from the coagulation bath (see the figure).

Our efforts to use the carbon nanotube fibers as artificial muscles provided another surprise (*14*). All fibers made by the Vigolo *et al.* (*2*) process swell in diameter by up to ~200% when immersed in water or an aqueous electrolyte, resulting in markedly decreased modulus and strength. Interbundle stress transfer within the fiber is apparently facilitated by residual poly(vinyl alcohol) from the spinning bath. Immersion in an aqueous solution transforms the poly(vinyl alcohol) into a gel with little binding capability, causing the fibers to partially expand. The fiber swelling remains above 50% even if the precursor ribbon-shaped nanotube mesh is washed in water for several days, apparently because the poly(vinyl alcohol) is strongly bound to the nanotubes. Fortunately, the swelling and degradation in aqueous liquids can be avoided by simply annealing the as-spun fibers at temperatures as low as 400°C for 1 hour while maintaining a vacuum (*14*). Thereafter, the carbon nanotube fibers provided actuator performance exceeding that reported for carbon nanotube sheets (*7*).

The brilliant work of Vigolo *et al.* (*2*) provides the first spinning process that can be modified to provide fibers comprising mostly single-wall carbon nanotubes. I am confident that by building upon their discoveries, it will be possible to devise an economically viable process for spinning strong nanotube fibers. However, at the current price of purified single-wall carbon nanotubes (~$1000/g), single-wall carbon nanotube fibers are only attractive for devices requiring little material. The HiPco nanotubes, which will become commercially available in small quantities this fall, may eventually remove this cost barrier. These tubes perform particularly well in the Vigolo *et al.* process (*2*), require no purification, and are believed to be scalable to economic volume production at a much lower price than present methods.

Once economic production of inexpensive high-quality single-wall nanotubes can be realized, large devices and strong composites may become commercially viable. Until then, the universe of nanotube applications will be largely restricted to the smaller (but no less interesting) world of nanoscale devices and nanothickness coatings.

References and Notes

1. *The Man in the White Suit* is a 1951 movie starring Alec Guinness (Ealing Studios).
2. B. Vigolo *et al.*, *Science* **290**, 1331 (2000).
3. G. Gao, T. Çagin, W. A. Goddard, *Nanotechnology* **9**, 184 (1998).
4. D. A. Walthers, *Appl. Phys. Lett.* **74**, 3803 (1999).
5. B. I. Yakobson, R. E. Smalley, *Am. Sci.* **85**, 324 (1997).
6. C. Niu *et al.*, *Appl. Phys. Lett.* **70**, 1480 (1997).
7. R. H. Baughman *et al.*, *Science* **284**, 1340 (1999).
8. M. S. Dresselhaus, K. A. Williams, P. C. Eklund, *MRS Bull.* **24** (no. 11), 45 (1999).
9. Conversion of mechanical energy to electrical energy uses the inverse of the electrochemical actuator effect (*7*).
10. See the Defense Sciences Office web site, www.darpa.mil/dso, under Programs/Synthetic Multifunctional Materials.
11. R. Haggenmueller, H. H. Gommans, A. G. Rinzler, J. E. Fischer, K. I. Winey, *Chem. Phys. Lett.*, in press.
12. H. H. Gommans *et al.*, *J. Appl. Phys.* **88**, 2509 (2000).
13. A. Rinzler *et al.*, *Appl. Phys. A* **67**, 29 (1998).
14. A. Lobovsky *et al.*, unpublished data.
15. P. Nikolaev *et al.*, *Chem Phys. Lett.* **313**, 91 (1999).
16. I thank the authors of (*2*) for describing their spinning process to me; A. A. Zakhidov and R. E. Smalley for useful comments; and R. E. Smalley, M. Bronikowski, P. Willis, D. Colbert, and K. Smith for the gift of HiPco nanotubes used in collaborative research. Supported by DARPA grant MDA972-00-C-0032.

