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Secretion and endocytosis at the interface of plants and fungi

K. Mendgen, U. Bachem, M. Stark-Urnau, and H. Xu

Abstract: Secretion products and the uptake of material from the outside of the protoplasts of plants and microbes may influence the mutual interaction in host–parasite systems. Organelles involved in secretion by rust fungi have been characterized using immuno-electron microscopy. Proteins with the C-terminal histidine – aspartic acid – glutamic acid – leucine tetrapeptide (HDEL) signal sequence accumulated in tubular vesicular complexes of the ER in the parasitic phase of the fungi. The tubular complexes were differentiated from smooth cisternae that probably correspond to the Golgi equivalent. In the plant, material was secreted into the papilla and at the same time, endocytosis of 1,3- β -glucans by clathrin-coated vesicles occurred at the penetration site of the monokaryotic appressorium. During further development of monokaryotic haustoria, different wall layers were produced around these haustoria. Components of those layers were plant cell wall constituents most of which were also detected in the Golgi apparatus. One layer was rich in callose, which is supposed to be synthesized at the plasma membrane. Obviously, endo- and exo-cytosis occur permanently by host and parasite during fungal development within the plant.

Key words: *Uromyces*, Golgi, secretion, endocytosis, high-pressure freezing, *Saccharomyces*, *Vigna sinensis*, *Vicia faba*.

Résumé : La sécrétion de substances et l'absorption de matériels à partir de l'extérieur de protoplastes de plantes et de microbes peuvent influencer les systèmes d'interactions mutuelles entre les hôtes et leurs parasites. À l'aide de la microscopie électronique et du marquage immunologique, les auteurs ont caractérisé les organelles impliqués dans la sécrétion de rouilles fongiques. Chez le champignon, au cours de la phase parasitaire, des protéines avec la séquence signalétique HDEL s'accumulent dans les complexes tubulo-vésiculaires du réticulum endoplasmique. Les complexes tubulaires se différencient à partir de citernes lisses qui correspondent probablement à la structure équivalente à l'appareil de Golgi. Chez la plante, au site de pénétration par l'appressorium, monocaryote, du matériel est sécrété dans les papilles et au même moment, on observe l'endocytose de 1,3- β -glucans par des vésicules enveloppées de clathrine. Au cours des autres étapes du développement de l'haustérie monocaryote, différentes couches pariétales sont déposées autour des haustéries. Les composantes de ces couches sont des constituants de parois cellulaires de plantes dont la plupart peuvent également être décelées dans l'appareil de Golgi. Une couche est riche en callose, laquelle est supposément synthétisée sur la membrane plasmique. Il y a donc des preuves que des endo- et des exo-cytoses sont constamment effectuées par l'hôte et le parasite au cours du développement fongique dans la plante.

Mots clés : *Uromyces*, Golgi, sécrétion, endocytose, congélation sous haute pression, *Saccharomyces*, *Vigna sinensis*, *Vicia faba*.

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Introduction

Secretion of enzymes or toxins by host or pathogen and the uptake of material from the outside of the protoplast such as signal molecules have a major influence on the outcome of the interaction between plants and their fungal parasites. During the establishment of host–parasite combinations, exo- and endo-cytotic processes seem to undergo interesting modifications to support or to suppress the interaction. In this review, we discuss general aspects of the secretory pathway

and evaluate modifications that occur during fungal infection of plants.

Proteins and secretion

Proteins destined for secretion carry a hydrophobic signal peptide at the amino terminus (85). This signal sequence binds to a signal recognition particle in the ER lipid bilayer (10). From this entry point (69), most membrane and soluble proteins enter the ER lumen where *O*-glycosylation and N-linked core oligosaccharide attachment may occur (34, 36). Resident proteins of the ER, Golgi, and other endomembrane compartments are actively retained or diverted from this bulk flow by specific targeting signals (40). One retention signal for soluble ER resident proteins is a C-terminal histidine – aspartic acid – glutamic acid – leucine (HDEL)

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tetrapeptide (66). Such ER proteins seem to be involved in assembly and correct folding of enzymes (23). The ER compartment, where proteins with this retention signal accumulate, appears to be modified in rust infection structures (see The endoplasmic reticulum (ER) of *Uromyces viciae-fabae*).

Vesicle transport

The subsequent transport of secretory proteins occurs in vesicles. Vesicles exist for transport from ER to Golgi, from Golgi to the vacuole, or from Golgi to the plasmalemma. Vesicle docking and fusion to the target membrane is, in collaboration with a membrane fusion complex, fuelled by GTP and ATP hydrolysis (60, 93). The specificity during recognition of the right membrane is mediated by a complementary set of proteins on the donor organelle and on the target organelle (43, 79). These basic functions appear highly conserved in eucaryotic cells. There is no evidence yet for modifications during infection of plants.

The Golgi and the trans-Golgi network

After processing in the ER, secretory proteins are post-translationally modified in cis-, medial-, and trans-Golgi cisternae. The modifications include the transformation of N-linked high mannose oligosaccharides to complex glycans (19). Morphology of and enzymatic activities in the different cisternae may differ in plants, animals, or fungi. In plants, most cell wall carbohydrates are synthesized in trans-Golgi cisternae (15). During infection of plant cells, the dictyosomes accumulate around fungal structures, such as haustoria, and molecules synthesized in trans-cisternae contribute to the encasement. Modifications in the arrangement of cytoskeletal elements (24a, 48a) or local Ca^{2+} fluxes (16a) in the infected cell may redirect secretion of vesicles towards penetrating fungal structures (see The host-parasite interface during haustorium formation).

