

REVIEW

Neurospora at the Millennium

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This is an opportune time to evaluate the contributions of *Neurospora* to genetics and biology in the recent past and to anticipate what its role might be in the years ahead. The turn of the century marks the 75th anniversary of the first genetic experiments with *Neurospora*. Although historical accounts of *Neurospora* genetics usually begin with the 1941 paper of Beadle and Tatum, genetic analysis had in fact been initiated 16 years earlier by B. O. Dodge (see Robbins, 1962). It was Dodge who identified the two mating types and used them to demonstrate Mendelian segregation in individual asci. Dodge quickly recognized the potentialities of the organism for genetic research. His enthusiasm was largely responsible for the adoption of *Neurospora* by geneticists and for its development as a model organism. Now, 75 years after Dodge's identification of the first gene, over 1000 loci have been characterized and mapped in the seven linkage groups (Perkins *et al.*, 2000).

Within a few years of Beadle and Tatum's initial work, and in large part because of it, many prokaryotic microbial models emerged in molecular genetics. These include *Escherichia coli* and *Salmonella*, together with the T series of virulent bacteriophages and the temperate phages P22, P1, and λ . Many of their attributes made them more

suitable for work on fundamental questions such as DNA replication, molecular recombination, and regulation. However, *Neurospora* workers continued to make fundamental contributions in such areas as meiotic recombination, metabolic organization, mitochondrial biogenesis and function, cell biology, heterokaryosis, fungal sexual development, and chromosome mechanics. It soon became apparent that eukaryotes differed markedly from prokaryotes in many respects and that numerous eukaryotic features could not be investigated at all in prokaryotes. The need for a eukaryotic microbial model was clear, and *Neurospora* was well qualified to assume such a role. The *Neurospora* work led to an increased appreciation of the lifestyle of mycelial fungi and to the development of other fungal models, such as *Aspergillus nidulans*. These models have increased in importance as molecular approaches were applied to fungal pathogens and industrial fungi. A new field has emerged—fungal genetics and biology, embodied in biennial meetings at Asilomar and indeed, this journal.

In recent decades, the spectacular contributions of *Saccharomyces* have tended to eclipse what has been accomplished with other fungi. The shadow is passing as post-genomic molecular and cell biologists seek to explore the diversity of mechanisms of regulation and development and the evolution of complex systems. The filamentous fungi have continued to make important contributions, both because of their similarities to the yeasts and because of their differences. The filamentous species are similar to *Saccharomyces* in their basic biochemistry and cell biology, but they are phylogenetically quite distinct from yeast and more complex developmentally. Aside from their in-

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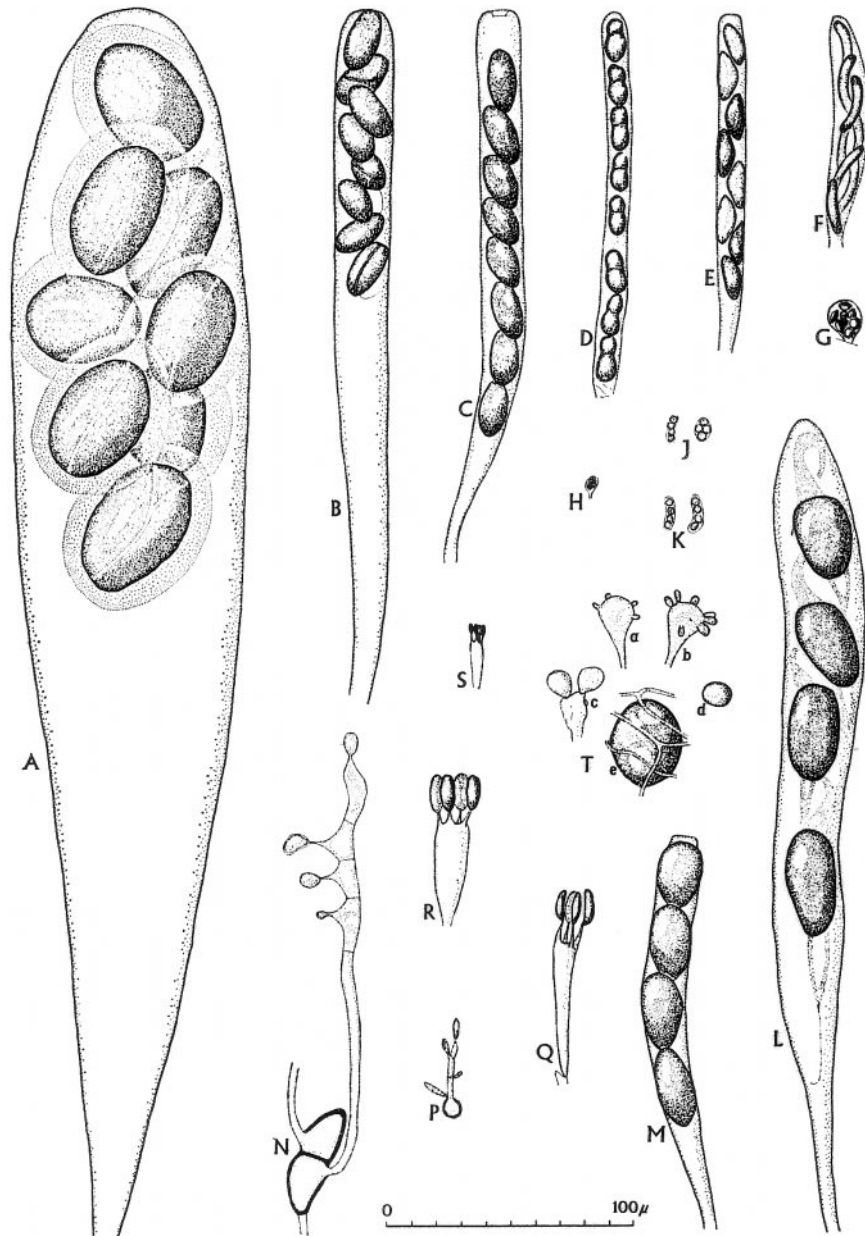


