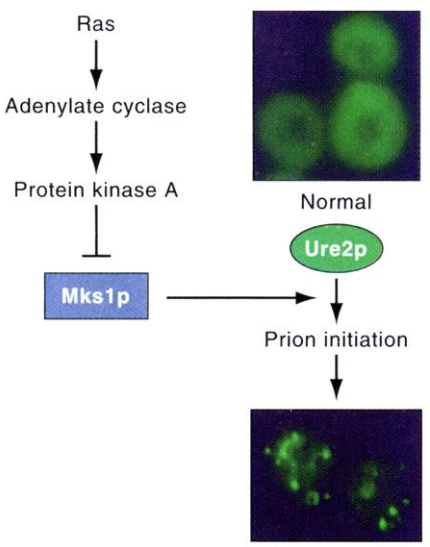
MEETING AMERICAN SOCIETY FOR MICROBIOLOGY

Microbes Display Their Versatility at ASM Meeting

LOS ANGELES—About 12,000 scientists gathered here from 21 to 25 May for the 100th annual meeting of the American Society for Microbiology (ASM). This year's lineup boasted presentations on a wide array of topics—everything from the body's defenses against microbial pathogens to bacterial involvement in geological processes.

Triggering Prion Formation in Yeast

Although tainted beef can cause mad cow disease and inherited mutations can lead to similar forms of human brain degeneration, neither factor triggers most cases of these conditions, known as transmissible spongiform encephalopathies (TSEs). The diseases, named for the spongy appearance of the patients' brains, are apparently caused by the abnormal deposition of infectious proteins, or prions. Most often, though, prions arise spontaneously in people who haven't been exposed to contaminated meat and don't carry an inherited mutation in the suspect gene. Researchers now have a new clue about what spurs the formation of these abnormal proteins—at least in yeast, which can also develop prionlike deposits.



Getting started. Under the influence of the protein Mks1p, Ure2p forms insoluble prion deposits (bright green spots). Ras pathway activity can block Mks1p function, however.

At the meeting, Reed Wickner, a yeast geneticist at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland, reported that a yeast gene called *Mks1p* is required for the generation of these misbehaving proteins. (The results also appear in the 6 June issue of the *Proceedings of the National Academy of Sciences*.) It's unlikely that the same protein sparks mammalian prion deposition, says Byron Caughey, a TSE biochemist at Rocky Mountain Laboratories in Hamilton, Montana. But, he adds, the new result "encourages us to look for a similar sort of player that might be related to TSE disease." Finding such a player might eventually lead to new therapies for inhibiting prion diseases.

Although nonprion proteins have long been thought to affect prion biology, only very recently have researchers started to pin them down, and only in yeast. Wickner and postdoc Herman Edskes have been studying a yeast protein called Ure2p. In its normal form, Ure2p is involved in nitrogen metabolism. But like mammalian prion proteins, Ure2p can clump into insoluble fibers that prevent it from performing its normal activities, and the researchers exploited this phenomenon to measure prion formation. Cells in which Ure2p is in the prion form can import a compound, ureidosuccinate (USA), that is brought in by machinery whose production would be blocked by normal Ure2p.

Searching for a trigger that initiates Ure2p prion formation, Wickner and Edskes decided to test Mks1p, a protein known to control Ure2p activity. Using the USA test, they found that Ure2p prion formation is undetectable in cells lacking Mks1p. But the incidence of prion-forming cells rose to above normal when the researchers genetically engineered yeast to make slightly more than the normal amounts of Mks1p. Together, these results show that the protein is required for the spontaneous generation of the Ure2p prion, says Wickner.

"This gene is the first one required for forming the prion seed," says Susan Liebman, who studies yeast prions at the University of Illinois, Chicago. It may not be the last, however. Liebman's team has evidence for an activity that is required for generating another yeast prion, but has not yet identified the gene responsible for it.

Once the first Ure2p prion forms, however, Mks1p is apparently not required for spreading prions to unrelated cells and to offspring. Edskes and Wickner transferred cytoplasm from cells that were carrying the Ure2p prion into yeast strains that either could or could not make Mks1p. After multiple generations—by which time any Mks1p from the original donor strain would be diluted to nothing—cells lacking Mks1p were still producing prions. The result indicates that Mks1p is not needed for prion propagation.

