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Chapter

Role of the Cytoskeletal Actomyosin Complex in the Motility of Cyanobacteria and Fungal Spores

Elena Sánchez-Elordi, Eva María Díaz, Carlos Vicente and María Estrella Legaz

Abstract

This study demonstrates the involvement of the cytoskeleton in the movement of cyanobacteria and fungal spores to their hosts to establish a state of symbiosis or pathogenicity. The term symbiosis sensu lato is referred not only to commensalism and mutualism but also to the parasitic aberrations. The establishment of association implies that the endohabitant can move on a wet surface until finding an entry point in the exohabitant surface. In aqueous media, the exohabitant secretes glycoproteins that form a chemoattraction gradient for the invading cells. In lichens, the gradient consists of fungal lectins whose function is to recognize a compatible green alga or cyanobacterium. In the case of pathogens, the secreted proteins usually are a mixture that includes false quorum and chemoattractant signals, and cell wall digestive enzymes. The results indicate that fungal lectins and defense proteins bind to specific cell wall receptors for signaling the activation of cytoskeleton, causing successive cycles of cell contraction-relaxation that permits the migration of the endohabitant. In this study, different biochemical and microscopy techniques have been used. The mechanisms through which the cytoskeleton carries out these cycles of cell contractionrelaxation are described, being this a remarkable advance compared to previous results.

Keywords: actin, chemotaxis, cytoskeleton, lichens, motility, myosin, *Nostoc*, pathogens, *Sporisorium*

1. Introduction

The main interactions between plants include epiphytism, mutualism, commensalism, and parasitism, although the frontier between these types of association can be confusing [1]. For example, most epiphytes do not negatively influence their phytophores since they absorb water and nutrients directly from the atmosphere [2]. It is the case of many bromeliads or the crassulaceae *Aeonium arboreum*, growing on the *Phoenix dactylifera* stipe without damaging it (**Figure 1A**), although in some cases, drift toward parasitism is evident, as has been demonstrated by Montaña et al. [3] for epiphytic Bromeliads growing upon *Cercidium praecox*. Many

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lichens are also epiphytic, although they can behave as hemiparasitic if the phytophore is vitally weakened by environmental circumstances, such as drought or severe air pollution (**Figure 1B**). Examples include *Evernia prunastri* growing on *Quercus rotundifolia* [4] or on *Betula pendula* [5]. In other cases, nonlichenized fungi are decidedly parasites (**Figure 1C**).

In this respect, lichens, traditionally considered as an example of mutual symbiosis, exhibit a characteristic that can lead to a decided parasitism: the specificity between symbionts. The fungus selectively chooses individuals from an algal species from its surroundings to form the thallus, while those from other different species will be rejected. This implies that a fungus susceptible to lichenization is able to discriminate between compatible or incompatible algae: the former will form the association while the latter will be eliminated [6]. The argument can be further complicated: if the algae that make up the association split up inside an established thallus, the newly hatched algae may not be recognized as compatible and should therefore be removed (**Figure 1D**), unless they are able to set up the appropriate recognition systems in time.

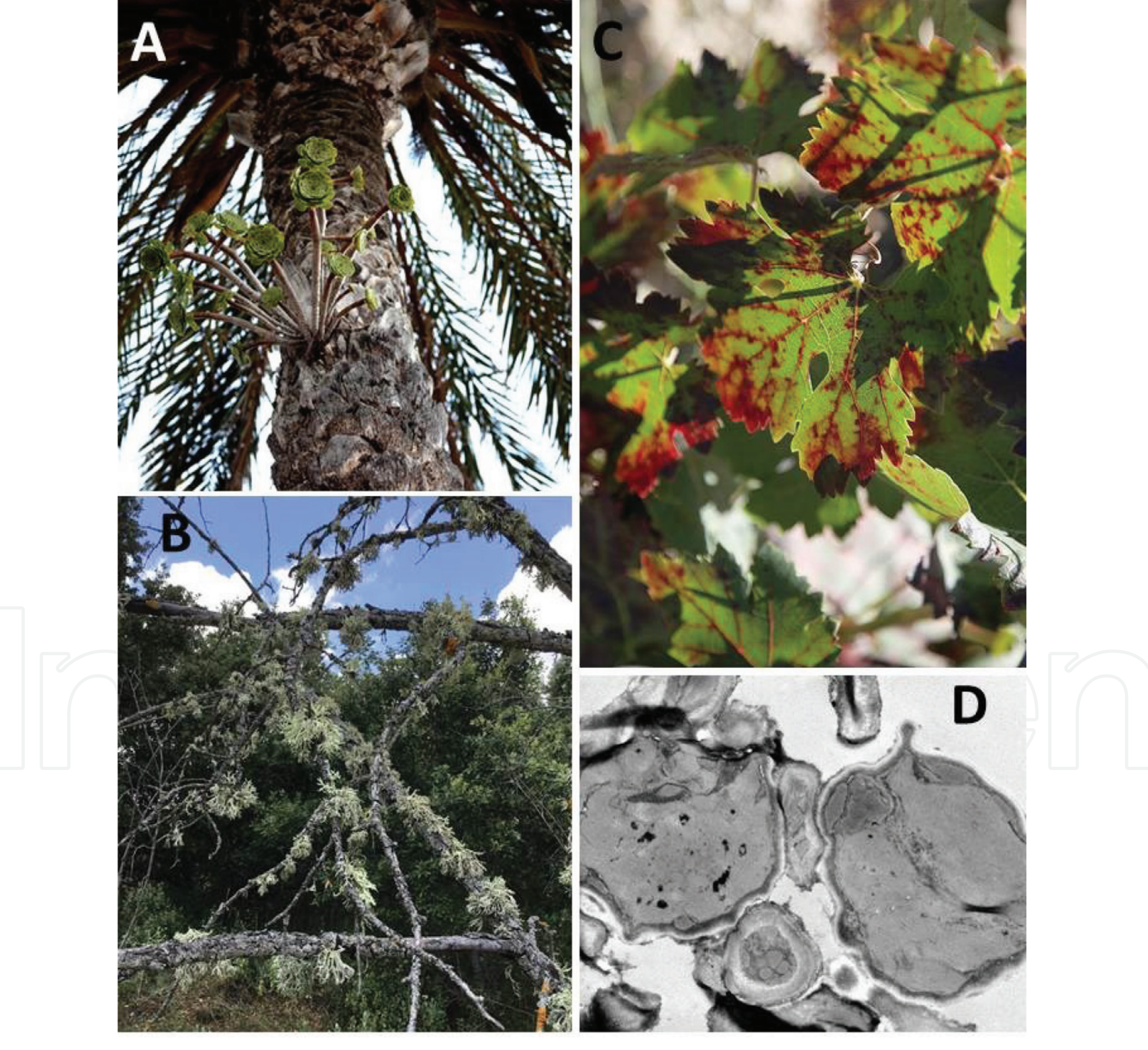

Figure 1.
(A) Aeonium arboreum*, a crassulacean species epiphytically growing on the stipe of* Phoenix canariensis*. (B) Hemiparasitic action of a dense population of epiphytic lichens that defoliated branches of their oak substrate. (C) Red spots on the leaves of* Vitis vinifera*, symptoms of the disease called tinder. The causal agents are fungi from* Stereum hirsutum *and* Phellinus igniarius *species. (D) Parasitic drift of* Xanthoria parietina *mycobiont on its phycobiont,* Trebouxia*, devoid of the receptor for recognition lectin.*

Lichen thalli can be reproduced by propagules containing some compatible algal cells surrounded by fungal hyphae. But there is also the possibility that a free-living fungus may find compatible algal cells in its environment. These algae, living in an aqueous film that covers the substrate (soil, rock, tree trunk), can move toward the fungal mass that would envelop them after being recognized. A similar situation is established when a single-cell organism (bacteria, fungal spore) is deposited on the wet surface of a plant, and the higher organism must discriminate whether it is an epiphytic, potential endosymbiont, or decidedly pathogenic microorganism. In the latter two cases, the cells must move in the water film until a suitable point of penetration is found.

Therefore, two main problems arise to explain the mechanisms used to establish this type of interspecific relationship: how unicellular organisms, potential endobionts, move toward the points of contact or entry and how they are recognized by the potential exohabitant when it reaches this position.

Lichens generally secrete glycoproteins to the environment depending on the availability of water [7]. Since most of these glycoproteins were enzymes, it was long time assumed that secretion was a function of the chemical composition of the substrate. This secretion might be taken as a kind of exocellular digestion of the compounds in the medium in order to be internalized into the thallus as simpler structures. However, using the lichen *Xanthoria parietina* growing on different substrates, rock or tree branches, it was found that the composition of the substrate did not influence the secretion of particular enzymes, which resulted in an exclusive function of the water availability and the degree of hydration of the thalli [8].

The aim of this study is to investigate the mechanism by which both prokaryotic and eukaryotic cells that do not have motile organs can move in liquid media thanks to the properties of their actomyosin cytoskeleton.

