTA activity [67]. Thus, the purified protein displayed enzymatic activities similar to those of other fungal PLBs, for example the PLBs from *Penicillium chrysogenium* [68] (former *P. notatum*) or *S. cerevisiae* [37,39,69,70].

Extracellular phospholipases as virulence factors in *C. albicans* pathogenesis were investigated by several groups: Ibrahim et al. [71] compared the extracellular phospholipase production of *C. albicans* blood isolates with those of commensal isolates and found that the former strains generated significantly more of the secreted activity *in vitro*. Furthermore, they demonstrated that high phospholipase secretion correlated with higher invasiveness in an epithelial cell assay and increased virulence in mouse models of disseminated candidiasis. Secreted phospholipase activities of *C. albicans* and other *Candida* species were compared *in vitro* using egg-yolk agar based methods and [72,73] as is the case with secreted proteinases, *C. albicans* showed the highest activities. However, lack of specificity of the egg-yolk agar methods for phospholipase determination has to be considered since secreted lipases (triacylglycerol hydrolases) might generate false-positive results. Furthermore, phospholipase gene and protein expression of a given strain could be differentially regulated *in vitro* versus the relevant *in vivo* situation. Hence, for functional characterization of PLBs as true virulence-associated factors, proof of the molecular version of Koch's postulates is desirable [74,75]. Two groups simultaneously published the sequence of the *C. albicans* phospholipase B1 (Ca*PLB1)* gene in 1998 [76,77]. Ca*PLB1* encodes a protein with 605 amino acids (M_r 66.5 kDa) with significant similarity to other fungal PLBs. While disruption of the gene did not reveal any difference in viability of the corresponding mutant *in vitro* or in its adherence to human endothelial and epithelial cells, the null mutation caused significant attenuation in virulence in a mouse model of systemic candidiasis [76]. This study used only a single *plb1* null mutant clone generated with the so-called *URA3* blaster technique [78] without the necessary revertant control. Therefore, the phenotype was confirmed in a second report by reintegration of an intact copy of the Ca*PLB1* gene to corroborate that the observed virulence phenotype was due to the ca*plb1*Δ mutation and not to secondary effects generated during the disruption procedure [79]. A mouse model for hematogenously disseminated candidiasis as well as an infant mouse oral-intragastric infection model demonstrated attenuation in the virulence of ca*plb1*Δ mutants.

Isolation of two other putative PLB genes and genome sequencing soon made it clear that the *C. albicans* genome harbors a relatively large PLB gene family of five members [40,41]. Ca*PLB2*, a PLB gene closely related to Ca*PLB1*, was isolated by Sugiyama *et al*. [80], however, gene disruption mutants which would enable functional characterization have not been

generated. Naglik *et al.* investigated by reverse-transcriptase polymerase chain reaction (RT-PCR) the secreted aspartyl proteinase (SAP) gene expression as well as the expression of Ca*PLB1* and Ca*PLB2* of *C. albicans* in whole stimulated saliva or vaginal swabs from 137 individuals that were *C. albicans* carriers or had candidiasis [81]. The study found that Ca*PLB1* expression correlated with human oral infection, while Ca*PLB2* expression while detected in some specimens did not correlate with infection or carrier status.

A third gene, Ca*PLB5* isolated by our group encodes the most distantly related protein to CaPlb1 within the *C. albicans* PLB family [41]. Ca*PLB5* encodes a 754 amino acid protein (theoretical M_r 81.4 kDa) with a signal sequence for secretion and a C-terminal hydrophobic sequence with a GPI anchor attachment site. While a short serine/threonine-rich domain in the C-terminal portion is common in other PLBs, the long (approx. 90 amino acid) S/T-rich N-terminal extension found in CaPlb5 appears exceptional. Searching current fully-sequenced genomes, this N-terminal extension is only found in PLBs from species very closely related to *C. albicans*, such as *Debaryomyces hansenii* (genome sequence published by [82]); neither the PLBs of other hemiascomycetous yeasts (e.g. *S. cerevisiae, C. glabrata*), archiascomycetes (*Schizosaccharomyces pombe*), euascomycetes (e.g. Aspergilli, *N. crassa*) nor of basidiomycetes such as *Cr. neoformans* (for review on genomics and evolution in yeasts see reference [83]) encode a similar domain. As this S/T-rich region provides multiple sites for O-glycosylation, it is possible that the protein assumes an extended rod-like structure that might protrude from the cell wall, as described for Epa1 adhesin in *C. glabrata* [84]. Epa1, however, is tethered to the cell wall via a processed GPI-anchor remnant. Therefore, assignment of a functional role to the N-terminal S/T-rich region and GPI-anchoring in CaPlb5 requires further study.

