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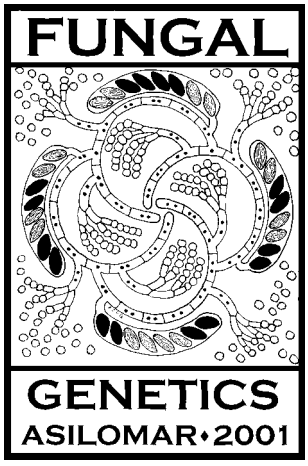
# MEETING REPORT
# The Fungi in Century 21

## The XXI Fungal Genetics Conference
## Pacific Grove, California, March 13–18, 2001

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## SOME HISTORY

The study of the filamentous fungi has had a diffuse history, a path that began with both taxonomic and practical interests. As humans began to appreciate the tastes and dangers of wild mushrooms, scientific classification of the higher fungi began. In the latter half of the 19th century, taxonomists and cytologists described the sexual stages and the life cycles of many forms. The genetic basis of mating systems began to be appreciated in the early 1900s, led by A. Blakeslee, H. Kniep, B. O. Dodge, and others even as many fungi were put to industrial and pharmaceutical uses. Beadle and Tatum's exploitation of *Neurospora* spp. for the first frontal attack on the genetic basis of cell function started a new era. It is understandable that this work continued as a major tributary of molecular biology, more related to study of genetics and cell function than as a rallying point for the unification of fungal genetics and biology. Instead, "mycology," as we used to call it, continued on many parallel paths, with a focus on the fungi as taxonomic entities, foods, animal and plant pathogens, agents of spoilage and fermentation, and providers of antibiotics.

By 1955, many workers on *Neurospora* and *Aspergillus nidulans* had become committed to these organisms, intoxicated by the ease with which they could be used to solve biochemical questions with genetic techniques. The *Neurospora* community was already tightly knit. David Bonner organized the first of the biennial *Neurospora* conferences in 1961, a meeting now considered the first of our present Fungal Genetics Conferences. By the late 1970s, as molecular biology became identified largely with phage and bacteria, *Neurospora* and *A. nidulans* came unwittingly to serve as models for studies of filamentous fungi, rather than of life itself. The emergence of yeast in the 1970s drove this point home forcefully, and the promise of the filamentous fungi to contribute in a major way to molecular biology seemed to have faded. If important contributions of these organisms were made, it was because they were, like most experimental organisms, suited to particular lines of work and because of the accumulated lore and libraries of mutants.

Research with filamentous fungi was resurrected in its modern form by the advent of molecular techniques in the late 1970s. The development of transformation techniques in yeast in 1978 led quickly to similar methods in filamentous fungi. This enabled many filamentous species to become prominent subjects of interest once again to general biologists. *Neurospora* and *Aspergillus*, after their considerable early contributions, were almost swamped by the

diversity of organisms now open to study. Recombinant DNA techniques had an important effect on the filamentous fungal community: the new techniques provided molecular biology as the *lingua franca* of the new field. The entrenched and insular mycological specialties no longer resisted overdue changes, which soon came into effect.

In 1986, the biennial *Neurospora* Information Conference (which had previously adopted the *A. nidulans* community) opened its meetings to all those working with filamentous fungi and named it the Fungal Genetics Conference. The reconstituted meeting drew more scientists, and in addition to *Neurospora* and *Aspergillus*, 12 more species were represented in the abstracts; 142 people attended. In 1999, 104 species appeared in the much thicker program; 649 people attended. Between those two meetings, a striking willingness on the part of all of us to hear about the wider world of filamentous fungi emerged. The biotechnology industry began to drive the interests of the community, with many former academics now leading new commercial enterprises based on fungal molecular biology. The journal *Experimental Mycology* was renamed *Fungal Genetics and Biology* in 1996, and the Fungal Genetics Stock Center now ties the larger community together with the Fungal Genetics Newsletter, a web site, and many sources of information and stocks.

The XXI Fungal Genetics Conference of 2001, held March 13–18, 2001 at the Asilomar Conference Grounds in Pacific Grove, California, showed us how far and how rapidly the unification of the area had proceeded. The meeting brought together 670 scientists. It was sponsored by the Genetics Society of America (GSA) and had support from the National Institutes of Health, the National Science Foundation, the Burroughs Wellcome Fund, and a host of commercial enterprises (named in the Acknowledgments) that defrayed many incidental expenses of the meeting. The scientific program was organized by **Rowland Davis** (Univ. Calif., Irvine) and **Marie-Josée Daboussi** (Univ. Paris Sud) with considerable support from **Marc Orbach** (Univ. Arizona, arrangements), **Nancy Keller** (Univ. Wisconsin, Chair of the Fungal Genetics Policy Committee), **Kevin McCluskey** (Fungal Genetics Stock Center, communications, program preparation, abstract coordination), and **Anne Marie Mahoney** (GSA representative, registration). The 4 full days included four morning plenary sessions, 20 concurrent sessions in the late afternoons, and approximately 500 posters, displayed in the evening. A summary of the major elements of the meeting follows.