Phytophthora infestans has a typical Golgi body, and pectinmethylesterase is processed in Golgi cisternae and transported in vesicles towards the hyphal tip for secretion (20). In Ascomycetes and Basidiomycetes, the Golgi compartments are not well defined yet (see The Golgi equivalent of the higher fungi).

Subsequent sorting to the vacuoles occurs in the trans-Golgi cisternae or the closely related trans-Golgi network (45). The targeting of plant and yeast proteins depends on the recognition of elements within the polypeptide sequence or structure (40). Obviously, there is not one single targeting signal for all plant vacuolar proteins. Sorting information can be contained within C- or N-terminal or internal regions of the mature protein (4). Targeting information for transport of proteins to the plasma membrane or to the specialized membrane around haustoria is still unknown.

Endocytosis

Secretory vesicles fusing with the plasma membrane increase the cell surface. As a consequence, endocytosis is needed to internalize surface membrane material. The full magnitude of this process can be appreciated from turnover times for the plasma membrane, which ranges from 10 min for a secretory cell to 3 h for an elongating epidermal cell (81). Two kinds of endocytosis, fluid-phase endocytosis and receptor-mediated endocytosis seem to occur in plants (52). Fluid-phase endo-

cytosis in plants obviously contributes to the return pathway from the plasma membrane to the cell interior (63, 64a). Markers of fluid-phase endocytosis have been criticized very often and proof of its occurrence is missing in undisturbed plant cells (64). In hyphae, they can be useful to follow intracellular transport (75). Receptor-mediated endocytosis is similar in animals and plants (73). It starts by binding of specific ligands to membrane-spanning receptors on the cell surface (77). In plants, clathrin interacts with the cytoplasmic tail of the receptor via adaptor proteins (41). Finally, receptor ligand complexes become clustered into coated pits (67). Clathrin aggregation causes the invagination of coated pits, which results in the formation of coated vesicles. With the coated vesicles, the plasma membrane material returns to the sorting station, the trans-Golgi network. In rust infected plants, during wall penetration (see Endocytosis during the penetration) and around haustoria (see Evidence for endocytosis at the host-parasite interface), numerous coated pits indicate increased endocytotic activity.

The secretory apparatus in plant pathogenic fungi and in yeast

The endoplasmic reticulum (ER) of *Uromyces viciae-fabae*

During infection structure differentiation rust fungi produce and secrete cell wall degrading enzymes step by step (14) to invade the host plant (55). During a metabolically independent growth phase, germ tubes, substomatal vesicles, or infection hyphae exhibit ER with either parallel (stacked ER) or single cisternae. They are irregularly distributed, except for some distinct regions within germ tubes where the ER is preferentially located in the cell cortex (39). The onset of the biotrophic growth phase in planta (i.e., haustorial mother cell differentiation) is characterized by a novel ER subcompartment. The ER cisternae within haustorial mother cells, haustoria, and intercellular hyphae form tubular ER, which is more dilated than regular ER. This ER subcompartment has been designated tubular-vesicular complex (TVC) (47, 48, 87). Serial sections reveal that the TVC is formed at terminal parts of the regular ER. The TVC represents the major fraction of the ER in the biotrophic growth phase. Preliminary observations suggest that the TVC may be induced by the host plant, since haustorial mother cells formed on the thigmo-inductive polyethylene membranes do not develop TVC (F. Scheffold and K. Mendgen, unpublished data).

Each cellular structure is correlated with a distinct function. Therefore it was proposed that the TVC must be ER with special tasks (87). We tested several antisera against luminal or membrane-bound marker proteins of the ER to find differences between regular ER and TVC. Western blotting revealed that luminal ER proteins of *Uromyces viciae-fabae* bear the C-terminal peptide HDEL as retention signal. We found by immuno-electron microscopy that these proteins accumulate severalfold in the TVC as compared with normal ER and the nuclear envelope. Additionally, it was shown by ELISA that in TVC-forming cells, e.g., haustoria, the concentration of luminal ER proteins is increased relative to cells without TVC, such as germinated urediospores (U. Bachem and K. Mendgen, unpublished data).

We suggest that the accumulation of luminal ER proteins in fungal structures produced within the plant cell is an indi-

Fig. 1. The Golgi equivalent in yeast (*Saccharomyces cerevisiae*) after feeding the cells with 0.01% neutral red. **Fig. 2.** Small vesicles are close by (arrows). The samples were high-pressure frozen, freeze substituted with 2% osmic acid in acetone, and embedded in Epon-Araldite (Fig. 1, $\times 31\,500$; Fig. 2, $\times 63\,000$). **Figs. 3–5.** Immunolocalization of clathrin in *Saccharomyces cerevisiae* with a mixture of eight monoclonal antibodies against the heavy chain of the yeast clathrin, obtained from Sandra Lemmon, Cleveland, Ohio. Vesicles (Fig. 3, $\times 96\,000$; Fig. 4, $\times 75\,000$) and plasma membrane intrusions (Fig. 5, $\times 43\,750$) were labelled. The samples were high-pressure frozen, freeze substituted and embedded in LR white resin.

cator for stress, constituted by the plant. One stress response in any organism is the induction of heat-shock proteins (33). The major protein within the *Uromyces* TVC that was identified by the techniques described above is a member of the heat-shock protein family, the binding protein (BiP). BiP is induced by stress factors, such as aberrant proteins (13, 23, 35). Aberrant proteins are considered to be the primary inducers of the heat-shock response (22, 65).