Using the MPA^R flipper [85,86] technique with a dominant selection marker [87] Ca*PLB5* null mutants were constructed in a wild-type strain of *C. albicans*. Phenotypic analysis of the ca*plb5* null mutants revealed loss of cell-associated phospholipase A_2 activity and significant decrease in liver colonization in an experimental mouse model for disseminated candidiasis [41]. Two additional phospholipase B genes are present in the *C. albicans* genome: Ca*PLB3* and Ca*PLB4*. Their functional roles remain to be determined. The individual PLB gene family members are expressed at different levels and show differential induction of transcription depending on the environmental conditions [41]. High expression levels are generally seen in hyphal or pseudohyphal forms of the fungus. Consequently, induction in the presence of phospholipids is most pronounced in the yeast form [41]. *In vivo* the individual members of the PLB family may contribute to varying degrees to pathogenesis at diverse host sites invaded and colonized by *C. albicans*. As with the SAP family in *C. albicans* [88], we can expect a high degree of regulation and functional diversity among the PLB family members.

Patatin-like domains – not just potatoes

Patatin is a soluble 386 amino acid storage protein predominantly localized as an inactive enzyme in the plant vacuole of potato tubers. Activation of patatin occurs upon pathogen infection or environmental stress. The protein accounts for about 40% of the soluble protein fraction in mature potato tubers; it possesses general esterase activity and in particular phospholipase A activity [89,90]. Structural analysis revealed that patatin contains an active site with a Ser/Asp catalytic dyad and glycine-rich motif (G-X-G-X-X-G) similar to human cytosolic phospholipase A_2, however, it does not present structural features (i.e. a flexible lid domain) suggestive of interfacial activation, nor a Ca^{2+}-dependent (C2) lipid-binding domain like $cPLA_2$ [91,92]. Recent studies have revealed that patatin-like domains can be found in several proteins of microorganisms, including pathogens. An interesting example is the exoenzyme U (ExoU), a potent cytotoxin of *Pseudomonas aeruginosa* which is encoded on a pathogenicity island and delivered into eukaryotic cells via type III secretion [93]. Expression

of the toxin in *S cerevisiae* caused vacuolar fragmentation, accumulation of palmitic acid and changes in phospholipid profiles [94]. Susceptibility of ExoU activity to PLA inhibitors and the structural similarities with patatin and PLAs demonstrated that the toxin is an intracellular phospholipase. A eukaryotic host cell factor is required for activation of the ExoU phospholipase and thus provides selectivity for its cytotoxic action on eukaryotic cells [95].

Fungi also produce patatin-like proteins. For example, in *S. cerevisiae* the triacylglycerol (TAG) lipases ScTgl3p, ScTgl4p, and ScTgl5p involved in the mobilization of TAG from lipid storage particles contain patatin-like domains [96,97]. ScNTE1, a homologue of human neuropathy target esterase (NTE), is a deacylating enzyme (phospholipase and lysophospholipase) involved in phosphatidylcholine turnover that also harbors the domain. Homologues of the aforementioned lipases with patatin-like domains can be found in pathogenic fungi like *C. albicans*(see Table 1 and Fig. 2). It remains to be determined whether these enzymes have a role in pathogenesis or virulence. Interestingly, patatin-like proteins have been identified in plants, e.g. in tobacco leaves, that exhibit PLA_2 activity and are induced during the hypersensitive response of the plant towards pathogen attack [100,101]. The patatin-related PLA_2 enzymes are believed to release precursors for oxylipins which are important plant defense molecules.

