PERSPECTIVES: MICROBIOLOGY

Looking for a Few Good Mutants

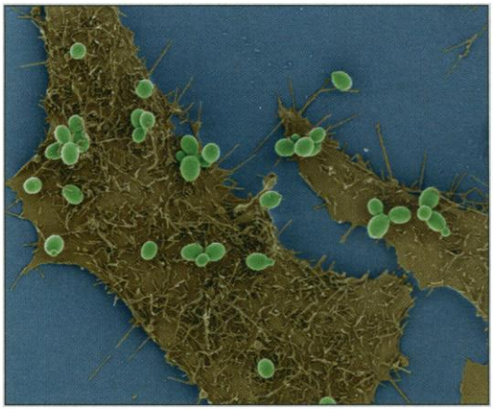
William E. Goldman

Medically important fungi have been steadily attracting more attention from microbiologists interested in infectious disease research, prompting a stream of talent and technology transfer from the better-studied bacterial systems. Still, strategies for discovering and validating virulence-related genes (that is, fungal genes implicated in disease) are largely confined to genetic approaches that were built around the molecular tools available for bacteria: mutagenesis by transposons (small pieces of DNA that insert randomly throughout the genome), gene disruption by homologous recombination, expression of cloned genes on plasmid vectors. The practical and genetic obstacles posed by fungal pathogens (principally the difficulties in generating defined mutants) have made the discovery of virulence genes a matter of selective guesswork. On page 578 of this issue, Cormack *et al.* describe the first rationally designed mutant hunt to identify a virulence-related gene in a fungus pathogenic for humans (*1*). Their search yielded a new gene, *EPA1* (epithelial adhesin 1), which encodes a lectin that enables the yeast *Candida* to adhere to epithelial cells. In successfully adapting a strategy originally developed for screening bacterial mutants, the authors had to bypass a formidable set of genetic barriers in their search for nonadherent *Candida* mutants.

Their choice of model system was *Candida glabrata*, a yeast that causes mucosal and systemic infections in humans similar to those caused by its better-known relative, *Candida albicans*. In contrast to the asexual diploid *C. albicans*, *C. glabrata* is an asexual haploid, which makes genetic manipulation far simpler. For example, as there is only one copy of each gene, it is possible to generate single mutations that completely eliminate a gene's function; gene disruptions require only one allelic replacement event; and mutations can be complemented by a single copy of the wild-type gene.

Mutants can be generated in fungi with chemical mutagens or with ultraviolet light, but a better method is the creation of insertion mutants in which genetically tagged DNA is introduced at random sites in the genome. With these mutants, the physically marked insertion can be easily analyzed and flanking sequences simply retrieved by cloning, allowing quick identification of the mutated region. In their parallel study recently published in *Genetics* (*2*), Cormack and Falkow observed that *C. glabrata*, like many fungi, allows integration of genetically tagged DNA at nonhomologous sites throughout its genome. In *C. glabrata*, most of these events appear to be simple insertions that have no duplications or rearrangements, with a strong preference for integration within promoter regions. This method of introducing DNA insertions throughout the genome was exploited as an effective mutagenesis system, allowing the authors to tackle their goal of identifying a virulence gene important for adherence.

Their objective was straightforward but not simple, and the same obstacles have been faced by many groups trying to identify mutants that lack virulence-related traits. The complexity of these phenotypes means that screening for individual null mutations scattered throughout a fungal-sized genome is far too labor-intensive. Instead, Cormack and colleagues chose to adapt the strategy of signature-tagged mutagenesis, originally developed by Holden and colleagues for *Salmonella typhimurium* (*3*). This approach permits large-scale screening of pools of transposon insertion mutants using the polymerase chain reaction (PCR) to follow the fate of unique DNA sequence "tags" carried by the transposons. Holden's group refined this strategy in their work on *Staphylococcus aureus* (*4*) by creating a master set of 96 tagged transposons that could be easily amplified by PCR and matched to tags immobilized in an array on a master hybridization filter; this allows reuse of the same tags to screen the fate of large numbers of insertion mutants. Cormack *et al.* have taken this one step further, introducing each of the 96 oligonucleotide tags directly into a single innocuous site within the genome of *C. glabrata*. This created a master set of 96 individually tagged strains that could then be mutagenized by any technique. As there is no insertion mutagenesis technology available for most fungi, this approach of pretagging strains has tremendous potential for expanding the signature-tagged mutagenesis strategy to other fungal pathogens. In the case of *C. glabrata*, the authors' parallel development of a mutagenesis system allowed them to generate 9600 insertion mutants from the original 96 tagged strains (that is, 100 different insertion mutants for each tagged strain). The mutants could then be screened for a nonadherent phenotype in a standard adherence assay.