2. Secreted proteins

In the early stages of the establishment of lichen symbiosis, parasitic attack of the mycobiont (the fungal partner) against a variable number of photobiont cells (algae or cyanobacteria) can occur, which can be attenuated, according to Ahmadjian [9], by subjecting the neo-association to conditions of deprivation of organic nutrients. In this way, the fungus must keep a vital and active population of green cells, on whose photosynthetic products it depends to maintain its chemoorganic metabolism. This parasitic attack is carried out by invasion of the photosynthetic cells by fungal haustoria or by secretion of proteins that cause changes in genetic expression, structure disorganization, and cell death. These actions require proteins such as arginine methyltransferase, arginase, dioxygenases, or chitinases, according to Joneson et al. [10], secreted by the fungus *Cladonia grayi* in contact with the single-celled green alga *Asterochloris* sp. The appearance of chitinase as a secreted protein during the first stages of recognition has been explained as a defensive reaction of the algal partner against the fungus that attempts parasitism, which means that for the association to be successful, the secretion and production of this enzyme must be avoided [11].

In the case of fungal recognition of an algae considered genetically incompatible, the contact ends with the disorganization of the photosynthetic apparatus and the enzymatic rupture of the cell wall, with the loss of protoplast and death of the cell [12]. When the fungal-secreted arginase does not find a specific receptor in the algal cell wall, the enzyme penetrates the cell wall and activates its own β-1,4-glucanase

up to 10 times above its normal physiological level, causing total digestion of specific areas of the cell wall. Such a drastic response contradicts the assertion of Wang et al. [13] when they state that *Endocarpon pusillum* mycobiont interacts with their photobiont, *Diplosphaera chodatii*, by means of secreted small proteins much weaker than those that produce pathogenic fungi.

Another model of interaction between individuals, studied in our laboratory, is the pathosystem *Saccharum officinarum*-*Sporisorium scitamineum*. Plant invasion by the pathogen causes the production of at least 5–6 defense proteins, among which a dirigent protein [14], secreted arginase, β-1,3- and β-1,4-glucanases, chitinase as well as a sixth protein that acts as a positive chemotactic factor have been identified [15]. The actions that these secreted proteins carry out on the spores of the pathogen are varied. On one hand, arginase secreted by the plant causes a false quorum effect on the fungal teliospore population. The quorum effect exists by itself. The teliospores themselves secrete authentic quorum signals to increase the population of cells at the points of invasion in such a way as to ensure the survival of a sufficient number of them in the event that the plant emits effective defense factors. The false quorum signal causes the teliospores to form large aggregates over which the hydrolytic enzymes of the plant, chitinase, and glucanases would act [16].

Therefore, the behavior of the former inhabitant against a process of recognition of compatibility in the symbiosis or defense against a pathogen presents molecular similarities, but a very different characteristic in each case. For lichens, the mycobiont secretes a protein (a lectin) able to discriminate between compatible and

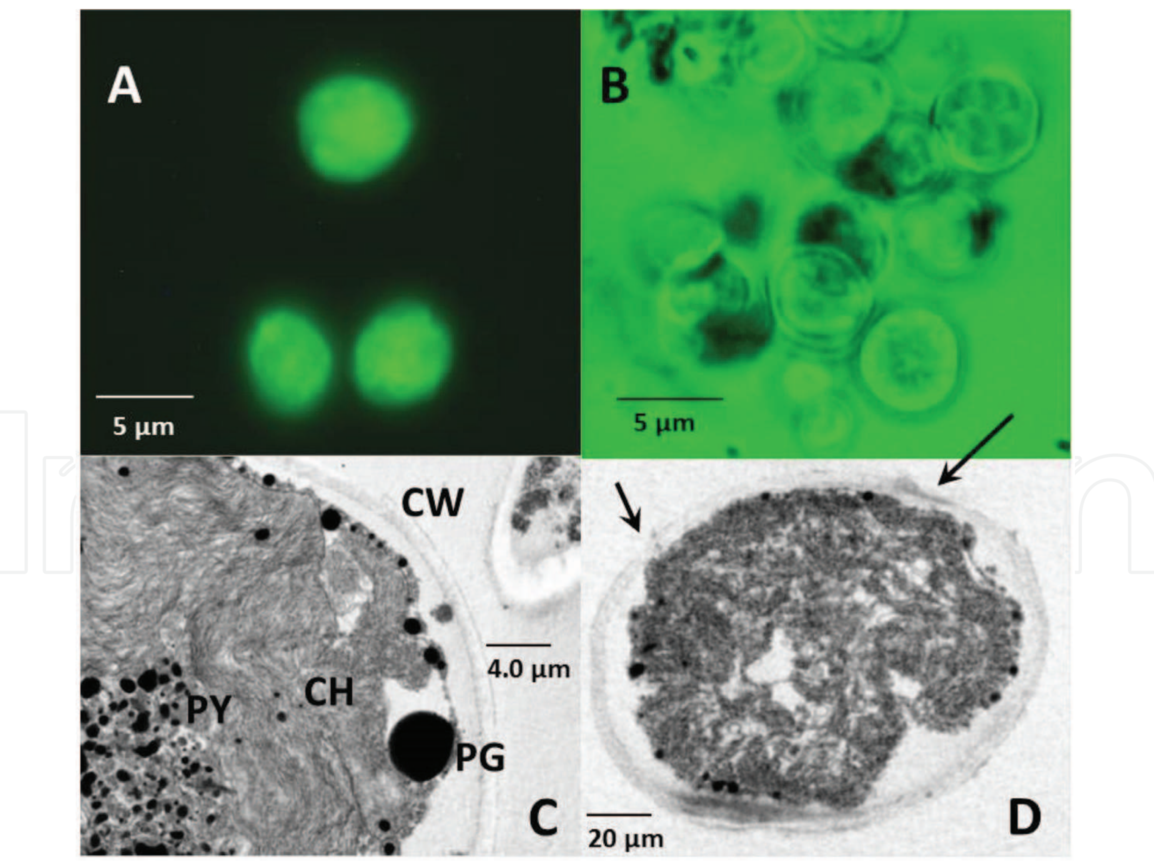

Figure 2.
(A) Trebouxia *cells isolated from* X. parietina *after the binding of the fluorescent lectin isolated from the compatible mycobiont. Fluorescence is superficially located on the algal cell wall. (B) The same algal cells lacking the specific lectin receptor. (C) Transmission electron micrograph of* Trebouxia *cells corresponding to (A). The integrity of the cells permits to distinguish the intact cell wall (CW), the chloroplast (CH) showing the complex lamellae system, the pyrenoid (PY), and one plastoglobuli (PG). (D) Transmission electron micrograph of* Trebouxia *cells corresponding to (B). The chloroplast has been disorganized, pyrenoid disappears, and the cell wall shows zones partially digested (black arrows).*

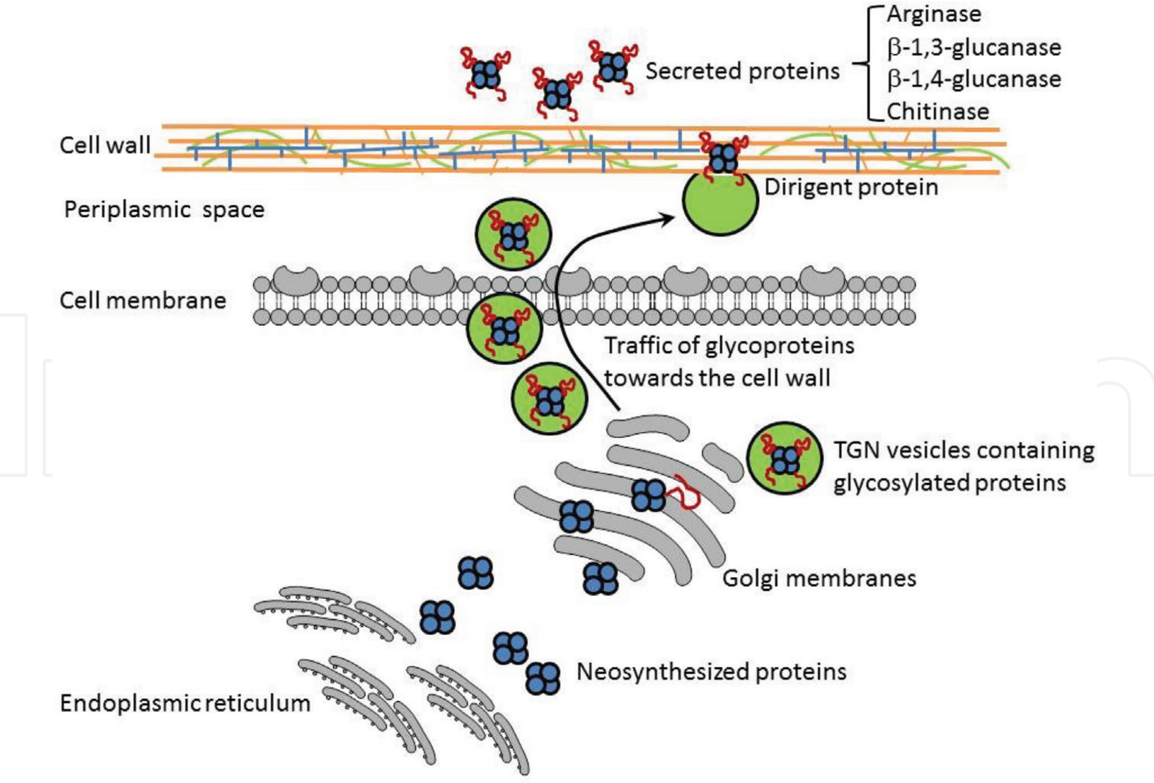

Figure 3.
Defense proteins produced by sugarcane cells are synthesized in the endoplasmic reticulum, glycosylated in the Golgi cisternae, and internalized in the trans*-Golgi network (TGN) vesicles to be transported to periplasmic space, crossing the cell membrane, to deposit them on the inner surface of the cell wall or to be secreted outside the cells.*

incompatible algae [17]. Only in the latter case, the secreted protein behaves as an aggressive factor (**Figure 2**). In the case of host-pathogen interactions, the proteins secreted by the host are always defense proteins (**Figure 3**). To carry out these actions, the potential endohabitant, symbiont or pathogen, must possess receptors for the secreted proteins that transmit the signal of compatibility or resistance to the cell machinery when they receive the recognition protein.