CONCLUSION

Fungal enzymes with phospholipase A_2 activity represent a heterogeneous group of proteins with some typical active site features embedded in a variety of structural backgrounds. Especially the multi-functional PLB enzymes are very common hydrolases in fungi, usually as components of the secretome, reflecting their importance in membrane homeostasis, acquisition of nutrients and/or interaction with other microorganisms or host cells. The potentially important role of fungal PLA_2 enzymes in mobilization of arachidonic acid precursors during contact with mammalian host cells opens up an intriguing field of study. The possibility that fungi interfere with the host's lipid-mediated signaling cascade not only by liberation of precursors, but also by generation of pro- or anti-inflammatory signaling molecules such as prostaglandins presents an interesting new insight into the complex interactions of fungi with their host.

Acknowledgements

The authors would like to thank the Volkswagen Foundation, Germany (1/74643) for financial support. This work was also supported by NIH/NIDCR grants P01 DE16839 (N. Agabian) and R21 DE014705 (G. A. Köhler) as well as UCSF/ARI-CARC grant CC02-SF-002 (N. Agabian). E. Haas-Stapleton was supported by the University-wide AIDS Research Projects (UARP) Postdoctoral Fellowship F04-SF-211 of the University of California.

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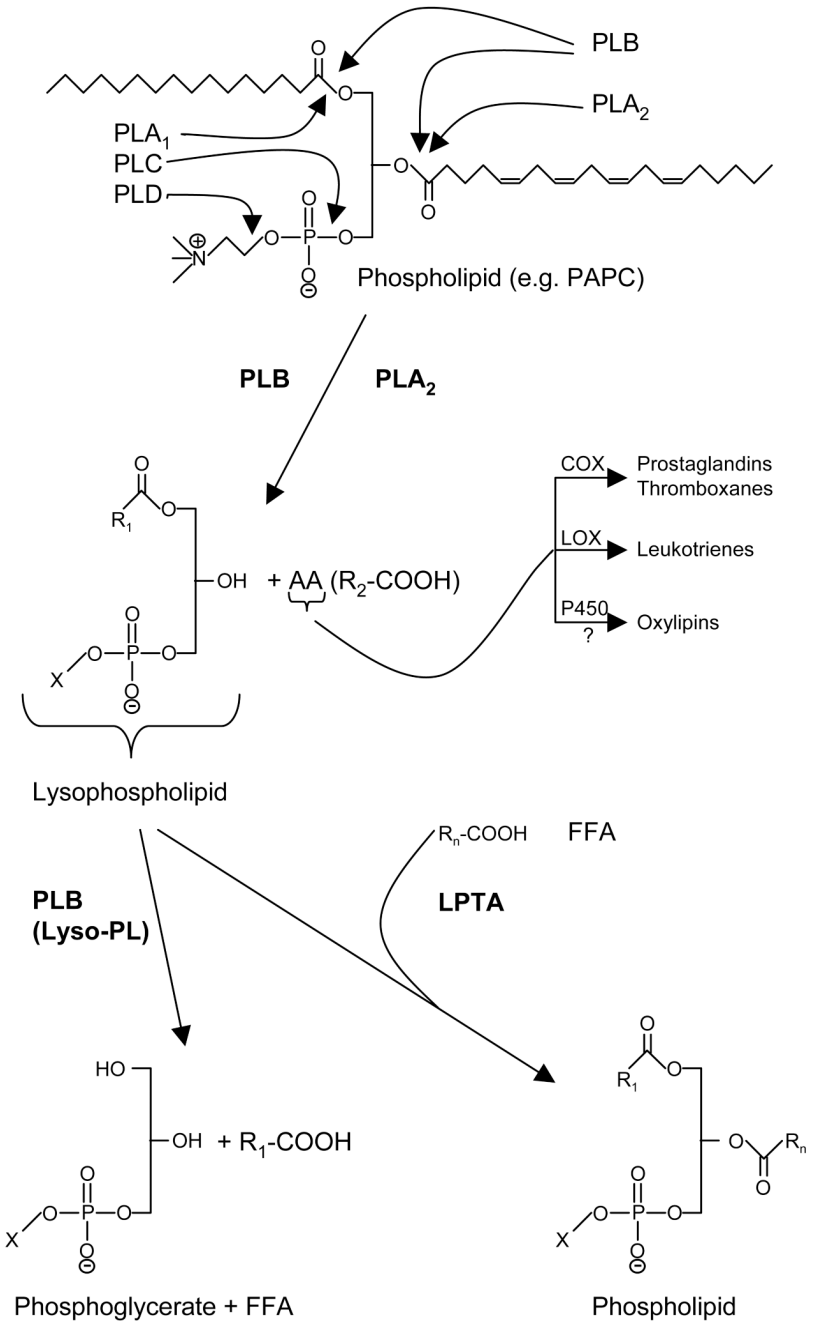

