As the boundaries continue to blur between p53 and its relatives, compelling differences remain. For example, despite their similarities, the three genes seem to have very different functions. Whereas deletion of *p63*[15,16] and *p73* (F. McKeon, personal communication) has dramatic developmental consequences in mice, *p53* null mice develop normally[1] (with some interesting exceptions). But p53 null mice are highly prone to tumours. That p53 is unique in serving as a tumour suppressor is supported by the fact that, in human cancers, loss or mutation of *p73* or *p63* seems to be infrequent[2]. Additionally, only p53 is susceptible to inactivation by the SV40 T antigen, the adenovirus E1B 55K protein and the human papillomavirus E6 protein. Finally, although Mdm2 binds to p53 and p73 (Fig. 1), only p53 is degraded as a result of this interaction[2].

Why might p53 alone be a tumour suppressor? There are several possible answers, all supported by published reports[2]. First, the tissue distribution of p63 and p73 is more restricted than that of p53. Second, the downstream targets of p53 and its relatives may differ, and perhaps p53 is more effective in inducing key apoptosis-related targets under some circumstances. Third, there may be a broader range of upstream regulators that can signal to p53. And fourth, interactions between some forms of p53 and its relatives have been documented. For instance, tumour-derived p53 mutants can repress transactivation and apoptosis induced by p73, and amino-terminally-truncated forms of p63 can repress wild-type p53 (ref. 2). So, cross-talk between p53 and its relatives may contribute to their different roles in tumorigenesis.

It has been suggested that p53 is the most recently evolved member of this family. We might speculate that, with the development of more complex multicellular organisms, a need arose for a more versatile stress-response factor — one that can respond to and transmit a more diverse set of signals to a more complex set of targets. Whatever the explanation for the differences among p53 family members, there is much work to be done to find out how these genes regulate important processes in cells, and why only p53 suppresses tumour formation. □

Eileen White is at the Howard Hughes Medical Institute, Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, Cancer Institute of New Jersey, and Rutgers University, 679 Hoes Lane, Piscataway, New Jersey 08854, USA.
e-mail: ewhite@mbcl.rutgers.edu
Carol Prives is in the Department of Biological Sciences, Columbia University, Sherman Fairchild Center for the Life Sciences, 816 Fairchild Building, New York, New York 10027, USA.
e-mail: clp3@columbia.edu

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Evolutionary genetics

No sex please, we're fungi

Ian R. Sanders

According to evolutionary theory, the advantage of sex is that recombination shuffles together new combinations of genes, thereby producing genetic variation and allowing deleterious mutations to be purged[1–3]. But some extremely successful organisms are both asexual and ancient[4]: the very existence of such 'scandalous' asexuals[5] flies in the face of theory. Among them are counted the arbuscular mycorrhizal fungi, the Glomales, studies of the genome structure and organization of which now reveal some remarkable phenomena. The papers concerned appear in *Fungal Genetics and Biology*[6] and *Gene*[7]. Most notably, they identify a striking degree of divergence in the ribosomal sequences of individuals among the Glomales.