PERSPECTIVES: MICROBIOLOGY

Turning Up the Heat on *Histoplasma capsulatum*

Bruce S. Klein

At least 70,000 fungal species inhabit our planet, yet remarkably few of them cause disease in humans. This happy coexistence may be set to change, however, as opportunistic fungal species (such as *Candida albicans* and *Aspergillus fumigatus*) and pathogenic fungi (such as *Histoplasma capsulatum*) take advantage of patients who are immunosuppressed either because of treatment with toxic cancer drugs or because of a primary infection with, for example, human immunodeficiency virus. The AIDS epidemic itself has changed the epidemiology of fungal diseases (*1*) with a dramatic increase in the number of opportunistic fungal infections—for example, the incidence of cryptococcal meningitis caused by the fungus *Cryptococcus neoformans* has increased 1000-fold in New York City alone.

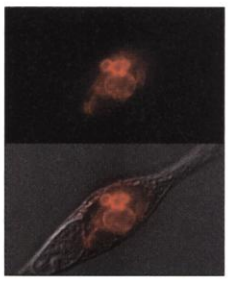
Dimorphic fungi—the silent majority of pathogenic fungal species—exist as either a free-living mycelial (mold) form in soil or as a parasitic yeast form that inhabits its cells such as macrophages within a mammalian host. The mold forms are saprophytes that absorb nutrients from dead organic matter in the soil and produce infectious spores. When inhaled by mammals, these spores are induced to undergo a morphogenetic transformation into the yeast form by the warmer temperature of the mammalian respiratory tract. Although this morphogenetic switch is essential for the dimorphic fungus to become parasitic, relatively little is known about the identity and regulation of the virulence genes that direct this transition (*2, 3*). With a cunning combination of molecular genetics techniques, Sebghati *et al.* (*4*) identify a crucial virulence gene encoding a calcium binding protein (CBP1) in the systemic dimorphic fungus *H. capsulatum*. As they report on page 1368 of this issue, disruption of the *CBP1* gene resulted in decreased survival of *H. capsulatum* yeast in cultured macrophages (the host cells that they normally parasitize) and in a mouse model of respiratory infection.

Histoplasmosis, the respiratory disease caused by *H. capsulatum*, is found worldwide and is endemic in the Ohio and Mississippi River Valley of the United States where most people are infected by age 20. The majority of infections are mild, but the illness progresses further in up to 10% of infected individuals producing life-threatening symptoms, such as inflammation of the membranes covering the heart (pericarditis) and fibrosis of major blood vessels. The seriousness of histoplasmosis grabbed public attention when noted songwriter and performer Bob Dylan was hospitalized with *H. capsulatum*–induced pericarditis several years ago. This systemic dimorphic fungus establishes a latent (silent) infection that can be reactivated (as happens in up to 40% of AIDS patients), necessitating long-term treatment.

In addition to its clinical importance, several other features make *H. capsulatum* an appealing target for basic investigation. The fact that the yeast form is haploid (it has a single set of chromosomes) permits recessive traits to be readily unearthed because only one copy (not two) of a target gene needs to be inactivated to see an effect. The life-style of *H. capsulatum* yeast parasites inside macrophages offers clues to secrets about the life-styles of other intracellular parasites. Upon infecting a mammalian host, *H. capsulatum* spores become transformed into yeast and are phagocytosed (engulfed) by macrophages and other cells of the reticuloendothelial system (see the figure). The yeast forms reside within the macrophage cytoplasm in phagosomes (phagocytic vesicles surrounded by host cell membranes) or in phagolysosomes (organelles formed by the fusion of phagosomes with lysosomes). Infection of macrophages by *H. capsulatum*

