Presidential Address

New angles in mycology: studies in directional growth and directional motility[1]

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Mycology is changing as an era of extensive genome sequencing comes of age and provides vital information that enables questions to be addressed about fungi in all the major taxonomic groups. As technology transfer facilitates what was once only possible for a very small number of model species, it becomes possible to explore the biology and biodiversity of fungi as a whole. The availability of genome sequence information and reverse genetic technologies allows hypotheses that emerge from biological observations to be tested. Genomic and post-genomic technologies will underline the importance of fungi as excellent models for the study of fundamental biological phenomena. Two enduring areas of research in my own laboratory are described that are now being extended using post-genomic approaches. These projects relate to how fungal hyphae extend and guide their tips and secondly how plant pathogenic oomycete zoospores are guided on their journey to the plant surface.

Fig. 1. Neil A. R. Gow. President 2001–2003.

NEW DIRECTIONS IN MYCOLOGY

My impressions of our discipline from the viewpoint of the activities of the British Mycological Society, recent publications, and scientific meetings and symposia over the last two years, is that we should be optimistic about the future of mycology. It must be acknowledged that mycology is a smaller discipline than some of its sister microbiological subjects, like bacteriology, virology, parasitology, etc., and it has never enjoyed any preferential treatment – nor should it. Despite representations that mycology needs specific investment, we have to compete for funds on a level playing field with other areas of biology and we succeed only by the efforts, status and quality of research by mycologists. Mycology is also changing in its perspective, reinventing itself, and that is a healthy development that will sustain its vitality. Protozoology was, not so long ago, a much smaller community than it is today. However, the massive global problems of parasitic diseases such as malaria, sleeping sickness and shistosomiasis coupled with modern methodolgies, genomics and molecular biological tools has led to huge investment of resources and an increase in both the interest and quality of work in this area. There is no reason why mycology should not also now cash in on the biodiversity and biological importance of fungi and see a similar renaissance of interest and financial support. However, to do this, the discipline will have to embrace the opportunities that

Table 1. Fungal and straminipile genome projects, funded, underway or completed.

Organism	Phylogenetic group	Primary significance
Rhizopus arrhizus	zygomycete	industrial
Ashbya gossypii[a]	ascomycete	industrial/model system
Aspergillus nidulans	ascomycete	food
A. fumigatus	ascomycete	human pathogen
A. flavus	ascomycete	mycotoxin producer
A. niger	ascomycete	industrial
A. oryzae	ascomycete	industrial
A. fischeri	ascomycete	occasional pathogen
A. fennelliae	ascomycete	mycotoxin producer
A. clavatus	ascomycete	occasion pathogen and toxin producer
Candida albicans[a]	ascomycete	human pathogen
C. dubliniensis	ascomycete	human pathogen
C. glabrata	ascomycete	human pathogen
Coccidioides immitis	ascomycete	human pathogen
Debaromyces hansenii	ascomycete	saprophyte, biological control agent
Hansenula poymorpha	ascomycete	biotechnology expression system
Histoplasma capsulatum	ascomycete	human pathogen
Kluyveromyces lactis	ascomycete	biotechnology
Magnaporthe grisea	ascomycete	plant pathogen
Neurospora crassa[a]	ascomycete	model system
Pneumocystis carinii	ascomycete	human pathogen
Saccharomyces cerevisiae[a]	ascomycete	model system/industrial
Schizosaccharomyces pombe[a]	ascomycete	model system
Trichoderma reesei	ascomycete	industrial – enzyme production
Paracoccidioides brasiliensis	ascomycete	human pathogen
Yarrowia lipolytica	ascomycete	model polymorphic fungus
Coprinopsis cinereus	basidiomycete	model system
Cryptococcus neoformans serotype A	basidiomycete	human pathogen
C. neoformans serotype B	basidiomycete	human pathogen
C. neoformans serotype D (2 strains)	basidiomycete	human pathogen
Phanaerochatae chrysosporium	basidiomycete	industrial
Schizophyllum commune	basidiomycete	wood rotting fungus and model system
Ustilago maydis[a]	basidiomycete	plant pathogen
Phytophthora infestans	oomycete	plant pathogen
P. sojae	oomycete	plant pathogen

