4 Control of the Response to Biotic Stresses

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4.1 Summary

The cytoskeletal network of plant cells is a dynamic structure, changes in whose organization respond to external stimuli. An attack of pathogenic microbes represents an external stress that seriously threatens plant survival. Recently, it has been found that cytoskeletal elements, such as microtubules and microfilaments, are involved in plant defence reactions, especially in response to fungal penetration. Tubulin and actin inhibitors suppress the polarization of plant defence-related responses, such as massive cytoplasmic aggregation, deposition of papillae and accumulation of autofluorescent compounds at the sites of fungal penetration. Simultaneously, these inhibitors allow non-pathogenic fungi to penetrate successfully into non-host plants. Thus, microtubules and microfilaments, through the temporal and spatial regulation of molecules and/or organelles in the host cell, seem to control resistance responses against attempts of fungal penetration. On the other hand, plant cytoskeletal elements seem to play a critical role in the cell-to-cell spread of plant pathogenic viruses. In the tobacco mosaic virus, the movement protein P30 forms filaments that colocalize primarily with MTs. This association of P30 with cytoskeletal elements may play a critical role in intracellular transport of the P30-viral RNA complex through the cytoplasm to and possibly through plasmodesmata. These findings strongly suggest that the cytoskeleton plays a central role in both plant defence mechanisms and in the pathogenicity of microbes. The possibility of enhancing plant resistance to pathogens via an artificial manipulation of cytoskeletal elements will be discussed.

4.2 Significance of the plant cytoskeleton for pathogen resistance

The cytoskeleton, consisting of microfilaments and microtubules, is a highly conserved subcellular structure whose organization changes dramatically during cell cycle, development and adaptation. In plant cells, a dynamic reorganization of the cytoskeletal network has been observed during cell division (Katsuta and Shibaoka 1992) and cell wall synthesis (Hardham et al. 1980). The cytoskeleton can respond by adaptive reorganization to a variety of external stimuli such as gravitropic stimulation (Nick et al. 1991), low temperature (Quader et al. 1989), hor-

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mone treatment (Ishida and Katsumi 1991) and wounding (La Claire 1989) and this adaptive reorganization of the cytoskeleton in response to these abiotic stresses has been investigated in great detail. Biotic stresses, including pathogen attack, represent strong stimuli that pose serious threats to plant survival. However, little is known about the behaviour of the plant cytoskeleton and its possible function during the response to biotic stresses.

In mammals, phagocytosis of macrophages, which is important for host defence mechanisms as well as for tissue repair and morphogenetic remodelling, is driven by the reorganization of actin microfilaments (Caron and Hall 1998). Recently, a dynamic reorganization of cytoskeletal elements, such as microtubules and microfilaments, has been observed in several plant-fungus interactions, indicating that the cytoskeleton is involved in plant defence.

In contrast there is strong evidence that plant cytoskeletal elements may play a critical role in the pathogenicity of plant viruses. Recent studies show that the movement protein of the tobacco mosaic virus interacts with microtubules and microfilaments in vivo. This association of the movement protein with cytoskeletal elements suggests that the plant virus exploits and usurpates functions of the cytoskeletal network, in analogy to mammalian cells, where certain bacterial pathogens exploit the cytoskeleton of the host cell for internalization (reviewed in Higley and Way 1997; Dramsi and Cossart 1998).

A similar exploitation of the host cytoskeleton might occur during nodule formation induced in Fabaceae by symbiotic bacteria of the genus *Rhizobium*, where a reorganization of the plant cytoskeleton is thought to play an important role during nodule ontogeny. In this process, nodulation factors secreted by *Rhizobium* are possibly involved in the control of the cytoskeletal changes.

Altogether, these examples demonstrate the importance of cytoskeletal elements for both plant defence mechanisms and microbial pathogenicity and symbiosis. The first part of this chapter will therefore describe the dynamic reorganization of the cytoskeleton and discuss the possible role of the cytoskeleton during defence responses with focus on fungal pathogens. The second part deals with the role of the cytoskeleton during viral pathogenesis and bacterial symbiosis. The last part gives an outlook of the possibility of an enhancement of plant disease resistance through artificial manipulation of cytoskeletal elements.