## GENOMICS

In the session on comparative and functional genomics, chaired by **Ralph Dean** (N. Carolina State Univ.) and **Donald Natvig** (Univ. New Mexico), speakers demonstrated the progress in genomic analysis of diverse fungi. These included both pathogens and nonpathogenic model fungi with emphasis on pathogenic mechanisms and fundamental cell biology, respectively. Reporting on the genome of the rice blast fungus, *Magnaporthe grisea*, **Dean** described efforts to map the genome with BAC clones, genomic and expressed sequence tags (ESTs). The similarities to the genome of *Neurospora crassa* (see below) were not surprising, but unexpected divergences in gene content, gene order, and synteny were uncovered. One point made was echoed repeatedly during the meeting: the need to study a number of genomes to infer their individuality of organization and content and their evolution. **Melanie Cushion** (Univ. Cincinnati), working on the small genome (7 Mb) of the AIDS-related, opportunistic pathogen *Pneumocystis carinii* (newly adopted as a fungus), indicated that the organism has much in common with *Schizosaccharomyces pombe* and *Cryptococcus neoformans.* Indeed, several genes homologous to those active in meiosis in yeast and some cytological evidence for a synaptonemal complex suggest that *P. carinii* might have a sexual phase. **Cushion** used EST approaches to try to identify the metabolic capacities of the organism and thus to search for drug targets unique to the pathogen. Subtelomeric gene families have been detected, and sterol biosynthetic genes have been identified that may aid in development of drug therapies appropriate to the organism. **Brett Tyler** (Univ. California, Davis) discussed the genomes of the many plant pathogens of the *Phytophthora* group, of which one of the smallest (ca. 62 Mb) is that of *P. sojae.* Considerable repetitive DNA was found as BAC clones were characterized, some with unique assortments of the repetitive DNA. Curiously, the unique-sequence DNA and the repetitive DNA are not randomly distributed. There appear to be repeat-rich and repeat-poor regions, which encourages an early EST approach to the functional genome. The promise of determining the basis (and evolution) of pathogenesis of the group through these approaches is clear. **Fred Dietrich** (Duke Univ.) discussed the analysis of the filamentous cotton pathogen, *Ashbya gossypii*, with one of the smallest (9.5 Mb) eukaryotic genomes and its comparison to that of *Saccharomyces cerevisiae.* The comparison was based on both homology and synteny. The genomes are unexpectedly similar de-

spite their different forms and capacities for pathogenesis, indicating that *A. gossypii* may be a quite useful model for studies of both cell biology and pathogenesis. Finally, **Ulrich Schulte** (Heinrich-Heine Univ., Dusseldorf) presented a close look at the sequence of chromosomes II and V from *N. crassa*, amounting to 14 Mb of the 38 Mb of the entire genome. The 3000 genes here, many of which are not recognizable by homology, suggest that the entire genome might have approximately 13,000 genes (compared to 6000 for *S. cerevisiae*). Annotation efforts proceed well here as an early start on annotating the full *N. crassa* genome, the sequence of which was released recently.

The related concurrent session on the *N. crassa* genome, organized by **Bruce Birren** (Whitehead Inst., MIT) and **Matthew Sachs** (Oregon Grad. Inst.), was something of a high point. The full sequence of the 38-Mb genome had been released for free use by the Whitehead Institute at MIT, working in collaboration with a number of investigators at other institutions, and funded by NSF. The sequence has 10-fold coverage and 1705 contigs (368 supercontigs). It was obtained by a shotgun approach and was released on Feb. 14, 2001. Within a month the web site had 30,000 hits, extending the model status of the organism into general genomic work on filamentous fungi. The genome is accessible at http://www.genome.wi.mit.edu/annotation/fungi/neurospora/. The sequence shows excellent correspondence for chromosomes II and V to the sequence prepared by the **Schulte** group (above), and the annotation phase—and organizing means for the community to collaborate in the effort—are under way. At the same session, **Tom Adams** of Cereon Corp. described how their *A. nidulans* genomic sequence (29 Mb, 3-fold coverage, and over 11,000 contigs) can be accessed (http://www.cereon.com), with some restrictions, by academic and nonprofit institutions.

**Jean-Paul Latgé** (Pasteur Inst.) and **Neil Gow** (Univ. Aberdeen) organized another related concurrent session on fungi in medicine and genomics of human pathogens. In this session, the status of sequencing the genome of the three most important human fungal pathogens was discussed. The stage of sequencing varies with the fungus: 10-fold coverage of *Candida albicans* nears completion at Stanford University, 4.5-fold coverage of *C. neoformans* is at mid-term at Stanford and TIGR, and a fledgling sequencing project of *Aspergillus fumigatus* has started at TIGR and the Sanger Institute. The completion and annotation of these genomes will lead to a burst of comparative and functional genomic studies among human pathogens. Comparative genomics of ascomycete-like fungi, including the plant pathogen *A. gossypii*, a close relative of *S. cerevisiae*, and that of *C. albicans*, may provide novel insights into the evolution of plant and human pathogenesis generally. Several speakers presented preliminary data showing that functional analysis (e.g., random mutagenesis, reporter protein libraries) could be combined with studies of the genome, proteome, and transcriptome to generate new approaches to the biology of these pathogens. Such approaches promise to lead to new drug targets, a major preoccupation of medical mycologists. Molecular techniques indicated that many populations of the so-called "asexual fungal pathogens" were actually recombining in nature. Genomics is therefore providing new horizons of investigation, from cell biology and drug design to population genetics.