FIG. 1. Asci and basidia from various fungi that have been used in genetic studies. Drawn to scale. Ascus C is *Neurospora crassa*. Ascus M is *N. tetrasperma*. The others are: (A) *Ascobolus immersus*. (B) *Ascobolus magnificus*. (D) *Venturia inaequalis*. (E) *Bombardia lunata*. (F) *Glomerella cinguata*. (G) *Aspergillus nidulans*. (H) *Ophiostoma multiannulata*. (J) *Saccharomyces cerevisiae*. (K) *Schizosaccharomyces pombe*. (L) *Podospora anserina*. (N) *Puccinia graminis*. (P) *Ustilago maydis*. (Q) *Cytidia salicina*. (R) *Coprinus fimetarius*. (S) *Schizophyllum commune*. (T) *Cyathus stercoreus*. From Emerson (1966).

trinsic interest, the filamentous fungi provide information that is either unattainable or difficult to obtain from yeast. Euscomycetes such as *Aspergillus*, *Neurospora*, *Cochliobolus*, *Magnaporthe*, and *Gibberella* and basidiomycetes such as *Coprinus*, *Schizophyllum*, and *Ustilago* are all in a

unique position to complement and extend the rapid growth of knowledge emerging from the study of *Saccharomyces*, the "minimal eukaryote" (see Fig. 1).

The sexual phase of *Neurospora* was described and the genus was named by Shear and Dodge (1927). The orange

vegetative phase had long been known as a prolific contaminant of bakeries, a constituent of edible Javanese cakes called oncham, an early colonist of burned-over vegetation, and a common colonist of sugar cane stubble and the residues of sugar-cane processing. Heterothallic species of the genus are exemplified by *Neurospora crassa*, the species most commonly used in experimental work. Growth is hyphal in the multinucleate, syncytial mycelium of the vegetative phase, which conidiates prolifically. There are two genetically determined physiological mating types, and matings can occur only between strains of opposite mating type. Genes at the complex mating-type locus are called idiomorphs in recognition of their nonhomologous base sequences. Individual strains can function both as a female and as a fertilizing parent. Differentiated female structures, the protoperithecia, send out specialized hyphae, the trichogynes, which seek out and fuse with cells of opposite mating type, from which they transport fertilizing nuclei into the protoperithecium. Mating culminates in the production of perithecia within which as many as 200 asci are formed. Each ascus contains the four products of a single meiosis. A postmeiotic mitosis before ascospore formation results, in *N. crassa*, in four pairs of sister ascospores arranged in a linear order that reflects the events of meiosis.

The main long-term contributions of *Neurospora* have been described by Horowitz (1991), Perkins (1992), and Davis (2000). In the present article, we begin with a brief narrative of *Neurospora* research in the earlier years and go on to describe in more detail some significant advances in the past decade. Because previous work was fully documented in the 1992 review, few references will be given to original publications before that date. Information on subjects for which reviews are not cited will generally be found in Davis (2000).

CONTRIBUTIONS IN THE DECADES BEFORE 1990

Recombination

Ease of culture, speed of growth, haploidy, and the ability to determine the genotypes of all four products of individual meioses made *Neurospora* attractive to geneticists. Recombination studies showed that the mechanism of meiotic recombination resembles that in the animals and plants conventionally studied by geneticists. An outstanding novel contribution was Mary Mitchell's use of

Neurospora mutants to provide the first well-verified example of gene conversion. This and other studies of intragenic recombination in *Neurospora* led directly to intense, definitive studies of conversion in asci of *Sordaria*, *Asco-bolus*, and *Saccharomyces*.

Cytogenetics

The behavior of chromosomes during meiosis and ascus development was described by McClintock (1945) and Singleton (1948, 1953), who showed for the first time that both meiosis and mitosis in fungi were typically eukaryotic. Their description of the pachytene karyotype and the first chromosome rearrangements opened the field of fungal cytogenetics (reviewed by Perkins and Barry, 1977). Patterns of aborted ascospores in crosses of strains heterozygous for standard and aberrant sequence facilitated the recognition and diagnosis of chromosome rearrangements. Insertional and terminal rearrangements provided a ready source of meiotically generated partial-diploid segregants that carry duplications of genetically defined chromosome segments.

Biochemical and Molecular Genetics

The simplicity of the nutritional requirements, the simple life cycle, and the ease of genetic analysis led Beadle and Tatum (1941) to use *Neurospora* in their search for mutants affecting intermediary metabolism. Their success in obtaining nutritional mutants—a finding that was entirely novel—initiated the explosive development of microbial and biochemical genetics, the precursor to what we now call molecular biology. Use of auxotrophs and selective platings led to the demonstration of gene exchange in bacteria (Lederberg and Tatum, 1946). Lederberg's first research experience had been with *Neurospora*. *Neurospora* was adopted in many laboratories in the years following 1941, yielding a wealth of information about general metabolism, mitochondrial function and biogenesis, formal genetics, chromosomal aberrations, and unique aspects of the fungal lifestyle such as heterokaryosis.

