fact tubulin binding domains and whether there are any common structural motifs in microtubule-associated proteins. Studies of the expression and structure of tau proteins in Alzheimer's disease should also provide important clues to the etiology of the neurofibrillary tangles.

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A Mechanism for Surface Attachment in Spores of a Plant Pathogenic Fungus

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Rice blast disease is caused by a fungus that attacks all above-ground parts of the rice plant. In a study of the means by which the fungus attaches to the hydrophobic rice leaf surface, it was found that spores (conidia) of the rice blast fungus *Magnaporthe grisea* have a mechanism for immediate and persistent attachment to various surfaces, including Teflon. This attachment occurs at the spore apex and is blocked by the addition of the lectin concanavalin A. Microscopy of hydrated conidia shows that a spore tip mucilage that binds concanavalin A is expelled specifically from the conidial apex before germ tube emergence. Ultrastructural analysis of dry conidia shows a large periplasmic deposit, presumably spore tip mucilage, at the apex. The results indicate a novel mechanism for the attachment of phytopathogenic fungal spores to a plant surface.

THE ASCOMYCETE *Magnaporthe grisea* Barr [*Pyricularia* sp. (*1, 2*)] causes the devastating plant disease called rice blast (*3*). Rice blast disease occurs in most of the major rice growing regions of the world, where severe epidemics result in substantial crop loss and lead to potential economic disaster. Because stably blast-resistant rice cultivars have not been developed, the disease is primarily controlled by cultural practices and fungicide application (*4*). We have begun to investigate the early stages of the infection process to identify cellular components or processes as targets for disease control measures. One of the early steps in any host-parasite interaction is the attachment of the parasite to the host. A traditional view of fungal attachment to plants is that the fungal spores become lodged or entrapped on the leaf surface, and that active fungal attachment does not occur until the formation of fungal hyphae and infection-specific cell types (*5*). We present evidence that the spores of a fungal pathogen have a mechanism for rapid and persistent attachment to surfaces prior to germination.

Blast lesions that develop on an infected plant produce numerous conidia that are released in moist air and may inoculate neighboring plants. A conidium germinates with the emergence of a hypha (germ tube) that later forms an infection structure termed an appressorium (*6*). Early stages of infection-related morphogenesis can be observed on an artificial surface (Fig. 1a) (*7*). On glass, conidia produce germ tubes within 3 hours and do not form appressoria. Conidia germinated on Teflon-PFA film (*8*) also produce germ tubes within 3 hours. Continued incubation results in the formation of appressoria. Artificial surfaces that are conducive to appressorium formation may have properties similar to the rice leaf surface (*9*). We have found that the rate of germ tube production and appressorium formation by *M. grisea* conidia on Teflon-PFA film is similar to that reported for rice leaf surfaces (*10*).

To determine when *M. grisea* first attaches to a surface, we counted the number of germinating conidia that could be flushed from Teflon-PFA film by pipetting (*7*). Approximately 90% of the conidia are resistant to removal from the surface 20 minutes after deposition (Fig. 1b). From this result we conclude that conidia can adhere to a surface prior to germ tube emergence. The addition of concanavalin A (Con A) antagonizes this early adhesion (Fig. 1b) but does not interfere with germ tube emergence.

To examine conidial adhesion directly we used a flow cell that permits the observation and video recording of conidia on a variety of surfaces under the influence of increasing hydraulic shear generated by a pump (*11*). The results of an experiment performed with conidia germinating on glass or Teflon-PFA film are shown in Fig. 1c. Conidia attached to Teflon-PFA film are able to resist higher flow rates than conidia attached to glass. These results demonstrate that conidial attachment occurs prior to germ tube formation and suggest that this attachment is substantially stronger to Teflon-PFA film, a surface conducive to appressorium formation.

We observed the response of attached conidia to the flow in the chamber (Fig. 1d). When the pump is off, conidia are oriented randomly with their apexes tethered to the surface. When the pump is turned on, hydraulic force aligns the conidia with their apexes opposing the direction of flow.

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Many conidia reverse their orientation if the direction of flow is reversed. We speculate that conidial shape and apical attachment are hydrodynamically favorable for resisting the flow of water.

Observation of freshly harvested conidia by phase-contrast light microscopy revealed a faint image of a substance at the conidial apex. Because Con A antagonizes conidial adhesion, we labeled conidia in suspension with fluorescein isothiocyanate (FITC)–conjugated Con A (Fig. 2, a and b). We named the substance at the conidial apex that binds Con A spore tip mucilage (STM). STM release occurs rapidly and independently from contact with a surface after spores are hydrated. We propose that the release of STM is the mechanism by which the conidia of the rice blast fungus attach to a surface. We infer that the same mechanism is used by the fungus to attach to a rice leaf surface prior to germ tube emergence and infection structure formation.