Figure 1. Mechanism of action of phospholipases (A, B, C, D)

The positions of hydrolytic activities of different types of phospholipases on a phospholipid (PAPC) are depicted. PLA_2 or PLB enzymes form lysophospholipids whose remaining esterified fatty acid can be removed by PLBs or other lysophospholipases. Lysophospholipase/transacylase (LPTA) activity commonly found in PLBs may esterify a free fatty acid (FFA) to a lysophospholipid resulting in a phospholipid. Arachidonic acid (AA) in the *sn*-2 position of PAPC can be mobilized by PLA_2 or PLB enzymes and function as a precursor for eicosanoids which are generated by the indicated enzymatic activities.

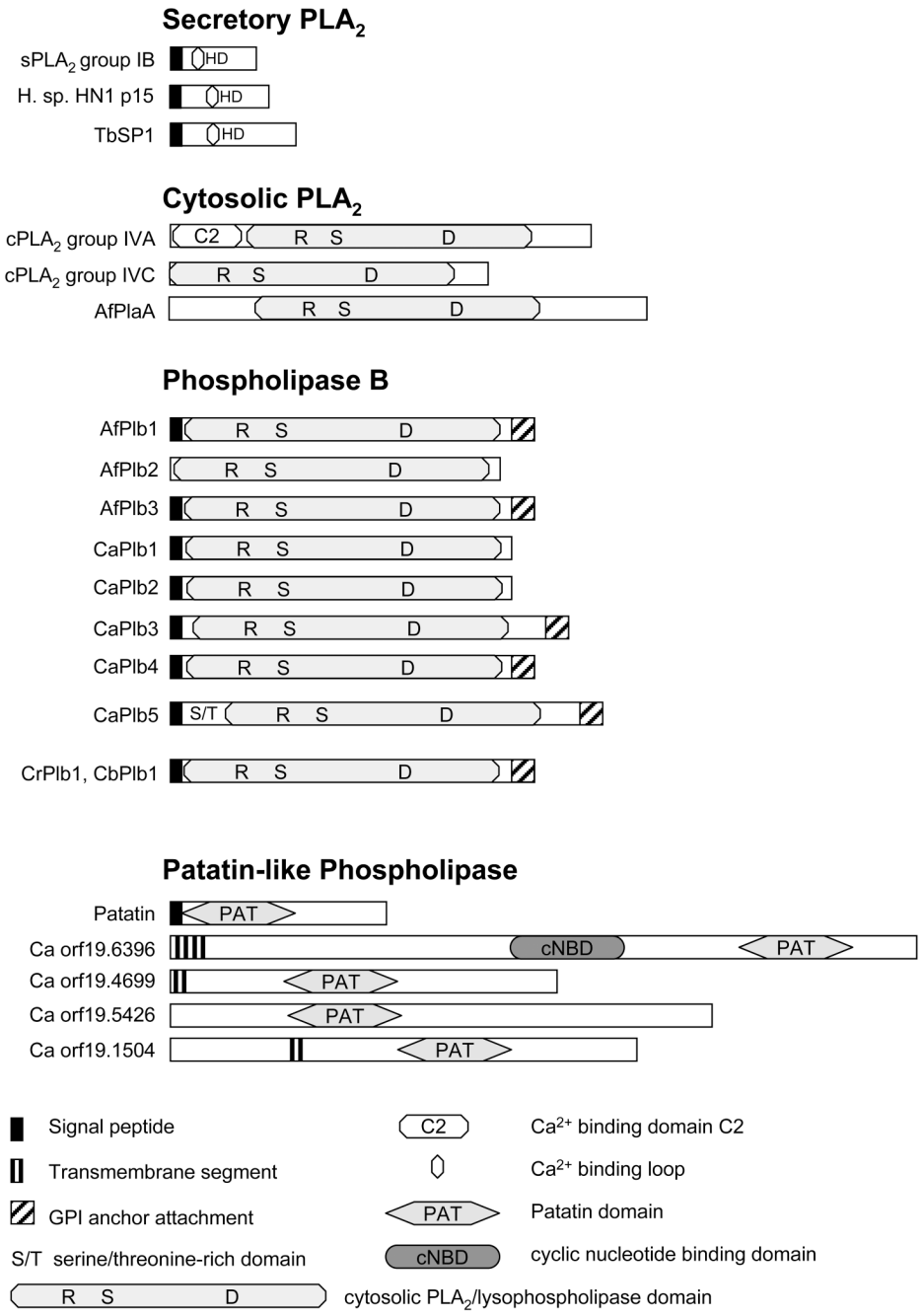

Figure 2. Domain structure of PLA_2 enzymes

Putative fungal orthologues of secretory ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$) enzymes are shown in comparison to the human enzymes are shown. Additionally, the structural domains of fungal PLB enzymes and patatin-like phospholipases are depicted.

Table 1

PLA_2, PLBs and patatin-like phospholipases in fungi[1]

Fungal species and gene name[2]	Size in aa	Other fungi with possible orthologues	Closest human homologue[3]	PLA_2 grouping[4]	Experimentally confirmed enzymatic activity	References
Secretory PLA_2 ($sPLA_2$) with a catalytic histidine						
Tuber borchii TbSP1	211	*A. oryzae, Coccidioides immitis, Giberella zeae, Helicosporum* sp., *Magnaporthe grisea, Neurospora crassa*	$sPLA_2$	group XIV	PLA_2 (Ca^{2+}-dependent)	[7,14]
Helicosporum sp. HN1	169					
p15						