Regulation of enzyme activities became an early preoccupation of workers with *Neurospora* at a time when studies of regulation were at the forefront of work in bacteria. Global regulatory systems governing nitrogen, phosphorus, and sulfur metabolism became models for eukaryotes, particularly with the discovery of regulatory cascades connecting the external stimulus to the change in

gene action. Much of this has been reviewed by Davis (2000).

In the early years, the study of genes and their protein products depended on the availability of mutant alleles or naturally occurring allelic differences. Mutants were examined phenotypically, mapped genetically, and used in studies of recombination and complementation. Fine-structure genetic mapping matured by way of amino acid sequencing and finally DNA sequencing. Studies of the mutational process provided information on reversion, suppressor mutation, and photo-reactivation and on the action and relative effectiveness of different mutagens. Mutagen-sensitive mutants contributed to the understanding of DNA repair mechanisms (reviewed by Schroeder *et al.*, 1998; Inoue, 1999). Duplicated DNA sequences were found to be inactivated premeiotically by a process called RIP² (repeat induced point mutation), an important novel discovery. RIP, which occurs in haploid nuclei prior to formation of the diploid zygote, results in severe mutational degradation, by GC to AT transitions, of both copies of the duplicated segment (reviewed by Selker, 1990).

The advent of transformation techniques, developed in 1979, was followed by restriction fragment length polymorphism (RFLP) mapping shortly thereafter. The ability to transform opened the way for molecular investigations of gene structure, gene expression, and evolution. By 1985, DNA-mediated transformation of *N. crassa* had become routine. Many of the problems that had reached their technical limits in the 1970s took on a new life, and a number of new areas opened up.

Genome Organization

The haploid genome was shown to contain approximately 43 Mb of chromosomal DNA, with 54% guanine + cytosine pairs; individual chromosomes range from 4 to 10.3 Mb. The seven linkage groups, which had previously been identified cytologically with individual chromosomes, were assigned to separate DNA molecules using pulsed-field gel electrophoresis (Orbach *et al.*, 1988; Orbach, 1992). Little repetitive DNA was found, mainly the genes specifying ribosomal RNA. Telomeres were shown to have a DNA sequence identical to that of humans.

The longstanding study in *Neurospora* of the mitochondrial genome and its introns, and of mitochondrial biogen-

esis, has contributed substantially, with studies of yeast mitochondria, to our understanding of the organelle in all aerobic eukaryotes (reviewed by Davis, 2000). Mitochondrial plasmids were discovered and characterized.

Most of the genes concerned with steps of the same biochemical pathway were found to be scattered about the genome, unlike many such groups in bacteria. The organization of genes in *Neurospora* is unsurprising, but an important feature of certain related genes was first appreciated in *Neurospora*. Two or more of the enzyme activities of some pathways, notably tryptophan, histidine, pyrimidine, and arginine synthesis, and the common aromatic amino acid sequence, are catalyzed by single proteins. When a few multifunctional loci or gene clusters were discovered, it was thought at first that they might be similar to bacterial operons. This was proved incorrect when the *aro* "cluster-gene" product was shown to be a single polypeptide chain that carried multiple enzyme activities.

Developmental and Cell Biology

Studies in *Neurospora* provided new information on the compartmentation of metabolic activities and small molecules in mitochondria, vacuoles, and microbodies, furthering knowledge of the integration of biochemical activities in eukaryotic cells.

In the 1960s, investigators were emboldened to use mutants in an attempt to analyze developmental features of *Neurospora* such as the mycelial growth habit and differentiation of vegetative spores. Strains with colonial growth or derangements of conidial formation were obtained. Abnormal morphology in some mutants was found to be associated with certain enzyme deficiencies in glycolysis and related reactions (reviewed by Brody, 1973; Mishra, 1977). These mutants gave promise that developmental processes could be related to well-characterized biochemical steps. While this hope was not realized, interest in growth and morphology persisted. Investigations of growth and differentiation were resumed strongly in the 1980s. The study of morphogenesis was reinitiated by isolating cDNAs specific for certain developmental stages or certain times in the circadian cycle. This work has flourished in the recent past, as we indicate below.

Linear growth of wild-type *Neurospora* can exceed 4 mm/h, perhaps the highest of any fungus. This proved advantageous for quantitative studies of growth and for a variety of other studies, including that of circadian rhythms. During normal mycelial growth, fusion among

² Abbreviations used: RFLP, restriction fragment length polymorphism; RIP, repeat induced point mutation; V-ATPase, vacuolar ATPase.

cells of the thallus is common. Heterokaryon formation between genetically different mycelia is readily observed. Heterokaryons have been used to examine hyphal fusion, complementation (both intergenic and intragenic), and vegetative incompatibility, which was shown to be induced by the mating-type idiomorphs or by allelic differences in a number of other genes that are polymorphic in the wild. Individual genes responsible for vegetative incompatibility were conveniently identified by their expression in heterozygous partial diploids. Studies of the attraction and fusion of trichogynes to cells of the opposite mating type, which are normally incompatible in vegetative confrontations, provided evidence for the involvement of pheromones and for nullification of the incompatibility response governed by the mating-type idiomorphs.

