been the key to their success is the use of high-throughput experiments to assess various zeolite compositions: the window of compositions that yielded ITQ-33 is narrow, and outside the common range usually used to prepare zeolites. This demonstration that new materials can be discovered within such a narrow compositional window should lead to the wider use of high-throughput technology in the search for further zeolites.

The role of hexamethonium in the formation of ITQ-33 is intriguing. To date, the general strategy has been to prepare increasingly large organic molecules possessing the rigidity, solubility and stability needed to 'direct' the crystallization of new materials. Typically, the size and shape of the resulting pores corresponds to the size and shape of the organic molecule. ITQ-33, however, is different: hexamethonium is small and flexible, and there is no obvious fit between it and the resulting pore structure. It could be that the hexamethonium molecules pack in such a way as to provide an exact fit for the voids; this is the case, for instance, with VPI-5, which is stabilized by a chain of water molecules that perfectly fit the interior of the pores[6]. Hexamethonium is a simple and relatively inexpensive reagent, and its use bodes well for making ITQ-33 viable for practical application. Other zeolites prepared with organic compounds of similar complexity are used in the petrochemical industry and as additives in catalytic converters.

Another exciting aspect of the latest work[1] is that the structure of ITQ-33 was 'predicted' by algorithms that generate framework structures consistent with the geometrical requirements of a zeolite[7]. In the past year, roughly half of the reported zeolite structures have been previously 'discovered' by these algorithms. It is possible to search the large structural databases generated by these programs for structures with hitherto unavailable properties. An example is given in Figure 2, in which a computer-generated framework with 18- and 24-ring pores is compared with ITQ-33 and other known zeolites. The advent of these powerful algorithms will help in solving the structure of microporous materials, and can make the synthesis of zeolites more 'directed' and perhaps more successful.

Although ITQ-33 has all the characteristics of a good acid catalyst, much work remains to be done to make it practical. The amount of germanium and fluoride required must be minimized or eliminated to reduce manufacturing costs. Better ways of recovering the organic director and recycling it could further increase its potential. Substitution of other atoms in the framework, such as titanium or tin, could expand the range of properties to catalytic reactions such as oxidation and Lewis-acid catalysis.

More generally, ITQ-33 may help us to gain a better understanding of the adsorption processes that occur at the interface between the microporous (pore diameter less than 2 nm) and mesoporous (pore diameter 2–100 nm) scales. It is at this length scale that the transition between monolayer and multilayer adsorption occurs and where the assumptions of classical adsorption theories can break down. The problem can be approached from the other side, and there are, indeed, mesoporous silicas with ordered and highly uniform pore sizes in the 2-nm range[8]. These materials are, however, difficult to prepare with uniform pores below 2 nm. ITQ-33 bridges these two length scales; and because it is crystalline, and all its pores are — except for defects — identical, one should be able to relate atomic structure precisely to the adsorption isotherms of simple gases. This information could help in the future to interpret adsorption isotherms of other non-crystalline materials that have substantial porosity at the micro–meso transition.

Finally, the discovery of ITQ-33 raises the question of whether we need materials with even larger cavities. Some of the unique properties of zeolites arise from the large curvature of their pores. As the pores get larger, the interaction of adsorbates with the pore walls increasingly resembles the interaction with a flat surface. At some point, the zeolite pore will start to look like the surface of layered aluminosilicates such as clays (albeit without their characteristic hydroxyl groups). Yet perhaps it is not catalysis or separations where the large-pore materials of the future will find use. Instead, it may be in such niches as sensors or photonics[9], or where the low-dielectric constant of such materials, arising from their porosity, can be exploited in the manufacture of improved microelectronic devices. The challenge remains to make structures with less and less in them. ■

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EVOLUTIONARY BIOLOGY

A kingdom revised

Tom Bruns

An international consortium of researchers has produced an impressive new tree of life for the kingdom Fungi. The results are a testament to cooperation between systematists with different expertise.