[a] Completed whole genome projects. The genome projects listed above are being carried out in genome sequencing centres around the world. Further information can be obtained simply by entering the name of the fungus in any web search engines. At the time of writing the *Aspergillus nidulans* and *A. fumigatus* genome projects are near completion. Most of these genomes aim to obtain sequence information representing between 3X to 10X coverage. The Fungal Genome Initiative at the Whitehead Center for Genome Research at M.I.T. See (http://www-genome.wi.mit.edu/seq/fgi/condidates.html) (http://www-genome.wi.mit.edu/annotation/fungi/fgi/nominated.html) for lists of genomes that have been suggested and prioritised for sequencing. This site invites new fungal genomes for consideration for sequencing by the research community. Additional non-public genome data bases exist that may eventually enter the public domain.

are presented to it and consequently mycology may look quite different in the future. No doubt old traditions will be diluted, but exciting new directions can already be seen to be taking the subject forward. At this time there are more than 30 genome projects underway or complete (Table 1) representing fungi from all the major groups (Bennett & Arnold 2001). This huge resource will make it easier to find genes of interest in related species that do not have their own genome database. I am not advocating that genomics is in any way sufficient to ensure a new platform for mycology, but it is self-evident that the availability of this type of information is an excellent way to short-circuit the painfully slow process of finding genes in organisms of interest. Gene sequences only facilitate understanding of biology and they do not necessarily in themselves generate good questions, hypotheses, or good science. Clear hypothesis-driven research is not encoded in the gene sequences, but it can surely take advantage of

them. The scientific meetings I have attended this year that were hosted by or involved the British Mycological Society pulsated with exciting developments, often in previously neglected fungal species, and only the most determined pessimist could attend these and worry about the future. It is more likely that we are entering a golden era of mycology, in which one of the great-untapped resources of biodiversity and some of the most genetically amenable and biologically important and interesting species will come to the fore. We will continue to learn from model fungi such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Neurospora crassa*, *Ustilago maydis*, and other species. If we combine this information with genomics, functional analysis, mathematical, biophysical, and structural biology, then we can enjoy new and exciting opportunities to explore the biology of many species of fungi that have much to offer but have, in the past, been inaccessible to systematic analysis.

My own group's work has focussed on a limited number of fundamental questions for many years, but the approaches we have used to address these questions have changed. In particular the application of molecular genetics and genomics to these questions has been vital. A central theme to this work has been how directionality of fungal growth is achieved. We have studied these phenomena in fungi that elaborate hyphae and in straminipiles ('oomycetes'), which liberate swimming zoospores that swim towards and infect plants.

ZOOSPORES AND DIRECTIONAL SWIMMING

Jim Deacon (University of Edinburgh) introduced me to zoosporic organisms, and his enthusiasm for them has never left me. These cells erupt dramatically and rapidly from sporangia in a time frame that enables the whole process to be readily observed under the microscope. They then swim remarkably quickly, at over 100 μm s^{-1}, seeking out a landing site for further development through the process of encystment and germination. All species of *Phytophthora*, and many of *Pythium*, are plant parasites of massive economic importance (Gow *et al*. 1999, van West, Appiah & Gow 2003). Their zoospores are agents for dispersal and the location of new plants to infect. These organisms are straminipiles, which in truth are taxonomically within the golden algae (*Chrysophyta*) and are very distantly related to *Fungi*, although they generate hyphae, mycelia, and other structures that resemble the vegetative and reproductive structures of fungi (van West *et al*. 2003).

One question that has interested my group is one that many have considered to be self-evident. However, our data have suggested a surprising result. How do zoospores locate a new plant and what cues guide them to the plant surface? The answer that has been in the text books for more that 30 years is that they employ chemotaxis to home in on nutrient gradients that exist around plant roots and other plant surfaces (e.g. Zentmyer 1961). We have focussed our work on the root as a convenient target for plant pathogenic *Phytophthora* and *Pythium* species. Clearly the notion that such organisms use chemotaxis to find a root is an entirely reasonable hypothesis since plant roots are well known to be the site of effusive nutrient exudation. Moreover, many microorganisms are well-known to have sophisticated arrays of chemoreceptors and signalling mechanisms that result in alteration of flagellation to achieve directional swimming. The classic example is in bacterial chemotaxis, for which we have a detailed knowledge of the biochemistry and physiology of chemotaxis as regulated by protein methylation and phosphorylation (Webre, Wolanin & Stock 2003).