The sexual phase offers unique opportunities for developmental studies. The ascus is a single giant cell within which meiosis and postmeiotic mitosis occur in a common cytoplasm, where no partitioning occurs until ascospore walls are formed (documented photographically by Raju, 1980). Mutants that affect ascus development in various ways were observed microscopically and compared with wild type (reviewed by Raju, 1992). Studies of the programming of ascus development took advantage of related species, especially the four-spored *Neurospora tetrasperma* (Raju and Perkins, 1994). In this species, the heterokaryotic ascospores normally contain nuclei that differ in mating type, and the opposite mating types are vegetatively compatible.

Natural Populations

Neurospora from nature was studied using collections begun systematically in 1968 (reviewed by Perkins and Turner, 1988). Strains obtained by sampling populations from many parts of the world provided information on ecology, geographical distribution, variation, and speciation. Criteria that used crossing behavior to define and distinguish species were developed. Molecular differences were used to study genetic polymorphisms, showing that intrapopulation variation in this haploid eukaryote is as great as that in *Drosophila* and humans and that a large proportion of the species variation is present in local populations. Wild strains proved to be a valuable source of variants for laboratory investigations and especially for the study of vegetative incompatibility (reviewed by Glass and Kulda, 1992) and meiotic drive. Strains that contain meiotic drive factors called Spore killers (*Sk*) were discovered. When a "selfish" *Sk* element is heterozygous in a cross,

ascospores that do not carry it are killed (reviewed by Turner and Perkins, 1991; Raju, 1994).

RECENT DEVELOPMENTS— THE PAST 10 YEARS

The 1990s were highly productive, with many significant advances beyond those described in the last detailed review (Perkins, 1992).

Genome Organization

Genome projects are now under way. Expressed sequence tags (ESTs) have been obtained that identify genes expressed at different stages of the vegetative or sexual phases of the life cycle or during different intervals of the circadian cycle. Over 2000 different genes have been identified in this way (Nelson *et al.*, 1997; Dolan *et al.*, 2000). More than half of these have no known homologs in the yeast genome or elsewhere (Nelson and Natvig, 2000; Braun *et al.*, 2000). Physical maps of the genome are being constructed (Arnold, 2000). Genome sequencing is progressing in Germany (Mewes *et al.*, 2000; <http://www.mips.biochem.mpg.de/proj/Neurospora/>) and at the Whitehead Institute, Massachusetts Institute of Technology, where an award by the National Science Foundation provides for ninefold coverage, assembly, and annotation. The results will be distributed in a public database. *Neurospora* will thus provide the first publicly available genome sequence for a filamentous fungus. The DNA sequence will complement already existing physical and genetic knowledge of the genome. At the same time, genetic mapping has progressed substantially using classical markers (Perkins, 2000), RFLP markers (Nelson *et al.*, 1998; Nelson and Perkins, 2000), and chromosome rearrangements (Perkins, 1997).

Recombination

Understanding of meiotic recombination has been advanced by high-resolution experiments using molecular markers. The recombinator site *cog* has been cloned and two alleles have been sequenced (Yeadon and Catcheside, 1995a). Intragenic recombination appears to be initiated at *cog⁺* (Yeadon and Catcheside, 1998), which is 3' of the *am* locus (Bowring and Catcheside, 1991). Intragenic recombination has been studied using simultaneously both

closely linked RFLP markers and more distant classical genes to flank the *am* gene (Bowring and Catchside, 1996, 1998) and the *his-3* gene (Yeadon and Catchside, 1998). Conversion tracts are frequently interrupted in both these studies. Although about one-third of gene conversions at *his-3* are accompanied by a crossover, this apparent association is tenuous at *am*, where recombination frequencies are much lower. These observations cast doubt on the widely held assumption that both conversion and reciprocal crossing over arise from the same event. Evidence has been obtained that conversion events at *am* stimulate crossing over nearby (Bowring and Catchside, 1999). Studies with closely linked molecular markers show that the genetic criteria previously used to establish the order of intragenic sites is flawed when differentially spaced conventional mutants are used as flanking markers (Bowring and Catchside, 1995).

With the cloning of large fragments of DNA, physical and recombination distances could be compared. In general, the values lie between 30 and 80 kb per genetic map unit, with much less recombination around the centromeres (e.g., Mautino *et al.*, 1993; Centola and Carbon, 1994).

Disruption of Duplicate Genes

RIP has been used extensively for gene disruption. Null mutations of a RIP-inactivated essential gene can be recovered in progeny when a strain that carries two copies of the wild-type allele is crossed to a dominant mutant that induces meiotic nondisjunction (Metzenberg and Grote-lueschen, 1992). The RIP-induced null mutation is sheltered in one component of viable heterokaryotic ascospores that result from nondisjunction (see, e.g., Harkness *et al.*, 1994).