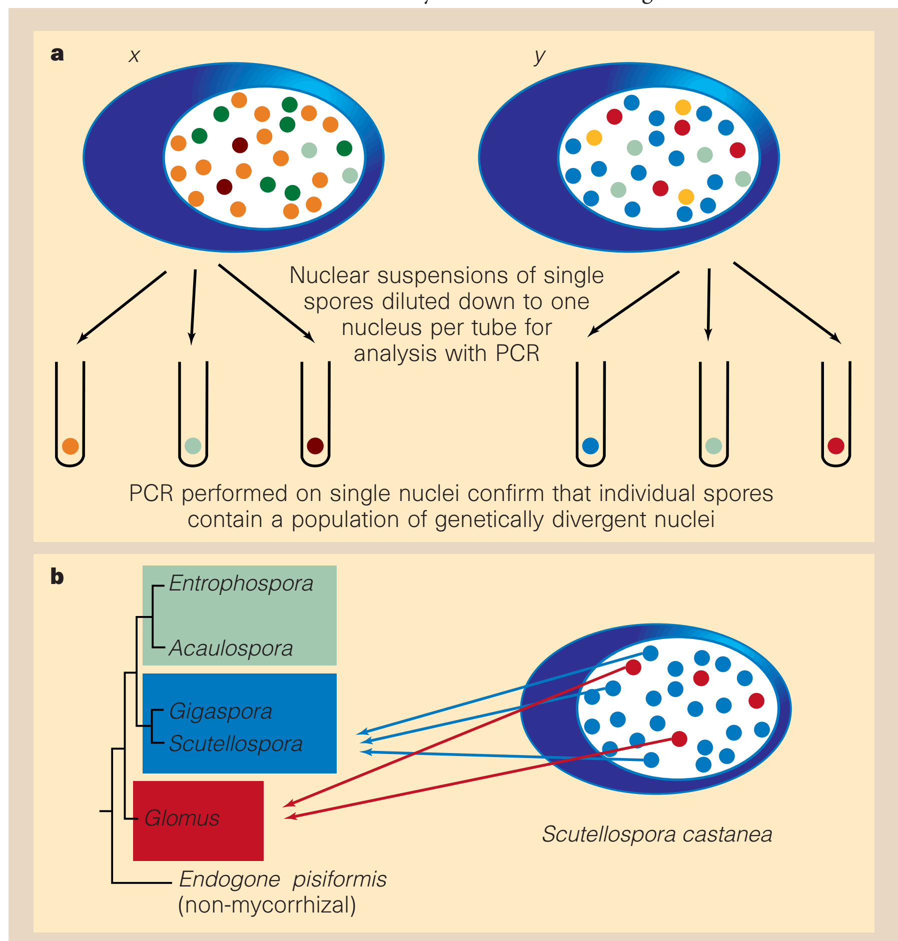

Figure 1 **Genetics of asexual mycorrhizal fungi of the order Glomales.** a, **Hijri *et al.*[6] demonstrate that different spores of *Scutellospora castanea* (*x* and *y*) contain different sequences of ribosomal DNA, but that each spore does not have the same complement of sequences. Further, they show that rDNA differs between nuclei from the same fungal individual, which may be because lack of recombination has allowed the multiple copies of rDNA to diverge.** b, **Hosny *et al.*[7] find that different sequences of the 18S gene from an isolate of *S. castanea* group into two different Glomales genera, *Scutellospora* and *Glomus*. The Glomales consists of three different families: Glomaceae (red), Gigasporaceae (light blue) and Acaulosporaceae (green), and the first two are thought to have diverged 353–367 million years ago[15]. The discovery of rDNA sequences in *S. castanea* that match genera in two different families suggests that genetic diversity in these fungi may have been increased by acquisition of DNA from their ancestors.**

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These fungi form symbioses — mycorrhizas — with plant roots which help plants to acquire mineral nutrients from the soil and also determine plant biodiversity and ecosystem function[8]. They are truly ancient, having remained largely morphologically unchanged since plants first colonized the land around 400 million years ago[9]. No sexual stage in the Glomales life cycle has been observed.

Polymorphism in the ribosomal DNA encoding the internal transcribed spacer (ITS), and the 5.8S and 18S subunits, occurs inside individual spores of fungi of the genus *Glomus*[10,11]. This is unusual because it is widely thought that sequences of multiple copies of ribosomal DNA are kept the same by a process known as concerted evolution[12], in which the joint evolution of two or more related genes occurs as if they constitute a single locus. It is on the assumption of concerted evolution that the ribosomal sequences are so widely used for taxonomy and phylogenetics[13]. The existence of different sequences of ribosomal genes in *Glomus* implies that recombination does not occur, because the mechanisms that keep the sequences of rDNA copies the same operate most frequently during recombination events.

Both of the new studies[6,7] looked at another fungus within the Glomales, *Scutellospora castanea*, and worked with exactly the same isolate[14] which was maintained in culture in the roots of plants. Using the polymerase chain reaction (PCR), six different ITS sequences and 13 of the 18S gene were picked out, respectively, from genomic DNA and from clones in an *S. castanea* genomic DNA library. This does not mean that variation is limited to six different sequences of each. Hijri *et al.*[6] confirmed that several different ITS sequences (including those of the 5.8S gene) occur in a single spore, but that each spore of this isolate does not have the same complement of the different sequences (Fig. 1a).

The Glomales are coenocytes — that is, many nuclei are enclosed within one cell wall — and it could be that different rDNA sequences occur on different nuclei in which the rDNA sequences have diverged due to a lack of recombination. To test this possibility, Hijri *et al.*[6] diluted nuclear suspensions from single spores to an expected one-nucleus-per-sample and performed PCR with specific primers that amplify the different rDNA sequences. Their results indicate that, indeed, different rDNA sequences are carried on different nuclei (Fig. 1a); so it seems that an individual of these fungi is, in genetic terms, actually a population of discrete nuclei.

Perhaps more astounding, however, is just how divergent the ribosomal gene sequences are. Hosny *et al.*[7] carried out a phylogenetic analysis of 18S sequences from *S. castanea* and from species of each of the other Glomales genera. Most sequences grouped in the genus *Scutellospora*, but two others from the same *S. castanea* isolate were so divergent that they clustered in the genus *Glomus* (Fig. 1b).

A contaminant in the genomic library, perhaps? It seems not, because the *Glomus*-like rDNA sequences were successfully amplified again from single spores of this isolate of *S. castanea*. According to previous phylogenetic analyses of the Glomales, the family known as Glomaceae, which contains the genus *Glomus*, diverged from the other mycorrhizal fungi that were later to form the Acaulosporaceae and the Gigasporaceae (containing the genus *Scutellospora*), between 353 and 367 million years ago[15].