In the context of zoospores, however, there were some observations that did not seem to fit this viewpoint. For example, the site of maximum nutrient exudation occurs in the meristematic region and zone of elongation of roots, behind the root cap. However, not all zoospores are attracted to this region, indeed some are actively repelled from it (Zentmyer 1961, Hickman & Ho 1966, Miller, Shand & Gow 1988, Gow 1993, Gow *et al*. 1999). Other positive evidence for chemotaxis came from direct experimentation in which capillaries were loaded with single nutrient sources or with a cocktail of individual nutrients or plant exudates. The result was that the zoospores accumulated at the mouth of the pipette or source of nutrients. There is no doubt that chemotaxis is partly responsible for this accumulation, but it is apparently not the whole answer. Unlike bacteria, zoospores become quickly immobilised and encyst when they encounter a changing environment. Nutrients and ions such as Ca^{2+}, and other compounds (Gow *et al*. 1999) all cause them to shed their flagella and generate an adhesive cyst wall in preparation for germination. Therefore, immobilisation of zoospores can give the appearance that their accumulation was achieved *via* chemotaxis. In addition, we have shown that aggregates of zoospore cysts can attract swimming zoospores, in what appears to be a signal relay system similar to that in other organisms such as *Dictyostelium discoideum*. This is a species-specific phenomenon, with cyst aggregates of one species attracting zoospores of the same type, but not of another (Reid, Morris & Gow 1995). There is little evidence for any specificity in zoospore chemotaxis (Deacon & Donaldson 1993), as is the case with many, but not all, bacteria. Instead, they seem to be attracted to all roots and most nutrients, so there is little help here in understanding why they accumulate only at specific parts of the root. Therefore, chemotaxis is at best an incomplete explanation for the observations that different zoospore species are attracted to very specific and differing sites of plant root surfaces. Are there any other explanations that suit the data better?

As a postdoctoral scientist I trained with Franklin Harold in the field of fungal electrophysiology, applied to the area of cell polarity in straminipile and fungal hyphae (Gow 1984, Gow *et al*. 1984, Kropf *et al*. 1984, Gow & Gooday 1987, McGillivray & Gow 1987). This work exploited the insights and technological inventions of Lionel Jaffe who had devised and fabricated ultrasensitive vibrating electrodes that enabled ionic circulations to be mapped around cells and tissues of a wide variety of types (Jaffe & Nuccitelli 1974, Jaffe 1981, 1985). These vibrating electrodes are sensitive on the nanovolt scale and represent the equivalent of an electron microscope in the voltmeter world. Our work, and others on fungal hyphae, showed that this circulation was ever-present at the tips of growing hyphae, but that it was not relevant to the process that polarises tip growth (Schreurs & Harold 1988, Youatt, Gow & Gooday 1988, Harold 1990, De Silva *et al*. 1992). However, I was struck by the fact that all living tissues that had been examined with these electrodes generated

ionic circulations and therefore extracellular and intracellular electrical fields through which the ionic circuit flowed (Jaffe 1981, 1985). Roots were no exception (Weisenseel, Dorn & Jaffe 1979, Behrens, Weisenseel & Sievers 1982, Miller *et al.* 1988, Miller & Gow 1989a, Hamada *et al.* 1992). Indeed, the size of the electrical fields generated by roots is quite large, in the region of 5–100 mV cm^{-1}, which is much higher than that around a fungal hypha. An obvious experiment was to attempt to assess whether zoospores were electrotactic. Using chambers that allowed electrical fields to be applied without generating potential artefacts due to toxic electrode products, we showed that zoospores are indeed electrotactic in electrical fields that were comparable in strength to those generated by plant root (Morris, Reid & Gow 1992, Morris & Gow 1993). Some species, such as *Phytophthora palmivora*, had zoospores that swam towards the positive anodic pole, while others, such as *Pythium aphanidermatum*, produce cathodotactic zoospores (Morris & Gow 1993). Since we had the electrophysiological means to determine where the anodic and cathodic parts of plant roots were to be found, we could readily test the hypothesis that this endogenous root-generated electrical field influenced the zoospore accretion pattern.