RIP frequently generates DNA-sequence signals for *de novo* methylation. The nature of these signals remains elusive, but detailed analysis of one short region with extensive RIP mutations suggests that the signals are redundant and degenerate and in some cases quite short, with many factors contributing to the actual pattern of methylation and its spread into contiguous regions (Miao *et al.*, 2000). Evidence was also obtained for maintenance methylation in *Neurospora* (Singer *et al.*, 1995). Further analysis of methylation resulting from RIP led to the discovery of an unexpected connection between histone acetylation and DNA methylation (Selker, 1998). Mutants defective in DNA methylation (*dim* mutants) have been isolated (see Foss *et al.*, 1998). Mutations in *dim-2*, which encodes a DNA methyltransferase (E. Kouzminova and

E. U. Selker, personal communication), result in loss of all detectable methylation, at least in the vegetative phase. (No known mutation in any other eukaryote completely abolishes DNA methylation.) Viability of the *dim-2* mutant indicated that DNA methylation is not essential in *Neurospora*. The mutant has been used to demonstrate that methylation can either interfere with gene expression (Irelan and Selker, 1997; Rountree and Selker, 1997) or promote it indirectly (Cambareri *et al.*, 1996), that methylation can inhibit transcript elongation *in vivo* (Rountree and Selker, 1997), and that gene silencing in the vegetative phase does not rely on DNA methylation (Cogoni *et al.*, 1996).

Silencing

Vegetative silencing of genes ("quelling"), which is reversible, can occur when additional copies of a gene are introduced into the cell by transformation (Romano and Macino, 1992; Pandit and Russo, 1992; reviewed by Irelan and Selker, 1996). Both the introduced and the resident copies are affected. Silencing is posttranscriptional and is dominant in heterokaryons (see Cogoni *et al.*, 1996; Cogoni and Macino, 1997, and references therein). This demonstrates a nuclear interaction via the cytoplasm. Quelling-deficient mutants in which transgene-induced gene silencing is impaired have been used to show that quelling requires three gene products: a protein homologous to RNA-dependent RNA polymerase (Cogoni and Macino, 1999a), a RecQ DNA helicase known to be involved in repair and recombination in other organisms (Cogoni and Macino, 1999b), and a homolog of the *Caenorhabditis elegans rde-1* gene-product, which controls the degradation of double-stranded RNA (Catalanotto *et al.*, 2000).

Silencing may also occur during the sexual phase. One of the more original advances has come from studies with an ascus-dominant gene, *Asm-1*, which affects ascospore maturation. Deletion of the *Asm-1* gene blocks maturation of all the ascospores of a cross, even if one of the parents carries the wild-type allele, *Asm-1*⁺. However, a frameshift mutation affects only those spores carrying the mutant allele, *Asm-1*. More striking is the fact that the dominant mutant phenotype prevails even in crosses between parents, each of which carries the normal allele, if the two copies of the *Asm-1*⁺ gene happen to be in different locations in the genome (Aramayo and Metzenberg, 1996). The dominant effect is attributed to transvection, in which certain genes must pair prior to Metaphase I if meiosis is to yield viable products. This discovery demonstrated

clearly that transvection occurs in an organism very different from *Drosophila*, where it was first described. The *Neurospora* results can be interpreted as due to silencing of unpaired segments and of all segments that are homologous to them in both parental nuclei.

Transposable Elements

Only one active transposon is known in *Neurospora*, but inactive DNA sequences have been found that represent different transposon families, and these bear unmistakable hallmarks of RIP (Kinsey *et al.*, 1994; Cambareri *et al.*, 1998; Margolin *et al.*, 1998; Bibbins *et al.*, 1998). Except for one DNA-intermediate element (Yeadon and Catchside, 1995b), the relic copies of transposable elements discovered so far appear to be retrotransposons. These are often severely degraded, an attribute ascribed to premeiotic inactivation by RIP. Evidence has accumulated that RIP serves as a genome defense system (see Selker, 1997). Characterization of centromeric DNA (Centola and Carbon, 1994) has revealed the presence of complex repeats reminiscent of the centric heterochromatin of *Drosophila* (Cambareri *et al.*, 1998). Defective transposable elements of several types are present among the repeats, and these show evidence of having been inactivated by RIP.

Mutation and DNA Repair

Neurospora continues to advance knowledge in the area of mutagenesis and DNA repair (Inoue, 1999). Study of the UV-sensitive mutant *mus-18* identified a novel DNA endonuclease that initiates an excision repair pathway rather different from other known DNA-repair mechanisms (Ishii *et al.*, 1991; see Yasui and McCready, 1998, for review). A UV-sensitive mutant, *mus-38*, is impaired in the previously known, highly conserved nucleotide excision repair pathway (Ishii *et al.*, 1998). Curiously, this pathway had eluded detection for some years, owing in part to the early misidentification of the *uvs-2* mutant as defective in the classical system.

Ectopic integration of transforming DNA was shown to be accompanied frequently by new gross chromosome rearrangements, many of which have breakpoints associated with vector DNA (Perkins *et al.*, 1993). Methods have been developed for obtaining targeted, homologous integration (e.g., Margolin *et al.*, 1997).

