

From elicitors to lipid-transfer proteins: a new insight in cell signalling involved in plant defence mechanisms

Jean-Pierre Blein, Pierre Coutos-Thévenot,
Didier Marion and Michel Ponchet

Elicitors and lipid-transfer proteins are small cysteine-rich lipid-binding proteins secreted by oomycetes and plant cells, respectively, that share some structural and functional properties. In spite of intensive work on their structure and diversity at the protein and genetic levels, the precise biological roles of lipid-transfer proteins remains unclear, although the most recent data suggest a role in somatic embryogenesis, in the formation of protective surface layers and in defence against pathogens. By contrast, elicitors are known elicitors of plant defence, and recent work demonstrating that elicitors and lipid-transfer proteins share the same biological receptors gives a new perspective to understand the role played by lipid binding proteins, mainly the early recognition of intruders in plants.

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Jean-Pierre Blein

UMR 692 INRA/Université de Bourgogne, Laboratoire de Phytopharmacie et de Biochimie des Interactions Cellulaires, INRA, BP 86510, 21065 Dijon Cedex, France.
e-mail: blein@epoisses.inra.fr

Pierre Coutos-Thévenot

Université de Poitiers, Laboratoire de Physiologie et Biochimie Végétale, UMR CNRS 6161, 40 Avenue du Recteur Pineau, 86022 Poitiers, France.

Didier Marion

Laboratoire de Biochimie et Technologie des Protéines, INRA, BP 71627, 44316 Nantes Cedex 03, France.

Michel Ponchet

IPMSV, INRA, BP 2078, 06606 Antibes Cedex, France.

Lipids fulfil many different key functions in the physiology of plants, for example, the surface layers (cutin and suberin) are made up of hydrophobic polyesters of fatty acid derivatives, which protect plant organs against biotic and abiotic stress. Lipids in the form of triglycerides are a source of carbon and energy (e.g. in storage organs such as seeds and fruits). As phospholipids and glycolipids associated with proteins and sterols, lipids form membranes to fulfil the necessary compartmentation of metabolic cellular pathways. Lipids, and especially their derivatives, are also involved in many important cell-signalling pathways. All these functions are strongly regulated through dynamic processes that require lipid trafficking at cellular, extracellular and membrane levels. In biological membranes, specialized regions called lipid rafts could play an essential role in lipid turnover [1] and modulation of protein activity [2]. Because lipids are hydrophobic, lipids involved in trafficking or long-distance signalling have to be transported by soluble macromolecules and supramolecular structures (lipid-binding proteins and lipoprotein vesicles) [3].

Lipid-protein complexes are involved in many metabolic pathways but they could also operate in cell signalling in a different manner, as lipids or proteins alone do. Attempts to describe functional water-soluble lipid complexes in plants led to the discovery of a family of small hydrophilic proteins called non-specific lipid-transfer proteins (nsLTPs) [4] because they transfer membrane lipids, *in vitro*, without specificity. In spite of intensive work on their structure and diversity at the protein and genomic levels, the precise biological role of these proteins remains unclear, even though they are probably involved in somatic embryogenesis [5], the formation and reinforcement of plant surface layers [6], and defence against pathogens [7,8]. However, recent work concerning elicitors and nsLTPs [9] sheds new light on the roles played by lipid-binding proteins during plant-microorganism interactions.

Dual, well hidden activity of elicitors

Among the elicitors of plant defence mechanisms, elicitors are unique [10]. These small proteins [11] are secreted by the phytopathogenic Chromista *Phytophthora* [12] or *Pythium* [13] and trigger classical plant responses to elicitor treatment [10]. In addition to the features they share with other elicitors, elicitors exhibit a peculiarity: they have a sterol carrier activity [14,15]. This is probably the main function of these proteins because *Phytophthora* and *Pythium* do not synthesize the sterols that are required for their reproduction [16]. Elicitors might act as shuttles, trapping the sterols from the host and then triggering active phases of sexual and asexual reproduction. These physiological and morphological changes probably need a complex signalling system involving specific receptors at the oomycete side (Fig. 1a).