## PATHOGENESIS

The study of fungus–host interactions, including pathogenesis and symbiotic relationships, had three major themes. These were represented in the plenary session on Fungal–Host Interactions, chaired by **Regine Kahmann** (Max-Planck-Instl., Marburg). The first concerned the various toxic compounds produced by plant pathogens, with attention to the cyclic polypeptides, polyketides, and tricothecenes. Of interest was the resistance of the pathogen to its own toxin. **Jonathan Walton** (Michigan State Univ.) reported on the toxic, cyclic peptide of *Cochliobolus carbonum*, which targets histone deacetylase of the host. The pathogen has an "intrinsic factor" that appears to protect one of its own enzymes, but, in addition, one form of the *C. carbonum* enzyme, owing to posttranslational modification, is resistant to the mycotoxin. With respect to the function of the three histone deacetylases in *C. carbonum*, the situation is complex, and one may infer a scenario in which histone deacetylases function as coactivators for gene expression rather than as repressors or silencers.

Another major theme emerged in the study of the signal transduction pathways in fungi as they attacked specific hosts. **Jim Kronstad** (Univ. Brit. Columbia) and **Joseph Heitman** (Duke Univ.) reported in the plenary session on the basidiomycete yeasts, *Ustilago maydis* and *C. neoformans*, pathogens on corn and humans, respectively. In both cases, mating between cells of the two mating types yields a filamentous dikaryon that can invade the host, exercise its pathogenic mechanisms, become pigmented, and sporulate. Again in both cases, mating appears to require a protein kinase A (PKA)-related signal transduc-

tion pathway via adenyl cyclase and cAMP. In addition, the haploid cells respond to pheromones by way of a MAP kinase cascade, similar to that found in *S. cerevisiae.* Current work on the genomes, particularly the mating type regions, is progressing with the hope of seeing the ways in which the elements of these cascades, described initially in *S. cerevisiae,* have been recruited for new and broader uses in the pathogens.

Studies of arbuscular mycorrhizal fungi and nematode-trapping fungi, both of which have taken advantage of molecular techniques, represent a third theme, namely, the physiology of fungal interactions with hosts or prey. **Maria Harrison** (S. R. Noble Foundation, Ardmore, OK) described the obligate biotrophic mycorrhizal fungi (*Glomus* spp.) as they grow in association with roots, acquiring carbohydrates, among other things, from the plant and delivering phosphate and perhaps other elements to the plant. The fungal spores in the soil germinate and invade the plant roots, forming inter- and intracellular relationships with the cortical cells. In the latter, they become highly branched, forming arbuscules that have a large surface area in contact with the plant cytoplasm. This invasive growth initially calls up a phytoalexin response of the plant, which is diverted by the fungus to other products. Of interest to **Harrison's** laboratory is the differentiation between the intraradicle and the internal (arbuscular) part of the fungus in the host (*Medicago truncatula*) on the one hand and the peripheral mycelium on the other. She showed that phosphate transport genes were active in the latter, and not in the former, and EST analysis continues with the hope of describing the variation in gene activity in the two compartments of the mycelial continuum.

Molecular approaches have been taken by **Anders Tunlid** (Univ. Lund) to *Arthrobotrys oligospora* and *Monacrosporium haptotylum,* which trap free-living nematodes with a mycelial network or adhesive knobs, respectively. Random DNA mutagenesis generated mutants affected in the formation of the specialized trapping structures, and one such gene was recovered. The second approach to identify genes involved reverse genetics to analyze a serine protease expressed during colonization. Homologous recombination was very efficient, allowing it to be used to disrupt the protease gene. However, these were not much affected in their ability to trap nematodes, suggesting that the function of this enzyme may not be related to the trapping process but rather in making more nutrients available. An EST project for the different cell types has been initiated and is expected to yield considerable knowledge of the cellular events during the sensing, trapping, and digestion processes. Of particular interest is that one of the knob-producing fungi attacks *Caenorhabditis elegans,* which puts the molecular events underlying infection in both organisms within reach.