Metabolism and Transport

Individual metabolic pathways have continued to receive attention. Studies of the *arg-6* gene, which encodes a multifunctional precursor of two enzymes located in mitochondria, show that *arg-6* is a product of gene fusion and that a signal sequence between the N-terminal and the C-terminal domains was doubtless derived from the evolutionary precursor of the latter. Insertion of the enzyme into mitochondria leads to cleavage of the precursor at specific sites in the linking, signal peptide (Parra-Gessert *et al.*, 1998). In the same pathway, Wang and Sachs (1997) have used detailed *in vitro* studies of the *arg-2* gene to demonstrate a novel translational regulatory mechanism, sensitive to arginine, that involves arginine-regulated ribosome stalling. Studies of the polyamine pathway have culminated in the general picture of how the key enzyme of the pathway, ornithine decarboxylase, is transcriptionally regulated by four regions of the DNA in locations distributed from far upstream to downstream of the coding region (Hoyt *et al.*, 2000). Studies of lipid metabolism have clarified the individual steps of unsaturated fatty acid synthesis and the involvement of specific genes (e.g., Goodrich-Tanrikulu *et al.*, 1995). Comparable studies of carotenoid synthesis have revealed a multisubunit, five-step desaturase, the initial substrate of which is the colorless precursor, phytoene (Hausmann and Sandmann, 2000).

Major advances have come in detailed knowledge of circuits governing the transport and metabolism of nitrogenous compounds (Feng and Marzluf, 1998), phosphate (Peleg *et al.*, 1996), and sulfur compounds (Kumar and Paietta, 1998), and particularly the control, fate, and localization of regulatory proteins. Similarly, work on heat-shock responses (reviewed by Plesovsky-Vig, 1996) has led to a more systematic knowledge of heat-shock proteins and their homology with counterparts in other organisms. Plesovsky-Vig and Brambl (1998) have described the probable role of some small heat-shock proteins in the stabilization of an enzyme of glycolysis, required during recovery.

Mitochondria and Mitochondrial Plasmids

Mitochondrial membranes and other features of mitochondria have been studied intensively. Workers in yeast and *Neurospora* have standardized the nomenclature and have compared the proteins and processes of protein import into the organelle (Pfanner *et al.*, 1996). Studies with *Neurospora* have progressed to the point that the prepro-

tein translocase (TOM complex) has been purified from the outer mitochondrial membrane. The complex is effective in translocating mitochondrial intermembrane proteins when inserted into artificial membranes, and the complex has been shown by electron microscopy to contain centers interpreted as pores that represent protein-conducting channels (Künkele *et al.*, 1998).

Neurospora was used to show that mitochondrial tRNA synthetase mediates RNA self-splicing (Wallweber *et al.*, 1997). Two mitochondrial plasmids are retroelements that share properties of RNA viruses and mitochondrial introns. The novel transcriptases they encode possess characteristics suggesting how present-day reverse transcriptases and DNA polymerases could have evolved (Wang and Lambowitz, 1993).

Mitochondrial plasmids that are present in natural populations were shown to belong to discrete families (Yang and Griffiths, 1993; Arganoza *et al.*, 1994). New examples of plasmids that cause senescence have been discovered (Yang and Griffiths, 1993; Marcinko-Kuehn *et al.*, 1994; He *et al.*, 2000; reviewed by Griffiths, 1992, 1995, 1998).

Circadian Rhythms and Photobiology

N. crassa has become a preeminent model for studying circadian rhythms. In an extension of work pioneered by J. F. Feldman, the gene *frq* (*frequency*) was shown to encode a central component of a molecular feedback loop in which the product of *frq* negatively regulates synthesis of its own transcript, resulting in oscillation in the formation of conidia (Aronson *et al.*, 1994; Dunlap, 1993). The control is indirect, since the FRQ product acts not on its own promoter, but on the WC-1/WC-2 complex produced by the light-transduction genes *white-collar-1* and *-2* (Dunlap, 1999). Resetting the clock occurs when induction of *frq* by light overcomes negative autoregulation, resulting in phase delay or advance, depending on the time of day (Crosthwaite *et al.*, 1995). The *frq* gene and the *white-collar* genes *wc-1* and *wc-2*, which encode photore-sponse regulators, specify interconnected feedback loops. The FRQ protein and the WC-1/WC-2 complex have an antagonistic relationship that, with rhythmic induction and degradation kinetics of certain components, lend stability to the circadian rhythm (Crosthwaite *et al.*, 1997; Lee *et al.*, 2000). Increasingly sophisticated models of the clock appear as the work continues (Merrow *et al.*, 1999; Mc-Watters *et al.*, 1999). In conditions of lipid starvation, entrainable and free running rhythmicity can persist even in absence of the *frq* and *wc* gene products (Lakin-Thomas and Brody, 2000). This suggests that *frq*, *wc-1*,

and *wc-2* can be bypassed under these conditions. Thus, they may not represent the entire core repertoire of this complex system. A variety of clock-controlled genes have been identified, the inactivation of which does not alter rhythmicity (Bell-Pedersen *et al.*, 1996). For reviews of the *Neurospora* clock work, see Bell-Pedersen (1998), Loros (1998), Dunlap (1999), and Lakin-Thomas (2000).

Significant contributions have been made to the molecular genetics of a related subject, photobiology, with the identification and characterization of photomutants and genes regulated by blue light (reviewed by Lauter, 1996). The two *wc* genes are global regulators of photoresponses, encoding blue-light-activated transcription factors and participating in the blue-light signal transduction pathway (Ballario and Macino, 1997; Schwerdtfeger and Linden, 2000). A gene homologous to archaeal rhodopsins provided the first example of an opsin in eukaryotes other than animals. The *Neurospora* gene-product, NOP-1, is a photochemically reactive member of the archaeal rhodopsin family (Bieszke *et al.*, 1999a,b).