Heat-shock proteins may protect the cells since their induction is correlated with tolerance to stressful treatments (35, 65). We assume that the ER of TVC-forming cells protects the rust fungus against stress metabolites produced by the host plant.

The induction of stress proteins in the ER may be an unspecific response that occurs simultaneously with more specific detoxification reactions. In the plant pathogen *Nectria haematococca*, a protein of the cytochrome P-450 family active in demethylation of the pea phytoalexin pisatin has been identified (84). The participation of such monooxygenases in intracellular detoxification of hydrophobic organic compounds is widespread (86). In hepatocytes, such compounds induce proliferation of smooth ER (70), where microsomal cytochrome P-450 proteins are more concentrated than in the rough ER (83). Morphologically, the TVC is comparable to this drug-induced smooth ER (44). TVC may contain the enzymes needed to detoxify phytoalexins produced by peas and beans during rust infection (18).

The Golgi equivalent of the higher fungi

In many fungal species organelles have been detected that

seem to functionally correspond to the Golgi apparatus in animal and plant cells. In Ascomycetes and Basidiomycetes, these organelles do not exhibit the typical dictyosome stack. They are called Golgi equivalents because they consist of only one single cisterna, which may vary in structure between different species or cell types.

To identify fungal Golgi equivalents, different ultrastructural approaches have been taken. The Golgi equivalents in some fungi were identified by carbohydrate-specific stains (1, 54). Concanavalin-A binding sites specific for mannose and (or) glucose have been detected in Golgi equivalents of *Magnaporthe grisea* (7). Neutral red, generally used as a lysosomal probe (29), is transported via the Golgi equivalent to the vacuole in bakers yeast (*Saccharomyces cerevisiae*) and labels single smooth cisternae (Figs. 1 and 2). Immunocytochemical studies indicate that the Golgi of bakers yeast and of fission yeast (*Schizosaccharomyces pombe*) contains transferases typical for dictyosomes (3, 68).

Based on biochemical and molecular methods, the Golgi equivalent of *Saccharomyces cerevisiae* is the best characterized of all fungi. According to these data, the bakers yeast Golgi equivalent consists of at least three functionally distinct subcompartments (88). However, immuno-cytochemical proof for this result is missing. As in most fungi, the Golgi equivalent of yeast is typically consisting of single cisternae, which seem to be randomly distributed. We were not able to identify trans-Golgi cisternae. However, a mixture of monoclonal antibodies against heavy chain of the yeast clathrin (44) labelled cytoplasmic vesicles in the neighbourhood of the Golgi equivalent, but not the cisternae itself (Figs. 3, 4).

Figs. 6–9. Electron micrographs of coated pit (Fig. 6, $\times 125\,000$), coated vesicle (Fig. 7, $\times 125\,000$), partially coated reticulum (Fig. 8, $\times 56\,000$), and multivesicular body (Fig. 9, $\times 125\,000$), labelled by a polyclonal antibody specific for β -1,3-glucans in *Vicia faba*, inoculated with basidiospores of *Uromyces vignae*, from Xu and Mendgen (91).

In addition, clathrin signals were obtained over coated pits (data not shown) and over plasma membrane intrusions (Fig. 5). Additional studies are needed to clarify whether single Golgi cisternae contain all typical Golgi enzymes or whether different populations of Golgi cisternae exist. In *Fusarium oxysporum*, three populations of smooth cisternae were observed (73a).

Some plant responses during fungal penetration

In early infection phases, such as appressorium formation, and before penetration of the host cell wall, the plant cytoplasm aggregates below the fungus (9, 49, 56, 90). Subsequently, a papilla is formed beneath the penetration area (24, 50), and the staining properties of the cell wall around the penetration hypha are modified (37, 53, 59, 61, 78, 91). Products of enzymatic degradation of the cell wall may diffuse to and contact the plasmalemma and thus could induce

the cytoplasmic aggregation as suggested by Kunoh et al. (49). Such degradation products are known to be active elicitors. Galacturonides, xyloglucans, hemicelluloses, xylans, N-linked glycoproteins (2, 25), and even 1,4- β -glucans (51) can elicit defence reactions. However, such macromolecules are unable to pass directly through the plasma membrane. They could be involved in a signal pathway starting from the plasma membrane or taken up by endocytosis and influence the plant metabolism in a still unknown way.

Endocytosis during the penetration

We have observed coated pits and coated vesicles in high numbers at the penetration site of the plant cell in a nonhost interaction (basidiospore of *Uromyces vignae* in *Vicia faba*) (91). Partially coated reticula and multivesicular bodies were also found in the vicinity of the penetration site (Figs. 6–9). The demonstration of clathrin around the coated pits and coated vesicles indicates endocytosis into plant cells (Figs. 10 and 11). Immuno-cytochemical detection of 1,3- β -glucans

Figs. 10 and 11. Coated pits and coated vesicles in *Vicia faba* labelled by a polyclonal antibody against plant heavy chain clathrin obtained from D. Robinson, Göttingen, Germany. Coated pit (Fig. 10, $\times 120\,000$) and coated vesicle (Fig. 11, $\times 120\,000$), from Xu and Mendgen (91).

within the coated pits, coated vesicles, partially coated reticula, and multivesicular bodies indicates that this material from the paramural space between the cell wall and the plasmalemma gathers in the area of the coated pits and is taken up by the cell. Receptors for 1,3- β -glucans have been described on the membrane of soybean cotyledones (92) and on the surface of soybean protoplasts (11, 16, 74). Uptake of other carbohydrates into the plant cell has been demonstrated by electron- and light microscopy (30, 42, 52, 63). However, the fate and the effects of such wall debris once they have reached the multivesicular bodies or the vacuole remain to be elucidated.