STM release does not appear to require protein synthesis or respiration (*12*) and thus STM may be stored in dormant conidia. Thin sections of hydrated or unhydrated conidia prepared by freeze substitution (*13*) were examined by transmission electron microscopy. Unhydrated dormant conidia contain a prominent deposit of material in the periplasmic space at the conidial apex (Fig. 2c). Electron micrographs of hydrated spores show the presence of STM as a wispy material that appears to have been released through rupture of the outer spore wall (Fig. 2d). These observations suggest that STM is stored in a compact, unhydrated form in the periplasmic space of dormant conidia.

We have demonstrated that conidia of the rice blast pathogen *M. grisea* have a mechanism for surface attachment. Moist air, rain, or dew would cause the hydration and rapid extrusion of stored STM which could then attach conidia to hydrophobic plant surfaces. This attachment mechanism may provide the fungus with several important advantages. It allows conidia to attach rapidly without the expenditure of metabolic energy. Apical attachment of the pyriform conidia allows them to resist the flow of water. STM release occurs in the presence of water which is required for subsequent events in infection (*5*).

A mechanism for conidial attachment has not been demonstrated for any other phytopathogenic fungus. There have been several reports of a sheath surrounding germ tubes of other phytopathogenic fungi (*14–17*). Experiments similar to the ones we have described should determine whether or not these fungi or others (*18, 19*) have the same attachment mechanism. Like many other