Moreover, the ability to load sterols is crucial because the formation of a sterol-elicitor complex is required to trigger the biological responses of tobacco cells and plants induced by elicitors [17]. Sterol loading from the plant plasma membrane allows elicitors to bind to their receptor, which then activates plant defence mechanisms (Fig. 1a). This dual involvement of a lipid-protein complex in alerting the plant (avirulence factor) and in pathogen development (virulence factor) perfectly illustrates the precarious equilibrium established between plant and pathogen. A weak displacement in this balance leads to either a compatible (disease) or an incompatible interaction. For example, *Phytophthora* downregulates the expression of elicitor genes *in planta*, minimizing plant responses and thus facilitating their spread [18].

When elicitors meet lipid-transfer proteins

Plant nsLTPs and oomycetous elicitors: from structure to lipid binding

In all plant species and organs, lipid-transfer activity coincides with proteins displaying molecular masses <10 kDa. These nsLTPs form a multigene family (>40 genes in *Arabidopsis* genome) and are ubiquitous

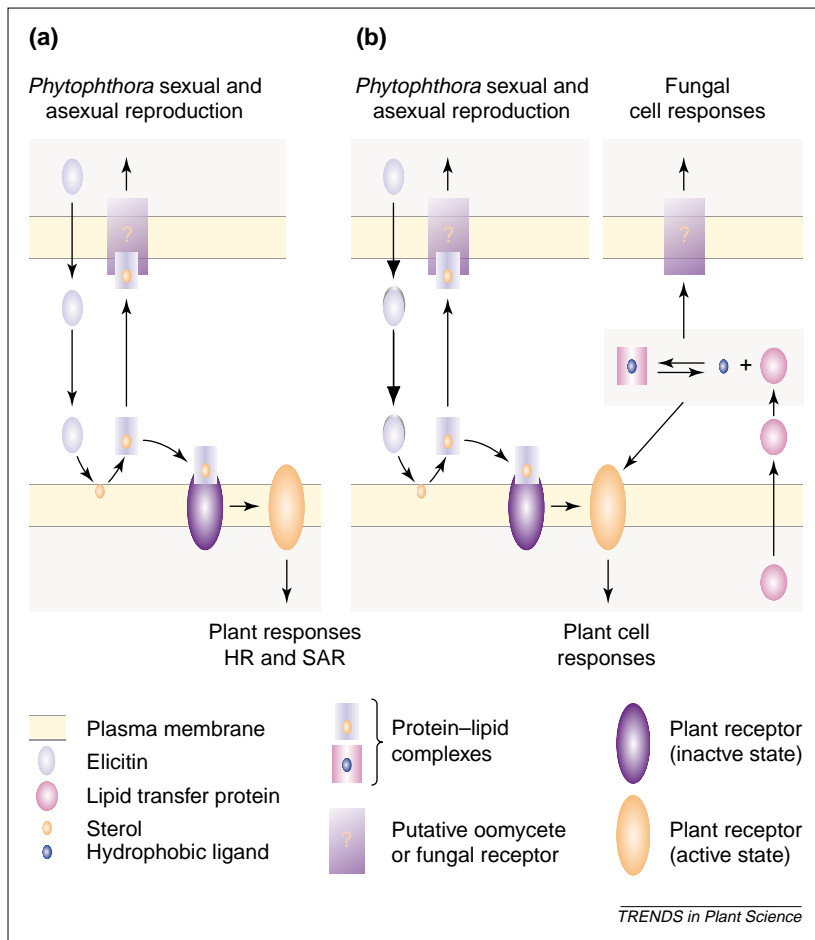


Fig. 1. Possible early events in plant–oomycete and plant–fungus dialogue: first steps involving the formation of a protein–lipid complex and its recognition by specific receptors. (a) During plant–*Phytophthora* interaction, elicitins act as shuttles between *Phytophthora* and plant cells. Elicitin–sterol complexes can be recognized by both *Phytophthora* and plant cells and then trigger biological responses (b) involving elicitins and lipid-transfer proteins. Lipid-transfer proteins and complex exogenous hydrophobic ligands are secreted by plant cells, which trigger biological responses. Abbreviations: HR, hypersensitive response; PM, plasma membrane; SAR, systemic acquired resistance.

in higher plants (>200 sequences belonging to ~60 species in data banks). Two families have been isolated and characterized, and are referred to as nsLTP1 and nsLTP2, with molecular masses of ~9 kDa and 7 kDa, respectively. All are characterized by a conserved motif of eight cysteines, which are involved in intramolecular disulfide bonds and define the nsLTP signature. The three-dimensional structure was determined for the lipid-free and lipid-bound major proteins of cereal seed nsLTP1 [6, 19]. This fold is characterized by a four-helix bundle surrounded, in part, by C-terminus turns with a saxophone-like shape (Fig. 2a).