On page 818 of this issue, James and colleagues[1] provide a landmark study in fungal evolution. Before now, the only broadly sampled phylogenetic trees of the fungi were based on sequences of a single gene — that encoding the small-subunit (18S) ribosomal RNA. Broad sampling of species is essential, because under-sampling is known to adversely affect the construction of evolutionary trees. However, the quantity and quality of data are equally important, and the 18S data were insufficient to provide strong statistical support for many key branches in the evolutionary trees. In any case, a single-gene tree is always questionable, because different genes can give different views of evolutionary history.

James *et al.*[1] addressed these problems by collecting sequence data from two additional ribosomal RNA genes, and three protein-coding genetic loci, for a carefully selected sample of 199 species. The results of the combined analyses, outlined in Figure 1, are quite similar to those seen with the earlier 18S data, but statistical support for some key branches in the tree has improved. This will be a relief to those who have followed the 18S data closely; it means that the new data have produced incremental shifts, not major alterations, in our understanding of fungal evolution.

The fungi, animals and plants are thought to have diverged from each other roughly a billion years ago. They are the only three eukaryotic kingdoms of life that developed multicellularity in terrestrial environments. Like plants and animals, the fungi had to adapt to terrestrial environments from ancestors that were aquatic. But the fossil record for fungi is much the worst; most of them are microscopic with relatively simple morphologies. For these reasons the evolutionary patterns within the fungi were poorly understood before the advent of nucleotide sequence data. It was known that most fungi lacked zoospores, motile cells that are propelled by flagella in water. Therefore the Chytridiomycota, the one aquatic group of fungi that contains flagella, was assumed

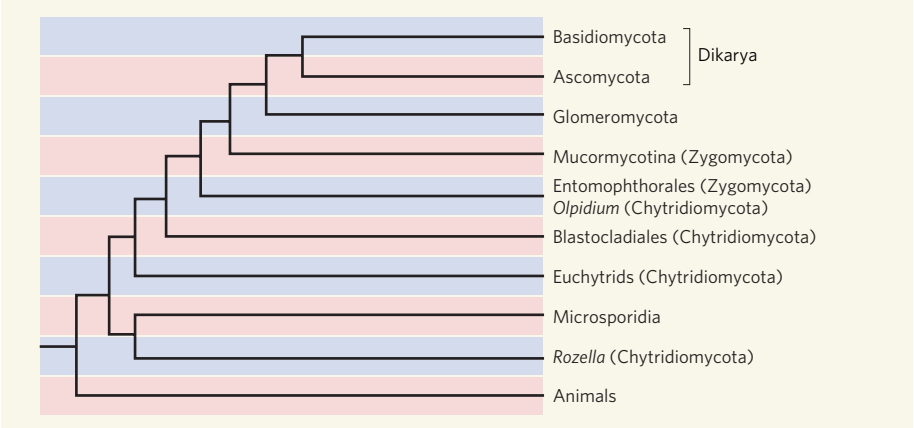

Figure 1 | The main branches of the kingdom Fungi. This highly simplified evolutionary tree shows the traditional phyla — Ascomycota, Basidiomycota, Glomeromycota, Zygomycota and Chytridiomycota. The Ascomycota and Basidiomycota are united as the dikarya, fungi in which part of the life cycle is characterized by cells with paired nuclei. Their closest relatives seem to be the Glomeromycota, a group that was previously included within the Zygomycota. Neither the Zygomycota nor the Chytridiomycota are monophyletic groups; instead they seem to be 'paraphyletic grades' that are grouped only by shared primitive morphologies. Also shown are the microsporidia and *Rozella* branches, which seem to be basal to the all other fungi. (Note that all of these branches are still in need of stronger statistical support. James and colleagues' much more detailed tree[1] appears on page 820.)

to be primitive. This has turned out to be correct but the details of the relationship are complicated.