4.3
Dynamic reorganization of the cytoskeleton during fungal attack

4.3.1
Reorganization of cytoskeletal network during attempts of fungal penetration

When plant cells perceive stimuli during fungal penetration attempts, visually detectable changes of cytoplasm are commonly observed including a movement of the nucleus towards the encounter site (Pappelis et al. 1974; Gross et al. 1993), cytoplasmic aggregation (Bushnell and Bergquist 1975; Kunoh et al. 1985), formation of cell wall appositions (papilla) beneath penetration sites (Aist 1976), alterations in the arrangement of cytoplasmic strands (Kitazawa et al. 1973; Kobayashi et al. 1993) and changes in the velocity of cytoplasmic streaming (Tomiyama 1956; Kobayashi et al. 1990). As motive force and track for the movements of organelles and cytoplasm the plant cytoskeleton is expected to play a role in these defence-related responses (Kamiya 1981; Williamson 1986; Seagull 1989).

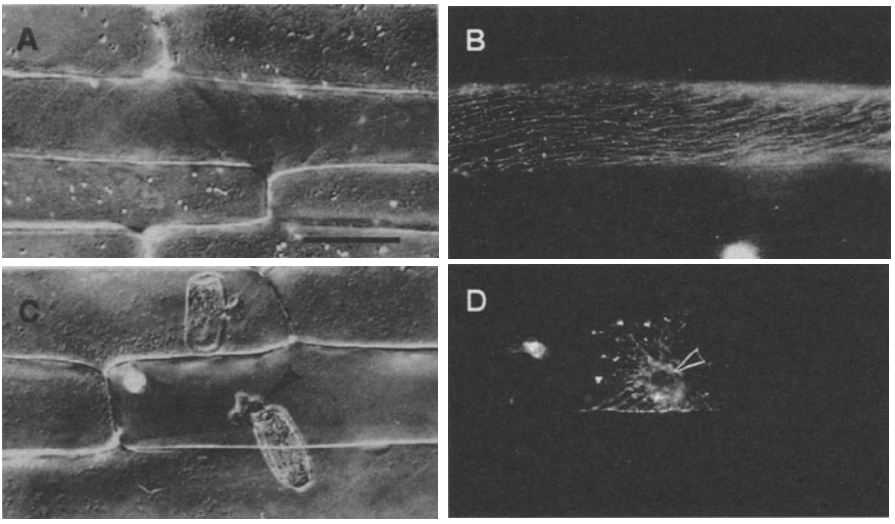

Fig. 4.1A-D. Rearrangement of microtubules in barley coleoptile cells inoculated with the non-pathogenic *E. pisi.* **A** and **C**: Differential interference contrast images. **D**: Epifluorescence images of immunostained microtubules. **A** and **B**: oblique orientation of microtubules in epidermal cells of uninoculated coleoptiles. **C** and **D**: microtubules 24 h after inoculation with numerous short and condensed microtubule bundles forming a radial array at the site of attempted penetration (**arrowheads**). **Bar** = 50 μm.

A dynamic reorganization of the cytoskeleton during attempted infection has been reported for several plant-fungus systems. In the interaction between barley coleoptiles and the non-pathogenic powdery mildew, *Erysiphe pisi*, microfilaments and microtubules are rearranged in the coleoptile during attempted penetration of the fungus (Kobayashi et al. 1991, 1992). When appressoria of the non-pathogenic fungus initiated penetration attempts, microfilaments and microtubules reorganized into a radial array directed towards the site of penetration (Fig. 4.1). In cultured parsley cells challenged by the non-pathogenic *Phytophthora infestans*, a localized depolymerization of microtubules occurred simultaneously with a rearrangement of microfilaments at the penetration site (Gross et al. 1993). Kobayashi et al. (1994) reported that such cytoskeletal rearrangements were characteristic for incompatible interactions between host and pathogen. In interactions between flax and flax rust fungus, *Melampsora lini*, radial arrays of microfilaments and microtubules at the encounter site were limited to incompatible interactions suggesting that this response followed the gene-for-gene rule. Conversely, major rearrangements of cytoskeletal components during interaction with pathogenic fungi have been observed in the cowpea and *Uromyces vignae* (Škalamera and Heath 1998) and the onion and *Botrytis allii* (McLusky et al. 1999) systems suggesting that the cytoskeleton is involved in host resistance as well as non-host resistance.