The most interesting structural feature is the presence of a large internal tunnel following the long axis of the protein, whose size is variable depending on the lipid-free nsLTP1s. The tunnel can adapt its volume to bind one or two monoacyl lipids, diacylated lipids or a wide variety of hydrophobic molecules. However, nsLTPs cannot load sterol or molecules with a rigid backbone [20–22], suggesting that plasticity of the tunnel and flexibility of the hydrophobic molecules are necessary for binding. Finally, loading of lipids within nsLTP1 is also controlled by the surface pressure of targeted membranes (i.e. the packing of membrane components) so that, in a normal physiological situation, nsLTP1 should not load membrane lipids [23, 24]. Therefore, protein plasticity, lipid flexibility and, to some extent, lipid packing limit the non-specificity of plant lipid-transfer proteins (LTPs).

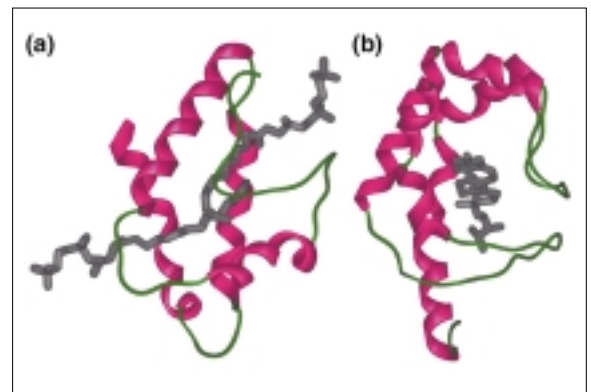


Fig. 2. Structures of lipid-transfer proteins and elicitins. Three-dimensional structures of (a) wheat lipid-transfer protein complexed with two molecules of lyso-myristoyl phosphatidylcholine in a head-to-tail orientation (Protein Data Bank 1BWO) and (b) cryptogein complexed by ergosterol (Protein Data Bank 1BXM). Lipidic ligands are shown in grey.

Elicitins are 10 kDa monomeric proteins displaying an α -helix fold stabilized by three disulfide bonds (Fig. 2b) that do not share sequence identity or a cysteine signature with nsLTPs. The elicitin fold provides a hydrophobic cavity with a higher specificity for sterol, although it is also capable of loading fatty acids [14, 15]. Surface-pressure measurements show that, in contrast to nsLTP1s, elicitins can easily penetrate the outer layer of membranes (D. Marion, unpublished). The energy barriers for interaction with cell membranes and the ligand specificities are necessarily different between nsLTPs and elicitins to interact with their target (plant or oomycetous chromista, Fig. 1b).

From non-specific lipid binding to functional polymorphism of plant nsLTPs

Although many data are available on nsLTPs, their real function is still a matter of discussion. All the structural, biochemical and physiological information has confirmed that nsLTPs are not involved in the intracellular lipid trafficking – the role they were initially thought to have – but that, instead, the key role of nsLTPs is in plant resistance to biotic and abiotic stresses [6–8, 19]. This justifies the classification of nsLTPs among the pathogenesis-related proteins. Two main basic mechanisms are probably involved, which agrees with the extracellular location of nsLTPs [25–28]: (1) the formation of hydrophobic protective layers (cutin and suberin); and (2) the inhibition of fungal growth. In a plant, both functions could be fulfilled by one nsLTP isoform or by several different ones. From a structural point of view, the plasticity of the hydrophobic tunnel is obviously an advantage for the formation of hydrophobic layers because they are composed of a large variety of saturated and unsaturated fatty acid derivatives with hydroxy, carboxy and/or keto functions [29]. Based on the binding properties of fatty acid derivatives, a role has been proposed for nsLTPs in cutin-monomer transport during cuticle formation [25, 30]. Although the precise role of nsLTPs in the formation of cutin layers is unknown, they could play