Signal Transduction

Extensive information has been obtained on the expression of genes under light, circadian, or developmental control (see Lauter, 1996; Bell-Pedersen *et al.*, 1996; Ebbole, 1996). Genes that specify α -1, α -2, and α -3 subunits of heterotrimeric GTP binding proteins have been isolated and characterized (Turner and Borkovich, 1993; Baasiri *et al.*, 1997; Kays *et al.*, 1998, 2000). Numerous additional genes that encode putative signal transduction proteins have been identified (Margolis and Yanofsky, 1998; see also Perkins *et al.*, 2000), making it likely that various signal cascades will be well defined in the near future.

Vegetative Growth, Differentiation, and Morphogenesis

Neurospora has recently proved to be as useful in the study of hyphal growth as it was to biochemical genetics. Work in this area has been facilitated by the availability of many genetically well-characterized morphological and biochemical mutants. Hyphal growth has been studied for many years and from many standpoints (reviewed in Davis, 2000). Attention has now focused on three major issues: cell wall formation, the activity of cytoskeleton and molecular motors, and the relevant signal transduction systems. The β -glucans and chitin components of the cell

wall are becoming better understood with the cloning of genes affecting β -glucan synthase activity (Enderlin and Selitrennikoff, 1994) and those encoding chitin synthase (Yarden and Yanofsky, 1991; Beth-Din and Yarden, 2000).

The hyphal tip, which contains a cluster of vesicles called the *Spitzenkörper*, is the site of deposition of cell wall material by exocytosis of these apical vesicles. The "hyphoid" model of vesicle distribution from this cluster accounts well for the shape of the tip (reviewed by Bartnicki-Garcia, 1990; Riquelme *et al.*, 2000). The biochemical events involve ion gradients and a tip-high calcium gradient (reviewed by Jackson and Heath, 1993; Silverman-Gavrila and Lew, 2000). The newest work has begun to unravel the cytoskeletal elements at the tip, which have until now been poorly described. Heath's laboratory has discovered a spectrin-containing membrane skeleton and has shown that actin is required at the tip, for normal growth, but microtubules are not (Degoussée *et al.*, 2000; Heath *et al.*, 2000). Moreover, a SNARE protein is found at the tip in a gradient that approximates the gradient of wall deposition in earlier studies (Gupta and Heath, 2000) even though it does not conform closely to the hyphoid equation of Bartnicki-Garcia.

Early studies of the proton gradient across the plasma membrane (reviewed by Slayman, 1977) flourished with characterization of the plasma membrane ATPase and cloning of the corresponding gene, *pma* (reviewed by Rao and Slayman, 1996). Interest in ATPases widened with the discovery and characterization of a distinct, multisubunit vacuolar ATPase (V-ATPase) and the cloning of most of the genes that contribute to it (reviewed by Margolles-Clark *et al.*, 1999). The role of the vacuole in growth and morphology is beginning to become clear, with studies of inhibitors of vacuolar function and their possible effects in the distribution of calcium, which is a major player in the control of growth and branching (Bowman *et al.*, 1997; reviewed by Bowman and Bowman, 2000).

New light has been shed on regulatory genes that affect morphology. Mutants known from the earliest days of *Neurospora* genetics have sometimes contributed. Several genes that served as morphological markers in constructing the first fungal genetic maps in the 1930s have now been cloned, sequenced, and characterized functionally. These include *fluffy* (Bailey and Ebbole, 1998) and *crisp-1*, both of which affect conidiation. *crisp-1* proved to be the structural gene for adenylyl cyclase (Kore-Eda *et al.*, 1991). The antagonistic roles of cAMP and calcium in hyphal branching had become clear in earlier days (reviewed in Davis, 2000), and studies have now been extended to the action of calcineurin (Prokisch *et al.*, 1997),

protein kinases (Yarden *et al.*, 1992), and protein phosphatases (Yatzkan and Yarden, 1995). A coherent picture of the control of hyphal growth and branching can be expected to emerge from the extension and integration of these studies.

Morphological mutants called *ropy* were shown to be defective in specifying subunits of dynein and related molecular motors (Plamann *et al.*, 1994). Mutations at *ropy* loci are selectable as suppressors of the morphological mutant *cot-1*. Similarly, the *mcb* (*microcycle blastoconidiation*) mutant, which affects growth polarity, acts as a suppressor of the morphological mutant *crisp* (Bruno *et al.*, 1996). The lack of polarity is correlated with a rate of secretion of extracellular enzymes in *mcb* cultures at the high level characteristic of the hyphal tip in wild-type cultures (Lee *et al.*, 1998). Another molecular motor, kinesin, which drives organelle movement on microtubules in a direction opposite from the dynein-activated movement, is represented by a distinct form (N-kin) in *Neurospora* (Seiler *et al.*, 1997). Curiously, double mutants lacking activity of both dynein/dynactin and kinesin are still viable, although they grow slowly (Seiler *et al.*, 1999). Other molecular motors that endow hyphae with specialized means of moving particular organelles are therefore thought to exist in *Neurospora*.

Recent work has helped solve the mystery of the hexagonal crystals, often called Woronin bodies, that are found in many filamentous fungi. These bodies clog septal pores upon a sudden loss of turgor, preventing excessive loss of protoplasm from burst or cut hyphal tips. They were originally identified as ergosterol crystals, but later studies showed that they are composed largely of protein. Isolation of these bodies and the constituent protein allowed Jedd and Chua (2000) to clone the gene *hex-1* and to show that its aggregated product is an inclusion in an unusual peroxisome. Disruption of the gene *in vivo* results in cytoplasmic bleeding at damaged hyphal tips.