4.3.2 Possible role of the cytoskeleton in defence responses against fungal penetration

The actual role of the cytoskeleton during the manifestation of the non-host resistance is not yet elucidated. Generally, the cytoskeleton controls cell shape, cell motility, cell migration and cell polarity through the spatial and temporal regulation of molecules and organelles in the cell. In plants, the cytoskeleton participates in diverse cellular processes such as cytoplasmic streaming (Kamiya 1981), motility of organelles (Williamson 1986), cell wall synthesis (Hardham et al. 1980) and cell division (Katsuta and Shibaoka 1992). In addition to these well-known functions, the cytoskeleton has been discussed in many eukaryotic systems in the context of signal transduction pathways (reviewed in Tsukita et al. 1997; Gundersen and Cook 1999). Recent evidence supports this possibility for plants as well. PI 3-kinase, an important modulator protein for phosphatidylinositol (PI)-mediated signal transduction, is closely associated with the cytoskeleton in carrot cells (Xu et al. 1992), and Clarke et al. (1998) reported that the actin-binding protein profilin functions as stimulus-response modulator that translates signals into alterations of cytoplasmic architecture in pollen of *Papaver rhoeas*.

In this context, the following three possibilities can be perceived with respect to the potential role of the cytoskeleton in plant defence responses (Kobayashi et al. 1996):

1. Polarization of defence-related reactions.
2. Signal transduction for defence responses.

3. Cell-to-cell communication for spreading information between neighbouring cells.

Cytoplasmic aggregation and papilla formation are generally observed in plant cells that are subject of fungal penetration attempts. Ultrastructural study of cytoplasmic aggregates shows the presence of ER, Golgi apparatus and mitochondria beneath penetration sites (Bushnell and Zeyen 1976). The papilla that is formed in the centre of such cytoplasmic aggregates has been thought to represent an important defence reaction (Aist 1976; Heath and Heath 1971). In addition to the accumulation of organelles, it is observed that various defence-related compounds become localized around fungal penetration sites. In onion cells that have been inoculated with *Botrytis allii*, the fluorescent phenolic compound feruloyl-3-methoxytryamine accumulated at sites of attempted penetration. Localization of peroxidase and polarization of microfilaments at the same sites suggests that the polarization of microfilaments may contribute to the cross-linking of phenolic compounds into the cell wall through directed transport of the peroxidase. In barley cells, a similarly polar accumulation of protein, polysaccharide and fluorescent materials could be observed at fungal penetration sites, and this polar accumulation was completely blocked by treatment with inhibitors of cytoskeletal polymerization and depolymerization (Fig. 4.2, Kobayashi et al. 1997b). Simultaneously, treatment with these inhibitors effectively increased the penetration efficiency of the non-pathogenic fungus, *E. pisi*. *E. pisi* hardly succeeded in penetrating into barley, wheat, cucumber and tobacco cells in the absence of the inhibitors, whereas up to more than 60 % of appressoria could penetrate successfully into cells of these non-host plants and form haustoria upon treatment with cytochalasins (Kobayashi et al. 1997c). These results strongly suggest that a major role of the cytoskeleton in the defence reaction against fungal penetration attempts consists of a guided redistribution of defence-related compounds and organelles in attacked cells.

Several results provided evidence for an involvement of the plant cytoskeleton in the hypersensitive reaction. The hypersensitive reaction in barley coleoptile cells that were challenged by an incompatible race of *Erysiphe graminis hordei* was partially inhibited by the actin polymerization blocker cytochalasin B (Hazen and Bushnell 1983). Similarily, Škalamera and Heath (1998) reported that hypersensitive cell death in cowpea cells was inhibited by cytochalasin E during infection with the cowpea rust fungus (*Uromyces vignae*). In the flax and flax rust fungus (*Melampsora lini*) system, a rapid hypersensitive response developing about 24 h after inoculation normally inhibits fungal development and invasion in an incompatible interaction. However, in the presence of the microtubule polymerization blocker oryzalin, the occurrence of hypersensitive cell death was delayed and its frequency reduced (Kobayashi et al. 1997a).