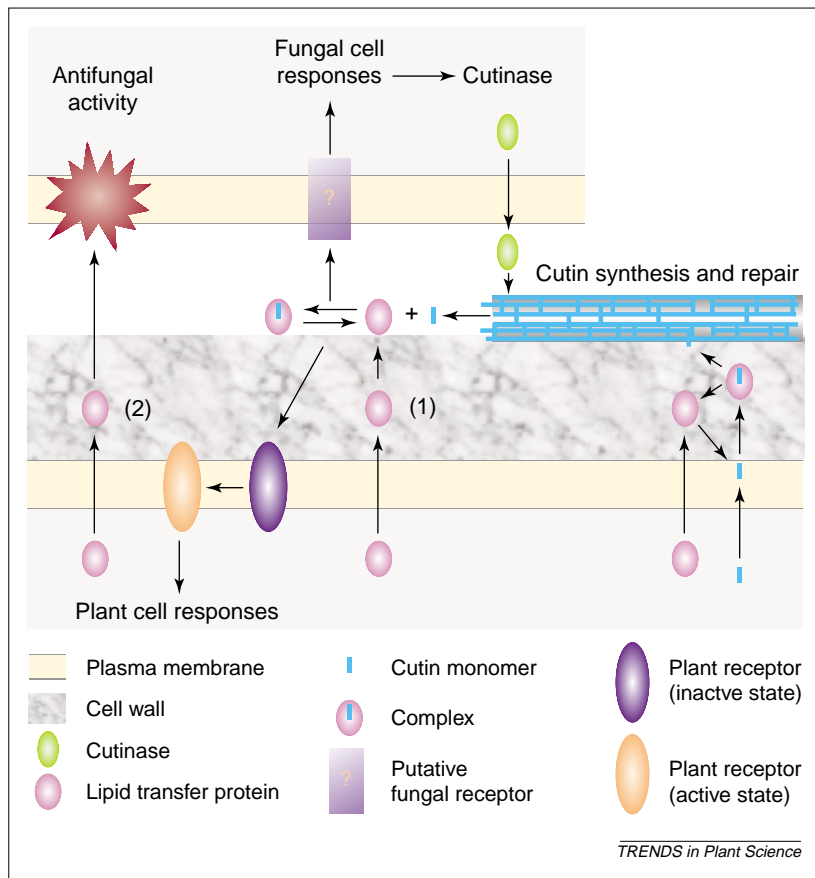


Fig. 3. Possible mechanisms behind the roles of non-specific lipid-transfer proteins (nsLTPs) during plant-pathogen interactions. This is a general case in which no protein elicitor is secreted by the fungal pathogen (e.g. *Fusarium* pathogens). The constitutive production of cutinase by the fungus induces a release of cutin monomers that are bound by the constitutively produced nsLTPs (a) located in the plant surface layers. This cutin-monomer-nsLTP complex can bind to the plasmalemma receptor and trigger plant defence responses, including synthesis of different nsLTPs (b) and other pathogenesis-related proteins that inhibit fungal growth. Overproduced nsLTPs might contribute to cutin repair and growth inhibition of fungi. In addition, the cutin-monomer-protein complex might bind to a fungal receptor to induce the overproduction of cutinase. The balance between the plant and fungal defence responses will ultimately lead to the death of one of the involved partners.

a role in transporting hydrophobic monomers to the extracellular polymerization hydrophobic-hydrophilic cutin-cell-wall interface where they are usually found [6,19] (Fig. 3).

Concerning the antifungal activity of nsLTPs, it must be emphasized that a protein from onion seeds with the nsLTP cysteine signature but low sequence identity has a fold that is highly similar to that of cereal seed nsLTP1s [31,32]. In this nsLTP1-like protein, the hydrophobic tunnel is interrupted by bulky aromatic tryptophan and phenylalanine residues so that it cannot bind and transfer lipids. However, it interacts strongly with membranes and increases their permeability and is, *in vitro*, an efficient antifungal agent [31,32]. Therefore, proteins of the nsLTP family can have different functions depending on the presence or absence of an internal lipid-binding site.