These experiments suggest that prion initiation and propagation in yeast are controlled by different genes. "The same distinction might apply to mammalian systems, but it hasn't been possible to approach the question experimentally," says David Harris, a cell biologist at Washington University School of Medicine in St. Louis. Exactly how Mks1p fosters Ure2p prion formation remains unclear. But the discovery also provides an intriguing link to one of the cell's major growth control pathways, named the Ras pathway after one of its prominent components.

Ras activity leads to the inactivation of Mks1p, and Wickner and Edskes showed that a form of Ras that's stuck on "on" decreased the incidence of prion formation more than 750-fold in yeast. This result suggests, says Wickner, that "the general control systems of the cell are impinging on the prion system." If so, Liebman says, "there is going to be a complicated control of prion generation."

No one knows if something similar occurs in humans, but if it does, Liebman adds, "maybe it will be possible to engineer those controls in our favor, once we understand what they are."

Pumping Iron?

Few cells can outdo the macrophage in sheer destructive potential. These immune cells literally eat microbial invaders for breakfast, first engulfing and encasing them in membranous intracellular sacs called phagosomes and then unleashing a host of weapons that tear the microbes apart. Most microbes are goners if a macrophage engulfs them, but some—including those that cause leprosy and leishmaniasis—have developed strategies for surviving in the phagosome's noxious environment. For example, they prevent the phagosome from picking up enzymes and other harmful chemicals that would otherwise destroy them. In an evolutionary arms race, however, the macrophage has evolved countermeasures to keep even these microbes in check. Now, new work reveals the mechanism of one of the weapons the cells deploy in this war of attrition.

Decades ago, researchers identified a

CREDIT: R. WICKNER AND H. EDSKES

mouse gene called *Nramp1* that confers resistance to some phagosome-dwelling microbes, and although they isolated it several years ago, they couldn't pin down its exact function. At the ASM meeting, Philippe Gros, a mouse geneticist at McGill University in Montreal, described results suggesting that the Nramp1 protein contributes to the demise of the microbes by pumping metal ions such as manganese out of the phagosome, depriving them of essential nutrients. "It's been recognized for a long time that certain genetic loci have a major role in nonspecific immunity, but their actions have been pretty mysterious," says Ferric Fang, a molecular microbiologist at the University of Colorado Health Sciences Center in Denver. "This is a direct demonstration of the molecular mechanism by which a fundamental system of innate immunity is working."

Researchers had suspected that Nramp1 might pump metals ions out of the phagosome. It's in the right location, dwelling in the membrane that surrounds the phagosome. And Nramp1's amino acid sequence resembles that of other proteins known to transport metals such as manganese and iron. But they had trouble proving what Nramp1 does, partly because they had no way to measure the flow of molecules across the phagosome membrane. Gros and his colleagues, with Sergio Grinstein at the University of Toronto, solved this problem.

First, the researchers allowed macrophages from normal and Nramp1-deficient mice to engulf tiny beads coated with Fura-6, a chemical that fluoresces orange except when manganese ions are present. After introducing the metal into the cells, they found that the fluorescence declined much more quickly in the Nramp1-deficient phagosomes than in normal ones, suggesting that the protein either keeps manganese from entering the phagosomes or removes it from them.

To distinguish between these possibilities, the researchers loaded the two types of macrophages with Fura-6–coated beads whose fluorescence had already been blocked by manganese, and then measured how long it took for fluorescence to reappear. The phagosomes of cells containing normal Nramp1 glowed more quickly and to a greater extent than did those of Nramp1-deficient cells. Together, the experiments showed that Nramp1 ejects manganese from the phagosome. "It's never been demonstrated so convincingly that a host mechanism for getting rid of pathogens is nutritional deprivation in the phagosome," says Samuel Miller, a microbiologist at the University of Washington, Seattle. "This work shows that pumping ions out of the phagosome is important."

Manganese may not be the whole story, however. Gros suspects that Nramp1 may also transport iron, which, like manganese, is essential for microbial growth. Indeed, several bacteria, including *Mycobacterium tuberculosis*, carry genes that resemble *Nramp1*, and experts propose that the two cousin molecules might even fight it out for essential cations in the phagosome.