Both the 18S data and the new multigene analyses show that the Chytridiomycota is paraphyletic — that is, it does not include all the descendants of its most recent common ancestor. But James *et al.*[1] show that a minimum of four independent losses of flagella has occurred; thus one of the key adaptations to the terrestrial environment has actually happened multiple times. Surprisingly, they show that one chytrid, *Olpidium brassicae* (Fig.1), may lie within the Entomophthorales, a group that includes insect parasites that lack flagella and that is usually considered a subgroup of the Zygomycota. *Basidiobolus*, traditionally a member of the Entomophthorales, had been placed within the Chytridiomycota by 18S data, but is now moved back by the multigene analysis to its more traditional place.

An interesting example of multigene support concerns the placement of the Glomeromycota. These fungi form mutualisms called mycorrhizae with the roots of most plants, and they had been considered to be members of the Zygomycota. The 18S data consistently depicted them as a distinct group closely related to the Ascomycota and Basidiomycota, but there was no statistical support for this placement. The multigene data, however, provide at least bayesian statistical support for the latter relationship (Fig. 1).

The most surprising result concerns *Rozella* (Fig. 2), an obscure genus that is parasitic on other Chytridiomycota. Together with the microsporidia, an enigmatic group of animal parasites, *Rozella* seems to be basal to all other sampled fungi (Fig. 1). There was no reason to expect this, and in this sense the result is reminiscent of the finding by plant systematists that an obscure tropical genus, *Amborella*, is the sister group to all other flowering plants[2]. These types of result again underline the importance of which species are sampled.

The placement of the microsporidia themselves is another notable result. On the one hand, the analyses with 18S sequences originally put them at the base of the eukaryotic tree, distant from fungi, animals and plants. But this conclusion turned out to be erroneous owing to a confounding factor known as long-branch attraction. Other studies using protein-coding genes had previously placed them in the fungi, but the exact relationship was unclear because of limited sampling within the kingdom[3,4]. James and colleagues[1] have now improved the sampling dramatically, and show that the microsporidia must be either at the base of the fungal tree, within the Chytridiomycota, or within the Entomophthorales; in addition they were able to reject eight previously theorized placements within the fungi or outside the kingdom.

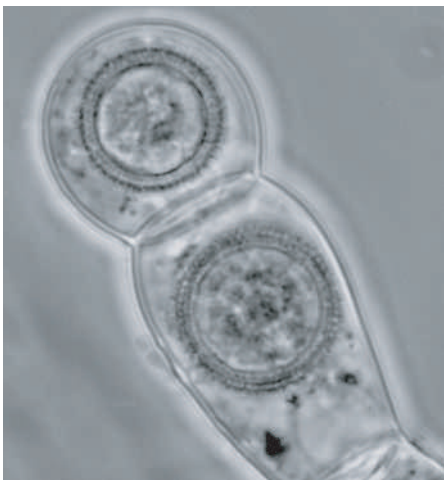

Figure 2 | *Rozella allomycis*. This parasite of other members of the same phylum, the Chytridiomycota, seems to be one of the most primitive fungi. Its resting sporangia (spore-producing bodies) are approximately 18 μm across and are shown within a hypha of its host chytrid, *Allomyces*.

There is still room for improvement in two key areas: branch support and taxon sample. Even with six gene loci, many branches remain unsupported or supported only by bayesian statistics, which may give overly optimistic assessments. For many branches it may be possible to increase support by adding additional data, and genomics will be a major contributor. Data from 29 complete fungal genomes were included in the analysis, but this sample is highly biased towards serious pathogens and model genetic systems. With the cost of sequence acquisition dropping, the number of sequenced fungal genomes will increase, and it may be possible to distribute this effort more evenly across the kingdom to provide a better evolutionary sample.

