COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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Fungal genomics goes industrial

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A functional genomics study of the rice blast fungus unravels the genetic basis of pathogenicity.

Rice blast is one of the plant world's most devastating diseases, each year destroying enough rice to feed 60 million people[1,2]. In a recent issue of *Nature Genetics*, Jeon *et al.*[1] present a high-throughput functional genomics study of the rice blast fungus, *Magnaporthe oryzae*. Their results, which involved the generation of >20,000 mutants of *M. oryzae*, constitute the largest individual study of fungal pathogenicity published to date and reveal many new gene functions required for rice blast disease.

Fungi are responsible for the rusts, mildews, blasts and wilts that afflict many of our most important crop species, including the cereals that sustain much of the planet's human population. In addition, fungal diseases of humans are on the rise as a consequence of the widespread use of immunosuppressive therapies for treating chronic illnesses and the prevalence of autoimmune disorders. Taken together, the human, social and economic costs of fungal infections provide a powerful incentive to investigate the mechanisms that diverse fungi deploy to cause disease.

There are, however, significant obstacles to studying pathogenic fungi using the tools of molecular genetics and genomics[3]. Many fungal species lack sexual reproductive stages, which prevents the use of conventional forward genetics, and most fungi exhibit very low frequencies of DNA-mediated transformation, prohibiting complementation studies[3]. Other important pathogenic species—the rust and powdery mildew fungi, for example—cannot be cultured at all outside a living host.

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Despite these difficulties, much has been learned about fungal pathogens in recent years, mainly by the careful deployment of reverse genetic analysis. Researchers have begun to define the major signaling pathways associated with initial plant infection[4,5], the processes by which fungi are perceived by resistant plant varieties[6] and the manner in which fungi invade plant and animal tissues to cause disease[4,7]. These studies have relied on selective study of model fungal species for which genetic resources have been developed and have primarily used targeted gene replacement to define gene function[3]. Above all, the study of fungal biology has been revolutionized by the rapid sequencing of >30 species[8]. These sequencing efforts have presented a new challenge: how to determine the functions of each of the ~10,000 genes of a fungal species in a high-throughput manner.

The work of Jeon *et al.* represents a significant step forward in achieving this aim for *M. oryzae.* A large library of mutants was generated using insertional mutagenesis with *Agrobacterium tumefaciens* (**Fig. 1**). *A. tumefaciens* is, of course, the workhorse of plant molecular biology, but it has become clear in recent years that it can be a highly efficient method for introducing DNA into filamentous fungi[9], with reported frequencies of up to 800 transformants per experiment (compared with <40 transformants per μg of DNA in a conventional fungal-transformation experiment). Although insertional mutagenesis in fungi has been used previously to identify novel determinants of pathogenicity[2,5], the scale of this study is unprecedented.

Jeon *et al.* carefully cataloged the mutants and stored them in multiwell plates. A set of high-throughput screens was then deployed to characterize growth- and virulence-associated phenotypes. These included the ability of each mutant to sporulate, to form specialized infection structures called appressoria[4] and to cause rice blast disease when inoculated on rice leaves. Although analysis of plant infection used a detached leaf assay in single tubes rather than whole plants, this was still a large-scale effort, because extensive technical replication was necessary to confirm the ability of each mutant to cause rice blast disease symptoms.

The real power of the method, however, lies in the analysis of insertion sites, which show good coverage of T-DNA insertions across the *M. oryzae* genome[10] and saturation of ~61% of the genome[1]. A relational database was generated to provide a key to all of the insertion lines of *M. oryzae* created. Two hundred two new pathogenicity loci have so far been identified. This is about twice as many as were previously known, with the existing knowledge having been gained during the previous 18 years of molecular genetic studies.

What are the lessons learned from this work, and how might others who study fungal diseases benefit? First and foremost, the existing mutant collection has been replicated and safely archived so that it can be made available to the worldwide community of researchers studying rice blast. Second, the informatic resources generated are now being interlinked in an effective way with the ongoing annotation program for the *M. oryzae* genome[10], making it a seamless process to go from analyzing a novel gene sequence to finding the corresponding insertion mutant.

The study of Jeon *et al.* highlights the need for similar industrial-scale studies of other important cereal pathogens for which genome sequence information is becoming available and in which there are well-organized communities of researchers already working. This is a key priority given the diversity of mechanisms deployed by fungal pathogens to invade their plant and animal hosts and the importance of developing durable disease control strategies. The ability to validate a virulence factor quickly in a large number of pathogenic species would allow academic research to be far more easily translated into target identification and validation for new antifungal drug development.