In addition to identifying Nramp1's natural substrate or substrates, researchers now want to determine whether Nramp1 plays a role in nonspecific immunity in humans, as it does in mice. Some results hint that it might, as several studies on populations in Africa and Vietnam have shown an association between the region of the genome that carries the *Nramp1* gene and susceptibility to leprosy and tuberculosis.

If Nramp1 does in fact contribute to host resistance to the human diseases, it might be possible to design drugs that combat the infections by helping Nramp1 keep metal ions out of the phagosome. After all, even good fighters can use better weapons.

Wringing Nutrition From Rocks

Microbes can squeeze just about anything from a stone—an ability that helps us as well as them. At the meeting, Jill Banfield, a mineralogist at the University of Wisconsin, Madison, presented evidence that microbes live on phosphate-containing crystals on rock surfaces, apparently by dissolving the mineral to obtain phosphate. The work, which reflects a growing interplay of geology and microbiology, might help explain how the tiny rock inhabitants get their phosphate, an essential nutrient for life. It could also lead to new insights about how soil fertility is established and maintained.

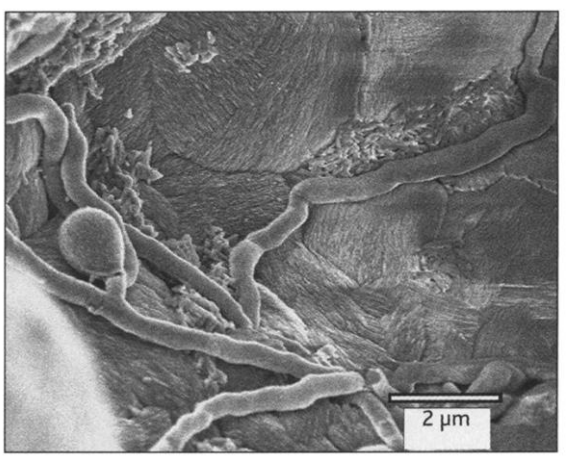
Because phosphate is locked up in insoluble minerals, its availability often limits the growth of living things in soil. And although scientists know that microbes play a role in releasing it and other essential chemicals, the extent of their involvement has been unclear. "Most geologists have focused on inorganic processes until recently," says Susan Brantley, a geochemist at Pennsylvania State University, University Park. "There's a huge deficit in our knowledge of [microbial] roles in element cycling."

To help fill that gap, Banfield and graduate student Anne Taunton made scanning electron micrographs of rock fragments from samples in the top 2 meters of the soil, where microbes are most plentiful. The researchers found that microbes are not evenly distributed. Instead, they cling to tiny spots of phosphate-containing minerals called lanthanide phosphates and don't seem to colonize other parts of rock fragments.

Banfield and Taunton also noted that the crystals remain intact at lower depths of soil, where microbes are sparse, but as one approaches the surface, microbe-occupied pits appear in the rock fragments as the phosphate is apparently dissolved away. Eventually the microbes disappear, too, leaving only empty holes. "The hypothesis is that microbes are inhabiting the pits and removing phosphate," Banfield says.

Banfield suggests that they dissolve the phosphate by releasing chemicals such as oxalate and carbonate. She and Taunton found that microbes cultured from soil samples release the chemicals and also showed that addition of oxalate and carbonate to lanthanide phosphate in solution increases the solubility of the phosphate. "One of the really important take-home messages is the effect of these low–molecular weight organic molecules that microbes make. They increase the rate of dissolution of these mineral phases," says Jim Fredrickson, a soil microbiologist at the Pacific Northwest National Laboratory in Richland, Washington.

Banfield and her colleagues are now trying to identify the organisms present in the soil. But while the extent and mechanism of microbial involvement in geological processes remains unclear, researchers are pleased with the growing interdisciplinary nature of the field. "What I find exciting is that a card-carrying geologist is in charge of this work," says Kenneth Nealson, an environmental microbiologist at the California Institute of Technology in Pasadena. "The field has never moved at the rate it should move, because it's never been populated by people who have a background in both areas. Now it's picking up." —EVELYN STRAUSS



In the pits. This micrograph shows fungi growing on aluminum phosphate in a rock fragment from soil.

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