3. Receptors

The nature of these receptors, both in lichen photobionts as well as in some sugarcane pathogens, has been investigated in our laboratory. The occurrence of a glycosylated urease located in the phycobiont cell wall of *X. parietina* has been identified as an arginase-lectin receptor [18]. This identity has also been extended to other lichen species, such as *E. prunastri* [6], *Leptogium corniculatum* [19], and *Peltigera canina* [20]. *X. parietina* and *E. prunastri* contain a green algae from the *Trebouxia* genus as chlorobiont, while *L. corniculatum* and *P. canina* are associated with *Nostoc* sp. (a cyanobacterium). Recently isolated photobionts from thalli of these four lichen species contained an active urease associated with the cell wall. However, this activity was completely inhibited when cell wall fractions isolated from phycobiont or cyanobiont cells were incubated for 2 h at 37°C with the corresponding, previously purified lectin. In addition, hydrolysis of the galactoside moiety of urease in intact algae with α-1,4-galactosidase releases high amounts of D-galactose and impedes the binding of the lectin to the algal cell wall. However, the use of β-1,4-galactosidase releases low amounts of D-β-galactose from the algal cell wall and does not change the pattern of binding of the lectin to its ligand [21]. The production of glycosylated urease is restricted to the season in which algal cells

divide, and this assures the recognition of new phycobiont produced after cell division by its fungal partner [22]. This should be interpreted as meaning that the polypeptide sequence of arginase (the lectin produced by the mycobiont) possesses an amino acid domain capable of stereochemically recognizing the remains of D-β-galactose in β-1,3 bonds of the glycosylated, algal urease.

This mode of binding a lectin to the polysaccharide moiety of its ligand by an affinity reaction equals, at the level of action mechanism, the secreted lichen arginases with other, well-known lectins from higher plants, such as concanavalin A (ConA) from *Canavalia ensiformis*, and ricin A (RCA) from *Ricinus communis*. Studies carried out by using α-methyl-mannose as a ligand suggest that the sugar forms seven hydrogen bonds with the peptide of ConA, four with –NH groups of Lys99, Tyr100, Arg228 and Lys229, and three with amino acids interacting with Ca^{2+}, Asn 14 and Asp208 [23]. On the other hand, Fontaniella et al. [24] showed that a commercial ConA was able to develop arginase activity that increased more than 40 times in the presence of 1.7 mM Mn^{2+}. Another similarity between ConA and fungal arginases lies in the fact that their activity as enzymes requires Mn^{2+}, while their activity as lectin is dependent on Ca^{2+} and both cations, at the level of biological activity, are mutually excluding. The comparison between crystalline structures of ConA-containing or not Ca^{2+} suggests that the cation pulls from Tyr12, Asp208, and Arg228 to conform the site to bind the specific sugar [25]. It is probable that the binding of Ca^{2+} to the specific domain for the cation changes the tertiary structure of the domain defined as site for the sugar binding and, for the same reason, the structure of the catalytic site for arginine. The ability to bind both cations together in order to develop their binding capacity to specific galactose ligands has been demonstrated for other lectins, such as that purified and crystallized from *Spatholobus parviflorus* [26].

According to this, Marx and Peveling [27] found that many cultured phycobionts isolated from several lichen species bind to commercial lectins, including Con A and RCA. In addition, Fontaniella et al. [24] found that ConA is able to bind to the cell wall of algal cells recently isolated from *E. prunastri* and *X. parietina* thalli. This binding involves a ligand, probably a glycoprotein containing mannose, which has been isolated by affinity chromatography. Analysis by SDS-PAGE of the purified ligand revealed that it is a dimeric protein composed by two monomers of 54 and 48 kDa. This ligand shows to be different from the receptor for natural lichen lectins, previously identified as a polygalactosylated urease.

The binding of sugarcane glycoproteins to their cell wall ligands in the bacterial endophyte *Gluconacetobacter diazotrophicus* [28] and in the bacterial pathogen *Xanthomonas albilineans* [29] results in cell recruitment (**Figure 4**) rather than a defense mechanism. Similar results on cytoagglutination were obtained using *Herbaspirillum rubrisubalbicans* treated with sugarcane glycoproteins of

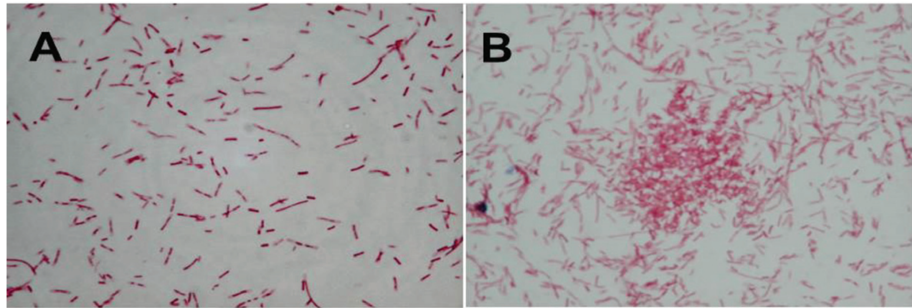

Figure 4.
Effect of secreted sugarcane glycoproteins on the cytoagglutination of Xanthomonas albilineans*. (A) Bacterial cells immediately after the contact with plant defense glycoproteins and (B) 3 h after the contact.*

mid- (MMMG) and high molecular mass (HMMG). MMMG were preferentially desorbed from the bacterial cell wall with sucrose and galactitol, whereas HMMG were mainly desorbed with glucose and mannose [30]. This would indicate that, against this bacterium, MMMG behaves as signal molecules that bind to their receptor, or receptors, using their polysaccharide moiety, whereas, on the contrary, HMMG would use their peptide moiety for binding to different receptors, similar to the action mode of the lectin ConA [24], from *Canavalia ensiformis*.

Surprisingly, receptors for both HMMG and MMMG do not behave as the typical adhesion receptors containing polysaccharides that bind by affinity to a specific peptide domain in the signaling molecule, the recognition of which implies the binding of this to selected carbohydrate moieties in their ligands [31]. In this case, the carbohydrate moiety of the signal molecule seems to be used to recognize a particular amino acid domain on the ligand (receptor) in an inverse way to that described for plant lectins and animal selectins. This fact suggests that HMMG and MMMG, with independence of their possible enzymatic activities [32], behave as true protein of resistance (PR), according to Su et al. [33], that would require ligands similar to toll-like receptors (TLRs), studied in animals [34].

The cytoagglutinating effect of sugarcane glycoproteins on smut teliospores was clearly reduced using invertase-digested glycoproteins. This suggested that the hydrolyzed glycidic moiety, which contains fructose residues polymerized as β-D-fructofuranosyl-1,2-β-D-fructose, could be involved in the process of binding since the extensive hydrolysis of β-(1 → 2) bonds impeded cell adhesion. To obtain experimental evidence of the presence of such cell-wall receptor, or receptors, glycoproteins were isolated from the cell wall of the fungal pathogen. These glycoproteins were separated by affinity chromatography through activated agarose columns to which sugarcane glycoproteins from different cultivars had been previously bound. Fungal cell-wall receptors retained by sugarcane glycoproteins were then recovered, desorbed by certain monosaccharides used as eluents [35]. Sugarcane HMMG and MMMG fractions exhibited a high affinity for N-acetyl-D-glucosamine, component of the cell wall of filamentous fungi. Interestingly, this binding mechanism differed, for example, from that described by Blanco et al. [27] for the cell wall receptors of *G. diazotrophicus*. In this case, glycoproteins bound through a domain β-(1 → 2)-fructofuranosyl fructose from its glycidic moiety to the bacterial cell wall receptors, which exhibited a binding site for this saccharide residue. Therefore, in the cases that HMMG or MMMG bound to their ligands using their polysaccharide moiety, either to bacterial cells or to fungal teliospores, they did not behave as lectins but as recognition factors using monosaccharide units or glycosidic bonds to bind to a particular domain of their ligands [36]. In addition, and as previously explained, HMMG and MMMG fractions behaved differently in their binding mechanisms to cell walls of *H. rubrisubalbicans*. These differences in the recognition mechanism could be interpreted as a discrimination factors between pathogens and endosymbionts.