A concurrent session, organized by **Anne Desjardins** (USDA, Peoria, IL) and **Gillian Turgeon** (Syngenta), explored a variety of mycotoxin-producing fungi, showing that many were made by products of gene clusters. In certain fungi, such as *Fusarium verticillioides,* self-resistance to mycotoxins requires an ABC transporter and other gene products. Most of these studies took full advantage of molecular techniques to relate the enzymes involved to the variant mycotoxins found in the pathogens. Another concurrent session, organized by **Richard Oliver** (Murdoch Univ., Perth) and **Anne Osbourn** (John Innes Ctr.), elaborated on the topic of signaling pathways. These were, in particular, the heterotrimeric G proteins and MAP kinase systems associated with the penetration of the host and the cAMP-related activities required in dimorphic species such as *U. maydis* for successful infection and the production of teliospores. Studies with a number of pathogens have clearly converged on these systems, and it should be possible in the near future to conclude, with the large number of pathogens now being studied, how similarly or how variably these systems actually operate in nature. Other topics in this session included the formation of the appressorium in *Magnaporthe grisea,* how turgor is generated, and a report on a gene that affects the subsequent penetration step. Of particular interest was the use of *M. grisea* mutants to address the question of tissue specificity.

One of three other sessions on pathogenesis and comparative and evolutionary genomics, convened by **Pierre deWit** (Wageningen Univ.) and **Rolf Prade** (Oklahoma State Univ.), brought together scientists working on model filamentous saprophytic fungi such as *A. nidulans* and model human and plant pathogenic fungi such as *Histoplasma capsulatum, Cladosporium fulvum, M. grisea,* and *U. maydis.* Technical issues such as strategies to sequence and determine synteny between fungal genomes and biological questions dealing with coevolution between plants and various types of plant pathogenic fungi were addressed. Much of the discussion concerned cloning and identification of avirulence genes and their products; other matters had to do with genome sequencing of several fungi, that of *U. maydis* having recently been completed (**Joerg Kaemper,** Max Planck Inst., Marburg).

A session on *Mycosphaerella graminicola,* convened by **Gerrit Kema** (Plant Res. International, Wageningen), explored one of the most important fungal diseases of wheat. The assembled group discussed isolation of an

avirulence factor, work on the population genetics of the species, biochemical genetics of ABC transporters, and pathways that might serve as antifungal drug targets. Progress on methodology, namely transformation strategies and approaches to gene disruption independent of sequence information, also arose in discussion. The session, which brought together most of the major investigators of this pathogen, led to discussion of research tools needed in the future, beyond current work on transformation and gene disruption strategies.

Finally, a highlight of the oomycete workshop, organized by **Brett Tyler** (Univ. California, Davis), was the rapid progress in identifying proteins involved in host–pathogen recognition. This included reports on cloning avirulence genes *Avr1a, Avr1b, Avr1k, Avr4,* and *Avr6* from *P. sojae* and *Avr1, Avr2, Avr11,* and *Avr4* from *P. infestans.* **Tyler** reported that two tightly linked genes, *Avr1b*-1 (secreted elicitor protein) and *Avr1b*-2 (transcriptional regulator of *Avr1b*-1), underlie the *Avr1b* phenotype. High-throughput genomics and proteomics are being used for identifying proteins secreted during infection by *P. sojae* and *P. infestans.* A secreted toxin-like protein that triggers cell death in a wide variety of plant species and, in the wide-host-range pathogen *P. cinnamomi,* a diverse multigene family that encodes secreted polygalacturonases were reported.

## CELL BIOLOGY

Considerable progress in understanding structural and functional aspects of cell structure was evident in the plenary session on Cell Biology, chaired by **Brent Heath** (York Univ., Toronto). We see here that studies of filamentous fungi are quite diverse, one of the attributes that makes them both interesting and yet somewhat behind yeast in molecular focus in the areas of cell polarity and organellar biogenesis. In the first talk, **Heath** reported recent studies of tip growth of *N. crassa,* which differs considerably from his prior studies of oömycetes. In *N. crassa,* spectrin-like molecules, rather than actin, appear to be a major player in the process, in conjunction with the tip-high, cytosolic calcium gradient. The maintenance of this gradient, interestingly, probably involves more the recirculation of organellar stores of calcium rather than the dependence upon influx at the tip. **Anne Ashford** (Univ. New South Wales) reported on the vacuole system of the mycorrhizal basidiomycete, *Pisolithus tinctorum.* The system displays a gradient from apical tubules to large subapical, spherical, interconnected structures, with regulated interconversions from one form to the other. Novel, localized interactions with the plasma membrane in the subapical regions regulate the system, with a role for microtubules throughout the hyphae. This distribution system can be uncoupled from tip growth. Evidently GTP-binding proteins are involved, one of which may be a dynamin-like protein. In a talk focusing on apical secretion, **Susan Assinder** (Univ. Wales) described vesicle production through the secretory endomembrane system of *A. nidulans.* This is mediated by coatamer complexes containing $\alpha$-COP subunits. Fusion of the *$\alpha$-COP* gene to GFP sequences enabled, for the first time, imaging of the organization of the Golgi apparatus in growing hyphae. This showed its apical concentration, consistent with polarized secretion. Brefeldin A alters both anterograde membrane flow from ER to Golgi apparatus and Golgi vesicle production. These techniques offer new methods to analyze the dynamics and organization of the endomembrane secretory system.