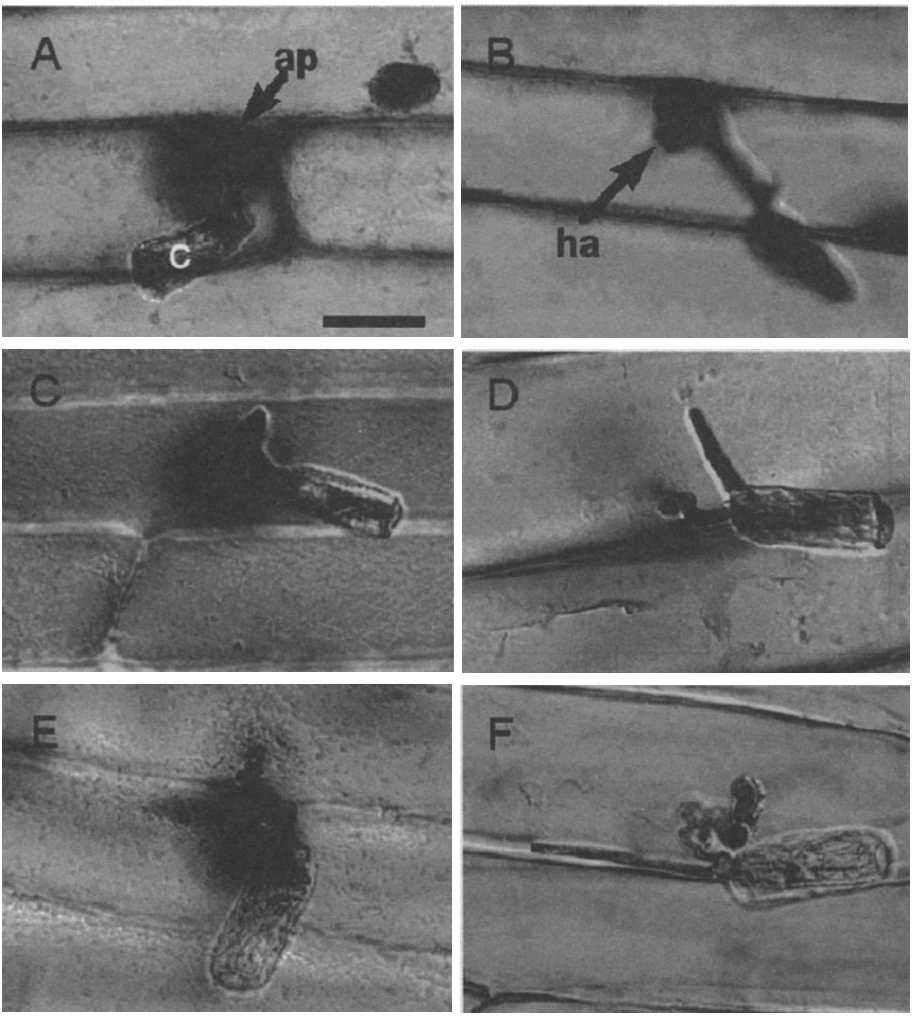

Fig. 4.2A-F. Bright-field micrographs showing the effects of the cytochalasin A on localized accumulation of defence-related materials in barley coleoptile cells 24 h after inoculation with *E. pisi*. **A, C, E** untreated controls. **B, D, F** treatment with 1 μgml^{-1} cytochalasin A. **A,B** Signal after staining with amido black visualizing protein accumulation. **C,D** Signal after Acid Schiff reaction visualizing carbohydrate accumulation. **E,F** Signal after staining with esorcinol blue visualizing callose. Note the absence of signals and the successful penetration of *E. pisi* and formation of haustoria in the cytochalasin-treated cells shown in **B,D** and **F**. **ap** Appressorium; **ha** haustorium. **Bar** = 50 μm.

The exact function of the cytoskeleton in the expression of the hypersensitive response remains to be elucidated, but it is likely that the plant cytoskeleton at least partially regulates signal perception or transduction in the pathway leading to the hypersensistive response via binding of proteins that function as signal

modulators (reviewed in Tsukita et al 1997; Zigmond 1996). In potato tissues treated with elicitors prepared from *Phytophthora infestans*, cytochalasin D and some inhibitors of the signal transduction cascade including staurosporine, ophiobolin and quinacrine inhibited the accumulation of rishitin, a potato phytoalexin, that is produced in potato tissues in response to the elicitor (Furuse et al. 1999). This result indicates that the factors involved in elicitor-induced signal transduction might be connected with actin cytoskeleton. Alternatively, the cytoskeleton might provide a rapid transport of these signals. In eukaryotic cells, external signals are received by receptors in or at the surface of cells, and the signals are transmitted to an intracellular target through a cascade of second-messenger modulation. Although the transmission of the signals is thought to be conveyed mainly by intracellular diffusion, the cytoskeleton provides tracks that might allow quick transmission of the modulated signals. Transfer of resistance from a cell that had been actually attacked by *E. pisi* to unchallenged adjacent cells was observed in barley (Kunoh et al. 1988). Recently, microfilaments have been detected as components of plasmodesmata in several plant species (White et al. 1994; Blackman and Overall 1998) raising the possibility that the cytoskeleton contributes to signal transmission between adjacent cells.