4. Cytoskeleton as the main responsible for displacement of *Nostoc* and *Sporisorium scitamineum* cells

Directed cell migration is a physical process that involves dramatic modifications in cell shape and, generally, adhesion to the extracellular matrix [37]. Chemoattractive displacement is typically linked to the reorganization of actin filaments in cells, since polarization is the triggering event of cell migration [38]. A ligand on cell surface must activate a signaling pathway that leads to contraction/

relaxation of the cytoskeleton. Then, cell polarizes and as a consequence, it moves to the chemoattractant source.

Moreover, many intracellular signaling molecules are involved in cell motility, such as MAPK cascades, lipid kinases, phospholipases, Ser/Thr and Tyr kinases, and scaffold proteins. Specially, GTP molecules play an essential role in both signal transduction and actin organization through Rho GTPases, which appear as the most important components of signaling cascade related to cell migration [38, 39].

Cell migration is the core to modern cell biology. However, progress has been hindered by experimental limitations and the complexity of the process. This has led to the popularity of *Dictyostelium discoideum*, with its experimentally friendly lifestyle and small, haploid genome, as a tool to dissect the pathways involved in migration. *Dictyostelium* has the potential to unlock many fundamental questions in the cell motility field [37]. Here, the involvement of the cytoskeleton in movement is analyzed in two very different systems, such as the compatible association fungus-alga in the lichen *Peltigera canina* and the plant-pathogen interaction between *Sporisorium scitamineum* and sugarcane plants.

4.1 Cytoskeleton reorganization in *Nostoc* cells in response to the binding of a fungal lectin

For symbiotic interaction, germinating hyphae of the mycobiont needs to meet a compatible photobiont cell, to recognize it, and to make contact [40]. When an isolated fungus and an isolated alga associate, the photobiont migrates toward its potential compatible partner, which implies that the cyanobiont would develop organelles to move toward the fungus. Displacement is particularly relevant in cyanolichens, in which the cyanobiont forms filaments inside the thallus, a segment of which can break off and migrate toward other locations [19]. The recognition process continues during thallus growth, since it is necessary that new generations of photobiont cells become involved in the association [9].

Lectins found in both prokaryotic and eukaryotic cells play an important role in cell interaction processes. Synthesis of fungal lectins with arginase activity and the occurrence of an algal receptor showing urease activity are absolutely required in the formation of lichen associations [41]. Urease on the algae cell wall acts as a ligand for fungal arginase, fixing it on the cell wall and preventing it to penetrate the cell [20]. So, lectins with arginase activity participate as recognizing proteins of compatible alga binding to a specific receptor on the cell wall. However, they penetrate and cause destruction of algae cells if the specific receptor does not exist [41]. This is the case of noncompatible interaction, as it is shown in **Figure 5**.

The search for the chemoattractant attracting photobiont cells leads to the discovery of the attractant properties of fungus lectin. In particular, chemotaxis of *Nostoc* cells from *P. canina* toward the lectin isolated from the same lichen species has been amply studied [42]. Many multicellular filamentous cyanobacteria move on solid surfaces by gliding, in absence of pili or fimbriae. It is the case of filaments and hormogonia of *Nostoc* [43]. This mechanism, which occurs in a parallel direction to the cell long axis, is associated with the production of polysaccharide slime and the attachment of the cell to a surface is needed. On the other hand, blebbing and the release of small vesicles by the cyanobacterial outer membrane have been observed in distantly related symbiotic and nonsymbiotic cyanobacteria such as *Nostoc*, the cyanobiont of *Peltigera* spp. [44]. Blebs are spherical membrane protrusions produced by contractions of the actomyosin cortex often considered to be a hallmark of apoptosis. However, blebs are also frequently observed during cytokinesis and migration in three-dimensional cultures and in vivo conditions [45].

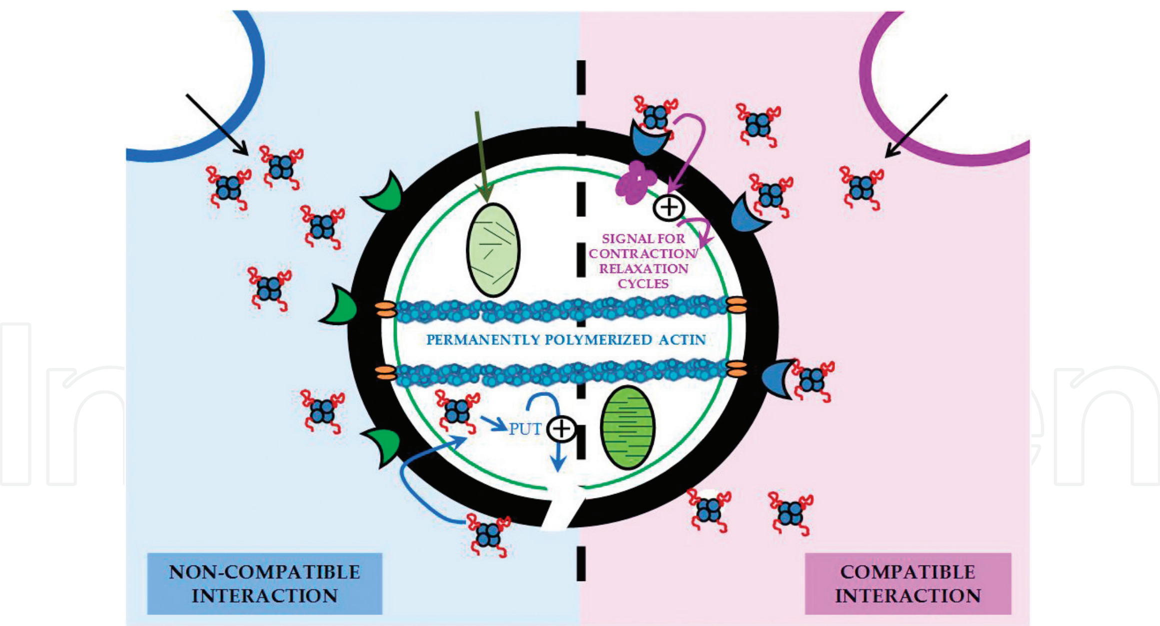

Figure 5.
On the right, recognition of fungus cyanobiont that leads to the cytoskeleton reorganization in Nostoc *cells after the lectin produced by compatible exosymbiont binds to a specific receptor in cell wall. In compatible interactions, integrity of photosynthetic apparatus is maintained. On the left, non-compatible symbiotic interaction. In this case, there is no ligand-receptor specificity. Internalized fungal arginase increases putrescine cytoplasmic levels, which activates glucanase that breaks down the cell wall. In noncompatible interaction, a disorganization of photosynthetic apparatus occurs. Representing: , the fungal lectin; , compatible exosymbiont; , noncompatible exosymbiont; , the specific receptor in* Nostoc *cell; , unspecific receptor in* Nostoc *cell; , the signal transducing proteins; , organized photosynthetic apparatus; , disorganized photosynthetic apparatus; , the F-actin; , the anchorage proteins; , the cyanobiont cell wall; and , the plasmatic membrane of cyanobiont.*

However, neither gliding nor blebbing can explain the invaginations observed by electron microscopy in one of the poles of *Nostoc* cells during the displacement [42], as can be seen in **Figure 6**. That is why the cytoskeleton has been revealed as responsible of migration of photobionts toward the fungus during a compatible interaction.

Some bacterial actin-like proteins or MreB have been already described in free-living cyanobacteria [46–47] but, contrary to that expected, chemotaxis assays of *Nostoc* displacement in presence of S-(3,4-dichlorobenzyl) isothiourea (A22), an inhibitor of MreB functionality, did not prevent the movement of cells toward the source of the lectin. Conversely, when *Nostoc* cells were incubated with the actin inhibitor phalloidin during chemoattraction assays, the drug inhibited chemotaxis by 50%. Also latrunculin A, which blocks actin polymerization, impedes *Nostoc* migration. The occurrence of F-actin fibers in *Nostoc* have also been found by immunocytochemical techniques associated with transmission electron microscopy.

Interestingly, when phalloidin was combined with blebbistatin, an eukaryotic myosin II inhibitor, the negative effect on displacement increases (78%), suggesting that blebbistatin may target a molecular target related to chemotaxis in cyanobacteria [42].

