First publ. in: The New Phytologist 173 (2007), 3, pp. 458-462
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Impact of genomics on fungal biology

IXth International Fungal Biology Conference & 16th *New Phytologist* Symposium Nancy, France, September 2006

Fungi represent an extremely diverse and complex class of organisms, and their categorization as 'lower eukaryotes' should by no means be mistaken as meaning low-end. At present, fungi serve as model systems for various aspects of molecular and cellular biology, for example cell cycle regulation, intracellular signaling, metabolic pathway analysis and transcriptional regulation (Feldbrügge *et al.*, 2004; Jiang, 2006; Oliver, 2006). They are also increasingly used on an industrial scale in the production of chemical compounds or in bioremediation (Grimm *et al.*, 2005; Tortella *et al.*, 2005). Some of the most recent and exciting advances within the field of fungal biology have been linked with genomic studies. To explore these, the IXth International Fungal Biology Conference & 16th *New*

Phytologist Symposium entitled 'Impact of Genomics in Fungal Biology' was held in Nancy, France (http://www.newphytologist.org/fungal-genomics/default.htm). The meeting brought together nearly 100 scientists, from all areas of fungal research, and highlighted a wide range of impacts that genome sequencing has and will have on our understanding of fungal biology.

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The diversity of fungi

First, it is important to emphasize just some of the important features of fungi. They exhibit a broad spectrum of shapes and sizes, and whilst most are characterized by the formation of hyphae exhibiting the characteristic tip growth, there are examples of fungi living as single cells (Wendland, 2001). There are even representatives that are able to switch between the different growth forms upon receiving specific molecular signals (Feldbrügge *et al.*, 2004). In terms of size, fungi range from single cells, of only a few micrometres in diameter, to the largest organism found on earth, belonging to an *Armillaria* species covering almost 9 km^2 (Ferguson *et al.*, 2003). As if this diversity is not enough, fungi are also characterized by a wide variety of different lifestyles, living solitarily or in association with other organisms in neutral, parasitic, or mutualistic relationships (Alexopoulos *et al.*, 1996). The most recognized association is mycorrhizal symbiosis, with > 90% of all plant species engaged in some kind of mycorrhizal relationship with fungi (Allen *et al.*, 2003). Adaptation to the different lifestyles might be the reason why so many fungi have acquired very specific pathways for metabolizing or producing different chemical compounds. Some 70 000 species of fungi have been described to date. However, estimates range up to 1.5 million for the total number of existing fungal species (Alexopoulos *et al.*, 1996). This means that, at present, we are only working with a small selection of the fungal species that are in existence, leaving plenty of room for the discovery of novel pathways and compounds, new regulatory elements or control elements, and so forth.

A brief history of fungal biology

Fungi have been used as a source of food and medicine throughout history: for example, *Saccharomyces cerevisiae* in the preparation of bread, beer and wine, and *Penicillium* for its antibacterial effects. Scientific research on fungi began with the introduction of the microscope in the 17th century and for a long time it was merely descriptive. Nevertheless, this created the starting point for our work with fungi today, and microscopy, in all its different variations, is still a very important tool. Another aspect that powered fungal research was the fact that many economically important plant diseases threatening animal and human food supplies are of fungal origin. A more recent discovery, in historic terms, was the growth-promoting effect of certain mycorrhizal fungi on almost all land plants. There has been a constant evolution of methods used to study fungi and consequently our views and understanding of fungal biology has changed over the years. While cytology was largely superseded by biochemical approaches towards the end of the last century, biochemistry now seems to be increasingly replaced by molecular, and more recently genomic, methods. The availability of some 50 fungal genomes, with about the same number 'in progress', paves the way for completely new scientific approaches as well as for improving traditional methods.

The far-reaching effects of genomics

Some of the highlights of the meeting in Nancy were the presentations, talks and posters dealing directly with recently completed genome analyses. Francis Martin (INRA, Nancy, France), for example, reported on the completion of the first genome of a fungus (*Laccaria bicolor*) engaged in a symbiotic interaction with a host plant. Of the approx. 20 000 genes identified to date, only *c.* 50% have been found to exhibit homology to existing database entries. It is therefore tempting to speculate that at least some of these 'novel' genes might be involved in establishing and/or maintaining the symbiotic relationship. One aspect discussed was the subset of genes whose products are likely to be secreted. In fungi living in close association with other partners, for example symbionts such as *L. bicolor*, but also obligate biotrophic parasites, such as the rust fungi, these secreted proteins establish the primary and most intimate contact between the two interaction partners. It will therefore be highly interesting to follow up on these secretome analyses. *L. bicolor* (Francis Martin), and *Magnaporthe grisea* (Ralph Dean, NCS University, Raleigh, NC, USA), for example, are estimated to secrete 10–15% of gene products. This is considerably more than the 5% secreted gene products reported for *Neurospora crassa*. However, it remains to be seen which direction further analysis of the sequence data, and experiments, will take. Daniel Ebole (Texas A & M University, College Station, TX, USA) reported on an 'in laboratory' approach to test the activity of proteins secreted from *Magnaporthe oryzae*. It might well be that *M. grisea*/*M. oryzae* will soon become a plant pathologist's alternative to the model fungus, *N. crassa*.