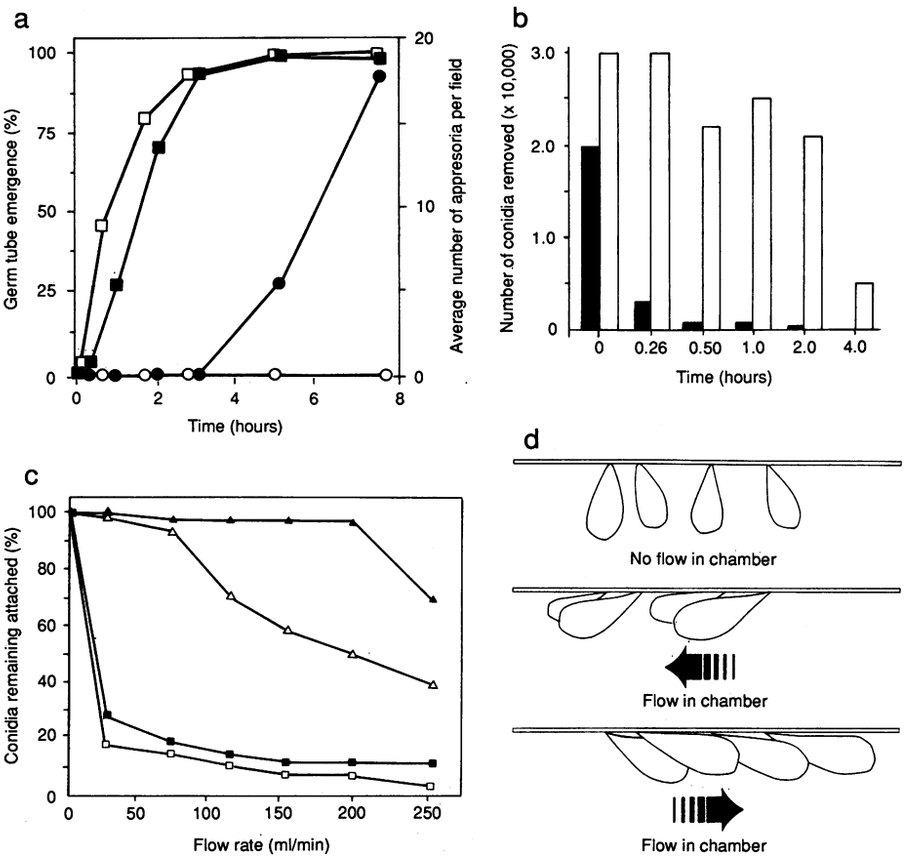

Fig. 1. Germination, appressorium formation (a) and early adhesion (b, c, and d) of *M. grisea* conidia. (**a**) Germ tube emergence on glass (□) or on Teflon-PFA film (■); appressorium formation on glass (○) or on Teflon-PFA film (●). After 24 hours of incubation, conidia germinated on glass formed an average of five appressoria per microscopic field. Conidia germinated on Teflon-PFA film formed an average of 217 appressoria per field. In the majority of cases one conidium differentiates to produce one appressorium. Germination experiments have been performed numerous times with the same results. The results of a typical experiment are shown. (**b**) The attachment of conidia to a Teflon-PFA surface in the presence of 10 m*M* phosphate buffer (solid bars) or in buffer containing Con A (1 mg/ml, Sigma) (open bars). The presence of the lectin Con A antagonizes early conidial adhesion, presumably by binding to STM. Experiments repeated twice on Teflon films and once on plastic cover slips gave similar results. Results from a single experiment with Teflon-PFA film are shown. A Con A concentration of 100 μg/ml was also effective at blocking spore attachment to Teflon film. (**c**) Spores deposited on Teflon-PFA film for either 35 minutes (△) or 75 minutes (▲) can resist higher flow rates than spores deposited on a glass surface for either 17 minutes (□) or 60 minutes (■) (*11*). Preliminary experiments with the flow chamber always showed conidia attaching preferentially to Teflon. Two complete experiments performed on different days gave the same result, although actual spore counts varied among the experiments. The results from one of these experiments is shown. (**d**) A diagrammatic representation of the response of attached conidia to shear force is shown here and described in the text.

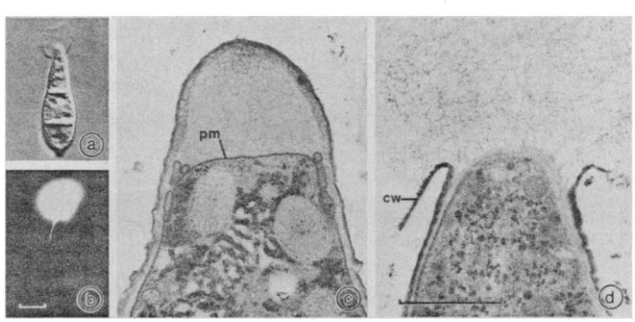

Fig. 2. Light (a and b) and electron (c and d) micrographs of *M. grisea* conidia before (c) and after (a, b, and d) hydration. (**a** and **b**) The same FITC-Con A–labeled conidium imaged with differential interference contrast optics and epi-fluorescence, respectively; scale bar, 5 μm. (**c**) A dry conidium apex contains a deposit outside the plasma membrane (pm). (**d**) When hydrated, the cell wall (cw) is broken at the apex and an extracellular fibrous matrix is released; scale bar, 1 μm. For light microscopy conidia were harvested in an aqueous suspension of FITC-Con A (1 μg/ml; Sigma) and viewed immediately. Electron microscope specimens were prepared by freeze substitution (*13*).

plant pathogenic fungi, *M. grisea* produces a mucilage around the growing germ tube and appressorium (*20, 21*). Conidial attachment may thus represent the first step in a multicomponent attachment process.