Candida glabrata, a yeast pathogen of humans, adhering to human epithelial cells in culture.

A key advantage of signature-tagged mutagenesis is that it allows a genuinely unbiased search for a mutant phenotype of interest, based solely on a functional screen tied to virulence. This type of screen is routinely done by infecting animals and has enabled the identification of virulence-related genes in *S. typhimurium*, *S. aureus*, *Vibrio cholerae*, *Streptococcus pneumoniae*, and *Yersinia enterocolitica* (*3–7*). In the Cormack study, the screen was based on the ability of wild-type *C. glabrata* to bind to a cultured human epithelial cell line (see the figure). Mutants that did not adhere to the epithelial cells were monitored for the loss of tagged strains. Of 16 totally nonadherent mutants, 14 had insertions affecting the expression of a single gene, *EPA1*. The adhesin encoded by *EPA1* appears to be a calcium-dependent lectin that most likely recognizes *N*-acetyl lactosamine residues of mammalian cell surface glycoproteins. The precise role of Epa1 in *C. glabrata* pathogenesis is still a mystery because the null mutant has no obvious phenotype in murine models of disease. It is possible that the role of this adhesin in human infection is not evident in the mouse model, or that the very high inoculation levels required for causing mouse disease mask a more subtle phenotype than Epa1 displays in natural host colonization.

This is not the first adhesin identified in *Candida* species, and it is likely that adherence to host tissues depends on a variety of surface molecules. The most recent-

The author is in the Department of Molecular Microbiology, Washington University, St. Louis, MO 63110, USA. E-mail: goldman@borcim.wustl.edu

ly discovered adhesin of *C. albicans* was reported earlier this year in *Science* by Sundstrom and colleagues (*8*). They demonstrated that Hwp1, a glycoprotein, is a substrate for mammalian transglutaminase and that this microbial adhesin covalently cross-links the pathogen to its host cell. *HWP1* was not discovered in a search for adhesin genes, but the function of its protein product in stable adherence became apparent through careful experiments and correlative observations. This has been the pattern for virulence gene discovery in fungal pathogens—candidate genes are identified on the basis of characteristics such as predicted biochemical properties, sequence similarities to known virulence determinants, transcriptional upregulation in models of infection, or functional complementation in heterologous systems (for example, genes that confer adhesive properties on *Saccharomyces cerevisiae*). Signature-tagged mutagenesis complements these approaches, allowing the identification of interesting genes according to the fate of randomly generated mutants in mixed infection experiments.

Mycologists should now be able to apply signature-tagged mutagenesis and related mutant screening strategies to the study of other fungal pathogens. Powerful molecular genetic systems are now in place for some of these fungi, and the most comprehensive sets of tools have been developed for *Candida* species, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Histoplasma capsulatum*. Within the past year, new developments have included novel strategies for fungal gene disruption (*9–11*), transformation with freely replicating plasmid vectors (*12, 13*), the use of reporter genes for studying transcriptional regulation (*14, 15*), and insertion mutagenesis (*2, 16*). The medically important fungi, although not as well studied as many bacterial pathogens, offer a fascinating smorgasbord of model systems for research into adherence, mechanisms of intracellular survival, tissue invasiveness, evasion of host defenses, phenotypic switching and variation, chronic and latent infections, modulation between saprophytic and parasitic lifestyles, and transmission. Even though signature-tagged mutagenesis has previously been used only in bacterial systems, it was originally conceived by the Holden lab as an approach for their ongoing studies of virulence genes in *A. fumigatus*. The paper by Cormack and colleagues returns signature-tagged mutagenesis to its Kingdom of destiny, and not a moment too soon for the rapidly emerging field of molecular mycology.

