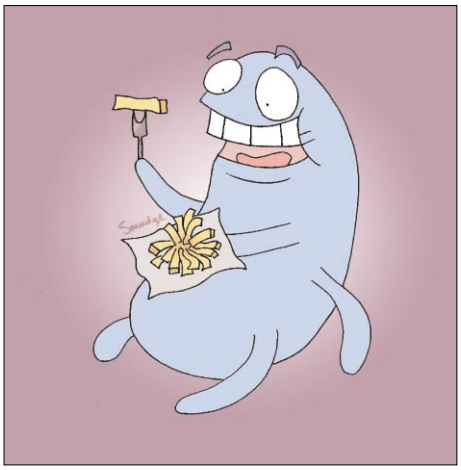
produced by microalgae in the University of Hawaii's strain collection, with the University receiving royalties for potential new drugs. Researchers have already isolated two anti-cancer candidates. Past efforts to harvest useful amounts of compounds from microalgae were thwarted because the microscopic algae were difficult to culture in large quantities. However, using massive, computer-controlled photobioreactors, Aquasearch grows microalgae on an enormous scale. *AV*
http://www.aqse.com/astax-3.htm

Chips with everything?



Researchers from Hoffman-La Roche have been using 'bacteria chips' to study the action of two currently popular antibiotics and their results suggest new directions for developing antibiotic drugs. Hans Gmuender and colleagues have taken advantage of so-called 'gene chips', tiny devices that monitor gene activity in a specific organism. Using a chip for *Haemophilus influenzae*, they monitored gene activity in the presence of novobiocin and ciprofloxacin. The two antibiotics produced clearly different gene 'signatures' on the bacteria chip, indicating that each affects a specific set of genes, which are distinguishable using genome technology. This technology will help in classifying and developing novel antibiotics to combat increasingly resistant bacterial strains. *CK*
http://www.eurekalert.org

In Brief compiled by Cathel Kerr (c.kerr@sol.co.uk) and Alexandra Venter (alexventer@hotmail.com)

Letters

Dismantling the *Cryptococcus* coat

Cryptococcus neoformans is an encapsulated yeast and an opportunistic fungal pathogen responsible for life-threatening infections of the central nervous system (CNS) in HIV-1-infected and immunocompromised individuals[1]. Key virulence factors of *C. neoformans* include the capsule, the probable antioxidant melanin and the ability to grow at 37°C. In the December 2000 issue of *Trends in Microbiology*, Tamara Doering reviewed the biosynthesis of the cryptococcal capsule and its role in virulence[2]. The *C. neoformans* capsule, a stable polysaccharide that ensheaths the fungal cell, is emerging as a formidable and highly regulated virulence factor. Capsule formation is induced by iron limitation or CO_2/HCO_3^- exposure, conditions found in the infected host[3,4]. Studies of murine pulmonary cryptococcosis have revealed that *C. neoformans* is a facultative intracellular pathogen and that polysaccharide accumulation contributes to its survival within macrophages[5]. Additionally, the capsule is a phagocytosis-evasion device and capsular antigens shed into the circulation are immunosuppressive by a variety of mechanisms[6].

The capsule structure is dynamic in nature and there is evidence to suggest that phenotypic switching can generate increasingly pathogenic variants. Studies of *C. neoformans* isolates from chronically infected patients revealed changes in capsular polysaccharide structure[7,8], and capsule synthesis can be altered *in vitro* depending on growth conditions[3]. It will be of considerable interest to identify the signals responsible for these modulations and to define the mechanisms of phenotypic switching. Another issue that remains to be addressed is the tissue tropism of *C. neoformans* cells for the CNS. The capsule might allow fungal cells to be transported into the brain by either promoting survival inside macrophages, or by mediating binding to immune effector cell surfaces, although as yet there is no evidence of a role for the capsule in adhesion.