Natural Populations

Information has been brought together on the more than 4600 cultures from natural populations that are now available from the Fungal Genetics Stock Center (Turner and Perkins, in press). These authors review the information that these strains have provided on species distribution, ecology, genetic diversity, population structure, and meiotic drive. The wild strains have continued to provide genetic variants for a variety of laboratory investigations. Surveys of the strains have revealed widespread occurrence of mitochondrial plasmids.

Neurospora tetrasperma

The pseudohomothallic species *N. tetrasperma* occurs naturally as a self-fertile heterokaryon carrying nuclei of both mating types. Investigations of *N. tetrasperma* from natural populations have related observations on heterokaryosis and crossing-over suppression in the mating-type chromosome to the unusual population genetics and evolution of this unique genetic system (Merino *et al.*, 1996; Gallegos *et al.*, 2000; Metzenberg and Randall, 1995; Raju and Perkins, 1994). In particular, Merino *et al.* (1996) showed that segments linked to mating type are different in the *mat A* chromosome than in the *mat a* chromosome of individual wild-collected, heterokaryotic *N. tetrasperma* strains, whereas autosomal markers are homogeneous in nuclei of the same heterokaryons. When strains from geographically distinct populations were compared, strikingly different evolutionary trees were obtained for the mating-type chromosomes than for the autosomes.

Mating Type

Substantial progress has been made in understanding the organization and function of genes at the mating-type locus, which are called idiomorphs rather than alleles in recognition of their lack of homology (Metzenberg and Glass, 1990). The *mat a* idiomorph contains a single open reading frame, while *mat A* contains three (Ferreira *et al.*, 1996). Both *mat A-1* and *mat a-1* appear to be essential for mating and for sexual development, while *mat A-2* and *mat A-3* increase fecundity but are not essential (Ferreira *et al.*, 1998). For reviews of mating type, see Staben (1996) and Coppin *et al.* (1997).

Heterokaryons and Vegetative Incompatibility

Genes responsible for vegetative (heterokaryon) incompatibility (*het* genes) have been cloned and sequenced, and with this, a start has been made to understanding the molecular basis of cell death in incompatible confrontations (Saupe *et al.*, 1996; Smith *et al.*, 1996, 2000a, 2000b; Shiu and Glass, 1999). The same multiple alleles of *het-c* that are found in *N. crassa* are present in other *Neurospora* species and in related genera, indicating derivation from a common ancestor and conservation during evolution (Wu *et al.*, 1998). The *tol* gene has been cloned and sequenced (Shiu and Glass, 1999). *tol* function is required for expression of mating type-mediated vegetative incompatibility, which is seen when *mat A* and *mat a* idiomorphs

are together in heterokaryons or in heterozygous partial diploids. However, a functional *tol* gene is not required for vegetative incompatibility reactions mediated by genes other than *mating type* (Leslie and Yamashiro, 1997). An active *tol* allele is normally present in the heterothallic outbreeding species *N. crassa*, where the gene was originally identified as a recessive mutation that suppressed *A + a* vegetative incompatibility. The species *N. tetrasperma*, which normally exists as a self-fertile (*mat A + mat a*) heterokaryon, was shown to possess an inactive *tol* allele, and the active and inactive *tol* alleles were interchanged between *N. crassa* and *N. tetrasperma* (Jacobson, 1992).

Heterokaryons are being used to produce heterodimeric molecules that incorporate components originating from genetically different nuclei. Intact antibody molecules are formed by heterokaryons in which the light chain is encoded by one nuclear type and the heavy chain is encoded by the other; the components assemble themselves in the cytoplasm and are secreted (Stuart, 1997, 1998).

PROSPECT

In the exciting years ahead, as genes are annotated following completion of the genomic sequence, the knowledge and the strains obtained so painstakingly during the past 75 years will be instrumental in relating sequence data to biologically meaningful problems.

Neurospora will continue to provide a healthy counterpart to *Saccharomyces*. The two organisms are phylogenetically and biologically quite distinct, with lineages diverging from a common ancestor 360 million years ago (Berbee and Taylor, 2000). They differ profoundly in life cycle, morphology, ecology, and chromosome complement. *Neurospora* is structurally more complex, with distinct, differentiated cell types. More than half of the expressed genes in *Neurospora* have no detectable homolog in yeast. *Neurospora* is perhaps more of a generalist, with genetic mechanisms that appear to be more representative of other eukaryotes. A more sophisticated picture of fungal genetics and biology should emerge from studies with both organisms.

Neurospora remains a powerful model, especially for the mycelial fungi. Future work with *Neurospora* should bring increased understanding of many aspects of fungal biology, ranging from genome organization, signal transduction, and hyphal growth to evolutionary history and

speciation. Because comparative genomics is largely independent of the techniques of classical genetics, much of what is learned from the *Neurospora* sequence can quickly be adopted in the study of diverse organisms. Availability of the genome sequence should be a boon to all students of fungi, empowering those who work with less tractable species to solve problems that would otherwise be too baffling. We look forward to the time when it can be said that the most important contribution of *Neurospora* in its later years has been to advance knowledge and propagate interest in all the fungi, a unique, widespread kingdom of organisms that is even now relatively unexplored.

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