Papilla formation and exocytosis

One of the most common plant reactions to fungal infection is the production of a papilla. Papillae mainly consist of callose, a 1,3- β -glucan polymer that is synthesized at the plasmalemma (46). Many membrane-bound vesicles are found in papillae (50). Immuno-cytochemical studies indicate that papillae contain many other substances such as pectin, polygalacturonates, xyloglucans, rhamnogalacturonans, arabinogalactan proteins, hydroxyproline-rich glycoproteins (Xu, Rodriguez-Galvez, and Mendgen, unpublished data), and thionin (17). Most of these substances are known to pass the ER and (or) the Golgi apparatus, suggesting, that exocytosis is involved in papilla formation.

The host – parasite interface

The host – parasite interface during haustorium formation

In later infection phases (57) obligately biotrophic pathogens such as the rust fungi associate closely with their host plant by means of haustoria. After penetration of the wall haustoria are formed within cells via invagination of the host plasma membrane. The haustorial protoplast is enclosed by an electron-dense fungal cell wall. The host – parasite interface between fungal cell wall and invaginated host plasma membrane (the extrahaustorial membrane) is defined as the extrahaustorial matrix (50). Sometimes the slightly electron-dense extrahaustorial matrix is ensheathed by additional layers of

plant cell wall-like material (28, 71). Thus, an encasement may separate the fungal haustorium from the plant protoplast. The formation of encasements seems to be a typical host response in incompatible host – parasite interactions (12, 31, 32, 82) but is also observed in compatible interactions (26, 89).

Secretion at the host – parasite interface

The interface of haustoria and their host cells has been investigated ultrastructurally and cytochemically (27). However, it has become apparent that processing and staining methods generally used for electron microscopy may have led to misrepresentation of a number of structural details (27) and can therefore not be used for the determination of the origin of components of the extrahaustorial matrix. Methods such as high-pressure freeze fixation (58) in combination with immunolabelling techniques allowed improved studies of the composition of the host – parasite interface, and by this means the origin of the constituents of the interface was revealed (80).

The extrahaustorial matrix of monokaryotic (M) haustoria (Fig. 12) was found to contain hydroxyproline-rich glycoproteins (HRGP) and arabinogalactan/arabinogalactan proteins (AG/AGP). HRGP and AG/AGP were also found in the trans-cisternae of the plant Golgi apparatus. Obviously, they were synthesized in the Golgi and secreted into the host – parasite interface. Encasement of haustoria started with the deposition of cellulose into the extrahaustorial matrix (Fig. 13, M. Stark-Urnau and K. Mendgen, unpublished data). Most M-haustoria had an encasement consisting of three additional layers. First, an electron-translucent layer containing HRGP, AG/AGP, and callose was formed on top of the extrahaustorial matrix. Then, an electron-translucent layer with numerous inclusions was formed. This layer contained both unesterified and methyl-esterified pectin and xyloglucans with fucosyl residues (Fig. 12). Methyl-esterified pectin and fucosyl residues on xyloglucans were also found in the trans-cisternae of the plant Golgi apparatus, indicating synthesis in the Golgi cisternae. The antibody directed against unesterified pectin did not label the Golgi apparatus, which may be due to the secretion of pectin in a methyl-

Fig. 12. Scheme of young and old M-haustoria: Monokaryotic haustorium (M-ha), fungal plasma membrane (*fpm*), fungal wall (*fw*), plant cell wall (*pw*), invaginated plant plasma membrane (*ppm*). The extrahaustorial matrix (*ema*) of all M-haustoria contained arabinogalactan proteins (AGP) and hydroxyproline-rich glycoproteins (HRGP) both, with a higher concentration close to the extrahaustorial membrane (*ehm*). The *ema* of encased haustoria also contained cellulose. This matrix was enforced with additional layers: an inner layer containing AGP, HRGP, and callose; a middle layer containing pectin and fucosylated xyloglucans; and an outer layer containing AGP and HRGP. At the tip of mostly young haustoria tubular clathrin-coated pits were formed. **Fig. 13.** Electronmicrograph of a young M-haustorium (*yha*) and an old M-haustorium (*oha*) of *Uromyces vignae* in *Vigna sinensis*. The extrahaustorial matrix (*ema*) of the old M-haustorium is labelled by a polyclonal antibody specific for cellulose ($\times 50\,000$).

esterified form that is later deesterified in the encasement (72, 76). Finally a slightly electron-dense layer with AG/AGP and HRGP encased the whole structure (Fig. 12). The demonstration of different layers containing different polysaccharides and glycoproteins shows that secretion by the infected host cell occurs step by step in a highly regulated fashion (80).

The deposition of host-derived polysaccharides into the host–parasite interface of symbiotic, compatible, and incompatible host–parasite interactions seems to represent a common phenomenon (5, 6, 8, 38, 62), though sequential secretion as described here has not been described before. Glycosylation during the secretion process itself seems similar to the general scheme described for plants (15).

Evidence for endocytosis at the host–parasite interface

In uredial infections of *Puccinia coronata* f.sp. *avenae* and *Puccinia graminis* f.sp. *tritici* complexes consisting of large and small tubules, formed around the extrahaustorial membrane, were observed (26, 27, 28). After processing by high-pressure freezing and freeze substitution of *Vigna sinensis* leaves infected with *Uromyces vignae*, tubular coated pits became visible on the extrahaustorial membrane (Fig. 12) mostly at the tip of M-haustoria. The pits were elongated and differed in this respect from coated pits generally observed in planta (21, 73, 91). Further studies of a possible involvement of the cytoskeleton in the shaping of these tubules are needed. Since the coat was recognized by a polyclonal antibody against plant clathrin (80), we interpreted this as an indication for endocytosis (52). Obviously, intensive membrane recycling processes seem to take place around M-haustoria where secretion and endocytosis are coupled events.