Despite tremendous progress in studies on capsule ultrastructure and biogenesis, the biosynthetic pathways involved remain largely uncharted. Although genetic analysis of acapsular mutants has identified several genes whose products are required for capsule production, relatively few genes have been isolated and characterized to date[9–11]. None of the Cap proteins has identity to known proteins but some clues about their function can be derived from analysis of their structures. Cap59 and Cap60 both contain a putative transmembrane domain, shown to be required for Cap59 function[9], and Cap60 has been localized to the nuclear envelope[11]. Complementing the genetics approach, biochemical identification of several enzymes involved in these pathways now permits the use of reverse genetics. Additionally, rapid advances in sequencing the *C. neoformans* genome will also be beneficial. Doering rightly points out that our understanding of capsule biosynthesis in *C. neoformans* will be aided by comparison with bacterial capsules and the abundant information on the synthesis of bacterial cell surface polysaccharides.

Another aspect requiring attention is the regulation of capsule production. The transcription factor homolog Ste12α (Refs 12,13) and the nutrient sensing Gα protein Gpa1 (Ref. 14) both play roles in controlling capsule induction, implicating the mitogen-activated protein kinase (MAPK) and cAMP signaling cascades in both capsule formation and virulence. Conservation of these cascades among fungi provides a starting point for identification of additional components of these virulence regulatory pathways. As the Stanford *C. neoformans* serotype D genome sequencing project (http://www-sequence.stanford.edu/group/C.neoformans/index.html) nears completion, future efforts should focus on development of gene-array technology to identify components and targets of these and other signal transduction pathways. We fully concur with the author that a combination of classical and modern molecular genetics will be central for understanding the mechanisms of capsule synthesis and its regulation.

Cletus A. D'Souza
Joseph Heitman*
Depts of Genetics, Pharmacology and Cancer Biology, Microbiology and Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710, USA.
*e-mail: heitm001@duke.edu

References

1 Casadevall, A. and Perfect, J.R. (1998) *Cryptococcus neoformans*, ASM Press
2 Doering, T.L. (2000) How does *Cryptococcus* get its coat? *Trends Microbiol.* 8, 547–553
3 Granger, D.L. *et al.* (1985) Virulence of *Cryptococcus neoformans*: regulation of capsule synthesis by carbon dioxide. *J. Clin. Invest.* 76, 508–516
4 Vartivarian, S.E. *et al.* (1993) Regulation of cryptococcal capsular polysaccharide by iron. *J. Infect. Dis.* 167, 186–190
5 Feldmesser, M. *et al.* (2000) *Cryptococcus neoformans* is a facultative intracellular pathogen in murine pulmonary infection. *Infect. Immun.* 68, 4225–4237
6 Murphy, J.W. (1999) Immunological down-regulation of host defenses in fungal infections. *Mycoses* 42 (Suppl. 2), 37–43
7 Goldman, D.L. *et al.* (1998) Phenotypic switching in the human pathogenic fungus *Cryptococcus neoformans* is associated with changes in virulence and pulmonary inflammatory response in rodents. *Proc. Natl. Acad. Sci. U. S. A.* 95, 14967–14972
8 Fries, B.C. *et al.* (1999) Phenotypic switching in *Cryptococcus neoformans* results in changes in cellular morphology and glucuronoxylomannan structure. *Infect. Immun.* 67, 6076–6083
9 Chang, Y.C. and Kwon-Chung, K.J. (1994) Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. *Mol. Cell. Biol.* 14, 4912–4919
10 Chang, Y.C. *et al.* (1996) The second capsule gene of *Cryptococcus neoformans*, *CAP64*, is essential for virulence. *Infect. Immun.* 64, 1977–1983
11 Chang, Y.C. and Kwon-Chung, K.J. (1998) Isolation of the third capsule-associated gene, *CAP60*, required for virulence in *Cryptococcus neoformans*. *Infect. Immun.* 66, 2230–2236
12 Yue, C. *et al.* (1999) The STE12α homolog is required for haploid filamentation but largely dispensable for mating and virulence in *Cryptococcus neoformans*. *Genetics* 153, 1601–1615
13 Chang, Y.C. *et al.* (2000) *Cryptococcus neoformans STE12α* regulates virulence but is not essential for mating. *J. Exp. Med.* 191, 871–882
14 Alspaugh, J.A. *et al.* (1997) *Cryptococcus neoformans* mating and virulence are regulated by the G-protein α subunit GPA1 and cAMP. *Genes Dev.* 11, 3206–3217

