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SECRETION IN THE PARASITIC PHASE OF RUST FUNGI

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INTRODUCTION

Rust fungi have a complex life cycle (see Littlefield 1981) and produce different spore forms. The basidiospores, aecio-spores and uredospores develop highly specialized infection structures in order to penetrate into the leaf. The germ tube of the dikaryotic uredospore follows the array of wax crystals on the cuticle in a typical way and grows towards a stoma (Fig 1a). When the tip of the germ tube contacts the lip of the stomatal opening (see Hoch and Staples 1987) appressorium formation begins. From the lower surface of the appressorium, an infection peg develops and penetrates into the substomatal cavity of the leaf where the substomatal vesicle with the infection hypha develops. After close contact with the parenchymatous host cells, a haustorial mother cell develops which, in turn, differentiates a haustorium in the host cell (Staples et al 1985).

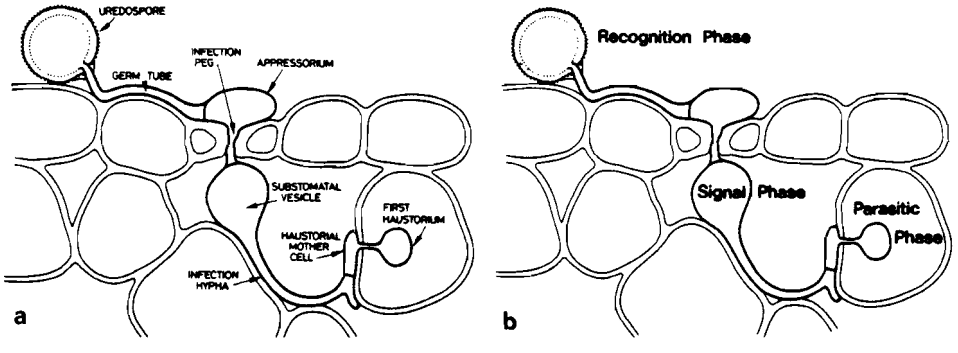

Fig. 1. Infection structures of the dikaryotic uredospore of most rust fungi.
a) morphological characterization
b) functional characterization (Mendgen 1988).

HOST RECOGNITION

This sequence of infection structures is the product of more or less specific interactions between the host and the parasite. In the recognition phase (Mendgen et al 1988), the germ tube recognizes (Fig 1b) the surface of the cuticle and the stomatal lip. This surface signal can be replaced by nearly any plastic surface (e.g. polyethylene) scratched with sand paper. During development of the substomatal vesicle and the infection hypha, more specific signals are exchanged between host and parasite. During this signal phase, the rust fungus suppresses defence reactions of the host plant such as the activity of phenylalanine ammonia-lyase (Yamamoto et al 1977; Moerschbacher et al 1988). Mainly the host plant promotes further fungal development and after differentiation of the haustorial mother cell, induces the first haustorium in the host cell (Mendgen 1982). Now, the parasitic phase of the rust fungus begins. Very specific interactions between host and parasite induce a flow of metabolites from host to parasite. This complex balance between the two partners can be disturbed eventually by the products of the host plant's resistance genes.

During the signal phase and the parasitic phase, the hyphae of the rust fungus exhibit special features. The surface of these hyphae contains ß 1 → 3 and ß 1 → 6 glucans (Mendgen et al 1985). In the electron microscope, brush like fibrils on the intercellular hyphae can be seen after freeze substitution of infected tissue (Mendgen et al 1988; Welter et al 1988). The fibrils may represent glycoproteins. They appear to be interwoven with the host wall. One can imagine that this material influences the host parasite interaction. There is not much information on how parasitic fungi secrete such wall material or enzymes or other molecules during growth in their host tissue. One reason might be that a typical Golgi body has not been observed yet in rust fungi.

SECRETION IN ANIMALS AND PLANTS

In animals, a well developed Golgi apparatus is responsible for secretion (Farquhar 1985; Burgess and Kelly 1987). This means a functional continuum of membranous cell compounds consisting of the nuclear envelope, endoplasmic reticulum, Golgi apparatus and a number of quite different vesicle types of very different size. Some of these vesicles finally merge with the plasma membrane. The Golgi apparatus itself consists of regions of stacked cisternae (dictyosomes) without ribosomes. The cisternae may occur singly, as flattened cavities or very often consist of a central plate-like region continous with a peripheral system of tubules and vesicles. The dictyosomes seem to be polarized. One pole is associated with the nuclear envelope or with the endoplasmic reticulum. It is called the proximal pole or the forming face. The opposite pole or the maturing face of the Golgi stacks seems to be involved with the formation of secretory vesicles. Here, an elaborate trans Golgi network is observed very often.

In plants, it is also assumed that a large part of the intracellular transport of macromolecules takes place via dictyosomes (Robinson and Kristen 1982). The Golgi apparatus of plants can be very similar to the structures observed in animals (Schnepf 1969; Sievers and Schnepf 1981). However, a biochemical and a structural analysis has indicated significant differences (Morré 1977; Jones and Robinson 1989). Because most plant cells secrete much smaller amounts of proteins, vesicle movement between ER and Golgi apparatus is considerably reduced. Also, the process of vesicle-mediated secretion and membrane recycling seems different from what has been observed in animal cells. Turgor pressure forces acting on the plasma membrane seem to induce unique types of plasma membrane configurations such as flattened vesicles with slit shaped membrane fusion sites and horse shoe-shaped membrane infoldings (Staehelin and Chapman 1987). There is a very close and characteristic association of ER cisternae and the plasmalemma. This may indicate a molecular recycling of membrane lipids from the plasma membrane to the ER (Craig and Staehelin 1988).