The usefulness of *Magnaporthe* as a model system was nicely demonstrated by both Nick Talbot (University of Exeter, Exeter, UK) and Marc-Henri Lebrun (CNRS-Bayer Cropscience, Lyon, France). Nick Talbot presented results

indicating that targeted mutagenesis of the *MgATG8* gene prevented conidial cell death and rendered the fungus non-pathogenic, clearly demonstrating a link between autophagic cell death and pathogenicity (Veneault-Fourrey *et al.*, 2006). Recognition of the pathogen by the host plant was the topic presented by Marc-Henri Lebrun. Most fungal *AVR* genes encode small peptides secreted into host tissues during infection; however, *ACE1* from *M. grisea* was identified as a large cytosolic polyketide synthase/nonribosomal peptide synthetase (Böhnert *et al.*, 2004). He presented evidence that, in this case, a metabolite from the metabolic pathway defined by *ACE1* is the reaction partner recognized by the *Pi33* resistance gene product. These presentations documented well the suitability of *Magnaporthe* as a model system. However, the mutant collection available for *N. crassa,* reported on by D. Ebole, might still give *Neurospora* a cutting edge advantage for heterologous expression studies.

In contrast to this work, a completely different aspect of large-scale sequence analysis was presented by Antonis Rokas (The Broad Institute, Cambridge, MA, USA). He reported on the use of the currently available eight *Aspergillus* genomes in the examination of *Aspergillus* evolution at a structural and a sequence level. No other genus currently provides such a broad spectrum of available sequences. Another interesting talk was that presented by Stephen Oliver (University of Manchester, Manchester, UK), who gave an outlook on the future development of functional genomic analyses and linking these to metabolic control analysis (Oliver, 2006). There were numerous other interesting talks, poster presentations and stimulating discussions that we cannot elaborate on here because of spatial constrains. However, during the meeting it became apparent that genomics has touched on and left its trail on all aspects of fungal research. Even if one does not have the advantage of having the genome of one's favorite research object available, input from the analysis of related species still helps a great deal. Yet, as much as we appreciate the input of some model organisms, such as *N. crassa* or *M. grisea*, we should not forget about the enormous diversity that the fungal kingdom provides. It is this diversity, at least in part, which makes fungi so interesting. There seems to be a focus on certain model species, such as *Arabidopsis thaliana* or *Medicago truncatula*, in the plant world. Yet, we think it would be wrong to take the same route with fungi. Generalization may help with some aspects, but obscure other vital ones. On the other hand, it would be highly desirable if the fungal community could get together more closely to generate a positive input on the selection of species to be sequenced in the future. Inevitably, there will be conflicts of interest, for the evolutionary biologist will have different concerns from, for example, an ecologist. However, our common goal should be to concentrate most available resources on the fungal kingdom to provide the basis for future research. For one thing is absolutely certain, while genomics is driving research right now, it is by no means the end point. Like cytology and biochemistry in the past, genomics, at one point in the future, will be superseded by other methodologies. There is no way to tell, at present, the direction that research will take in the future; however, it will undoubtedly be '… omic'. Transcriptomics, secretomics, transportomics are only some examples of things to come. All of these holistic approaches might require a new way of thinking and new experimental methodologies, because the vast amount of data might not be manageable using conventional techniques. Fungi should be at the forefront of model organisms because of their 'simple make-up', which makes new hypothesis fairly easily to test in the laboratory. Again, we think that strong emphasis should be put on the fact that 'not all fungi are created equal'. This aspect is beautifully highlighted by recent work of James Galagan and coworkers, who found that a comparison of *Aspergillus nidulans*, *A. fumigatus* and *A. oryzae* revealed only 68% average amino acid identity between any pair of species (Galagan *et al.*, 2005). The evolutionary distance between any pair of these three species would therefore be comparable to that between human and fish.

Another aspect we should all bear in mind is that sequencing is only the first step. Sequencing creates an abundance of data, but it is only 'GATC'. Proper curation and annotation of the data are urgently needed if we do not want to get lost in the jungle of information. We definitely have to keep going and make this data work for us in every possible way, be it systems biology, evolutionary biology, biochemistry or metabolomics.

Outlook

Fungal biology has come a long way since the first fungal spore conference (the predecessor of the fungal biology conference series) in 1966. Methods, techniques, short-term goals and faces may have changed over the years, but what is still keeping us going is our common goal to understand fungal biology and in doing so provide a major input in understanding the biology of life in general. A milestone for fungal biology, not unlike the introduction of the polymerase chain reaction (PCR) for molecular biology, was the availability of fungal sequences on a larger scale. As John Taylor (University of California, Berkeley, CA, USA) pointed out in his concluding remarks, 'None of us remains untouched by the power of genomics and for some of us, especially the younger ones, genomics already appears to have been there forever'.

The availability of a number of fungal genomes, together with the fact that more are in the pipeline, has revolutionized many aspects of fungal research and this has put fungal biology onto new starting grounds. It might enable us, for the first time, to put research on the biology of fungi in the lead over prokaryotic, animal, plant or medical research. The reason being that fungi are small, yet complex, eukaryotes. So much of the principles of eukaryotic life might be more

easily analyzed in fungi than in higher eukaryotes. What is even more important, the theories we developed on the basis of the sequences can be quickly and easily tested in the laboratory. A very important point, in this respect, is the fact that genomics generates a flood of information that no one can deal with alone. Therefore, it is vital to engage in new collaborative efforts in order to understand fully the complexity of fungal biology in the near future.

Acknowledgements

Last but not least, we want to take the opportunity to thank all the people involved in a fascinating and stimulating meeting. Thanks to Holly Slater, Helen Pinfield-Wells, Nick Talbot and Francis Martin for a meeting superbly organized, both scientifically and socially. Also thanks to all the chairpersons and speakers for some very interesting sessions. and also thanks to the many busy people in the background, largely from Francis' group, who made everything run smoothly and enjoyably.

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Key words: database curation, fungal diversity, fungal genomics, model organisms, sequence annotation.