We showed that anodotactic zoospores were attracted to anodic sites of roots and reciprocally that cathodotactic zoospores were attracted to cathodic sites but repelled from anodic sites (Fig. 2). It is not a perfect correlation, but a much better one than that offered by invoking chemotaxis. We were also able to show that induced or natural changes in the electrical field around a root predicted changes in where zoospores accumulated (van West *et al.* 2002). For example, treatment of roots with the toxin fusicoccin (which alters electrogenic ionic transport at the root surface) led to alterations in the electrical field and at the sites of *P. palmivora* and *P. aphanidermatum* accretion (van West *et al.* 2002). We also used focal electrical fields passed through a micropipette close to the root surface and showed that applied electrical field overwhelmed the local cues and recruited zoospores to surfaces that were otherwise repellent. Finally, we were able to take advantage of our first successes in molecular genetics of *Phytophthora*, in which we had successfully transformed *P. palmivora* with GFP (van West *et al.* 1999b). This provided a fluorescently-tagged strain that could be distinguished from unlabelled zoospores of *P. aphanidermatum* that were co-inoculated in liquid medium around a growing root. We then observed the spatial segregation of two populations of zoospores at the root surface; two species experiencing the same nutrient gradients, but accumulating at different places (Fig. 2). Our work, therefore, points to the surprising conclusion that these swimming cells take advantage of the electrical fields generated by the roots to enable a directional response to be mounted towards living plants (van West *et al.* 2002). Therefore, the homing response of zoospores is due to

multiple mechanisms, that include electrotaxis, chemotaxis, and induced encystment, and is not simply related to taxis in gradients of root exudates.

In the last year, 100 000 expressed sequence tags (ETSs) from *P. infestans* cDNAs have been sequenced by a Syngenta (Torto *et al.* 2003), and a genome sequencing project was announced in the same week this article was completed. The new direction for our work in this area is to take advantage of this information to identify genes that encode zoospore-specific functions and functions that relate to the swimming, encystment, and germination of *Phytophthora* zoospores. Functional analysis of *Phytophthora* genes is now possible (van West *et al.* 1999a, 2003, Shepherd, van West & Gow 2003) using gene silencing and other post-genomic technologies such as proteomics, and so we are now well-positioned to investigate these vitally important processes at the molecular level.

HYPHAE AND DIRECTIONAL GROWTH

A second area of directional growth is the problem of how hyphal growth is orientated during normal and tropic growth. Graham Gooday instilled in me the importance of physically watching your organism grow and divide under the microscope. My abiding experience as a research student was in carrying out time-lapse analysis of the growth of hyphae of the human pathogenic fungus *Candida albicans* in a hot room for half or whole day stretches (Gow & Gooday 1982). When I started working on *C. albicans*, almost nothing was known about its genetics. But, from the clinical point of view, it was already becoming apparent that changing medical practice was resulting in more and more patients undergoing treatments that opened the door for opportunistic fungal pathogens such as *C. albicans*. Consequently, research in this organism was gathering momentum and receiving significant investment.