4.4 Involvement of the cytoskeleton in viral pathogenesis

To establish a systemic infection, plant viruses must move locally from cell to cell and enter the phloëm, through which they will move over long distances to establish a systemic infection and produce disease. A virus-encoded product, the movement protein (MP), actively potentiates viral cell-to-cell spread through plasmodesmata, the cytoplasmic bridges that function as intercellular connections (Gibbs 1976; Deom et al. 1992; Citovsky and Zambryski 1993; McLean et al. 1993; Lucas and Gilbertson 1994). In this chapter, we focus on the involvement of the cytoskeleton in cell-to-cell movement of viruses. There are a number of excellent recent reviews which described the general mechanism of viral movement in detail (Hull 1991; Lucas and Gilbertson 1994; Maule 1994; Carrington et al. 1996; Lartey and Citovsky 1997; McLean et al. 1997; Lazarowitz and Beachy 1999).

The 30-kDa protein (P30) MP encoded by tobacco mosaic virus (TMV) has been most extensively studied with respect to the mechanism of cell-to-cell movement. P30 can bind RNA in vitro and increase the size exclusion limits of plasmodesmata in mesophyll cells (Citovsky et al. 1990, 1992; Deom et al. 1992; Moore et al. 1992). Since White et al. (1994) demonstrated that microfilaments are associated with plasmodesmata, the possibility that the cytoskeleton could be involved in viral cell-to-cell movements in plants had been considered. Moreover, the cytoskeleton is known to act as a trafficking system for intracellular transport, translocation vesicles, organelles, protein, and even mRNA to specific cellular locations (Williamson 1986; Vale 1987; Dingwall 1992; Singer 1992; Wilhelm

and Vale 1993; Bassell et al. 1994; Hesketh 1994). In animals, the cytoskeleton is involved in trafficking of parasite genomes to the nucleus and in the intracellular redistribution of viral proteins (Ben-Ze'ev et al. 1983; Pasick et al. 1994; Topp et al. 1994; Avalos et al. 1997; Li et al. 1998;). Therefore, it appeared plausible that associations between the P30-RNA complex and the cytoskeleton might be important in the spread of virus in plants.

Recently, this supposition was supported by the discovery that there are specific associations of P30 with microtubules and microfilaments during studies on the role of the cytoskeleton in directed transcytoplasmic movement and regulation of plasmodesmal function (Heinlein et al. 1995, 1998; McLean et al. 1995; Carrington et al. 1996;). Fusion proteins between jellyfish green fluorescent protein (GFP) and the MP coaligned with microtubules in infected tobacco protoplasts derived from the BY-2 cultured cell line (Heinlein et al. 1995 1998). Similarly, affinity-purified P30 polyclonal antibodies visualized a number of P30 filaments that were observed to colocalize with microtubules in both virus-infected and P30-transfected tobacco protoplasts (McLean et al. 1995). These coalignments were disrupted by low temperature (Heinlein et al. 1995; McLean et al. 1995) and by treatment with oryzalin and propizamide, which are known to disrupt microtubules, whereas no effect could be observed after treatment with the microtubule-stabilizing agent taxol (Heinlein et al. 1995). McLean et al. (1995) also examined a potential interaction between P30 and the actin cytoskeleton and could demonstrate that some of the P30 filaments colocalized with actin cytoskeleton. Furthermore, P30 appears to bind directly to actin and tubulin because P30 produced in overexpressors under control of the CaMV-35S promotor cosedimented with actin and tubulin in vitro. However, it is not known whether in vivo P30 interacts directly with the plant cytoskeleton or whether the binding to actin and tubulin is mediated by further proteins (McLean et al. 1995). Although treatments of cytochalasin showed a similar effect on the filamentous structure of P30, changes in the P30 network were much less pronounced than with cold treatment (McLean et al. 1995). Moreover, the colocalization between P30 filaments and microfilaments typically was less pronounced in number and extent than that seen between P30 filaments and microtubules (McLean et al. 1995). These results suggested that P30 protein associates mainly with microtubules and only partially with micro-filaments. A microtubule-binding activity could also be demonstrated in vitro for a further plant viral protein, the HSP70-related 65-kDa protein of the beet yellows closterivirus (Karasev et al. 1992).