Conclusions

The complex exchange of signals between smut (3a) or rust fungi and their respective host plants (32a) is accompanied

by a continuous flow of material into the host–parasite interface and, at the same time, a more or less pronounced uptake of molecules from this zone by both partners. These events start with the penetration process and possibly end after complete encasement and inactivation of fungal structures. The most important feature of biotrophy may be a balanced activity of host and parasite to support survival of both organisms.

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References

1. Akashi, T., Homma, M., Kanbe, T., and Tanaka, K. 1993. Ultrastructure of proteinase-secreting cells of *Candida albicans* studied by alkaline bismuth staining and immunocytochemistry. *J. Gen. Microbiol.* **139**: 2185–2195.
2. Aldington, S., and Fry, S.C. 1993. Oligosaccharins. *Adv. Bot. Res.* **19**: 1–101.
3. Ayscough, K., Hajibagheri, N.M.A., Watson, R., and Warren, G. 1993. Stacking of golgi cisternae in *Schizosaccharomyces pombe* requires intact microtubules. *J. Cell Sci.* **106**: 1227–1237.
- 3a. Bauer, R., Mendgen, K., and Oberwinkler, F. 1995. Cellular interaction of the smut fungus *Ustilago waldsteiniae*. *Can. J. Bot.* **73**: 862–866.
4. Bednarek, S.Y., and Rakhel, N.V. 1992. Intracellular trafficking of secretory proteins. *Plant Mol. Biol.* **20**: 133–150.
5. Benhamou, N. 1993. Spatio-temporal regulation of defence genes. In *Mechanisms of plant defense responses*. Edited by B. Fritig and M. Legrand. Kluwer Academic Publishers, The Netherlands. pp. 221–235.
6. Bonfante-Fasolo, P., Peretto, R., and Perotto, S. 1992. Cell surface interactions in endomycorrhizal symbiosis. In