Cytosolic PLA_2 ($cPLA_2$) with a catalytic serine						
Aspergillus fumigatus afplaA	842	*A. nidulans* (published), *A. oryzae, Chaetomium globosum, Co. immitis, G. zeae, M. grisea, N. crassa, Ustilago maydis*	$cPLA_2$ GIVC	closest to group IV	PLA_2	[24,102]
Phospholipase B enzymes (PLB)						
Aspergillus fumigatus		*Saccharomyces cerevisiae* and other fungi				
af*plb1*	633		$cPLA_2$ GIVC	closest to group IV	n.d.	[60]
af*plb2*	588					
af*plb3*	630					
Candida albicans		*S. cerevisiae* and other fungi				
Ca*PLB1*	605		$cPLA_2$ GIVC	closest to group IV	PLB, lyso-PL, LPTA	[76,77,79,103]
Ca*PLB2*	609				n.d.	
Ca*PLB3*	702				n.d.	[80]
Ca*PLB4*	632				n.d.	[40,41]
Ca*PLB5*	754				PLA_2	[40]
						[40,41]
Cryptococcus bacillisporus and Cr. neoformans		*S. cerevisiae* and other fungi				
			$cPLA_2$ GIVC	closest to group IV	PLB, lyso-PL, LPTA	[45,51]
Cb*PLB1*	634					
Cn*PLB1*	637					
Patatin-like Phospholipases						
C. albicans		*S. cerevisiae* orthologues (similar genes present in other fungi):		n.a.	n.d.	[40,104–106]
orf19.6396/13754	1386	*NTE1 (YML059C)*	Neuropathy target esterase (NTE)			[97]
orf19.4699/(12169)	682	*TGL3 (YMR313C)*				[96,98]
orf19.5426/12881	949	*TGL4 (YKR089C)*				
orf19.1504/9080	853	*TGL5 (YOR081C)*				

[1] Only published genes are shown

[2] gene designations in accordance to current conventions in the respective fungal species

[3] as determined by BLASTp analysis

[4] as described in references [2] D.A. Six and E.A. Dennis, Biochim Biophys Acta 1488 (2000) 1–19, [3] J. Balsinde, M.V. Winstead and E.A. Dennis, FEBS Lett 531 (2002) 2–6.