A model for intracellular transport of P30 can be proposed based on the observed colocalization of P30 with cytoskeletal components and on the well-known fact that both microtubule motor and actin-myosin systems appear to actively transport various mRNAs as RNA-protein complexes in animal cells (Wilhelm and Vale 1993). Since viruses tend to exploit cellular mechanisms, the interaction of P30 with both microtubules and microfilaments may mimic the transport of RNA-

protein complexes and organelles via microtubule motors and actin-myosin systems for long- and short-distance transport, respectively (Langford 1995).

The P30-RNA complex is predicted to be elongate and thin, because this would reduce diffusion within the cytoplasm while favouring organized or directed movement along cytoskeletal elements (Citovsky et al. 1992). According to their model, the cytoskeleton provides a track for the long unfolded P30-RNA complexes and facilitates linear, directed transport. P30 could be associated with the cytoskeleton either before, during, or after RNA complex formation. Then it would associate with microfilaments for short-distance unidirectional movement to and possibly through plasmodesmata because theses structures contain actin. Results of injection studies with fluorescent dextranes suggested that plasmodesmal gating could be controlled via microfilaments (Ding et al. 1996). Thus, P30 is proposed to interact with plasmodesmata-associated microfilaments usurpating them to be targeted and moved through plasmodesmata into the cytoplasm of adjacent cells. Since TMV-P30 can move between cells by itself (Waigmann and Zambryski 1995), P30 may be shuttled through plasmodesmata by the microfilaments extending between cells. The ability of P30 to increase the plasmodesmal size exclusion limit (Wolf et al. 1989, 1991; Waigmann et al. 1994) may also be related to its interaction with actin, because actin was found in the neck region of plasmodesmata, where the size exclusion limit is thought to be regulated (White et al. 1994). Summarizing, this evidence suggests that microtubules and filamentous actin may deliver complexes of MP with viral RNA to and through plasmodesmata (Heinlein et al. 1995; McLean et al. 1995; Carrington et al. 1996).

In plant cells, including BY-2 suspension cultured cells, microtubules are observed in the cortical cytoplasm at the cell periphery (cortical microtubules) and in association with the ER (Allen and Brown 1988; Hepler et al. 1990; Reuzeau et al. 1997). Interestingly, the association of MP with microtubules was most pronounced during the mid to late stages of infection and subsequent to its association with elements of the ER (Heinlein et al. 1998; Mas and Beachy 1998). It is unknown whether viral MPs harbour plasmodesmal targeting sequences. Based on analogies to nuclear import and the involvement of import receptors, such plasmodesmal targeting sequences are expected. Alternatively, viral MPs or viral replication complexes and MPs could form specific associations with subdomains of the cortical ER and/or cortical microtubules and microfilaments, which themselves are associated with plasmodesmata and act to guide the MPs toward these intercellular channels (Lazarowitz and Beachy 1999). In this case, microtubule and microfilament associated proteins, including molecular motors, and a functional characterization of the interaction between MPs, ER, and cytoskeleton becomes important (Lazarowitz and Beachy 1999).

4.5
Involvement of the cytoskeleton in rhizobial symbiosis

The symbiotic interactions between soil bacteria of the genera *Rhizobium*, *Azorhizobium* or *Bradyrhizobium*, which are referred to as rhizobia, and plants of the *Leguminosae* family result in the formation of nodules, new organs in which the bacteria reduce nitrogen into ammonia that can be subsequently utilized by the plant. The early stages of root nodule development are mediated by signal exchange between plant and rhizobia controlling altered gene expression on the bacterial part and cell growth, division and differentiation on the host part. In plants, cell shape and the direction of cell expansion depend on the correlation of cellulose microfibrils in the cell wall and plasma membrane-associated cortical microtubules with transverse microtubules maintaining cell elongation (see Chapt. 1; Giddings and Staehelin 1991; Williamson 1991). Similarly to actin microfilaments, the microtubular cytoskeleton changes its organization remarkably during cell division (Traas et al. 1987; Baluška and Barlow 1993;) and cell wall synthesis (Hardham et al. 1980; Seagull 1992; Goddard et al. 1994). Therefore, the cytoskeleton was expected to be involved in root nodule development.