**Gregory Jedd** (Rockefeller Inst.) described work revealing the nature, role, and biogenesis of the Woronin body of *N. crassa.* This organelle plugs septal pores upon loss of turgor of hyphae, such as might be caused by bursting of hyphal tips. Originally identified as ergosterol crystals, the organelle is in fact a modified peroxisome containing an inclusion body consisting of a specific protein. The protein, and its gene, *hex-1,* has been identified, sequenced and tagged with GFP. It forms Woronin body-like crystals *in vitro* and bears a peroxisome targeting signal that targets it to *S. cerevisiae* peroxisomes when expressed in that species. The gene encoding the protein is translated in two different forms, the minor form retaining an intron yielding a protein critical to the initial assembly of the membrane that surrounds the body. This protein also has domains that appear to confer an ability to bind to other membranes and thus putatively to play a role in mediating the interaction of the Woronin bodies with the plasma membrane lining the septal pores during the sealing response. Thus, this unique structure proves to be an interesting model for more general types of organelle assembly and possesses sophisticated properties that belie its morphological simplicity.

The final plenary talk in this session, by **Oded Yarden** (Hebrew Univ. Jerusalem), concentrated on the morphogenetic role of COT-1, a membrane-associated serine/threonine protein kinase of *N. crassa.* The *cot-1* mutants have a tight colonial phenotype, and the kinase appears to function in a general stress response system involving ion fluxes, osmoregulation, and glycerol synthesis, in addition

to having a role in normal morphogenesis. However, this stress response system apparently differs from a similar MAP kinase-based system in *S. cerevisiae*, indicating the importance of analysis of diverse species, even when closely related. Unexpected complexity of the system was revealed with searches of the *N. crassa* genome sequence. What was previously thought to be two isoforms of COT1 appear to be two different gene products, both recognized by the antibody made against a presumed unique protein. Analysis of their separate roles will be of interest in the future.

**Gero Steinberg** (Max Planck Inst., Marburg) and **Cees van den Hondel** (Univ. Leiden) chaired a concurrent session on polarity, cytoskeleton, and cell wall metabolism. It included talks on specific mechanisms by which microtubules and associated motors, kinesin and dynein, deliver components within the cell, thus having a major role in the establishment and maintenance of polarity. The ability to screen more broadly than previously for polarity mutants in *N. crassa* will accelerate the study of this attribute of fungal cells. Improved imaging systems coming into play will provide not only more data on membrane dynamics but will aid in phenotypic characterization of morphological mutants. The session had the virtue of including talks on many species, specifically *A. nidulans, N. crassa, U. maydis, S. pombe,* and *C. albicans,* which stimulated considerable discussion at the end.

A related concurrent session on organelles, organized by **B. Bowman** (Univ. California, Santa Cruz) and **R. Brambl** (Univ. Minnesota), explored specific targeting roles of mitochondrial translocase components, a peroxisomal ABC transporter required in the sexual cycle of *Podospora anserina,* and further studies of the function of the protein HEX-1 in the Woronin body. The SNARES required for apical vesicle fusion, identified by search of the genomes of both *N. crassa* and *S. cerevisiae,* reveal that each species has certain unique types (**G. Gupta**), possibly related to the differences in hyphal vs budding modes of growth. Finally, exploration of organelle imaging in fungi showed considerable progress, with observations in vitally stained material that conflict with impressions based on conventional cytology (**N. Read,** Univ. Edinburgh).

**Christophe D'Enfert** (Pasteur Inst.) and **Nancy Keller** (Univ. Wisconsin) chaired a session on signal transduction, separate from those devoted to pathogens. This area is now making considerable progress after a slow start. It is clear that cAMP-based, heterotrimeric G protein systems and MAP kinase pathways can be assigned some role in various processes, including morphology, spore germination and secondary product production, dimorphic switching, pH control, and cytokinesis. In some of these cases, a good outline of how the cascade has its effect is available; in others, much needs to be done to follow the cascade to its final phenotypic target.

Signaling in specific contexts was a theme of several other concurrent sessions. In the basidiomycete biology session, chaired by **Ursula Kües** (ETH, Zurich) and **Erika Kothe** (Friedrich-Schiller Univ., Jena), various aspects of the mating system were connected to signaling pathways. A dominant *ras* mutant affects not only hyphal growth and organization, but also the nuclear acceptance controlled by the B mating-type locus. In another species, a *ras* mutation relieves an inhibition of the formation of chlamydospores. MAP kinase components are found in the mating type loci of *C. neoformans.* The pheromone response pathways of *S. commune* involve G proteins with structures of great delicacy underlying their specificity in mating. Thus, the basidiomycetes offer many possibilities for the study of signaling in a coherent manner, together with opportunities for comparisons among related forms. A counterpart session on mating, heterokaryosis, and downstream effects, convened by **Louise Glass** (Univ. California, Berkeley) and **Tom Fowler** (Univ. Vermont), explored genes for transcription factors and pheromones important in mating and some of the interesting features of vegetative incompatibility in *N. crassa* and *P. anserina.* In the former, the allelic forms of compatibility factors form a heteromultimer activating the incompatibility response. In the latter, a prion-like modification of an incompatibility gene was deduced from genetic and physiological studies. The session on photobiology and clocks, chaired by **Luis Corrochano** (Univ. Seville) and **Deborah Bell-Pedersen** (Texas A&M Univ.), included talks on photoreceptors and downstream effects such as conidiation, on the new opsin-related genes *nop-1* and *opr-1* of *N. crassa,* and on the possibility that a nucleotide diphosphate kinase is a part of the signaling pathway, as it is phosphorylated after exposure of cells to blue light. The session had a number of papers related to the circadian clock. These studies are now beginning to look at physical associations among the protein components of the clock, WC-1, WC-2, and FRQ, and of these proteins and the promoters of their own and other genes. Finally, clock-controlled genes and their rhythms have been analyzed by various means including EST work, reporter-gene constructs, and microarrays. The comprehensiveness of the work on the clock, for which *N. crassa* remains a major model, is likely to lead to many insights into circadian rhythms, regulation, and signaling pathways.