There are other obstacles that hinder progress in studying pathogenic fungi. One priority is increasing the ease of gene replacements to validate gene functions. Only in this way can rapid analysis of signaling and metabolic pathways be carried out from the leads identified through insertional mutagenesis. Useful tools in this regard are fungal strains that can carry out only homologous recombination because the nonhomologous DNA repair

pathway is blocked by deletion of the ku70- or ku80-encoding genes[11]. However, there is a need for good inducible promoters, a more reproducible means of gene silencing that allows titration of gene expression levels and more systematic studies to determine protein-protein interactions during infection-related development.

Finally, there is still a need for high-quality genetic maps and detailed gene annotation programs, allowing conventional genetics to complement functional genomic studies. The power of conventional genetics to define essential gene functions, carry out suppressor screens and define synthetic lethal interactions, to name only three examples, has not been sufficiently exploited for the study of fungal pathogenicity. The availability of large well-defined mutant collections, such as that of Jeon *et al.*, and the advent of industrial-scale genomics have brought this longstanding aim within reach.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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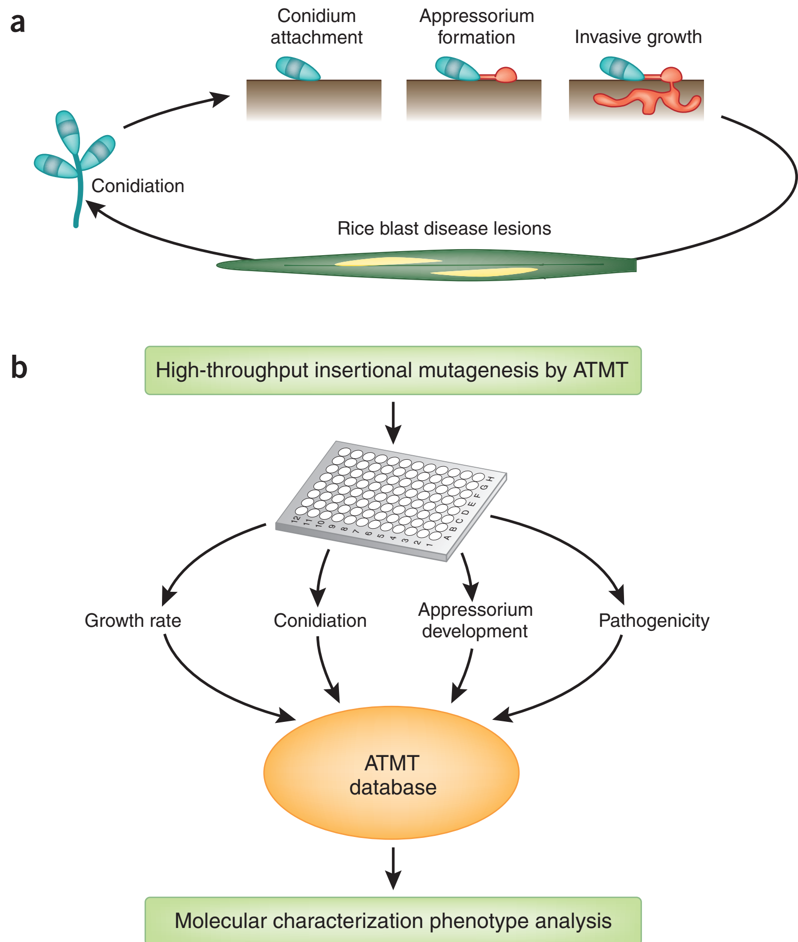

Figure 1 High-throughput genome-wide functional analysis of pathogenicity genes in the rice blast fungus *Magnaporthe oryzae*. (**a**) The fungus infects plants by forming specialized infection structures, called appressoria, that penetrate the rice cuticle, leading to tissue invasion and disease symptoms. (**b**) Jeon *et al.* used *Agrobacterium tumefaciens*–mediated transformation (ATMT) to create >20,000 mutant strains. These were grown in multiwell plates and analyzed for growth, conidiation, appressorium development and pathogenicity in a high-throughput plant infection assay. The resulting data were stored in a relational database and mutants were selected for in-depth phenotyping and molecular characterization of the insertional mutation. Two-hundred-two new pathogenicity genes were identified.