Rhizobia produce Nod factors (NFs), whose synthesis is under the control of nodulation (nod) genes that are transcribed in the presence of plant flavonoids. NFs are signal molecules involved in most of the early developmental responses, in growth responses elicited by the corresponding bacteria, such as root hair induction and deformations, in the invasion of plant tissues by means of tubular structures called infection threads and in the formation of a nodule meristem whose activity ensures nodule (Newcomb 1981; Brewin 1991; Roth and Stacey 1989; Hirsch 1992; Kijne 1992; Long 1996).

The examination of cell division patterns provides the opportunity to explore the effect of NFs on plant development such as a so-called cytoplasmic activation that includes the formation of phragmosomes which was shown to be highly site specific and to occur early in response to NFs in some plants such as *Vicia*, (Van Brussel et al. 1992). The current hypothetical model proposes that NFs bind to plasmalemma-located receptors (Bono et al. 1995; Niebel et al. 1997), followed by subsequent signal transduction. In alfalfa root hairs, *Rhizobium meliloti* NFs induce a depolarization of plasma membrane potential (Ehrhardt et al. 1992; Felle et al.1995), cytoskeletal changes (Allen et al. 1994) and calcium spiking (Ehrhardt et al. 1996). It has been shown that *Rhizobium leguminosarum* bv. *trifolii* NFs are specifically internalized into clover root hairs (Philip-Hollingsworth et al. 1997).

Yang et al. (1994) found that the inner cortical cells that showed phragmosome formation were induced to enter the cell cycle via passage through the G_1 and S phases and continued to divide, forming nodule cortex and nodule meristem. In contrast, the outer cells that had been invaded by the bacteria ceased division. Therefore, it appears that rhizobia exploit cell division directly in the inner cell

layers by inducing the formation of additional cells that will form the organ in which the bacteria will ultimately reside. In the outer cell layer, they seem to exploit, additionally, an indirect consequence of cell division, namely, increased cell wall synthesis and vesicular traffic. These cytoskeleton-dependent functions could help to render the usually inactive outer cortical cells as conductive to infection thread formation as are the tip-growing root hairs of the epidermis (Hirsch 1992; Kijne et al. 1992; Ridge 1992; Van Brussel et al. 1992; Yang et al. 1994).

The involvement of the plant cytoskeleton in early stages of nodulation has been suggested by studies demonstrating that cytoskeletal reorganizations occur at the tip of root hairs treated with NFs or in the root cortex of *Vicia hirsuta* that either had been infected by its specific symbiont or treated with NFs (Allen et al. 1994; Van Spronsen et al. 1995; Timmers et.al. 1998). Moreover, several of the symbiotic responses such as root hair induction and deformation, activation of cortical cells, oriented growth of an infection network in plant tissues, formation of nodulation-related division centres and cell enlargement (Truchet et al. 1991; Ridge 1992; Van Brussel et al. 1992; Ardourel et al. 1994; Yang et al. 1994) are dependent on the cytoskeleton. Thus, changes in cytoskeletal organization are likely to be involved in many of the symbiosis-related steps directing nodule development.

In the Leguminosae, the nodule meristem remains active for several weeks, thus leading to the formation of elongated indeterminate nodules comprising central and peripheral tissues. Timmers et al. (1998) provided experimental evidence for internalization of NFs and for architectural rearrangements of microtubules during nodule differentiation. In indeterminate nodules of alfalfa, the organization of microtubules in the central zone of the nodule changes tightly parallel to features of symbiotic differentiation that are related to cell infection, bacterial release, endopolyploidization, cell enlargement, spatial organization of cell components and organelle ultrastructure and positioning (Timmers et al. 1998). Their observations showed that in alfalfa nodules, these microtubular changes initiate in the nodule zone where rhizobial NFs are internalized in infected cells and that these changes strongly correlate with symbiosis-specific cell differentiation traits. Similar changes could be observed in different nodule types (Timmers et al. 1998),indicating that the link between cytoskeletal changes and rhizobial symbiosis might be general.

4.6 Prospects

Recent advances of plant molecular biology uncovered plant disease resistance genes at a molecular level. More than 20 resistance genes and their respective downstream genes have been cloned and their structure has been determined in detail (reviewed in Hammond-Kosack and Jones 1997). The defence-related signal cascade mediating race-specific cultivar resistance will be elucidated in the near future. Concerning applied science, this molecular understanding of defence responses should permit the development of disease-resistant crops with the help of molecular breeding techniques. As described above, the plant cytoskeleton plays an important role in plant defence mechanisms as well as viral pathogenesis and microbial symbiosis. As a highly conserved subcellular structure in higher plant cells, the cytoskeleton will provide new targets for molecular breeding of disease-resistant crops. Possible strategies to modify the cytoskeletal network in order to enhance plant disease resistance are as follows:

1. Regulation of cytoskeletal organization through modification of cytoskeletal regulators.
2. Control of expression of specific isotypes for tubulin (see Chapt. 7) or actin.
3. Structural modification of cytoskeletal proteins to change their affinity for other molecules.