The ability of *C. albicans* to undergo a reversible transition between yeast cells and more or less elongated pseudohyphal, and unconstricted true hyphae had been a source of attention for medical mycologists for many years (Gow 1997). It still is, and is likely to remain, a hot topic for years to come. It is astonishing how much progress has been made in the analysis of this phenomenon in recent years (Berman & Sudbery 2002, Calderone 2002). The *C. albicans* genome revealed a mating type locus which led first to the engineering of mating-compatible strains, and subsequently to the recognition of natural mating between compatible mating types of natural isolates (Hull & Johnson 1999, Hull, Raisner & Johnson 2000, Magee & Magee 2000, Miller & Johnson 2002). This serves as an example of how genomic information is revolutionising mycology. Lessons learnt from *Saccharomyces cerevisiae* have added to the speed of progress in *C. albicans*, but many *Candida*-specific projects have in turn fed back and taught us things about *S. cerevisiae*. My own
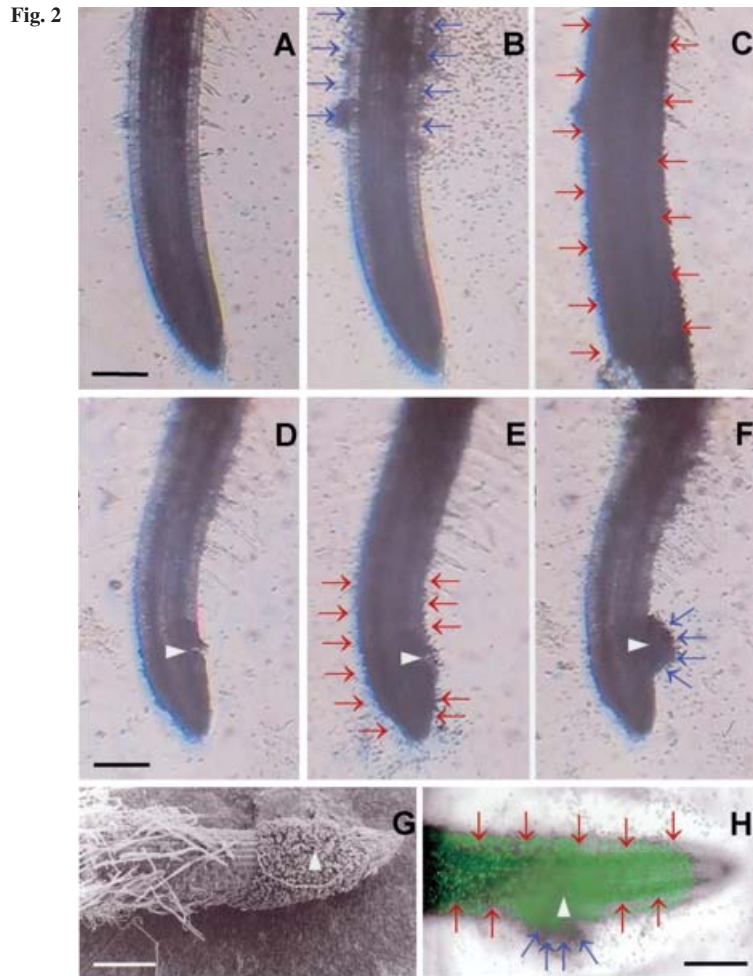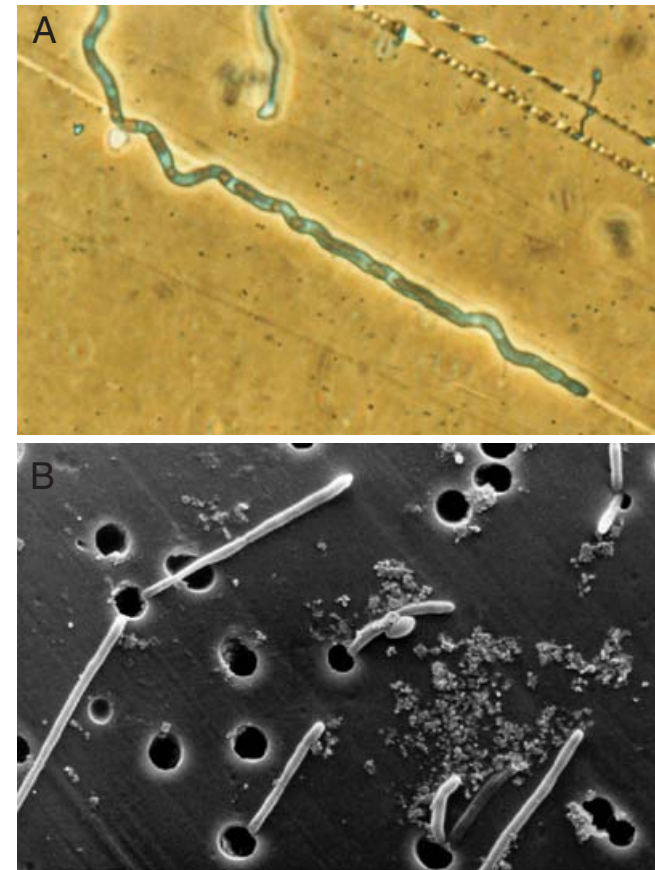
Fig. 2. Correlation of the pattern of zoospore accumulation around roots of rye grass and the local electrical field. The blue arrows indicate where zoospores of *Pythium aphanidermatum* accumulate and the red arrows indicate the accumulation of zoospores of *Phytophthora palmivora*. Zoospores of *Py. aphanidermatum* are cathodotactic while those of *Ph. palmivora* are anodotactic in exogenously applied electrical fields. In (A) the anodic (+) and cathodic (−) sites as measured with a voltage-sensitive vibrating microelectrode is shown. (B) and (C) are the same root, that was first exposed to (B) *Py. aphanidermatum* zoospores, (C) then cleaned and exposed to *Ph. palmivora* zoopores. In (D–F) zoospores of *Ph. palmivora* are first applied to a wounded root (white arrow head), then the root was wiped clean and exposed to zoospores of *Py. aphanidermatum*. (G) A SEM showing localised accumulation of *Py. aphanidermatum* zoospores around a wounded root. (H) Spatial segregation of zoospores of mixed populations of *Py. aphanidermatum* and *Ph. palmivora* zoospores, the latter being transformed with a plasmid containing green fluorescent protein (GFP). Bar = 100 μm. Reproduced from van West *et al*. (2002), with permission.