**Dick Weiss** (Univ. California, Los Angeles) and **Michael Hynes** (Univ. Melbourne) convened a session on metabolic organization and regulation, in which a trend toward integrating classical regulatory work on metabolism with studies of development, pathogenicity, and secondary metabolism was apparent. Amino acid biosynthesis and catabolism were prominent topics. Studies of arginine breakdown in both *A. nidulans* and *N. crassa*, amino acid biosynthesis and cross pathway regulation in *A. nidulans*, and the relation of cross pathway control to pathogenicity in *M. griseae* illustrated the complex regulatory interactions governing metabolic regulation in filamentous fungi. Novel techniques developed to characterize translational control mechanisms and the use of RNA fingerprinting to characterize differentially expressed genes in Ectomycorrhizae were described. Sophisticated work on pH responses in fungi reflect the importance of pH signaling via the *pal* genes in *N. crassa* and the C2H2 zinc finger protein, PacC, of *A. nidulans*. The GATA family of transcription factors in fungi turned up in studies of iron metabolism, nitrogen metabolism, gibberelin production, and light and circadian rhythms. Other transcription factors required in virulence, catabolism, melanin production, and other activities rounded out the session. There was a curious dearth of presentations on chromatin modifications in gene regulation, although work on histone deacetylases from *A. nidulans* and *Cochliobolus* was reported (the latter arising in the Pathogenesis plenary session).

## GENOME STRUCTURE AND MAINTENANCE

We have learned in recent years that the genome of fungi (and by extension ourselves) is by no means stable. In fact, it is downright precarious, maintained by a balance of error and damage on the one hand and repair and meiotic renewal on the other. The plenary session on Genome Structure and Maintenance, chaired by **Denise Zickler** (Univ. Paris Sud), began with **David Perkins** (Stanford Univ.), who outlined the types, the experimental history, and the current molecular uses and analysis of chromosomal rearrangements in *N. crassa*. He pointed to the possibility of discovering the mechanisms leading to the formation of aberrations, both natural and those caused by insertion of transforming DNA. Sequencing of small regions has led to detection of small, previously cryptic inversions that lead to differences among related species in gene order. Further work may uncover aberrations underlying the spore-killer factors, which are associated with localized recombination blocks. The role of aberrations in differentiation of fungal groups will be illuminated by study of broader syntenic relation of genes in related species.

Two similar approaches to chromosome behavior in *Coprinus cinereus* (**Miriam Zolan,** Indiana Univ.) and in *Sordaria macrospora* (**Zickler**) yielded complementary information about meiosis. In the first, **Zolan** sought mutants of genes involved in both repair and meiotic processes, taking advantage of the synchronous meioses in the mushroom and the white-cap phenotype of those deficient in the process. She obtained genes homologous to the *RAD* (sensitive to ionizing radiation) and *SPO11* genes of *S. cerevisiae*, involved in making and processing of double-strand breaks in leptotene, and another gene, *rad9* of *Coprinus*. The mutants showed that these genes' effects extended beyond the precise steps defined for them in yeast. The *rad9* gene encodes a cohesin, important in sister-chromatid cohesion. The protein also appears to play a role in the behavior or formation of the syntaptonemal complex, and thus the pairing of homologs. Some of the phenotypes of these genes in the pairing process could be explored with fluorescent *in situ* hybridization analysis, following probes specific for the two *B* locus "alleles" as they paired in meiosis.

**Zickler** described work on Spo76p (of *S. macrospora*) and BIMD (of *A. nidulans*), proteins belonging to a highly conserved protein family implicated in sister-chromatid cohesion and DNA repair. *SPO76* and *bimD* are functional homologs with respect to their roles in mitotic chromosome metabolism but not in meiosis. Both proteins localize to mitotic and meiotic chromosomes, except at metaphase(s) and anaphase(s). During meiotic prophase, Spo76p assembles into strong lines correlated with axial element formation, while BIMD stains all chromatin. This difference is correlated with differences in chromosome structure and/or function and may explain the lack of heterologous complementation of meiotic defects. *S. macrospora* forms synaptonemal complexes, whereas *A. nidulans* appears to lack these structures. Also, *A. nidulans* differs from *S. macrospora* by its lack of positive crossover interference. In *bimD6* germlings, mitotic nuclear divisions and the overall cellular program occur more rapidly than in wild type. Thus, BIMD, an abundant chromosomal protein, is a negative regulator of normal cell cycle progression. *bimD6* reduces the level of mitotic interhomolog recombination but does not alter the ratio between crossover and noncrossover outcomes. Moreover, *bimD6* is

normal for intrachromosomal recombination. Therefore, BIMD is probably not involved in the enzymology of recombinational repair *per se.* Spo76p is also required for meiotic interhomolog recombination, likely at postinitiation stage(s). She proposed that BIMD and Spo76p exert their diverse influences on cell cycle progression, chromosome morphogenesis, and recombination by modulating chromosome structure.