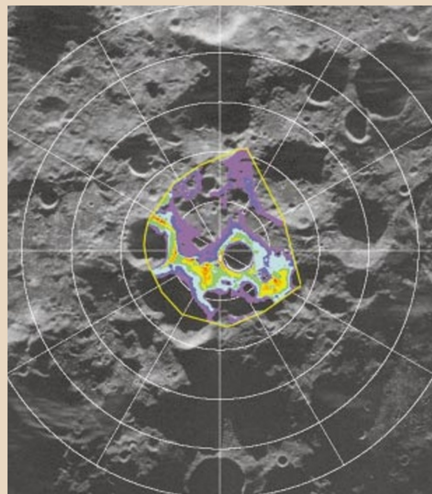
How then can we explain the observations reported in these two studies? The results support the idea that genetic drift — the random change of allelic frequencies in a population — has occurred in the absence of recombination, leading to genetically divergent nuclei. But at the same time it seems

Lunar exploration

Polar endeavours

Almost exactly 85 years ago, Sir Ernest Shackleton set out to cross the Antarctic continent. After his ship was frozen into the ice pack, the expedition waited and worked through the long polar nights, then the long polar days, before Shackleton managed to get them all rescued, a story that continues to generate popular books[1]. In honour of his exploits, Shackleton had a lunar crater (just to the right of centre in this image of the lunar south pole) named after him. That crater is now getting attention as a part of another story that involves ice, long days and nights — and perhaps, ultimately, human exploration.

Lunar scientists have long known that because the Moon's axis of rotation is almost perpendicular to its path around the Sun, the polar regions could have high points that are permanently sunlit, and crater floors that are permanently in shadow. This false-colour image (inside the yellow line, and overlaid on a radar image of the region) shows the percentage of the lunar day for which a given location is illuminated (white, orange and red receive the most illumination, the clear regions receive none), based on a newly reported analysis of images taken in 1994 by the Clementine spacecraft[2]. A separate radar study[3] has confirmed that many regions,



including the bottom of Shackleton Crater, are likely to be permanently shadowed. Conversely, some regions are sunlit most of the time and would be prime locations for lunar bases, or at least for solar arrays to support a polar base. For example, the white region at the left (poleward) edge of Shackleton Crater is sunlit more than 80% of the time, and there is a ridge 10 km away that is sunlit 90% of the time that the first spot is not.

A polar base would be more attractive if the permanently shadowed crater floors have managed to cold-trap water ice, which could be mined for life support or fuel. Data from the neutron spectrometer aboard the Lunar Prospector spacecraft[4] apparently showed abundant hydrogen at the poles, possibly, though not definitely, in the form of water ice; similarly, a Clementine radar experiment may[5] or may not[6] have provided evidence for ice.

At the end of July, with its funding expired and its fuel nearly gone, Lunar Prospector will be crashed into a crater near the pole (the larger crater above and to the right of Shackleton) and Earth-bound telescopes will search for evidence of water released by the impact[7]. The chances of the experiment detecting water are no better than 10%, but this is just the next step in a search for the conditions that might make exploration of the lunar poles possible.

Timothy D. Swindle

Timothy D. Swindle is in the Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721-0092, USA.
e-mail: tswindle@U.Arizona.EDU

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unlikely that the nuclei have also harboured rDNA sequences that have then remained conserved for 400 million years. Probably the most likely way to explain their occurrence is hyphal anastomosis (cross-connection), and the exchange of nuclei. Clearly, more information is now needed on DNA polymorphisms within and among spores of Glomales species from regions of DNA other than the multi-copy ribosomal genes. One possibility is that infrequent recombination occurs among nuclei. Using such sequences as markers in population-genetic models would enable a test of whether nuclei from a single spore form a recombinant population.

Meantime we are left with the distinct possibility that individuals in the Glomales contain a population of highly divergent nuclei that are subject to the accumulation of mutations and additional genetic material from distant sources. How do these fungi cope with the accumulation of deleterious mutations? How can they perform and regulate their cellular, developmental and metabolic processes? Phylogeneticists are faced with equally fundamental questions. Is concerted evolution in fact in operation? Is it valid to apply phylogenetic techniques to these organisms at all?