- Perspectives in plant cell recognition. Edited by J.A. Callow and J.R. Green. Cambridge University Press, Cambridge, Mass. pp. 239–255.
7. Bourett, T.M., Piccollelli, M.A., and Howard, R.J. 1993. Postembedding labeling of intracellular Concanavalin A-binding sites in freeze-substituted fungal cells. *Exp. Mycol.* **17**: 223–235.
 8. Bradley, D.J., Wood, E.A., Larkins, A.P., Galfre, G., Butcher, G.W., and Brewin, N.J. 1988. Isolation of monoclonal antibodies reacting with peribacteroid membranes and other components of pea root nodules containing *Rhizobium leguminosarium*. *Planta*, **173**: 149–160.
 9. Bushnell, W.R., and Bergquist, S.E. 1975. Aggregation of host cytoplasm and the formation of papillae and haustoria in powdery mildew of barley. *Phytopathology*, **65**: 310–318.
 10. Campos, N., Palau, J., Torrent, M., and Ludevid, D. 1988. Signal recognition particles are present in maize. *J. Biol. Chem.* **263**: 9646–9650.
 11. Cheong, J.-J., and Hahn, M.G. 1991. A specific, high-affinity binding site for the hepta- β -glucoside elicitor exists in soybean membrane. *Plant Cell*, **3**: 137–147.
 12. Cohen, Y., Eyal, H., and Hanania, J. 1990. Ultrastructure, autofluorescence, callose deposition and lignification in susceptible and resistant muskmelon leaves infected with the powdery mildew fungus *Sphaerotheca fuliginea*. *Physiol. Mol. Plant Pathol.* **36**: 191–204.
 13. Craig, E.A., Gambill, B.D., and Nelson, R.J. 1993. Heat shock proteins: molecular chaperones of protein biogenesis. *Microbiol. Rev.* **57**: 402–414.
 14. Deising, H., Rauscher, M., Haug, M., and Heiler, S. 1995. Differentiation and cell wall degrading enzymes in the obligately biotrophic rust fungus *Uromyces viciae-fabae*. *Can. J. Bot.* **73**. In press.
 15. Driouich, A., Faye, L., and Staehelin, L.A. 1993. The plant Golgi apparatus: a factory for complex polysaccharides and glycoproteins. *TIBS*, **18**: 210–214.
 16. Ebel, J., and Cosio, E.G. 1994. Elicitors of plant defence responses. *Int. Rev. Cytol.* **148**: 1–36.
 - 16a. Ebel, J., and Scheel, D. 1992. Elicitor recognition and signal transduction. In *Plant gene research*. Edited by T. Boller and F. Meins. Springer-Verlag, Wien. pp. 183–205.
 17. Ebrahim-Nesbat, F., Bohl, S., Heitefuss, R., and Apel, K. 1993. Thionin in cell walls and papillae of barley in compatible and incompatible interactions with *Erysiphe graminis* f.sp. *hordei*. *Physiol. Mol. Plant Pathol.* **43**: 343–352.
 18. Elnaghy, M.A., and Heitefuss, R. 1976. Permeability changes and production of antifungal compounds in *Phaseolus vulgaris* infected with *Uromyces phaseoli*. I. Role of the spore germination self-inhibitor. *Physiol. Plant Pathol.* **8**: 253–267.
 19. Farquhar, M.G. 1985. Progress in unraveling pathways of Golgi traffic. *Annu. Rev. Cell Biol.* **1**: 447–488.
 20. Förster, H., and Mendgen, K. 1987. Immunochemical localisation of pectinesterases in hyphae of *Phytophthora infestans*. *Can. J. Bot.* **65**: 2607–2613.
 21. Fowke, L.C., Tanchak, M.A., and Galway, M.E. 1991. Ultrastructural cytology of the endocytotic pathway in plants. In *Endocytosis and vesicle traffic in plants*. Edited by C.R. Hawes, D.E. Evans, and J.O.D. Coleman. Cambridge University Press, Cambridge, Mass. pp. 15–40.
 22. Georgopoulos, C., and Welch, W.J. 1993. Role of the major heat shock proteins as molecular chaperones. *Annu. Rev. Cell Biol.* **9**: 601–634.
 23. Gething, M.-J., and Sambrook, J. 1992. Protein folding in the cell. *Nature (London)*, **355**: 33–45.
 24. Gold, R.E., and Mendgen, K. 1984. Cytology of basidiospore germination, penetration, and early colonization of *Phaseolus vulgaris* by *Uromyces appendiculatus* var. *appendiculatus*. *Can. J. Bot.* **65**: 1989–2002.
 - 24a. Gross, P., Julius, C., Schmelzer, E., and Hahlbrock, K. 1993. Translocation of cytoplasm and nucleus to fungal penetration sites is associated with depolymerization of microtubules and defence gene activation in infected, cultured parsley cells. *EMBO J.* **12**: 1735–1744.
 25. Hahne, G., and Lörz, H. 1988. Release of phytotoxic factors from plant cell walls during protoplast isolation. *J. Plant Physiol.* **132**: 345–350.
 26. Harder, D.E. 1978. Comparative ultrastructure of the haustoria in uredial and pycnial infections of *Puccinia coronata avenae*. *Can. J. Bot.* **56**: 214–224.
 27. Harder, D.E., and Chong, J. 1991. Rust haustoria. In *Electron microscopy of plant pathogens*. Edited by K. Mendgen and D.-E. Lesemann. Springer-Verlag, Berlin. pp. 235–250.
 28. Harder, D.E., Rohringer, R., Samborski, D.J., Kim, W.K., and Chong, J. 1978. Electron microscopy of susceptible and resistant near-isogenic (*sr6/Sr6*) lines of wheat infected by *Puccinia graminis tritici*. I. The host–pathogen interface in the compatible (*sr6/P6*) interaction. *Can. J. Bot.* **56**: 2955–2966.
 29. Haugland, R.P. 1992. Handbook of fluorescent probes and research chemicals. Molecular Probes, Inc., Eugene, Oreg.
 30. Hawes, C., Coleman, J., Evans, D., and Cole, L. 1989. Recent advantages in the study of plant coated vesicles. *Cell Biol. Int. Rep.* **13**: 119–128.
 31. Heath, M.C. 1971. Haustorial sheath formation in cowpea leaves immune to rust infection. *Phytopathology*, **61**: 383–388.
 32. Heath, M.C. 1989. A comparison of fungal growth and plant responses in cowpea and bean cultivars inoculated with urediospores or basidiospores of the cowpea rust fungus. *Physiol. Mol. Plant Pathol.* **34**: 415–426.
 - 32a. Heath, M.C. 1995. Signal exchange between higher plants and rust fungi. *Can. J. Bot.* **73**(Suppl. 1). This issue.
 33. Heikkilä, J.J. 1993. Heat shock gene expression and development. I. An overview of fungal, plant, and poikilothermic animal developmental systems. *Dev. Genet.* **14**: 1–5.
 34. Helenius, A. 1994. How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. *Mol. Biol. Cell*, **5**: 253–265.
 35. Hendrick, J.P., and Hartl, F.-U. 1993. Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.* **62**: 349–384.
 36. Herscovics, A., and Orlean, P. 1993. Glycoprotein biosynthesis in yeast. *FASEB J.* **7**: 540–550.
 37. Hippe-Sanwald, S., Hermanns, M., and Somerville, S.C. 1992. Ultrastructural comparison of incompatible and compatible interactions in the barley mildew disease. *Protoplasma*, **168**: 27–40.
 38. Hippe-Sanwald, S., Marticke, K.H., Kieliszewski, M.J., and Somerville, S.C. 1994. Immunogold localization of THRGP-like epitopes in the haustorial interface of obligate, biotrophic fungi on monocots. *Protoplasma*, **178**: 138–155.
 39. Hoch, H.C., and Staples, R.C. 1983. Ultrastructural organization of the non-differentiated uredospore germling *Uromyces phaseoli* var. *typica*. *Mycologia*, **75**: 795–824.
 40. Höfte, H., and Chrispeels, M.J. 1992. Protein sorting to the vacuolar membrane. *Plant Cell*, **4**: 995–1004.