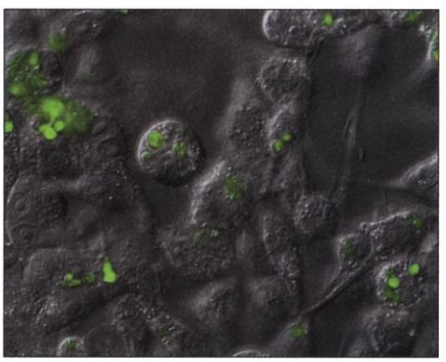
The author is in the Department of Pediatrics, University of Wisconsin–Madison, Madison, WI 53792, USA. E-mail: bsklein@facstaff.wisc.edu

yeast activates the release of powerful cytokines by antigen-specific T lymphocytes (*5*). In response to these cytokines, host macrophages are able to block the multiplication of yeast parasites by sequestering iron (which the parasites need to survive), by activating oxygen-dependent pathways of parasite destruction, and by promoting fusion of phagosomes with lysosomes containing proteolytic enzymes (*6*). But *Histoplasma* yeast are not to be so easily defeated—they possess their own defenses that enable them to persist (often in a latent state) for long periods of time within macrophage phagosomes. Their defense machinery interferes with the fusion of phagosomes and lysosomes, and blocks acidification of phagolysosomes, a step that may be necessary for activation of lysosomal enzymes. Furthermore, the yeast parasites release copious amounts of CBP1 into the calcium-poor environment of macrophage phagosomes—this protein mops up free calcium ions, delivering them to the yeast, which are then able to survive under calcium-limiting conditions.



Identification of virulence genes in systemic dimorphic fungi is possible with classical genetics approaches in which genes are exchanged through mating of mutants. However, there are drawbacks including the health hazard of handling molds and, most important, the fact that the sexual stages of dimorphic fungi are lost during laboratory culture. Hence, investigators have turned to molecular genetics (*7*)—through the recombinant techniques of genetic transformation and gene replacement, a target gene can be inactivated by replacing it with an engineered DNA plasmid containing a selection marker. Unfortunately, a principal obstacle to the success of this strategy is that the plasmid DNA either randomly integrates into the fungal genome (thereby inactivating genes other than the target gene) or is treated as foreign DNA by the fungus, which adds tandem repeat DNA sequences (telomeres) to the plasmid so that it cannot integrate into the genome at all. Generation of telomeric linear plasmids in the laboratory can be an advantage if one wants to construct extrachromosomal (episomal) vectors or to overexpress genes (*8*), but it continues to confound attempts to inactivate target genes in dimorphic fungi. Despite these problems, there have been a few successes (*9*) such as the disruption of the *URA5* gene in *H. capsulatum* yeast, although the frequency of mutants obtained in that study was very low (1 in 1000) (*10*).

Enter Sebghati and co-workers, who combine two low-efficiency processes (genetic transformation and gene replacement) to inactivate the *CBP1* gene of *H. capsulatum* with high efficiency. Their strategy adds a clever twist to the approach previously applied to *Cryptococcus neoformans* to disrupt a capsule-synthesis gene in which mutants were first positively selected for growth in medium without adenine and then were negatively selected by killing off wild-type cells with the toxic pyrimidine analog, 5-fluoro-orotic acid (*11*). Sebghati *et al.* engineered a telomere linear plasmid that contained two selection marker genes—one gene (which disrupts *CBP1* if it integrates properly with the fungal DNA) encoded a protein conferring resistance to the antibiotic hygromycin B; the other gene, *URA5*, enabled yeast to grow without uracil. Initially, growth in the absence of uracil enabled the selection of mutant yeast that contained the plasmid in an extrachromosomal location; these mutants were protected from illegitimate recombination of the plasmid with the fungal genome by the telomeres on the end of the plasmid. Subsequently, yeast were grown in medium containing hygromycin B to select for mutants with a correctly integrated plasmid; to ensure that the telomeric ends of the plasmid had been removed in these mutants and that the plasmid did not integrate illegitimately with retained *URA5* sequences, they were grown in medium containing 5-fluoro-orotic acid, which killed off any yeast that still had the *URA5* gene. With this strategy, the investigators determined that the frequency of yeast mutants with an inactivated *CBP1* gene was 33%, demonstrating that gene disruption by homologous recombination is efficient in *H. capsulatum*. Hopefully, this approach will enable disruption of other *Histoplasma* virulence genes and of target genes in other dimorphic fungi. And on the way, we will learn how much flanking DNA and sequence homology with the target gene are needed for even greater gene targeting efficiency.