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7. *Magnaporthe grisea*, strains 4091-5-8 and 0-42 (*2*), was grown and maintained as previously described (*2*). Conidia were harvested in 0.25% gelatin solution by gentle scraping of plate cultures, washed once by centrifugation at 2000*g*, suspended in 10 m*M* phosphate buffer, *p*H 6.5, and deposited in 100-μl portions containing 50,000 conidia on 2 by 2 cm squares of 10-mil Teflon-PFA film or on clean glass microscope cover slips and incubated at room temperature. The extent of conidial germination was determined by inverting spore suspensions onto a microscope slide and observing them at ×200 by phase-contrast light microscopy. The percentage of conidia with germ tubes was determined by counting the first 200 spores and recording the number of conidia with germ tubes. The average number of appressoria per field was determined for five microscopic fields observed at ×200. The extent of conidial attachment was determined by removing the 100-μl droplet of conidia from the Teflon-PFA surface at indicated times by pipetting. A further 100-μl droplet of sterile 0.25% gelatin solution was used to wash off any unattached conidia. The spores that had been removed by these two pipettings were combined, diluted, and plated in duplicate in complete medium. Colonies were counted after 1 week.
8. Teflon-PFA is tetrafluoroethylene copolymerized with perfluoroalkoxy pendant groups. Teflon polymers are chemically inert, have negligible moisture absorption, and are known for their nonstick characteristics. As thin films, these polymers have excellent optical clarity. Further details can be found in *Encyclopedia of Polymer Science and Technology* (Wiley, New York, 1976), vol. 1 (suppl.), pp. 260–267.
9. Rice leaf surfaces are covered by waxes and are resistant to wetting [S. Y. Zee, *Wheat and Rice Plants, a Scanning Electron Micrograph Survey* (Cosmos Books, Hong Kong, 1981), p. 58]. In this way they resemble the hydrophobic polymer surfaces that elicit appressorium formation.
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11. Conidia, grown and harvested as described above (*7*), were resuspended at 5×10^5 conidia per milliliter in sterile distilled water and were allowed to settle over the surface of glass microscope slides or similar sized strips of 10-mil Teflon-PFA film. The surfaces with attached conidia were placed inside a flow chamber (Du Pont Engineering Technology Laboratory) equipped with a port for microscopy, and the chamber was attached to a centrifugal pump and flowmeter. Hydraulic shear was generated by increasing the flow of water through the opening in the chamber (cross-sectional area, 0.8 by 25 mm). Conidia in the chamber were viewed by light microscopy, and the response of conidia to increased flow rates was recorded on video tape. The percentage of conidia remaining attached after incremental increases in flow rate was determined from the tape recordings.
12. Dilute conidial suspensions were prepared by gently harvesting conidia from culture dishes in 10 m*M* phosphate buffer, *p*H 6.5, containing either sodium azide (2 m*M*) or cycloheximide (300 μg/ml). A control suspension was prepared with phosphate buffer alone. Conidial suspensions were deposited in 100-μl portions onto plastic cover slips and allowed to incubate at 23°C for 20 minutes, labeled with Con A, and examined microscopically. All three conidial suspensions contained a majority (≥86%) of conidia with STM (Fig. 2a). After 2.5 hours of incubation, microscopic examination of control conidial suspensions showed uniform germ tube emergence. Suspensions containing sodium azide or cycloheximide did not produce germ tubes after 2.5 hours.
13. Electron microscope specimens were processed on squares of cellulose membrane substratum. For dry conidia, squares of dry substratum were gently wiped across the sporulating mycelium. For wet conidia, 20-μl droplets of conidial suspension (*11*) were held aseptically on hydrated substratum for 10 minutes. Specimens were frozen in liquid propane, substituted in 2% osmium tetroxide and 0.1% uranyl acetate in acetone, embedded in Quetol epoxy resin, and thin-sectioned [R. J. Howard and K. L. O'Donnell, *Exp. Mycol.* **11**, 250 (1987)].
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Perivascular Microglial Cells of the CNS Are Bone Marrow–Derived and Present Antigen in Vivo

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A crucial question in the study of immunological reactions in the central nervous system (CNS) concerns the identity of the parenchymal cells that function as the antigen-presenting cells in that organ. Rat bone marrow chimeras and encephalitogenic, major histocompatability–restricted T-helper lymphocytes were used to show that a subset of endogenous CNS cells, commonly termed "perivascular microglial cells," is bone marrow–derived. In addition, these perivascular cells are fully competent to present antigen to lymphocytes in an appropriately restricted manner. These findings are important for bone marrow transplantation and for neuroimmunological diseases such as multiple sclerosis.

THE CENTRAL NERVOUS SYSTEM (CNS) of mammals has long been considered an immunologically privileged site (*1*). In healthy animals the CNS is virtually devoid of lymphocytes. The blood-brain barrier also excludes immunoglobulin, and molecules of the major histocompatibility complex (MHC) necessary for restricted antigen recognition are nearly undetectable in the CNS (*2*). However, during immunopathological processes that involve the CNS, this organ must allow the entry of immunospecific effector cells and permit antigen recognition by T cells in an MHC-restricted fashion. In multiple sclerosis, there is a malfunction of mechanisms that ordinarily protect the CNS from immune attack, and an autoimmune disease results. One of the persistent questions central to our understanding of this disease, and CNS immunology in general, concerns the identity of the cell or cells in the nervous system that can present antigen to T cells. Experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis, has been extensively studied in an attempt to answer this question, but to date no specific cell type has been functionally identified in vivo as an endogenous CNS antigen-presenting cell (APC).

Three primary candidates for the role of APC in the nervous system are endothelial, astrocytic, and microglial cells. In vitro studies have shown that both the astrocyte and the endothelial cell can express MHC-encoded molecules on their cell surfaces and present CNS-related antigens to T lymphocytes in an MHC-restricted manner (*3*). Nevertheless, the possibility remains that these cells in vitro may be exhibiting secondary functions not normally performed in vivo. In both multiple sclerosis and experimental allergic encephalomyelitis (EAE), immunohistochemical studies have shown that class I and class II MHC antigens are exhibited by both endothelial cells and microglial cells (*4, 5*). Astrocytes appear to

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