Dismantling the *Cryptococcus* coat

Response from Doering

Drs D'Souza and Heitman make several interesting points on capsule synthesis in *Cryptococcus neoformans*. In response, I would like to emphasize that, as well as 'classical and modern molecular genetics' being required for understanding capsule synthesis, other approaches will be important. For example, biophysical and imaging techniques could help elucidate the organization of the cryptococcal capsule or the conformation of specific capsular polysaccharides. Although the former area might be informed and advanced by studies of mutants with defects in capsule construction, it is hard to imagine how the latter might be addressed by genetic approaches. Additionally, as D'Souza and Heitman point out, genetic analysis has clearly implicated several gene products in capsule synthesis, but we remain in the dark as to their functions. So, beyond the valuable information to be gleaned from gene-array technologies and genome sequence, I stress that this must be complemented by biochemistry, cell biology and biophysics for a full understanding of the complexities of capsule synthesis and regulation.

Tamara L. Doering
Dept of Molecular Microbiology,
Washington University School of Medicine,
St Louis, MO 63110-1093, USA.
e-mail: doering@borcim.wustl.edu

Dismantling the *Cryptococcus* coat

Response from D'Souza and Heitman

We fully concur! Genetics and molecular biology provide powerful approaches that have opened the door to the characterization of the components regulating capsule synthesis and function. That said, the cell biology and biochemical approaches that Dr Doering is pioneering in her elegant studies on capsule biosynthesis promise to shed much needed light on how the capsule is assembled. We applaud her efforts to wed genetics and molecular biology with cell biology and biochemistry, and we look forward to learning much from the studies she and her colleagues have embarked upon.

Cletus A. D'Souza
Joseph Heitman*
Depts of Genetics, Pharmacology and Cancer Biology, Microbiology, and Medicine,
Howard Hughes Medical Institute,
Duke University Medical Center,
Durham, NC 27710, USA.
*e-mail: heitm001@duke.edu

Pro-inflammatory programmed cell death

The ability of exogenous glycine to antagonize pathological ion fluxes through the plasma membranes of *Salmonella*-infected, ischemic or ATP-depleted host cells suggests the possibility that diverse stimuli ultimately evoke cell death using common effector pathways[1]. The biochemistry of rapid macrophage death induced by *Salmonella* most closely resembles necrosis, but the caspase-1 dependence of this cytotoxicity[1,2] clearly distinguishes it from any usual form of accidental cell death. Coupled with the observation that infection or invasion *per se* are not sufficient to trigger macrophage death, we embrace the notion that *Salmonella*-induced death is a programmed event, as discussed by Boise and Collins[3].

'Physiological cell death' was historically termed necrobiosis and, from the descriptions of coagulation necrosis and shrinkage necrosis, the latter was renamed apoptosis[4]. As detailed by Boise and Collins, apoptosis is a non-inflammatory program of cell death to remove unwanted cells. Correspondingly, we envision the evolution of a pro-inflammatory program to remove potentially dangerous cells, like the process that takes the life of *Salmonella*-infected macrophages. This is an alternative pathway from simply and quietly removing an unwanted cell in a developing organism – the screaming, alarm-ringing pro-inflammatory death of a potentially dangerous cell in an organism where the prompt recruitment of additional cells or cellular functions are necessary to promote a positive outcome for the host. We propose the term pyroptosis from the Greek roots *pyro*, relating to fire or fever, and *ptosis* (to-sis) to denote a falling, to describe pro-inflammatory programmed cell death. The observed caspase-1-activation or -dependence during cell death in the immune[4], central nervous[6–8] and cardiovascular systems[9] indicates pyroptosis could play a significant role in a variety of biological systems. As some pathogens might trigger pyroptosis to their advantage[10], further study of *Salmonella* will provide scientists with yet another opportunity to probe host cell functions.