41. Holstein, S.E.H., Daneker, M., and Robinson, D.G. 1994. Identification of a β -type adaptin in plant clathrin-coated vesicles. *J. Cell Sci.* **107**: 945–953.
42. Horn, M.A., Heinsteins, P.F., and Low, P.S. 1989. Receptor-mediated endocytosis in plant cells. *Plant Cell*, **1**: 1003–1009.
43. Hurtley, S.M. 1993. Membrane proteins involved in targeted membrane fusion. *TIBS*, **18**: 453–455.
44. Jones, A.L., and Fawcett, D.W. 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital. *J. Histochem. Cytochem.* **14**: 215–232.
45. Jones, R.L., and Robinson, D.G. 1989. Protein secretion in plants. *New Phytol.* **111**: 567–597.
46. Kauss, H. 1994. Callose synthesis. In *Membranes: specialized functions in plant cells*. Edited by M. Smallwood, P. Knox, and D.J. Bowles. JAI Press, Greenwich, Conn. pp. 609–631.
47. Knauf, G.M., and Mendgen, K. 1988. Secretion systems and membrane-associated structures in rust fungi after high-pressure freezing and freeze-fracturing. *Biol. Cell*, **64**: 363–370.
48. Knauf, G.M., Welter, K., Müller, M., and Mendgen, K. 1989. The haustorial host–parasite interface in rust-infected bean leaves after high-pressure freezing. *Physiol. Mol. Plant Pathol.* **34**: 519–530.
- 48a. Kobayashi, I., Kobayashi, Y., and Hardham, A.R. 1994. Dynamic reorganization of microtubules and microfilaments in flax cells during the resistance response to flax rust infection. *Planta*, **195**: 237–247.
49. Kunoh, H., Aist, J.R., and Hayashimoto, A. 1985. The occurrence of cytoplasmic aggregates induced by *Erysiphe pisi* in barley coleoptile cells before the host cell walls are penetrated. *Physiol. Plant Pathol.* **26**: 199–207.
50. Littlefield, L.J., and Heath, M.C. 1979. Ultrastructure of rust fungi. Academic Press, New York.
51. Lorences, E.P., McDougall, G.J., and Fry, S.C. 1990. Xyloglucan- and cello-oligosaccharides antagonists of the growth-promoting effect of H⁺. *Physiol. Plant.* **80**: 109–113.
52. Low, P.S., and Chandra, S. 1994. Endocytosis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**: 609–631.
53. McKeen, W.E., Smith, R., and Bhattacharya, P.K. 1969. Alterations of the host wall surrounding the infection peg of powdery mildew fungi. *Can. J. Bot.* **47**: 701–706.
54. McLaughlin, D.J. 1974. Ultrastructural localization of carbohydrate in the hymenium and subhymenium of *Coprinus*. Evidence for the function of the Golgi apparatus. *Protoplasma*, **82**: 341–364.
55. Mendgen, K., and Deising, H. 1993. Infection structures of fungal plant pathogens — a cytological and physiological evaluation. *New Phytol.* **124**: 193–213.
56. Mendgen, K., Keller, G., and Kuhn, C. 1987. Infektion und Wirtsreaktion beim Gerstenmehltau. Film C 1642, Institut für den Wissenschaftlichen Film, Göttingen.
57. Mendgen, K., Schneider, A., Sterk, M., and Fink, W. 1988. The differentiation of infection structures as a result of recognition events between some biotrophic parasites and their hosts. *J. Phytopathol.* **123**: 269–272.
58. Mendgen, K., Welter, K., Scheffold, F., and Knauf-Beiter, G. 1991. High pressure freezing of rust infected plant leaves. In *Electron microscopy of plant pathogens*. Edited by K. Mendgen and D.E. Lesemann. Springer-Verlag, Heidelberg. pp. 31–42.
59. Mims, C.W., Taylor, J., and Richardson, E.A. 1989. Ultrastructure of the early stages of infection of peanut leaves by the rust fungus *Puccinia arachidis*. *Can. J. Bot.* **67**: 3570–3579.
60. Nuoffer, C., and Balch, W.E. 1994. GTPases: multifunctional molecular switches regulating vesicular traffic. *Annu. Rev. Biochem.* **63**: 949–990.
61. O'Connell, R., and Bailey, J.A. 1986. Cellular interactions between *Phaseolus vulgaris* and the hemibiotrophic fungus *Colletotrichum lindemuthianum*. In *NATO ASI Series, Biology and molecular biology of plant–pathogen interactions*. Edited by J. Bailey. Springer-Verlag, Berlin. pp. 39–48.
62. O'Connell, R.J., Brown, I.R., Mansfield, J.W., Bailey, J.A., Mazau, D., Rumeau, D., and Esquerré-Tugayé, M.T. 1990. Immunocytochemical localization of hydroxyprolin rich glycoprotein accumulating in melon and bean at sites of resistance to bacteria and fungi. *Mol. Plant-Microbe Interact.* **3**: 33–40.
63. O'Driscoll, D., Hahn, C., Read, S.M., and Steer, M.W. 1993. Endocytotic uptake of fluorescent dextrans by pollen tubes grown in vitro. *Protoplasma*, **175**: 126–130.
64. Oparka, K.J., Wright, K.M., Murrant, E.A., and Allan, E.J. 1993. Fluid-phase endocytosis: do plants need it? *J. Exp. Bot.* **44**: 247–255.
- 64a. Owen, T.P., Jr., Platt-Aloia, K.A., and Thomson, W.W. 1991. Ultrastructural localization of Lucifer Yellow and endocytosis in plant cells. *Protoplasma*, **160**: 115–120.
65. Parsell, D.A., and Lindquist, S. 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* **27**: 437–496.
66. Pelham, H.R.B. 1989. Control of protein exit from the endoplasmic reticulum. *Annu. Rev. Cell Biol.* **5**: 1–23.
67. Pley, U., and Parham, P. 1993. Clathrin: its role in receptor-mediated vesicular transport and specialized functions in neurons. *Crit. Rev. Biochem. Mol. Biol.* **28**: 431–464.
68. Preuss, D., Mulholland, J., Franzusoff, A., Segev, N., and Botstein, D. 1992. Characterisation of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. *Mol. Biol. Cell*, **3**: 789–803.
69. Rapoport, T.A. 1992. Transport of proteins across the endoplasmic reticulum membrane. *Science (Washington, D.C.)*, **258**: 931–936.
70. Remmer, H., and Merker, H.-J. 1963. Enzyminduktion und Vermehrung von endoplasmatischem Retikulum in der Leberzelle während der Behandlung mit Phenobarbital (Luminal). *Klin. Wochenschr.* **41**: 276–283.
71. Rijkenberg, F.H.J., and Truter, S. 1973. Haustoria and intracellular hyphae in the rusts. *Phytopathology*, **61**: 281–286.
72. Roberts, K. 1990. Structures at the plant cell surface. *Curr. Opin. Cell Biol.* **2**: 920–928.
73. Robinson, D.G., and Hillmer, S. 1990. Coated pits. In *The plant plasma membrane*. Edited by C. Larsson and I.M. Moeller. Springer-Verlag, Berlin. pp. 233–255.
- 73a. Rodriguez-Galvez, E., and Mendgen, K. 1994. The infection process of *Fusarium oxysporum* in cotton root tips. *Protoplasma*. In press.
74. Schmidt, W.E., and Ebel, J. 1987. Specific binding of fungal glucan phytoalexin elicitor to membrane fractions from soybean *Glycine max*. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 4117–4121.
75. Shepherd, V.A., Orlovich, D.A., and Ashford, A.E. 1993. A dynamic continuum of pleiomorphic tubules and vacuoles in growing hyphae of a fungus. *J. Cell Sci.* **104**: 495–507.
76. Sherrier, D.J., and Vandenbosch, K.A. 1994. Secretion of cell wall polysaccharides in *Vicia* root hairs. *Plant J.* **5**: 185–195.
77. Smythe, F., Carter, L.L., and Schmid, S.L. 1992. Cytosol- and clathrin dependent stimulation of endocytosis