The study of gene silencing is a lively field, covering all eukaryotic groups, including plants, fungi, *C. elegans,* and mammals. **Carlo Cogoni** (Univ. Rome) reported on the "quelling" phenomenon in *N. crassa* in which DNA or RNA, introduced into cells, leads to variable, posttranscriptional gene silencing (PTGS) of the expression of the homologous nuclear gene. The phenomenon involves formation of double-stranded RNAs that ultimately lead to degradation of homologous mRNAs. Three *qde* loci whose mutants are defective in the process have been isolated. *qde-1* defines a new conserved gene family. The *qde-1* gene product is an RNA-dependent RNA polymerase, which supports models that implicate such an enzyme in PTGS. The homology of the *qde-2* gene with *rde-1* of *C. elegans,* involved in RNA interference and the mammalian translation initiation factor eIFGC2, suggests a possible connection of PTGS and translation or the recognition of mRNA by ribosomes. The *qde-3* gene is required for both activation and maintenance of gene silencing in *N. crassa* and belongs to the RecQ DNA helicase family. Interestingly, the *qde-3* mutants show a wild-type ability to repair DNA damage, suggesting a function other than DNA repair and recombination for RecQ. The QDE-3 protein may function in the DNA–DNA interaction between introduced transgenes or with an endogenous gene required for activation of gene silencing. Finally, PTGS also has promising applications, by providing an interesting tool for knockouts of gene expression in the developing field of functional genomics.

**Annie Sainsard-Chanet** (CNRS, Gif ) presented data on the long-standing problem of senescence in *P. anserina.* Senescence reflects increases in the proportion of mitochondrial DNAs carrying a large deletion. This takes place prematurely in strains carrying mutations of the *AS1* gene, encoding a cytosolic ribosomal protein. Some premature-death mutants escape senescence and were found to have deletion of part of a *COX1* exon (mitochondrial), leading in turn to a respiratory defect. To judge the role of a respiratory defect in senescence, mutants blocked in the nuclear *cox5* gene were used. These mutants did not suffer premature death and had stable mitochondrial DNA. They grew very slowly, using the SHAM-sensitive alternative oxidase (AO). If AO were overexpressed in these mutants, they achieved wild-type growth and displayed senescence. Similar overexpression in wild type ($cox5^+$) did not alter the senescence phenotype (even though the concentration of reactive oxygen species might be considerably higher). **Sainsard-Chanet** concluded that senescence correlates with efficient production and use of ATP rather than with AO expression or production of reactive oxygen species. It might be added that the mechanism of mitochondrial DNA rearrangements and deletions is still of considerable interest. In the concurrent session on DNA repair, correlation of these rearrangements with heavy metal availability in the mitochondrion (**Heinz Osiewacz,** J. W. Goethe Univ., Frankfurt) and the role of certain other genes, possible targets of *AS1* action (laboratory of **Marguerite Picard,** Univ. Paris Sud), were noted.

The concurrent session organized by **Hirokazu Inoue** (Saitama Univ.) and **Etta Käfer** (Simon Fraser Univ.) on DNA repair and mutation covered the analysis of mutants affecting these processes in *N. crassa* and *A. nidulans.* Working closely with what is known of these processes in budding and fission yeasts, the community has unraveled much of the confusion presented by mutation/repair mutants of the filamentous species. In particular, the classical and a new excision repair pathway have been clearly defined by mutations (*mus-38* and *mus-18,* respectively), and postreplication repair has been attributed to the *mus-8* and *uvs-2* genes of *N. crassa,* each having homologs in *A. nidulans* and yeast. Similarly, mutagenic repair, imparted by an error-prone polymerase, has been defined by mutation of the yeast *REV-3* homologs, *uvsI* of *A. nidulans* and *upr-1* of *N. crassa.* Less progress has been made in defining the agents of double-strand break repair in the filamentous forms, although homologs of relevant yeast genes (e.g., *MRE11*) have been detected. Finally, genes involved in DNA replication checkpoint control are beginning to be found in both *A. nidulans* and *N. crassa.*

Another concurrent session on accessory genetic elements, chaired by **Jack Kennell** (Southern Methodist Univ.) and **Sven Saupe** (CNRS, Bordeaux), concentrated on transposons and mitochondrial plasmids and introns. The senescence phenomenon arose again in studies by **Kennell** of *N. crassa,* who showed that it may begin with the integration of a mitochondrial plasmid (Mauriceville), with the overreplication of a variant plasmid, or with a nuclear cytochrome *c* mutation. Attempts to domesticate transposons, both fungal and plant, for use in various species have met with some success. The major motive is to use them as mutagenic agents and tags. A dsRNA virus

affecting virulence of *Cryphonectria infestans* and several possible cases of prions in other fungi were also discussed.