SECRETION IN FUNGI

In fungi, growth takes place mainly at the hyphal tip. This tip is also responsible for secretion of different types of molecules. Glucans and chitin are obviously synthesized at the plasma membrane (see Wessels 1987). Glycoproteins, e.g. elicitors (Kogel et al 1988) should follow the normal way of synthesis, processing, sorting and delivery.

Some "lower" fungi, such as *Pythium*, *Chytridium*, *Saprolegnia*, and *Phytophthora*, have a typical Golgi apparatus (Grove et al 1968, Taylor and Fuller 1981, Heath et al 1985, Förster and Mendgen 1987). The dictyosomes appear to pinch off vesicles that accumulate at special areas of the fungal protoplast or at the hyphal tip. The vesicles consist of different populations of size and their contents react with stains for carbohydrates. With immunological techniques, it was demonstrated that these vesicles, depending on their size, may have different contents (Gubler and Hardham 1988). Pectinmethylesterase was localized in the dictyosome, in three different types of vesicles and over the plasma membrane (Förster and Mendgen 1987). This labeling pattern suggests the "normal" way of protein glycosylation and secretion as described for animals.

In other fungi, the situation is different. *Saccharomyces cerevisiae* has a small, but still typical dictyosome (Baba and Osumi 1987). In *Schizosaccharomyces pombe*, only single tubular structures with vesicles or single fenestrated cisternae were detected after ultrarapid freezing and freeze substitution (K. Tanaka, pers. comm.). Also in other Ascomycetes and Basidiomycetes, such very small structures reminiscent of single Golgi cisternae have been detected: smooth cisternae (Howard and Aist 1979), centers of presumed Golgi activity (Hoch and Howard 1980), modified dictyosomes (Dargent et al 1982), Golgi-like bodies (Dahmen and Hobot 1986), Golgi equivalents (Roberson and Fuller 1988), Golgi cisternae (Newhouse et al 1983), or Golgi bodies (McLaughlin 1974; Mims et al 1988).

In the dicaryotic stage of the rust fungus *Uromyces*, the appearance of the Golgi equivalent seems to change during its development. In the germ tubes, small, variously shaped segments of inflated cisternae were detected (Hoch and Staples 1983). In infection hyphae, the Golgi equivalent consists of numerous small tubular-vesicular structures (Scheffold, unpublished result) which were called tubular-vesicular complex type 2 (TVC 2) (Welter et al 1988). TVC 2 is similar to the structures observed in germ tubes, but larger in size and the cisternae are more elaborated. Subsequently, in the parasitic phase (in intercelluar hyphae and haustoria), TVC 2 becomes less frequent (Knauf et al 1989). Now, a different, quite prominent structure, called tubular-vesicular complex type 1 (TVC 1), becomes obvious in intercellular hyphae and haustoria (Fig. 2). It consists of more or less big complexes of cisternae. In developing spores, TVC 1 covers most of the spore's cytoplasm. In areas where the tubules approach the plasmalemma, sometimes vesicles fuse with the plasma membrane suggesting secretion events. These events in the rust fungi (Knauf and Mendgen 1988) are different from secretion of vesicles in plants (Craig and Staehelin 1988).

Do these tubular-vesicular structures have the secretory functions of a Golgi apparatus? Are proteins glycosylated there and are the vesicles designated for secretion? In order to study these processes in the rust fungi, the following strategies are being pursued:

1. Monoclonal antibodies are raised against the surface of infection hyphae and haustorial mother cells. We are looking now whether these antibodies bind to glycoprotein common to tubular-vesicular complexes and to glycoproteins secreted on the hyphal surface.

2. Antibodies against pectinesterase from *P. infestans* also recognize such an enzyme in the rusts. Since the enzyme is glycosylated, we look for enzymes responsible for this. A GDP-Man:Dol-P mannosyltransferase and a galactosyltransferase have been detected (Welter, Fink and Frittrang, unpublished result). The first enzyme is localized in the ER Tanner and Lehle 1987), the latter may be localized in the

smooth cisternae of the dictyosome. With antibodies against these enzymes, we hope to characterize the Golgi equivalent in the rust fungi.

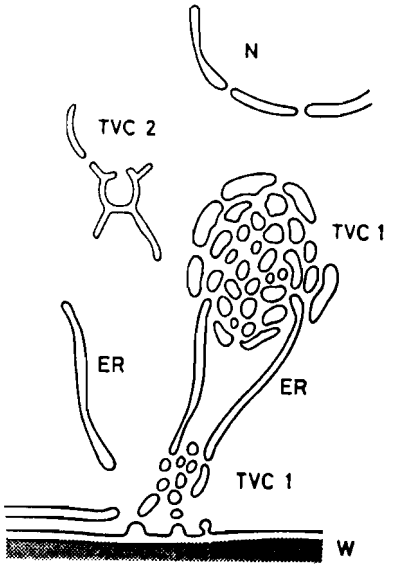

Fig. 2
Secretion in the parasitic phase of rust fungi
TVC 1= tubular-vesicular complex type 1
TVC 2= tubular-vesicular complex type 2
ER= endoplasmic reticulum
N= nucleus W= wall

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