Temporal and spatial control of defence responses appears to be highly important for an effective inhibition of pathogen attack. Little time is required for fungal pathogens to penetrate plant cell walls and to invade cells subsequently. In general, a hypersensitive reaction has to be triggered prior to a successful colonization by the pathogen in order to suppress the formation of lesions. Rapid responses of cytoskeletal network to microbial attack are therefore essential for a successful suppression of pathogen attack. The organization of the cytoskeleton is thought to be regulated by a number of binding proteins. Whereas such binding proteins have been isolated from yeast and animal cells, little is known about their plant counterparts. An exception is the actin-binding protein profilin that has been well characterized as a modulator of the actin cytoskeleton in both plant and animal cells (Sun et al. 1995; Steiger et al. 1997). Stable overexpression of birch pollen profilin in mammalian cells renders the actin network more resistant to depolymerizing agents (Rothkegel et al. 1996). This result indicates that the status of the cytoskeleton can be controlled through artificial regulation of cytoskeleton-associating proteins. Recently, small GTPases of the Rho family have emerged as key regulators of the actin cytoskeleton that appear to control coordinately different cellular activities through interaction with multiple target proteins (Hall 1998). Homologues of Rac, a member of the Rho GTPase family, have been cloned from several higher plants (Yang and Watson 1993; Borg et al. 1997; Winge et al. 1997). In fact, overexpression of a constitutively active (i.e. GTP-bound) form of a Rac gene results in an enhanced hypersensitive responses and in resistance to the blast disease in rice (Ono et al. 1999). Although it is not clear

whether this effect was caused via a modified regulation of the actin cytoskeleton, such Rac GTPases are prime candidates for the manipulation of cytoskeletal reorganization.

In general, the different actin and tubulin isotypes are highly conserved in terms of amino acid sequence. However, the existence of multiple genes coding for these cytoskeletal proteins raises the possibility that different isotypes can have different roles in vivo (Meagher 1991; Chasan 1992). For example, the small *Arabidopsis* genome contains six expressed genes for α- and nine for β-tubulins (Kopczak et al. 1992; Snustad et al. 1992). It has been shown for animal cells that certain tubulin isotypes from the same animal are not functionally exchangable, although they are often interchangable with the same isotypes from different organisms (Ludueña 1993). The promoter of the maize *Tuba3* α-tubulin gene is activated by colonization with an arbuscular mycorrhizal fungus, whereas the closely related *Tuba1* promoter is not (Bonfante et al. 1996). The *Arabidopsis ACT7* actin gene was the only isotype to respond to several external stimuli including hormone treatments, light regime and wounding (McDowell et al. 1996). In barley leaves challenged by the non-pathogenic powdery mildew fungus, *E. pisi*, only one among three of the major expressed actin isotype genes was found to respond to the fungal attack (Hattori, K. and Kobayashi, I. unpubl. data) These results indicate that specific isotypes of cytoskeletal proteins are associated with plant defence responses.By controlling the expression of these specific isotypes artificially, one might venture to enhance disease resistance.

Changes in amino acid sequence of cytoskeletal proteins can cause changes in the affinity for other molecules. Anthony et al. (1998) reported that a point mutation of α-tubulin caused dinitroaniline herbicide resistance in plants, indicating that the mutation resides in the drug-binding site and that the change in the amino acid resulted in decrease of affinity between a-tubulin and herbicides. Modification of the domains where viral movement proteins interact with cytoskeletal proteins is expected to block cell-to-cell transmission of virus particles. Structural analysis of the binding partners and yeast two-hybrid approaches are expected to uncover the relevant domains and structures to develop this strategy.

Plants had to evolve a network of complex defence mechanisms consisting of diverse mechanisms designed to combat a wide variety of pathogens. The cytoskeleton controls a variety of cellular activities and thus appears to play an important role in some defence-related responses. Pathogens often take advantage of the genetic conservation and functional plasticity characteristic of the plant cytoskeleton. However, this means that the cytoskeleton is a good target for approaches with the aim of protecting plants from various biotic stresses.

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