This means that, in the presence of compatible fungus, the binding of the lectin to its specific cell wall receptor would activate the signaling pathway that involves cytoskeleton reorganization. It must take place probably by means of GTPase activity, since the inhibition of chemotaxis produced by the combined action of phalloidin and blebbistatin is largely reversed by GTP and its analogs, GTP(γ)S and

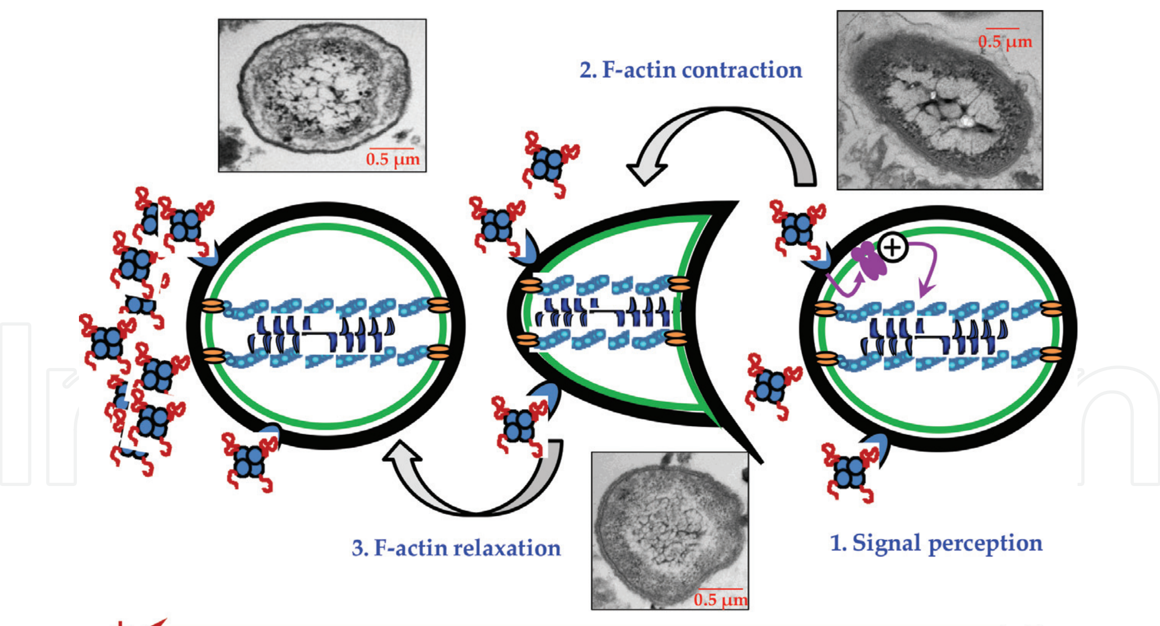

Figure 6.
Scheme of movement of Nostoc *cells during symbiotic interaction that explains how motility of lichen cyanobionts is due to contraction-relaxation episodes of the cytoskeleton. (1) Chemoattractant lectins released by fungus bind to specific receptors in photobiont cell walls. As a result, the transduction signal that implies cytoskeleton reorganization is activated. (2) Polar cell invaginations are produced by interaction of an ATPase with contractile ability, sensitive to blebbistatin, with F-actin cytoskeleton. (3) After this, depolymerization of F-actin is achieved at the opposite pole, repolymerization of which produces the cell advancement. Representing: , the fungal lectin; , the specific receptor in* Nostoc *cell; , the signal transducing proteins; , the actin monomeres; , the F-actin; , the contractile protein; , the anchorage proteins; , the cell wall; and , the plasmatic membrane. Ferritin-labelled F-actin can be seen in micrographs obtained by transmission electron microscopy (TEM).*

GDP(β)S, as well as by cyclic AMP [48]. On the contrary, when it is a noncompatible interaction, lectin penetrates into the cell, promoting putrescine synthesis. The diamine, which causes disorganization of photosynthetic apparatus, activates glucanase that breaks down the cell wall. Compatible and noncompatible interaction effect on cytoskeleton organization is schematized in **Figure 5**.

The absence of superficial elements (fimbriae, pili, or flagellum), related to cell movement, and the appearance of invaginated cells during or after movement, verified by scanning electron microscopy, support the hypothesis that the motility of lichen cyanobionts could be achieved by contraction-relaxation episodes of the cytoskeleton induced by fungal lectin [42]. However, other issues raised included (1) how cytoskeleton is reorganized during migration, (2) how is the mechanism of force generation of movement for cyanobacteria from *P. canina*, and (3) how it can be related to the invaginations previously observed by electron microscopy. The answers to all of these questions have led to elaborate a proposal of migration mechanism in cyanobacteria.

Figure 6 represents F-actin contraction/relaxing cycles in the *Nostoc* photobiont cells during migration following the lectin gradient. Firstly, binding of arginase molecules to cell wall receptors induces F-actin contraction by means of the activation of a signaling cascade where GTPases must play a main role. At the same time, contraction of filaments must be responsible for invagination appearance in one of the poles of the cell, which is followed by the actin depolarization at the opposite pole. This fact releases the tension from the actin-like cable bound to the membrane, and, finally, induces recovery of the spherical cell shape and movement of the cell [42].

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4.2 Cytoskeleton reorganization in *S. scitamineum* cells in response to the binding of sugarcane glycoproteins

In the early stages of smut disease, spore germination occurs on the internode surface of host stalks, followed by the formation of appressoria, mainly on the inner scale of young buds and on the bases of emerging leaves [49]. Penetration into the plant meristem takes place between 6 and 36 h after fungal cells are deposited on the surface [50]. Since the pathogens normally use the opened stomata of sugarcane leaves to penetrate, it is easy to think that the teliospores deposited at random on the surface of a leaf, far from stomata, should develop a mechanism of displacement toward the way of entry [51]. For this rationale, it is important to demonstrate the existence of these mechanisms and to study how they can be carried out.

Cytoskeleton reorganization in response to the binding of glycoproteins also occurs during *Sporisorium scitamineum*-sugarcane recognition. Moreover, displacement after recognition also results in cytoagglutination of smut teliospores in the same way that activation and chemotaxis of lichen photobionts induced by fungal lectins cause cell aggregation [15]. Interestingly, if glycoproteins are produced by sugarcane-resistant varieties, chemotaxis initially directed to plant invasion results in a “suicide” mechanism.

It has been proposed that at least three classes of glycoproteins exist in the mixture of sugarcane defensive glycoproteins produced by resistant cultivars: (i) a chemotactic glycoprotein, yet uncharacterized; (ii) a cytoagglutinating factor endowed with arginase activity, which also inhibits germination; and (iii) enzymatic proteins that mediate the breakdown of the teliospore cell wall. It has been demonstrated that agglutination of a lot of smut cells in a small region in contact with sugarcane glycoproteins confers resistance, since degradative activity also contained in these glycoproteins (β-1,3-, β-1,4-glucanase, and chitinase) can hydrolyze cell wall of many teliospores at the same time [15]. In this context, it must be pointed out that defensive agglutination depends necessarily on early chemoattraction of cells. For this reason, it is very interesting to go into some depth about how the teliospores movement is stimulated by sugarcane signals. Currently, it has been found that the early chemoattractive effect is fully relevant to trigger a successful defensive response [52]. Lower levels of chemoattractant power exhibited by glycoproteins released by nonresistant cultivars have been directly related to the minor capacity of these plants to defend themselves.

Brand and Gow [53] summarize the knowledge on spore movement in plant-pathogen interactions. The two most frequently proposed mechanisms are submicroscopical contractions of helically arranged fibrils within the cell walls and the occurrence of motile appendages in zoospores. Other species of pathogenic fungi produce spores that are capable of gliding in the same way that it occurs for many species of cyanobacteria. Gliding is a form of cell movement that differs from crawling or swimming in which it does not rely on any obvious external organ or change in cell shape and it occurs only in the presence of a substrate [54].

Light and electron microscopy images showed the absence of motile external structures in smut teliospores. However, in the same way that it occurs for *Nostoc*, the invaginations observed during the cellular displacement suggested that cytoskeleton could be the responsible of spore displacement after the contact with sugarcane glycoproteins. Indeed, chemotactic movement of teliospores was strongly inhibited by phalloidin, latrunculin A, and blebbistatin, and the presence of actin and myosin in *S. scitamineum* teliospores has been revealed by immunohistochemical techniques [52].

Teliospores do not need to develop lamellipodia in the direction of movement because they do not “crawl” on a substrate, but “swim” in solution because of the

rigidity of the cell wall. Therefore, invagination at the opposite pole would be the only mechanical requirement for cell motion [52]. Again as in *Nostoc* migration, a movement model has been proposed for smut teliospores displacement, which is schemed in **Figure 7**. Firstly, glycoprotein binding to its ligand on cell wall generates a signaling cascade that will trigger cytoskeletal remodeling (1). Translocation of actin filaments has been described as a consequence of the interaction of contractile myosin activity with F-actin cytoskeleton. It leads to an increase in the cell volume at the front of advance and the retraction of the opposite pole. At this pole, filaments must be anchored to cell membrane, since invaginations are observed (2). Finally, depolymerization of F-actin is achieved at the opposite pole, the repolymerization of which leads to cell advancement (3) [30, 52].