- in vitro by purified adaptors. *J. Cell Biol.* **119**: 1163–1171.
78. Snetselaar, K.M., and Mims, C.W. 1993. Infection of maize stigmas by *Ustilago maydis*: light and electron microscopy. *Phytopathology*, **83**: 843–850.
79. Söllner, T. 1993. SNAP receptors implicated in vesicle targeting and fusion. *Nature (London)*, **362**: 318–324.
80. Stark-Urnau, M., and Mendgen, K. 1995. Sequential deposition of plant carbohydrates and glycoproteins into the host parasite interface of *Uromyces vignae* and *Vigna sinensis*. Evidence for endocytosis and secretion. *Protoplasma*. In press.
81. Steer, M.W. 1988. Plasma membrane turnover in plant cells. *J. Exp. Bot.* **39**: 987–996.
82. Tahiri-Alaoui, A., Gianinazzi, S., and Dumas-Gaudot, E. 1993. *Chalara elegans*-infected tobacco roots: ultrastructural observations on in vitro host–fungus relationships. *J. Phytopathol.* **137**: 245–256.
83. Tashiro, Y., Masaki, R., and Yamamoto, A. 1993. Cytochrome P-450 in the endoplasmic reticulum. Biosynthesis, distribution, induction, and degradation. *In* Subcellular biochemistry. Vol. 21. Endoplasmic reticulum. Edited by N. Borgese and J.R. Harris. Plenum Press, New York. pp. 287–311.
84. Van Etten, H.D., Matthews, D.E., and Matthews, P.S. 1989. Phytoalexin detoxification: importance for pathogenicity and practical implications. *Annu. Rev. Phytopathol.* **27**: 143–164.
85. von Heijne, G. 1988. Transcending the impenetrable: how proteins come to terms with membranes. *Biochim. Biophys. Acta*, **947**: 307–333.
86. Waxman, D.J., and Azaroff, L. 1992. Phenobarbital induction of cytochrome P-450 gene expression. *Biochem. J.* **281**: 577–592.
87. Welter, K., Müller, M., and Mendgen, K. 1988. The hyphae of *Uromyces appendiculatus* within the leaf tissue after high pressure freezing and freeze substitution. *Protoplasma*, **147**: 91–99.
88. Wilsbach, K., and Payne, G.S. 1993. Dynamic retention of TGN membrane proteins in *Saccharomyces cerevisiae*. *Trends Cell Biol.* **3**: 426–432.
89. Woods, A.M., and Gay, J.L. 1987. The interface between haustoria of *Puccinia poarum* (monokaryon) and *Tussilago farfara*. *Physiol. Mol. Plant Pathol.* **30**: 167–185.
90. Xu, H., and Mendgen, K. 1991. Early events in living epidermal cells of cowpea and broad bean during infection with basidiospores of the cowpea rust fungus. *Can. J. Bot.* **69**: 2279–2285.
91. Xu, H., and Mendgen, K. 1994. Endocytosis of 1,3- β -glucans by broad bean at the penetration site of the cowpea rust fungus (haploid stages). *Planta*, **195**: 282–290.
92. Yoshikawa, M., Keep, N.T., and Wang, M.C. 1983. A receptor on soybean membranes for a fungal elicitor of Phytoalexin accumulation. *Plant Physiol.* **73**: 497–506.
93. Zimmerberg, J., Vogel, S.S., and Chernomordik, L.V. 1993. Mechanisms of membrane fusion. *Annu. Rev. Biophys. Biomol. Struct.* **22**: 433–466.