Fig. 3. Thigmotropism of hyphae of *Candida albicans* growing (A) on a scratched membrane and (B) on a Nuclepore membrane where hyphae are emerging from pores then reorientating to grow along the membrane surface.

research in *C. albicans* has been in several areas, including the study of chitin synthesis (Gow *et al.* 1994a, Munro *et al.* 2001, 2003, Munro & Gow 2001), cell wall glycosylation (Buurman *et al.* 1998, Thomson *et al.* 2000), virulence (Hube *et al.* 1997, Gow *et al.* 2003), molecular systematics (Nino-Vega *et al.* 2000, Tavanti *et al.* 2003) and the cell cycle and process of vacuolation (Barelle *et al.* 2003). Relevant to this article, however, is a study that started with a chance observation made while observing cells growing down the microscope.

In order to perform time-lapse analysis we had been growing *C. albicans* hyphae on the top of and under membranes, in order to generate flat surfaces on which growth could be observed (Sherwood *et al.* 1992, Gow 1994, Gow *et al.* 1994b, Sherwood-Higham *et al.* 1995, Perera *et al.* 1997, Watts *et al.* 1998). We observed what many plant pathologists already knew – the phenomenon of thigmotropism (contact-guidance), in which hyphal growth occurs in relation to underlying surface contours on the substratum (Fig. 3). Plant pathogens use this tropic response on leaf and shoot surfaces to grow along or across cortical grooves to find stomata, though which they infect via the induction of appressoria (Allen *et al.* 1991, Zhou *et al.* 1991, Read *et al.* 1992). Thigmotropism is, however, a property of a wide variety of cell types including, for example, pollen tubes, plant roots, neurites, and other cells that have to grow on or between other cell layers. We reasoned that thigmotropism in *C. albicans* may accomplish the goal of allowing the hyphae to invade human tissue at sites of weakened surface integrity or between the cells of epithelia or endothelia. In fact, it is rather difficult to observe exactly how hyphae navigate in living tissues and they exhibit a number of breaking-and-entry strategies including direct penetration into cells, and induction of their own phagocytosis, as well as growing between cell layers (Gow, Brown & Odds 2002, Gow *et al.* 2003). However, molecular genetics in *C. albicans* is now sufficiently advanced that these phenomena can now be investigated using a full repertoire of cell biological and molecular biological tools.

We showed that not only do hyphae bend when they encounter a grove or pore, but that growth on surfaces can also induce a helical pattern of hyphal development (Sherwood *et al.* 1992, Gow *et al.* 1994b, Sherwood-Higham *et al.* 1995, Perera *et al.* 1997, Watts *et al.* 1998). Since all these changes in growth direction have to be regulated by the cytoskeleton, we have tried to investigate the underlying mechanisms that regulate directionality of growth from growth *per se*.