Also in the case of *S. scitamineum* cells, GTPases seem to be relevant in the activation of the movement. GTP causes an enhancement of the inhibition exerted by blebbistatin during migration. However, a large reversion is achieved by addition of GTPγS, the poorly hydrolysable GTP analogue [30, 55] or GDPβS, a deactivator of Rho [56] GTPases, and a total reversion of inhibition can be observed by combining GTP and GTPγS. GTP slightly reverts latrunculin A effect probably at the Rho pathway. However, when GTP analogues, GTPγS and GDPβS are added to the incubation media in the presence of latrunculin A, no reversion of the chemostatic inhibition is observed. These results indicate that GTPγS behaves as a strong activator of the small Rho-GTPase and its downstream pathways, which results in its final switch-off. Conversely, GDPβS blocks this signaling cascade, likely by inhibiting GTP exchange on Rho. These results, that are *not a priori* easy

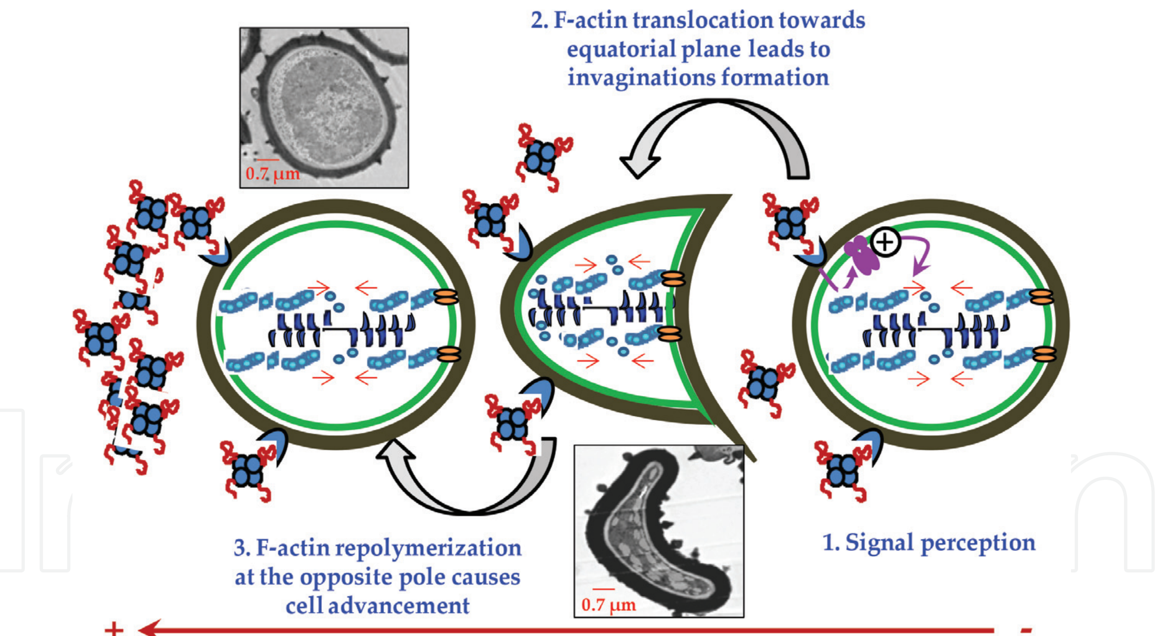

Figure 7.
Scheme of movement of S. scitamineum *cells during sugarcane-pathogen interaction that explain how motility of teliospores is due to contraction-relaxation episodes of the cytoskeleton. (1) chemoattractant glycoprotein released by sugarcane plants binds to specific receptors in fungal cell walls. As a result, the transduction signal that implies cytoskeleton reorganization is activated. (2) Polar cell invaginations are produced by interaction of myosin II with F-actin cytoskeleton. The translation of the actin filaments into the interior of the cell must begin at its less end, located in the equatorial plane of teliospore. This permits an increase of the cell volume in the front of advance and the retraction of the opposite pole, which produces cell invagination. (3) Repolymerization of F-actin in the front of cell produces the cell advancement. Representing: , the glycoproteins; , the receptor in fungal cell; , the signal transducing proteins; , the actin monomeres; , the F-actin; , the myosin; , the anchorage proteins; , the cell wall; , the plasmatic membrane; and ← the direction of retrograde flow. F-actin specific labeled ferritin can be seen in micrographs obtained by transmission electron microscopy (TEM).*

to understand, manifest that GTPases should participate in a meticulous regulation of actin organization.

Moreover, microtubules seem to be also involved in migration mechanism since nocodazole inhibits chemotactic displacement. Interestingly, assays revealed that the negative effect that this drug exerts on chemoattraction is related to a blockage of actin polarization. This demonstrates that actin and microtubules interact, participating together in the establishment of cellular polarity during migration (**Figure 8**). Microtubules-actin interactions regulate important processes in which dynamic cellular asymmetries need to be established such as cell motility, neuronal pathfinding, cellular wound healing, cell division, and cortical flow [57]. The presence of tubulin has also been demonstrated by immunohistochemical techniques in *S. scitamineum* cells [58].

It is obvious that cytoskeleton reorganization in fungal cells is also involved in germination, in addition to chemotaxis. This is because hyphae of filamentous fungi are very polarized cells and a continuous migration of vesicles from the teliospore cytoplasm through the hyphal cell body to the growing hyphal tip is necessary for organism development [59, 60]. It is clear that cytoskeleton plays a crucial role in polarity establishment in fungal cells during germination: microtubules support nuclei division and long-distance-transport functions in filamentous fungi, whereas actin microfilaments are required for localized targeting events [61]. Microtubule organization in *S. scitamineum* teliospores seems to be crucial for a successful germination [58]. *S. scitamineum* secretes its own arginase, which activates a signal transduction cascade that accelerates teliospore germination when it binds to its cell wall. Moreover, it has been recently suggested that microtubule stabilization during germination of smut teliospores could be triggered by the production of moderate levels of spermidine. *In vitro* microtubule polymerization assays in the presence of spermidine indicate that this polyamine interacts positively with cytoskeleton, probably by means of the positive charges of the molecule. Thus, polyamines are able to bind strongly to existing negative charges in different cellular components, such as nucleic acids, proteins, and phospholipids [62]. Spermidine could act in this way interacting and stabilizing the cytoskeleton.

So, polarization of cytoskeleton occurs during both teliospore movement and germination. Herein lies one of the most surprising discover about *S. scitamineum*-sugarcane interaction: glycoproteins from resistant to smut plants stimulate the

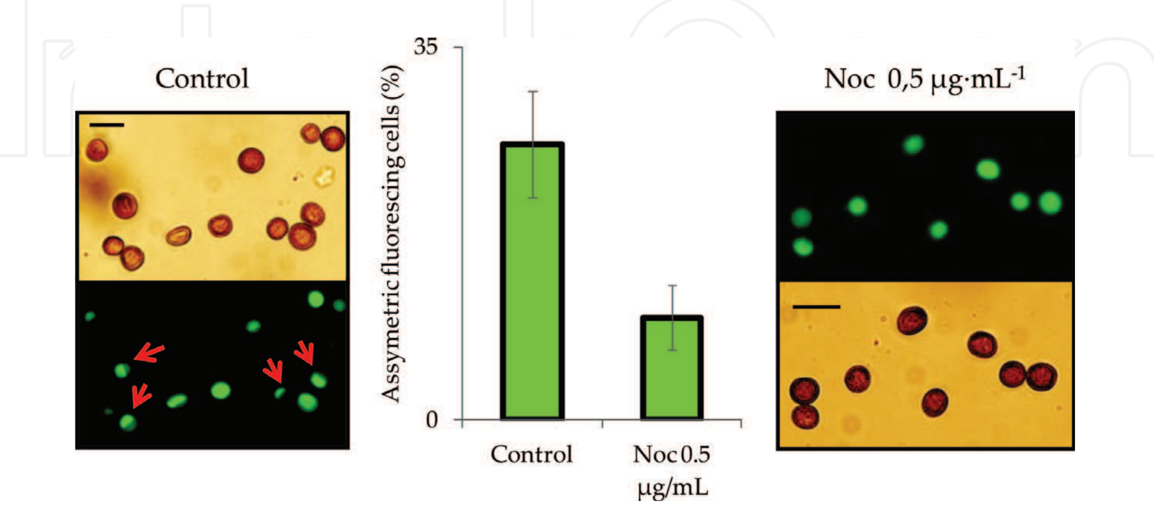

Figure 8.
Micrographs obtained by fluorescence optical microscope that show F-actin distribution in S. scitamineum cells in the absence (on the left) or in the presence (on the right) of nocodazole (Noc) 0.5 µg.mL-1, an inhibitor of microtubule polymerization. F-actin was detected using phalloidin labeled with fluorescein isothiocyanate (FITC). Red arrows indicate polarized cell. In the middle, percentage of cells with an asymmetric distribution of F-actin in the absence (control) or in the presence of Noc 0.5 µg.mL-1.

organization of the actin cytoskeleton to induce the movement of the teliospores toward the cytoagglutination points but stimulate its depolymerization for avoiding germination. Thus, after displacement as a consequence of cytoskeleton reorganization, the result is agglutinated cells without germinative capacity. Teliospore agglutination without germination triggered by sugarcane arginase becomes the result of a false quorum signal that prevents teliospore infection.