As to the second area for improvement, greater effort needs to be focused on sampling the environment for unknown fungal groups. It is estimated that the kingdom contains 1.5 million species, fewer than 5% of which have been described[5]. If most of the unknown species are members of well-known groups, then the current phylogenetic estimates should be largely unaffected by additional discoveries. However, some entirely new lineages have been recovered by sequence analysis of common but previously unsampled environments[6,7]: we can't predict how such discoveries will affect our perception of fungal evolution.

The cooperation among researchers that has resulted in the new paper[1] is almost as impressive as the product itself. Systematics can be a fairly balkanized field, with specialists defending their turf or their analytical methods against perceived competitors[8]. However, cooperation has always been common among fungal researchers because the field is woefully underpopulated. The James group included both traditional, morphologically based systematists, who contributed a wealth of knowledge on the organisms, and molecular systematists, who supplied the methodological and analytical techniques. Even Ralph Emerson, who died in 1979, made a notable posthumous contribution: it was his culture of *Rozella*, isolated in 1947, that made the sequence acquisition for this critical branch possible. This fusion of talents was essential to ensure that the broadest possible sample of fungi was selected, and that the data were collected and analysed rigorously. The results represent a proud moment for the field, and will be in the textbooks for some time to come. ■

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STRUCTURAL BIOLOGY

Enzyme target to latch on to

Malcolm A. Leissring and Dennis J. Selkoe

Insulin-degrading enzyme is implicated in diabetes and Alzheimer's disease, but few molecular tools exist that can probe its function. A study now reveals its unusual structure and may lead to an expanded toolbox.

Proteases are vital enzymes that have been targeted for the treatment of many diseases. One such protease, insulin-degrading enzyme (IDE), has strong links to diabetes and Alzheimer's disease but has nonetheless proved to be an elusive drug target, despite more than 50 years of intensive research. On page 823 of this issue, Shen and colleagues[1] reveal high-resolution crystal structures of IDE that open the door to the rational design of pharmacological modulators of this protease*. Crucially, the authors show that it might be possible to develop not just inhibitors, but activators as well.

IDE was discovered in 1949 by the physician and biochemist I. Arthur Mirsky[2]. Mirsky reasoned that inhibitors of IDE would be an ideal anti-diabetic therapy, as they would slow the degradation of insulin. In support of this approach, Mirsky found that liver extracts containing an inhibitor of IDE enhance the action of insulin when injected into rabbits[3]. Thereafter, Mirsky and many others sought to develop potent inhibitors of IDE as potential drugs. Despite these efforts, very few compounds that specifically inhibit IDE are available today, apart from substrates of IDE such as insulin itself. By revealing IDE's active site in unprecedented detail, the crystal structures provided by Shen *et al.*[1] may hold the key to realizing a potent and selective IDE inhibitor.

Recent discoveries, however, raise concerns about the wisdom of inhibiting IDE. Chief among them is the finding that IDE naturally degrades the amyloid-β protein that accumulates abnormally in Alzheimer's disease[4]. Here, it would be desirable to activate rather than inhibit IDE, a strategy that has already proven effective in mouse models of the disease[5]. Moreover, results from different animal models cast doubt on the concept of treating diabetes by chronically inhibiting IDE. A well-established rat model of diabetes was found to harbour mutations in IDE that reduce its ability to degrade both insulin and amyloid-β protein[6,7]. More recently, genetically modified mice that lack the gene for IDE were created. These mice had elevated insulin levels upon fasting, as predicted, but they also developed glucose intolerance, and they showed increased levels of cerebral amyloid-β protein[8]. These and other findings suggest that in some cases of diabetes (and perhaps also in some cases of Alzheimer's disease), there might be too little IDE activity rather than too much, with chronically elevated insulin levels perhaps leading to insulin resistance.

If this is true, IDE activators seem to be the logical therapeutic approach, especially for Alzheimer's disease. Current thinking suggests that activators would be difficult to achieve in practice, for the same reason that it is easier to break a machine than to improve its performance. But the work of Shen *et al.*[1] shows that IDE has unorthodox enzymatic properties that might permit activators to be developed after all.