Dialogue between LTPs and elicitors in defence signalling
Recently, it has been shown that nsLTP1s bind to high-affinity sites located on plant plasma membranes. Interestingly, these sites have been characterized as the

elicitor receptor [9]. Although there is no sequence identity and no folding homologies between these proteins, it is possible to superimpose some helices of nsLTP1s and elicitors in three-dimensional space [9], which could, in part, explain their affinity and competition for the same membrane receptor (Fig. 1b). However, the mode of binding to the receptor is different for elicitors and nsLTP1s, in agreement with differences in their activity. Elicitors induce hypersensitive cell death and nonspecific systemic resistance, which is related, at the cell level, to a complex cascade of signalling pathways [10]. These cellular responses are inhibited by nsLTP1 [9], which behaves as an elicitor antagonist. Whether nsLTP1s are needed to form a complex with lipids to interact with the receptor and to induce plant cell responses (agonist behaviour), as highlighted for elicitors [17], is an open question. It is known that some cutin monomers that can be released by the action of fungal cutinases can trigger plant defence responses [33]. Could the nsLTP-cutin-monomer complex therefore trigger a hypersensitive response or other plant defence responses?

Finally, the low sensitivity of some plants to elicitors might be the result of competition between endogenous nsLTPs and exogenous elicitor on the corresponding receptor, which has been observed on all plant cell membranes tested to date. This hypothesis is partly supported by a low level of soluble LTP content in tobacco, whereas plants that do not react to elicitors (e.g. tomato) contain ten times more of these proteins (M. Ponchet, unpublished). It might also be explained by differences in competition kinetics between elicitors and the endogenous LTPs from different plant species. To test this possibility, it would be particularly helpful to determine the binding characteristics of different nonhomologous LTPs in both lipid-free and lipid-bound forms.

During the host-pathogen interaction, we have mainly considered the plant signalling pathways. Another question concerns the triggered responses and signalling in pathogen cells. Could nsLTPs act as messengers to inform the pathogens? It is noteworthy that cuticle components stimulate a specific stage of fungal development for spore germination and appressorium formation. They can also induce fungal cutinase production [34] (Fig. 3). If there is a receptor on the membrane of pathogenic hyphae that can recognize nsLTP-cuticle-component complexes, this could determine further fungal responses – a new possible field for investigation.

These data address a major question about the structural motifs common to these protein families and involved in the recognition of exogenous and endogenous proteins in the plant and animal kingdoms. Could hypersensitive responses in plant defence and human allergy obey similar preliminary recognition processes? To illustrate this point, it is noteworthy that some LTPs are ubiquitous allergens of plant-derived foods [35,36], and it would be interesting to consider the allergenic properties of nsLTPs

further [35]. To some extent, nsLTPs trigger a hypersensitive reaction that could be compared to the hypersensitive response of plant cells to elicitors. Therefore, does a similar subtle recognition process occur on animal membranes (i.e. protein binding to a specific receptor and subsequent cell signalling response)? Interestingly, another plant allergen [37], the hydrophobic protein from soybean, which has no sequence identity with nsLTP1s except the cysteine signature, has a somewhat-similar fold with no internal cavity and no lipid-binding capability [38]. The folding homology of the soybean seed-surface protein [39], whose biological function is unknown, opens an interesting question about its possible interaction with the nsLTP1–elicitor receptor.

Conclusions

The recent investigation of plant defence relationships shows that lipid-binding proteins, secreted by the

plants and the oomycete should play a key role in the lipid-mediated dialogue between the pathogen and the plant. The balance between lipid specificity and non-specificity of the corresponding protein carriers has an obvious evolutionary advantage for the plant and for the oomycetes in offering a versatile on–off system for the many different lipid-mediated events that can occur in plant–pathogen interactions. It constitutes a new and important field of investigation for understanding the mechanisms underlying self–non-self recognition in diverse hetero- and autotrophic organisms. From the plant point of view, elicitors probably deregulate a complex natural equilibrium established between constitutive, induced and lipid-loaded LTPs, close to an agonist–antagonist receptor model. By using plant LTPs, we are beginning to understand the subtle molecular mechanism that determines competition and even symbiotic relationships in the plant kingdom.

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