5. Conclusions

Cytoskeleton reorganization is the trigger of displacement of *Nostoc* and *Sporisorium scitamineum* cells during exohabitant/endohabitant recognition. On one hand, movement of *S. scitamineum* teliospores occurs by means of continuous episodes of polymerization and depolymerization of the actin cytoskeleton, in collaboration with myosin. Fungal cells displace toward defensive sugarcane glycoproteins as part of a "suicidal behavior," since displacement finally results in cytoagglutination and cell death [15]. Chemotactic movement of teliospores was strongly inhibited by phalloidin and latrunculin A, which are involved in F-actin polymerization and depolymerization cycles, and by blebbistatin, which avoids the functionality of a contractile protein similar to a myosin II, responsible for the contraction-relaxation of the cytoskeleton. Migration of smut teliospores has been described as consistent with a jellyfish-like "swimming" mechanism.

On the other hand, interesting results presented by Díaz et al. [41] suggest a cytoskeletal-driven mode of cyanobacteria chemotaxis similar to those of eukaryotic cells responding to a chemoattractant gradient. It has been concluded that *Nostoc* chemotaxis toward arginase requires actin and myosin II-like proteins. Displacement implies a rearrangement of the cytoskeleton causing cell polarity, which is, in turn, inhibited by phalloidin and latrunculin A, as revealed by confocal microscopy.

F-actin reorganization in response to extracellular chemotactic signaling has been amply studied. Migration is typically linked to the formation of external structures that promote movement. However, similar results in such different systems (lichen and plant pathogen) indicate that this mechanism of cytoskeletal reorganization, which induces cell chemotaxis in absence of lamellipodia/filopodia formation, is conserved in different organisms for recognition between species.

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Author details

Elena Sánchez-Elordi[1], Eva María Díaz[1], Carlos Vicente[1,2]* and María Estrella Legaz[1]

1 Intercellular Communication in Plant Symbiosis Team, Faculty of Biology, Madrid, Spain

2 Complutense University, Madrid, Spain

*Address all correspondence to: cvicente@bio.ucm.es

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References

[1] Leung TLF, Poulin R. Parasitism, commensalism, and mutualism: Exploring the many shades of symbioses. Life Environment. 2008;**58**: 107-115

[2] Martin CE, Schmitt AK. Unusual water relations in the CAM atmospheric epiphyte *Tillandsia usneoides* L. (Bromeliaceae). Botanical Gazette. 1989;**150**:1-8

[3] Montaña C, Dirzo R, Flore A. Structural parasitism of an epiphytic bromeliad upon *Cercidium praecox* in an intertropical semiarid ecosystem. Biotropica. 1997;**29**:517-521. DOI: 10.1111/j.1744-7429.1997.tb00046.x

[4] Bouaid K, Vicente C. Effects of lichen phenolics on defoliation of *Quercus rotundifolia*. Sauteria. 1998;**9**: 229-236

[5] Monso MA, Legaz ME, Vicente C. A biochemical approach to the hemiparasitic action of the epiphytic lichen *Evernia prunastri* on *Betula pendula*. Annales Botanica Fennici. 1993;**30**:299-303

[6] Legaz ME, Fontaniella B, Millanes AM, Vicente C. Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. European Journal of Cell Biology. 2004;**83**: 435-446. DOI: 0171-9335/04/83/0-1 $15.00/0

[7] Legaz ME, Díaz-Santos E, Vicente C. Lichen sustrates and urease production and secretion: A physiological approach using four Antarctic lichens. Biochemical Systematics and Ecology. 1986;**14**:375-379

[8] Rodríguez M, Vicente C. Water status and urease secretion from two ecotypes of *Xanthoria parietina*. Symbiosis. 1991;**11**:255-262

[9] Ahmadjian V. The Lichen Symbiosis. New York: Wiley; 1993. 250 p

[10] Joneson S, Armaleo D, Lutzoni F. Fungal and algal gene expression in early developmental stages of lichen-symbiosis. Mycologia. 2011;**103**: 291-306. DOI: 10.3852/10-064

[11] Athukorala SNP, Piercey-Normore MD. Recognition- and defense-related gene expression at 3 resynthesis stages in lichen symbionts. Canadian Journal of Microbiology. 2015;**61**:1-12. DOI: 10.1139/cjm-2014-0470

[12] Molina MC, Stocker-Wörgötter E, Turk R, Bajon C, Vicente C. Secreted, glycosylated arginase from *Xanthoria parietina* thallus induces loss of cytoplasmic material from *Xanthoria* photobionts. Cell Adhesion and Communication. 1998;**6**:481-490. DOI: 10.3109/15419069809010796

[13] Wang YY, Liu B, Zhang XY, Zhou QM, Zhang T, Li H, et al. Genome characteristics reveal the impact of lichenization on lichen-forming fungus *Endocarpon pusillum* Hedwig (Verrucariales, Ascomycota). Genomics. 2014;**15**:34 http://www.biomedcentral.com/1471-2164/15/34

[14] Sánchez-Elordi E, Contreras R, de Armas R, Benito MC, Alarcón B, de Oliveira E, et al. Differential expression of SofDIR16 and SofCAD genes in smut resistant and susceptible sugarcane cultivars in response to *Sporisorium scitamineum*. Journal of Plant Physiology. 2018;**226**:103-113. DOI: 10.1016/j.jplph.2018.04.016

[15] Sánchez-Elordi E, Morales de los Ríos L, Díaz EM, Ávila A, Legaz ME, Vicente C. Defensive glycoproteins from sugarcane plants induce chemotaxis, cytoagglutination and death of smut teliospores. Journal of Plant Pathology. 2016;**98**:493-501

[16] Sánchez-Elordi E, Morales de los Ríos L, Vicente C, Legaz ME. Sugar cane arginase competes with the same fungal enzyme as a false quorum signal against smut teliospores. Phytochemistry Letters. 2015;**14**:115-122. DOI: 10.1016/j.phytol.2015.09.013 1874-3900

[17] Galun M, Kardish N. Lectins as determinants of symbiotic specificity in lichens. Cryptogamic Botany. 1995;**5**: 144-148

[18] Molina MC, Muñiz E, Vicente C. Enzymatic activities of algal-binding protein and its algal cell wall receptor in the lichen *Xanthoria parietina*. An approach to the parasitic basis of mutualism. Plant Physiology and Biochemistry. 1993;**31**:131-142

[19] Vivas M, Sacristán M, Legaz ME, Vicente C. The cell recognition model in chlorolichens involving a fungal lectin binding to an algal ligand can be extended to cyanolichens. Plant Biology. 2010;**12**:615-621. DOI: 10.1111/j.1438-8677.2009.00250.x

[20] Díaz EM, Sacristán M, Legaz ME, Vicente C. Isolation and characterization of a cyanobacterium-binding protein and its cell wall receptor in the lichen *Peltigera canina*. Plant Signaling and Behaviour. 2009;**4**: 598-603

[21] Sacristán M, Millanes AM, Legaz ME, Vicente C. A lichen lectin specifically binds to the β-1,4-polygalactoside moiety of urease located in the cell wall of homologous algae. Plant Signaling & Behavior. 2006;**1**: 23-27

[22] Sacristán M, Millanes AM, Vicente C, Legaz ME. Synchronic production of fungal lectin, phycobiont lectin receptors and algal division in *Evernia prunastri*. Journal of the Hattori Botanical Laboratory. 2006;**100**:739-751

[23] Derewenda Z, Yariv J, Helliwell JR, Kaeb AJ, Dodson EJ, Papiz MZ, et al. The structure of the saccharide binding site of Concanavalin a. The EMBO Journal. 1989;**8**:2189-2193

[24] Fontaniella B, Millanes AM, Vicente C, Legaz ME. Concanavalin A binds to a mannose-containing ligand in the cell wall of some lichen phycobionts. Plant Physiology and Biochemistry. 2004;**42**: 773-779. DOI: 10.1016/j.plaphy.2004.09.003

[25] Shoham M, Yonath A, Sussmann JL, Moult J, Traub W, Kalb J. Crystal structure of demetallized Concanavalin A: The metal binding region. Journal of Molecular Biology. 1979;**131**:137-155

[26] Geethanandan K, Abhilash J, Bharath SR, Sadasivan C, Haridas M. X-ray structure of a galactose-specific lectin from *Spatholobous parviflorous*. International Journal of Biological Macromolecules. 2011;**49**:992-998. DOI: 10.1016/j.ijbiomac.2011.08.021

[27] Marx M, Peveling E. Surface receptors in lichen symbionts visualized by fluorescence microscopy after use of lectins. Protoplasma. 1983;**114**:52-61

[28] Blanco Y, Arroyo M, Legaz ME, Vicente C. Isolation from *Gluconacetobacter diazotrophicus* cell walls of specific receptors for sugarcane glycoproteins, which act as recognition factors. Journal of Chromatography. A. 2005;**1093**:204-211. DOI: 10.1016/j.chroma.2005.07.019

