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Neurospora Genetics at Turn of the Century

Abstract

Research advances are described that have been made since a 1992 survey in Genetics (130: 687-701).

Neurospora genetics at the turn of the century

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Research advances are described that have been made since a 1992 survey in *Genetics* (**130**: 687-701).

The century's end also marks the 75th anniversary of the first genetic experiments with Neurospora. Although accounts usually begin with the 1941 paper of Beadle and Tatum, genetic analysis was in fact initiated 16 years earlier by B. O. Dodge. It was he who identified the two mating types and used them to demonstrate mendelian segregation in individual asci. Dodge quickly recognized the potentialities of the organism. 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. Horowitz (1991), Perkins (1992), and Davis (2000) have described the main long-term contributions of Neurospora and have evaluated their significance. Specific advances since the 1992 review are summarized below.

Neurospora crassa has become a preeminent model species for studying circadian rhythms. In an extension of work pioneered by J. F. Feldman, the gene *frq* (*frequency*) has been 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 daily oscillation (Aronson *et al.* 1994, Dunlap 1993). A variety of clock-controlled genes have been identified, the inactivation of which does not alter rhythmicity (Bell-Pedersen *et al.* 1996). 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). Photoresponse regulator genes (*white-collar-1* and *-2*) are essential in assembly or operation of the *frq* feedback loop (Crosthwaite *et al.* 1997). Demonstration that entrainable and free running rhythmicity can persist in the absence of *frq* and *wc* gene-products has suggested increasingly sophisticated models (Merrow *et al.* 1999, Lakin-Thomas and Brody 2000, McWatters *et al.* 1999). For reviews see Bell-Pedersen (1998), Loros (1998), Dunlap (1999), Lakin-Thomas (2000).

Extensive information has been obtained on genes the expression of which is under photo-, circadian, or developmental control. (See Lauter 1996, Bell-Pedersen *et al.* 1996, Ebbole 1995). Genes have been isolated and characterized that specify α_subunits of heterotrimeric GTP binding proteins (Turner and Borkovich 1993, Baasiri *et al.* 1997, Kays *et al.* 1998). Numerous genes that encode putative signal transduction proteins have been identified (Margolis and Yanofsky 1998).

Significant contributions have been made to the molecular genetics of fungal photobiology, with the identification and characterization of photomutants and genes regulated by blue light (reviewed by Lauter 1996). The two *white collar* 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 provides the first example of an opsin in eukaryotes other than animals; the gene-product is a photochemically reactive member of the archaeal rhodopsin family (Bieszke *et al.* 1999a).

The ascus-dominant expression of the ascospore-maturation gene *Asm-1* has been shown to result from failure of transvection, wherein chromosome rearrangements or ectopic placement of a gene disrupts pairing of allelic chromosomal genes during the sexual phase and results in a mutant phenotype, even in spores that carry the normal allele (Aramayo and Metzenberg 1996). This discovery provided a clear demonstration that transvection occurs in an organism very different from Drosophila..

Study of the UV-sensitive mutant *mus-18* has identified a novel DNA endonuclease that initiates an excision repair pathway completely different from previously 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).

Evidence has accumulated that RIP (repeat-induced point mutation) serves as a genome defense system (see Selker 1997). While only one active transposon has been found in Neurospora, sequences have been discovered that represent several different transposon families, with unmistakable hallmarks of RIP (Cambareri *et al.* 1998, Kinsey *et al.* 1994, Margolin *et al.* 1998, Bibbins and Connerton 1998). 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.

RIP has been used extensively for gene disruption. Null mutations of RIP-inactivated essential genes can be recovered by using a meiotic mutant that produces heterokaryotic ascospores (Metzenberg and Grotelueschen 1992; Harkness *et al.* 1994).

RIP was shown frequently to generate signals for de novo methylation. 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 protein 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 is thought to encode 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.) Identification of *dim-2* 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 ("quelling") does not rely on DNA methylation (Cogoni *et al.* 1996).

Reversible silencing (quelling) can occur when additional copies of a gene are introduced by transformation (Rom 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). Quelling-deficient mutants in which transgene-induced gene silencing is impaired have been used to show that quelling requires 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 *C. elegans rde-1*, which controls the degradation of double-stranded RNA (Catalanotto *et al.* 2000).

Mitochondrial tRNA synthetase has been shown to mediate RNA self-splicing. 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).

Regulated ribosome stalling has been demonstrated (Wang and Sachs 1997).

Vesicles from the outer mitochondrial membrane have been purified on a massive scale and the preprotein translocase (TOM complex) has been shown by electron microscopy to contain centers interpreted as pores that represent protein-conducting channels (Künkele *et al.* 1998).

Over 4600 cultures from natural populations throughout the world are now available for study (Turner and Perkins 2000). Wild-collected strains have provided information on species distribution, ecology, genetic diversity, population structure, and meiotic drive systems. They have also been a source of genetic variants for a variety of laboratory investigations.