References

1. B. P. Cormack, N. Ghori, S. Falkow, *Science* **285**, 578 (1999).
2. B. P. Cormack and S. Falkow, *Genetics* **151**, 979 (1999).
3. M. Hensel *et al.*, *Science* **269**, 400 (1995).
4. J.-M. Mei, F. Nourbakhsh, C. W. Ford, D. W. Holden, *Mol. Microbiol.* **26**, 399 (1997).
5. A. J. Darwin and V. L. Miller, *ibid.* **32**, 51 (1999).
6. S. Chiang and J. Mekalanos, *ibid.* **27**, 797 (1998).
7. A. Polissi *et al.*, *Infect. Immun.* **66**, 5620 (1998).
8. J. F. Staab, S. D. Bradway, P. L. Fidel, P. Sundstrom, *Science* **283**, 1535 (1999).
9. J. Morschhäuser, S. Michel, P. Staib, *Mol. Microbiol.* **32**, 547 (1999).
10. R. B. Wilson, D. Davis, A. P. Mitchell, *J. Bacteriol.* **181**, 1868 (1999).
11. T. T. Brandhorst, M. Wüthrich, T. Warner, B. Klein, *J. Exp. Med.* **189**, 1207 (1999).
12. J. P. Woods, E. L. Heinecke, W. E. Goldman, *Infect. Immun.* **66**, 1697 (1998).
13. A. Varma and K. J. Kwon-Chung, *Curr. Genet.* **34**, 60 (1998).
14. J. B. Patel, J. W. Batanghari, W. E. Goldman, *J. Bacteriol.* **180**, 1786 (1998).
15. M. Del Poeta *et al.*, *Infect. Immun.* **67**, 1812 (1999).
16. J. S. Brown, A. Aufauvre-Brown, D. W. Holden, *Mol. Gen. Genet.* **259**, 327 (1998).

PERSPECTIVES: DEVELOPMENTAL NEUROSCIENCE

Spontaneous Activity: Signal or Noise?

David A. McCormick

The brain is constantly active. From well before birth, till death's final hour, neurons in the central nervous system generate barrages of electrical discharges. Electrical activity that does not bear any obvious relationship to, for example, the task of processing sensory information or the generation of movement is commonly referred to as spontaneous. Although the appropriateness of this term is questionable, as it presupposes detailed understanding of the action potential discharges in a neuronal network, spontaneous electrical activity is clearly something that the brain does generate. For example, the first investigators to record from the cerebral cortex and thalamus of sleeping animals were surprised to find strong, rhythmic barrages of action potential activity. Indeed, the average rate of action potential generation during sleep could be significantly higher than that in waking animals. We now know that much of this activity is truly spontaneous in that it can be detected in brain slice preparations in vitro, despite the prior cessation of all activity and the lack of a clearly defined stimulus from the environment (*1*).

What is the function of this spontaneous activity? Is it an epiphenomenon of the neuronal circuitry that is irrelevant to the true task of the neuronal pathway, or does it have some other significance? In a report on page 599 of this issue, Weliky and Katz set out to answer this question by investigating functional neuronal connections in the visual system of awake baby ferrets before their eyes are open, when external sensory stimuli cannot be perceived (*2*).

Many processes in neurons, and even some aspects of the formation and refinement of neuronal circuits during development, are dependent on, or influenced by, action potential activity (*3*). During development, the basic connections that define the complex of neuronal circuits making up the nervous system are determined largely through genetic preprogramming that depends on a wide variety of molecular guidance cues (*4*). Refinement of these neuronal connections during development is highly sensitive to experience during a window of time referred to as the critical period (*5*). However, in many animals, especially primates, significant development of neuronal connections occurs before precise sensory experience (*6*). This is especially true of the visual system because no patterned information can reach retinal photoreceptors in utero. Nevertheless, many of the basic connections, for example, between the ocular dominance layers in the lateral geniculate nucleus (LGN) of the thalamus, and receptive field properties of neurons, such as orientation tuning in the primary visual cortex, are determined during fetal development. Although it is well known that competitive interactions between inputs before birth are involved in sharpening the terminal fields of axons (*7*), the underlying mechanisms are unclear. They may in part depend on the presence of action potentials, even in the absence of visual experience (*3*). For example, blocking retinal activity in one eye of the cat with an intraocular injection of the Na^+ channel poison tetrodotoxin in utero results in a competitive disadvantage in the formation of connections by that eye in the LGN; the competing eye then innervates more than its fair share of the LGN (*3*). If these connections form before patterned sensory

The author is in the Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510, USA. E-mail: david.mccormick@yale.edu