## FILAMENTOUS FUNGI: PRACTICAL MATTERS

Two concurrent sessions, one on new tools in manipulation of filamentous fungi and the other on the use of fungi in industry, drew considerable interest. The first, organized by **Geoffrey Turner,** covered functional analysis of genes by insertions and knockouts and by the monitoring of gene expression. A transpositional inactivation approach to speed work now dependent on cloning and gene disruption was described for *M. grisea* and other fungal species. A genomic library was subjected to *in vitro* transposition, followed by cloning and transformation of the fungus by homologous integration. Two other speakers described *in vivo* approaches. One used *Impala*, a *Fusarium oxysporum* transposon adapted to use in *A. nidulans* and *A. fumagatus*, with the problematic finding that the transposed elements generally avoided genes (as a smart transposon should seek to do). Tests of a similar approach with Tc1 of *C. elegans* in the fungus *U. maydis* may prove more useful for active genes. The frequency of homologous recombination in the yeast-like *A. gossypii* makes a knockout strategy based on homologous recombination useful, especially in view of its small genome (see above). In the matter of gene expression analysis, EST arrays using over 4000 sequences of *A. nidulans* are being developed for such studies. Arrays using DNA library materials are now being constructed in species in which sequencing has not progressed as far, such as *Histoplasma capsulatum*, and a strategy was described by which genomic and cDNA macroarrays (*sic*) can yield information on expression of industrially important genes. Still another approach, serial analysis of gene expression was described. Here, 14-mer tags are used to determine the frequency of given mRNAs (as cDNA) as a measure of expression; the latter are then identified in a cDNA library.

The session on industrial fungi, organized by **Debbie Yaver** (Novozyme Biotech) and **Linda Lasure** (Pacific Northwest National Lab.), included talks on transformation and expression systems. Improved transformation systems, including the survival of transforming DNA through sexual crosses, have emerged from work on the economically important basidiomycetes *Pleurotus ostreatus* and *Agarucus bisporus.* The techniques are being applied to study of mushroom viruses and improved mushroom crops. In biotechnological work, *Chrysosporium* cellulase production has been greatly improved, and mutants with low protease activities have been selected for use in producing heterologous human proteins. Work on the large penicillin biosynthetic cluster of *Penicillium chrysogenum* indicated that only 3 of 17 ORFs were essential for penicillin production. A report on a system for shuffling parts of any two variants of coding regions (up to 14 kb) placed between a *cog* (high recombination) site and the *his-3* gene of *N. crassa* was described. Finally, a metabolic engineering feat by which expandase (responsible for alteration of penicillin to cephalosporin) was accomplished by expressing the expandase gene of *Cephalosporium* in a *Penicillium* host was described. The process is now in commercial use.

## AND FINALLY

At the Saturday evening banquet, **Ron Morris** (Rutgers Univ.) gave a keynote talk covering the middle years of *A. nidulans* molecular genetics as he experienced it. The talk, addressed in part to the younger generation, sought to impress upon them the rigors of a kitless laboratory, where failed experiments required trouble-shooting rather than ordering from a different supplier. Recounting the isolation and analysis of mutants blocked in the cell cycle and their relation to germination, hyphal growth, and nuclear distribution was accompanied by the playing of a national top-10 song that he had written some years before. The fungal genetics community felt that they had gained more than scientific illumination in the years of his contributions, as **Morris** has propagated his interests through many scientific descendants, a song still in his heart.

## ACKNOWLEDGMENTS

This summary was prepared from notes and descriptions by the plenary and concurrent session chairs. I thank them very much for their efforts, both in organizing their sessions and in providing summaries. Any errors, however, are my own. I do not regret omitting names of the speakers in the concurrent sessions. This strategy exonerates them from my errors of reporting and shields them from blame in their making any unjustified or premature claims. No summaries were provided for 2 of the 20 concurrent sessions, and no report on them is included. Abstracts

of the talks and posters may be found at the Fungal Genetics Stock Center Web site, http://www.fgsc.net/html.

No citations of this summary with respect to scientific points should be made. Authors wishing original sources should consult the FGSC Web site for abstracts of plenary and poster presentations (http://www.fgsc.net/fungalgenetics2001/plenary2001.htm), the latter set representing most of the concurrent session talks.

The commercial sponsors, whose contributions greatly helped the atmosphere of the meeting and facilitated interaction among attendees outside the formal sessions, were Archer Daniels Midland, Genencor, Merck, Microbia, Monsanto, Novozymes Biotech, Inc. and Novozymes A/S, Paradigm Genetics, Pioneer Hi-Bred, Proteome, Sylvan, Syngenta, and Torrey Mesa Research Institute. The organizers and planners thank them warmly for their assistance.