The authors' crystal structures[1] reveal that IDE resembles a clam shell, with two bowl-shaped halves connected by a flexible hinge (Fig. 1). This configuration allows the protease to switch between 'open' and 'closed' states. Shen *et al.* show that extended hydrogen bonding between the two halves of IDE creates a 'latch' that tends to keep the protease closed (Fig. 1a). Notably, by introducing mutations to the enzyme that destabilize the hydrogen-bond latch, the authors were able to increase the protease's efficiency in cleaving a test substrate by as much as 40-fold (Fig. 1b). This improved efficiency was also seen in the degradation of insulin and amyloid-β protein.

So what is the mechanistic basis of the profound enzyme activation seen in the mutant IDE? This can be understood by considering a simple, two-step model[9] of the enzyme reaction. First, the enzyme and the substrate bind to each other in a reversible process to form an enzyme–substrate complex. Second, catalytic cleavage of the substrate occurs with concomitant release of the reaction products. Mutations that promote the open state of the protease — thus allowing it to bind substrate — could improve the efficiency of the reaction by accelerating the rate of the enzyme–substrate complex formation.

However, there is a second way that these mutations could activate the protease. In our simple model of the enzyme reaction, the second step actually includes at least two discrete processes: catalysis (that is, substrate cleavage) and dissociation of the products from the enzyme. This complication is usually ignored by assuming that the rate of product dissociation is rapid compared with that of catalysis, making catalysis the rate-limiting step. Although this assumption holds for many proteases, the new work suggests that IDE probably conforms to a more complex kinetic model, where catalysis does not lead automatically to product release. Instead, an additional step is required in which the

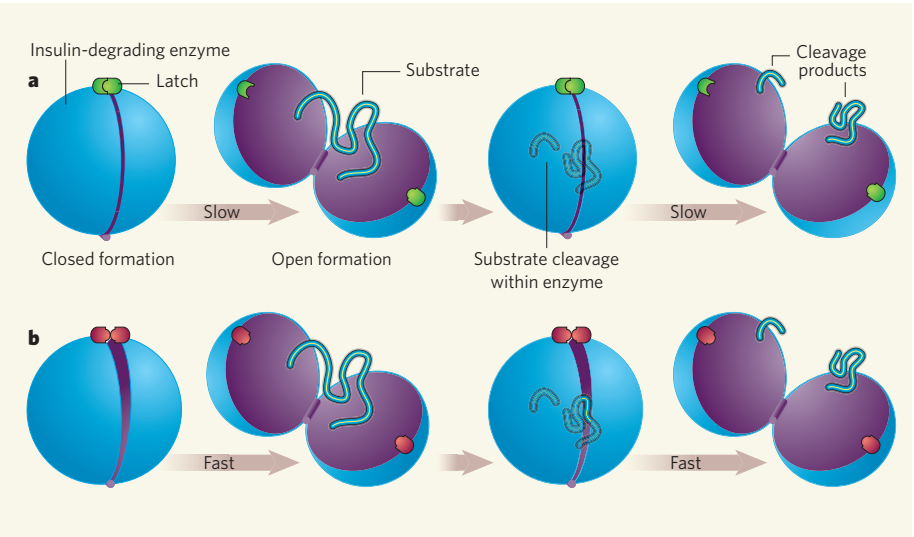

Figure 1 | Enzyme activation. a, Insulin-degrading enzyme (IDE) cleaves molecules implicated in diabetes and Alzheimer's disease. The crystal structures of IDE reported by Shen *et al.*[1] reveal a 'latch' mechanism (green) that holds the enzyme in a closed state, delaying entry of the substrate or exit of the cleavage products. **b**, Mutations (red) that disrupt the latch promote the open conformation of the enzyme. Such mutants accept substrates and release products more readily than naturally occurring IDE, and so are more active.

*This article and the paper concerned[1] were published online on 11 October 2006.