[29] Blanco Y, Sacristán M, Legaz ME, Vicente C. Isolation of specific receptors from *Xanthomonas albilineans* cell walls for lectins-like glycoproteins of sugarcane. Journal of Plant Interactions. 2006;**1**:107-114. DOI: 10.1080/17429140600768759

[30] Legaz ME, Sánchez-Elordi E, Santiago R, de Armas R, Fontaniella B, Millanes AM, et al. Metabolic responses of sugar cane plants upon different plant–pathogen interactions. In: Ahmad P, Ahanger MA, Singh VP, Alam P,

editors. Plant Metabolites and Regulation under Environmental Stress. London: Academic Press; 2018. pp. 241-280. DOI: 10.1016/B978-0-12-812689-9.00013-3

[31] Zihni C, Mills C, Matter K, Balda MS. Tight junctions: From simple barriers to multifunctional molecular gates. Nature Reviews. Molecular Cell Biology. 2016;**17**:564-580. DOI: 10.1038/nrm.2016.80

[32] Su Y, Wang Z, Xu L, Peng Q, Liu F, Zhu Li Z, et al. Early selection for smut resistance in sugarcane using pathogen proliferation and changes in physiological and biochemical indices. Frontiers in Plant Science. 2016;**7**:art 1133. DOI: 10.3389/fpls.2016.01133

[33] Su Y, Xu L, Wang Z, Peng Q, Yang Y, Chen Y, et al. Comparative proteomics reveals that central metabolism changes are associated with resistance against *Sporisorium scitamineum* in sugarcane. BMC Genomics. 2016b;**17**:800-820. DOI: 10.1186/s12864-016-3146-8

[34] Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. Biochemical and Biophysical Research Communications. 2009;**388**:621-625. DOI: 10.1016/j.bbrc.2009.08.062

[35] Millanes AM, Vicente C, Legaz ME. Sugarcane glycoproteins bind to surface, specific ligands and modify cytoskeleton arrangement of *Ustilago scitaminea* teliospores. Journal of Plant Interactions. 2008;**3**:95-110. DOI: 10.1080/17429140701861727

[36] Aguilar-Cuenca R, Llorente-Gonzalez C, Vicente C, Vicente-Manzanares M. Microfilament-coordinated adhesion dynamics drives single cell migration and shapes whole tissues. F1000Research. 2017;**6**(F1000 Faculty Rev):160. DOI: 10.12688/f1000research.10356.1

[37] King JS, Insall RH. Chemotaxis: Finding the way forward with *Dictyostelium*. Trends in Cell Biology. 2009;**19**:523-530. DOI: 10.1016/j.tcb.2009.07.004

[38] Vorotnikov AV. Chemotaxis: Movement, direction, control. Biochemistry. 2011;**76**:1528-1555. DOI: 10.1134/S0006297911130104

[39] Raftopoulou M, Hall A. Cell migration: Rho GTPases lead the way. Developmental Biology. 2004;**265**: 23-32. DOI: 10.1016/j.ydbio.2003.06.003

[40] Galun M. Lichenization. In: Galun M, editor. Handbook of Lichenology. Vol. II. Florida: CRC Press; 1988. pp. 153-169

[41] Insarova ID, Blagoveshchenskaya EY. Lichen symbiosis: Search and recognition of partners. Biology Bulletin. 2016;**43**:408-418. DOI: 10.1134/S1062359016040038

[42] Díaz EM, Vicente-Manzanares M, Sacristán M, Vicente C, Legaz ME. Fungal lectin of *Peltigera canina* induces chemotropism of compatible Nostoc cells by constriction-relaxation pulses of cyanobiont cytoskeleton. Plant Signaling & Behavior. 2011;**6**:1525-1536. DOI: 10.4161/psb.6.10.16687

[43] Hoiczyk E, Baumeister W. Envelope structure of four gliding filamentous cyanobacteria. Journal of Bacteriology. 1995;**177**:2387-2395. DOI: 10.1128/jb.177.9.2387-2395.1995

[44] Boissiere MC. Cytochemical ultrastructure of *Peltigera canina*: Some features related to its symbiosis. The Lichenologist. 1982;**14**:1-28. DOI: 10.1017/S0024282982000036

[45] Charras G, Paluch E. Blebs lead the way: How to migrate without lamellipodia. Nature Reviews. Molecular Cell Biology. 2008;**9**:730-736. DOI: 10.1038/nrm2453

[46] Usmanova A, Astier C, Méjean C, Hubert F, Feinberg J, Benyamin Y, et al.

Coevolution of actin and associated proteins: An alpha-actinin-like protein in a cyanobacterium (*Spirulina platensis*). Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology. 1998;**120**:693-700. DOI: 10.1016/S0305-0491(98)10065-2

[47] Guerrero-Barrera AL, García-Cuéllar CM, Villalba JD, Segura-Nieto M, Gómez-Lojero C, Reyes ME, et al. Actin-related proteins in *Anabaena* spp. and *Escherichia coli*. Microbiology. 1996; **142**:1133-1140. DOI: 10.1099/13500872-142-5-1133

[48] Díaz EM, Vicente-Manzanares M, Legaz ME, Vicente C. A cyanobacterial β-actin-like protein, responsible for lichenized *Nostoc* sp. motility towards a fungal lectin. Acta Physiologiae Plantarum. 2015;**37**:249-260. DOI: 10.1007/s11738-015-2007-4

[49] Waller JM. Sugarcane smut (*Ustilago scitaminea*) in Kenya. II infection and resistance. Transactions of the British Mycological Society. 1970;**54**: 405-414. DOI: 10.1016/S0007-1536(70) 80155-3

[50] Alexander KC, Ramakrishnan K. Infection of the bud, establishment in the host and production of whips in sugarcane smut (*Ustilago scitaminea*) of sugarcane. Proceedings of the International Society of Sugarcane Technologist. 1980;**17**:1452-1455

[51] Santiago R, Alarcón B, de Armas R, Vicente C, Legaz ME. Changes in the cynnamyl alcohol dehydrogenases activities from sugarcane cultivars inoculated with *Sporisorium scitamineum* sporidia. Physiologia Plantarum. 2012; **145**:245-259. DOI: 10.1111/j.1399-3054.2012.01577.x

[52] Sánchez-Elordi E, Vicente-Manzanares M, Díaz E, Legaz ME, Vicente C. Plant–pathogen interactions: Sugar cane glycoproteins induce chemotaxis of smut teliospores by cyclic contraction and relaxation of the cytoskeleton. South African Journal of Botany. 2016;**105**:66-78. DOI: 10.1016/j.sajb.2015.12.005

[53] Brand A, Gow NAR. Tropic orientation responses of pathogenic fungi. In: Pérez-Martin J, Di Pietro A, editors. Morphogenesis and Pathogenicity in Fungi. Berlin: Springer Verlag; 2012. pp. 21-41. DOI: 10.1007/978-3-642-22916-9

[54] McBride MJ. Bacterial gliding motility: Multiple mechanisms for cell movement over surfaces. Annual Review of Microbiology. 2001;**55**:49-75. DOI: 10.1146/annurev.micro.55.1.49

[55] Strange PG. Use of the GTPγS ([35S] GTPγS and Eu-GTPγS) binding assay for analysis of ligand potency and efficacy at G protein-coupled receptors. British Journal of Pharmacology. 2010; **161**:1238-1249. DOI: 10.1111/j.1476-5381.2010.00963.x

[56] Peterson DA, Peterson DC, Reeve HL, Archer SL, Wei EK. GTP (γS) and GDP(βS) as electron donors: New wine in old bottles. Life Sciences. 1999;**65**: 1135-1140. DOI: 10.1016/S0024-3205 (99)00347-1

[57] Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM. Conserved microtubule–actin interactions in cell movement and morphogenesis. Nature Cell Biology. 2003;**5**:599-609. DOI: 10.1038/ncb0703-599

[58] Sánchez-Elordi E, Baluška F, Echevarría C, Vicente C, Legaz ME. Defence sugarcane glycoproteins disorganize microtubules and prevent nuclear polarization and germination of *Sporisorium scitamineum* teliospores. Journal of Plant Physiology. 2016;**200**: 111-123. DOI: 10.1016/j.jplph.2016.05.022

[59] Fischer R, Zekert N, Takeshita N. Polarized growth in fungi – Interplay between the cytoskeleton, positional

markers and membrane domains. Molecular Microbiology. 2008;**68**: 813-826. DOI: 10.1111/j.1365-2958.2008.06193.x

[60] Baumann S, Pohlmann T, Jungbluth M, Brachmann A, Feldbrugge M. Kinesin-3 and dynein mediate microtubule-dependent co-transport of mRNPs and endosomes. Journal of Cell Science. 2012;**125**:2740-2752. DOI: 10.1242/jcs.101212

[61] Xiang X, Plamann M. Cytoskeleton and motor proteins in filamentous fungi. Current Opinion in Microbiology. 2003;**6**:628-633. DOI: 10.1016/j.mib.2003.10.009

[62] Gupta K, Dey A, Gupta B. Plant polyamines in abiotic stress responses. Acta Physiologiae Plantarum. 2013;**35**: 2015-2036. DOI: 10.1007/s11738-013-1.5239-4

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