Insidious invaders. The parasitic yeast forms of the fungal pathogen *H. capsulatum* inside the phagosomes of cultured host macrophages. (**Bottom**) Green fluorescence indicates yeast cells that are expressing the gene encoding CBP1, which captures calcium ions for the yeast, allowing them to live in the calcium-poor conditions of phagosomes. (**Top**) Red fluorescence indicates the location of secreted CBP1 protein within the phagosomes of infected macrophages. [Reprinted from (*13*) with permission]

With their yeast mutant in hand, Sebghati *et al.* then demonstrated that CBP1 scavenges calcium ions in the macrophage phagosomes and makes them available to the *H. capsulatum* yeast parasites, promoting their survival. The mold form of *Histoplasma* in soil does not make CBP1 and therefore cannot survive in a low-calcium environment. These findings complement earlier work with *Salmonella*, which showed that a protein for sensing and binding calcium ions was necessary for the survival of this pathogenic bacterium within host cell phagolysosomes (*12*). Sebghati and co-workers postulate that by scavenging calcium ions CBP1 impairs the acidification of phagolysosomes, thus inhibiting lysosomal enzymes that require a low pH for activity. If this hypothesis is correct, then CBP1 would connect two key cellular events—the regulation of pH and calcium ion concentration—making it clear why CBP1 is so crucial to intracellular survival of *Histoplasma* yeast.

Genetic tools are now available to force *H. capsulatum* to yield its secrets and to bring the virulence genes of this pathogenic fungus into clearer focus. With arrays of random clones of *H. capsulatum* genomic DNA now ready for analysis, new virulence genes will soon be identified. This endeavor should be aided by the sequencing of the *H. capsulatum* genome.

References and Notes

1. B. P. Currie, A. Casadevall, *Clin. Infect. Dis.* **19**, 1029 (1994); A. Casadevall, *Curr. Opin. Microbiol.* **3**, 337 (2000); S. M. Levitz, *Res. Immunol.* **149**, 489 (1998).
2. B. Maresca, G. S. Kobayashi, *Microbiol. Rev.* **53**, 186, (1989); E. J. Keath *et al.*, *Infect. Immun.* **57**,1384 (1989).
3. G. Medoff *et al.*, *Science* **231**, 476 (1986).
4. T. S. Sebghati, J. T. Engle, W. E. Goldman, *Science* **290**, 1368 (2000); J. W. Batanghari *et al.*, *Mol. Microbiol.* **27**, 531 (1998).
5. G. S. Deepe Jr., *Curr. Opin. Microbiol* **3**, 359 (2000); G. S. Deepe Jr., R. A. Seder, *Res. Immunol.* **149**, 397 (1998).
6. S. L. Newman, *Trends Microbiol.* **7**, 67 (1999); D. M. Retallack, J. P. Woods, *Microbes Infect.* **1**, 817 (1999).
7. D. M. Retallack *et al.*, *Microb. Pathog.* **28**, 169 (2000); S. Colonna-Romano *et al.*, *Microb. Pathog.* **25**, 55 (1998).
8. J. P. Woods, W. E. Goldman, *Mol. Microbiol.* **6**, 3603 (1992); J. P. Woods, W. E. Goldman, *J. Bacteriol.* **175**, 636 (1993); K. L. Fisher *et al.*, *Infect. Immun.* **67**, 3312 (1999).
9. T. T. Brandhorst *et al.*, *J. Exp. Med.* **189**, 1207 (1999); U. Reichard *et al.*, *Infect. Immun.* **68**, 5830 (2000).
10. J. P. Woods *et al.*, *J. Bacteriol.* **180**, 5135 (1998).
11. Y. C. Chang, K. J. Kwon-Chung, *Mol. Cell. Biol.* **14**, 4912 (1994).
12. E. Garcia Vescovi *et al.*, *J. Biol. Chem.* **272**, 1440 (1997).
13. S. Kugler *et al.*, *Cell. Microbiol.* **2**, 549 (2000).

14. I thank G. Deepe, J. Woods, and members of my laboratory for helpful comments.

CREDIT: BILL GOLDMAN