Relevant to this is another tropic response of hyphae that my group had described previously. When fungi are grown in an imposed electrical field they become aligned (McGillivray & Gow 1986, Crombie, Gow & Gooday 1990, Lever *et al.* 1994). A somewhat larger field has to be applied that that required to induce zoospore electrotaxis, but these hyphae still grow and branch happily while experiencing an electrical field. Galvanotropism has been observed in fungi from all

the major groups (s) and a clue about the mechanism came from the observation that the response was markedly attenuated in media that lacked calcium ions (Lever *et al.* 1994). This is particularly relevant because in other cell types, such as pollen tubes (Malhó *et al.* 1994, Malhó, Camacho & Moutinho 2000), *Neurospora* hyphae (Lorelei *et al.* 2002) and nerve cells (Davenport & Cater 1992), calcium-ion gradients have been observed associated with the regulation of tip-growth and cell orientation. In nerve cells calcium-indicating dyes showed that applied electrical fields induced a local calcium ion wave that correlated with changes in direction of the growth cone (Onuma & Hui 1988, Bedlack, Wei & Loew 1992, Davenport & Cater 1992).

In relation to thigmotropism, it was therefore possible that changes in cell orientation also involved local changes in calcium ion transport at the membrane surface. In *Uromyces appendiculatus* it had been shown that the cell membrane had stretch-activated (SA) cation channels that may serve as sensors for thigmotropic responses (Zhou *et al.* 1991, Gow 1993). In collaboration with Julia Davies, we were able to make patch-clamp recordings of cell membranes of yeast and hyphae of *C. albicans* and to show that these too had such SA channels. Organic and inorganic blockers of SA and voltage-activated calcium channels also attenuated the thigmotropic and galvanotropic responses (Watts *et al.* 1998, Gow *et al.*, unpubl.). Therefore, it seems as though both thigmotropism and galvanotropism are calcium-requiring responses in which calcium ion channels may serve as sensors of environmental cues such as topography and electrical fields. Most recently we have identified the genes encoding the calcium ion channel complex (Cch1/Mid1) in *C. albicans* and have generated isogenic null mutants to determine their influence on these processes. These mutants are attenuated in both thigmotropism and galvanotropism (Gow *et al.*, unpubl.). We have also begun to dissect out the underlying signalling mechanism by examining the behaviour of various signalling mutants in galvanotropism and thigmotropism assays. These ongoing experiments suggest that the mechanism of hypha orientation can be separated from that that is required simply to extend the apex.

SUMMARY

Both these studies on directionality of fungal responses started with basic observations, then pursued the mechanism at increasingly molecular levels to try to gain a foothold on the underlying biology. Neither straminipiles nor *C. albicans* is particularly easy to work with at the molecular level, and all the molecular tools that exist for their analysis have been developed within the period of my career working with these organisms. Therefore, they are examples of what should be a common feature of mycological research in the future, namely that generic and transferable

tools and technologies will be applied to new organisms that had previously been recalcitrant to investigation at the molecular level. The opportunity to explore fungal biology at a different level provides ample opportunity for exiting new science. This revolution is already underway. For example, it seemed inconceivable, only a few years ago, that the asexual fungus *C. albicans* studied for 100 years by so many people would finally be made to mate (Gow, Odds & Brown 2000, Hull *et al.* 2000, Magee & Magee 2000, Gow 2002, Miller & Johnson 2002) or that we would be learning about the complete pattern of gene expression of cells that had been phagocytosed by macrophages (Lorenz & Fink, 2001). Ten years ago, when Graham Gooday gave his Presidential Address to the Society, he summarised what was known about the biochemistry of the 'the chitin synthase enzyme' (Gooday 1995). We now know some fungi have as many as ten enzymes encoded in their genomes (Miyazaki & Ootaki 1997, Chitinis *et al.* 2002), and much is to be done in dissecting their individual roles in yeasts and filamentous fungi. These are just a few examples close to my own interests where genomics is setting the stage for mycological research in the future. The advances are equally impressive in other areas of mycology from systematics, evolution, and ecology to cell biology. Therefore, I find it easy to justify my optimism that there will be no shortage of new directions for mycologists in the future.

ACKNOWLEDGMENTS

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