The evolution of genomes in the absence of recombination is not yet understood. But perhaps the way that these asexual fungi have existed for 400 million years is in some way connected to the maintenance of high genetic diversity in their populations and their ability to have exchanged genetic material with their ancestors. What is clear is that evolutionary biologists and fungal geneticists are faced with a happily disconcerting puzzle. □

Ian R. Sanders is at the Botanisches Institut, Universität Basel, Hebelstrasse 1, 4056 Basel, Switzerland.
email: sanders@ubaclu.unibas.ch

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Alzheimer's disease

Pinning down phosphorylated tau

Michel Goedert

A defining characteristic of several neurodegenerative diseases, including Alzheimer's disease and Pick's disease, is the formation of filamentous deposits of a microtubule-associated protein called tau in an abnormally hyperphosphorylated form. The discovery of mutations in the *tau* gene in a condition known as 'familial frontotemporal dementia and parkinsonism linked to chromosome 17' has renewed interest in the mechanisms by which dysfunction of tau causes neurodegeneration.

On page 784 of this issue, Lu *et al.*[1] describe an intriguing interaction between phosphorylated tau and a prolyl isomerase, Pin1. Prolyl isomerases enhance the rate of *cis* to *trans* isomerization of the peptide bond on the amino-terminal side of proline. Pin1 is an essential nuclear protein belonging to the parvulin family of prolyl isomerases[2]. This group is distinct from two other prolyl isomerase families, the cyclophilins and the FK506-binding proteins, which are targets of the immunosuppressive drugs cyclosporine and FK506, respectively. Pin1 consists of a carboxy-terminal catalytic domain, as well as an amino-terminal protein–protein interaction region called a WW domain that specifically recognizes phosphorylated serine or threonine residues preceding a proline residue (the S/T–P motif)[2,3].

In its short history, Pin1 has generated much interest because it regulates entry and progression through mitosis. It does this by interacting with a large set of mitosis-specific phosphoproteins, most of which can be detected by a monoclonal antibody called MPM2 (ref. 3). Lu *et al.*[1] started off armed with the knowledge that MPM2 also recognizes hyperphosphorylated tau in the brains of people with Alzheimer's disease[4,5]. Moreover, during mitosis, tau is phosphorylated at a number of the S/T–P sites that are hyperphosphorylated in Alzheimer's[6,7]. This prompted the authors to examine whether phosphorylated tau interacts with Pin1. And they found that tau (phosphorylated either by a mitotic cell extract or by Cdc2 kinase) does, indeed, bind to the WW domain of Pin1.

The longest isoform of tau in the human brain has 17 S/T–P sites. Of these, Lu and colleagues found that only one — phosphorylated threonine 231 (T231) — was required for the interaction with Pin1. This residue is located upstream of the microtubule-binding repeats in a proline-rich region that is required for full activity of tau. The T231 residue is hyperphosphorylated in Alzheimer's disease and is also phosphorylated, to a certain extent, in the normal brain[8,9]. This residue can also be phosphorylated by glycogen synthase kinase-3β, but only after phosphorylation of serine 235 by cyclin-dependent kinase-5 or mitogen-activated protein kinase[10,11].

Lu and colleagues went on to gather more evidence for the interaction between tau and Pin1. First, using a pull-down assay, they showed that Pin1 binds to hyperphosphorylated tau from the brains of people with Alzheimer's disease, but not to tau from age-matched healthy brains. Tau from normal brain is notorious for the speed with which it is dephosphorylated after death[12], so it may be premature to conclude that Pin1 interacts only with hyperphosphorylated (pathological) tau.

Second, by immunoblotting, the authors detected endogenous Pin1 in the paired helical filaments (PHFs) from diseased brains. (The PHFs, which are composed of hyperphosphorylated tau, make up the pathological neurofibrillary tangles of Alzheimer's disease.) Third, using immunohistochemistry, Lu *et al.* found that recombinant Pin1 binds to pathological tau. Finally, the authors looked at localization of Pin1. In control brains they observed nuclear staining for endogenous Pin1, consistent with its known localization in non-neuronal cells. But in the brains of people with Alzheimer's disease, Pin1 staining was also associated with pathological tau in neuronal cells (although it is not clear what percentage of the tau-positive structures was also immunoreactive for Pin1).

The tau protein in PHFs from the brains of patients with Alzheimer's disease is phosphorylated at more than 20 residues, many (but not all) of which are S/T–P sites[13]. In healthy brains, tau is heterogeneously phosphorylated at between eight and ten of these residues[9]. Because of its abnormal hyperphosphorylation, tau from PHFs cannot bind to microtubules or promote microtubule assembly. Hyperphosphorylation of tau is believed to be an early event that precedes assembly into PHFs. Yet there is no experimental evidence linking hyperphosphorylation of tau to PHF assembly[14] — synthetic, paired helical-like filaments can, in fact, be produced in a phosphorylation-independent way.

To examine the functional effects of the interaction between Pin1 (Fig. 1, overleaf) and tau phosphorylated at T231, Lu *et al.* used recombinant tau phosphorylated by Cdc2 kinase, with or without added Pin1. As expected, phosphorylated tau could neither bind microtubules nor promote microtubule assembly properly. But in the presence of Pin1, biological activity of the phos-

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