Surveys of strains from nature have revealed the widespread occurrence of mitochondrial plasmids, which belong to discrete families (Yang and Griffiths 1993, Arganoza *et al.* 1994). New examples have been discovered of plasmids that cause senescence (Yang and Griffiths 1993, Marcinko-Kuehn *et al.* 1994, He *et al.* 2000; reviewed by Griffiths 1992, 1993, 1998).

Investigations with the pseudohomothallic species *N. tetrasperma* have revealed novel features of this unique genetic system (Merino *et al.* 1996, Gallegos *et al.* 2000, Metzenberg and Randall 1995, Raju and Perkins 1994).

Integration of transforming DNA was shown to be accompanied by new gross chromosome rearrangements, many of which have breakpoints associated with vector DNA (Perkins *et al.* 1993).

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, *mcb* (*microcycle blastoconidiation)* acts as a suppressor of the morphological mutant *crisp* (Bruno *et al.* 1996). Mutations of *mcb* affect growth polarity. Secretion of extracellular enzymes in *mcb* cultures is increased to the high level that is characteristic of the hyphal tip in wild type cultures (Lee *et al.*1998)

Understanding of meiotic recombination has been advanced by high-resolution experiments using molecular markers (e.g., T. Randall and D. R. Stadler, in preparation). The recombinator gene *cog* has been cloned and two alleles have been sequenced (Yeadon and Catcheside 1995). Intragenic recombination appears to be initiated at cog^L (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 Catcheside 1996, 1998) and the *his-3* gene (Yeadon and Catcheside 1998). In both cases, conversion tracts frequently are interrupted. 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. This observation casts 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 Catcheside 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 Catcheside 1995).

Substantial progress has been made in understanding the organization and function of genes at the *mating type* locus (now called idiomorphs in recognition of their lack of homology). The *mat a* idiomorph contains a single open reading frame, while *mat A* contains three (Ferreira *et al.* 1996). For reviews see Staben (1996), Coppin *et al.* (1997). Both *mat A-1* and *mat a-1* appear to be essential for mating and for sexual development, while *mat A-2* and *mat-A3* increase fecundity but are not essential (Ferriera *et al.* 1998).

Genes responsible for vegetative (heterokaryon) incompatibility (*het* genes) have been cloned and sequenced (Saupe *et al* 1996, Smith *et al.* 1996, 1999, 2000; Shiu and Glass 1999). The same multiple alleles of *het-c* that are found in *N. crassa* are also present in other Neurospora species and in related genera, indicating that they were derived from a common ancester and have been conserved during evolution (Wu *et al.* 1998).

The *tol* gene has been cloned and sequenced (Shiu *et al.* 1999). A functional allele of *tol* (*tolerant*) is required ... expression of the *mating-type* mediated vegetative incompatibility phenotype that results when *mat A* and *mat a* idiomorphs are together in heterokaryons or heterozygous partial diploids. Vegetative incompatibility reactions mediated by genes other than

mating type do not require the presence of a functional *tol* allele (Leslie and Yamashiro 1998). An active *tol* allele is normally present in the heterothallic outbreeding species *N. crassa*, and the gene was originally identified in that species as a recessive mutant that suppresses *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 (Jacobson 1992). The active and inactive *tol* alleles have been interchanged between *N. crassa* and *N. tetrasperma*.

Genes that served as morphological markers in constructing the first fungal genetic maps in the 1930's have now been cloned, sequenced, and characterized functionally (e.g., *crisp-1* (Kore-Eda *et al.* 1991), *fluffy* (Bailey and Ebbole 1998).

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 produced and secreted by one nuclear type and the heavy chain is produced and secreted by the other (Stuart 1997, 1998).

Genetic mapping has progressed substantially, using both classical and RFLP markers (Perkins 2000, Nelson *et al.* 1998, Nelson and Perkins 2000).

Genome projects are under way. Expressed sequence tags (ESTs) have been obtained that identify genes expressed at different stages of the life cycle or during different parts of the circadian cycle. More than half the expressed sequences show no similarity to genes previously identified in the yeast genome or elsewhere. Over 2000 different genes have been identified in this way (Nelson *et al.* 1997, Dolan *et al.* 2000). Over 50% of these have no known homologs in any organism (Nelson and Natvig 1000). Physical maps of the genome are being constructed (Arnold, 2000), and DNA sequencing of the genome is progressing (Mewes *et al.* 2000; *http://www.mips.biochem.mpg.de/proj/neurospora/*).

At the turn of the century, Neurospora is genetically and biologically the best known euascomycete. The rich resources of information, brought together by Davis (2000) and by Perkins *et al.* (2000), will speed progress in relating sequence data to biologically meaningful problems.

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