MINIREVIEW

Senescence in fungi: the view from Neurospora

Ramesh Maheshwari & Arunasalam Navaraj

Department of Biochemistry, Indian Institute of Science, Bangalore, India

Correspondence: Ramesh Maheshwari, Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India. Tel.: +91 80 2334 1045; fax: +91 80 360 0814; e-mails: fungi@biochem.iisc.ernet.in and ramesh.maheshwari01@gmail.com

Present addresses: Ramesh Maheshwari, S3/A2 4th Main, 17th Cross, Malleswaram, Bangalore 560005, India. Arunasalam Navaraj, Department of Hematology & Oncology, University of Pennsylvania Medical School, Philadelphia, PA 19104, USA.

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Abstract

Some naturally occurring strains of fungi cease growing through successive subculturing, i.e., they senesce. In Neurospora, senescing strains usually contain intramitochondrial linear or circular plasmids. An entire plasmid or its part(s) integrates into the mtDNA, causing insertional mutagenesis. The functionally defective mitochondria replicate faster than the wild-type mitochondria and spread through interconnected hyphal cells. Senescence could also be due to spontaneous lethal nuclear gene mutations arising in the multicellular mycelium. However, their phenotypic effects remain masked until the nuclei segregate into a homokaryotic spore, and the spore germinates to form a mycelium that is incapable of extended culturing. Ultimately the growth of a fungal colony ceases due to dysfunctional oxidative phosphorylation. Results with senescing nuclear mutants or growth-impaired cytoplasmic mutants suggest that mtDNA is inherently unstable, requiring protection by as yet unidentified nuclear-gene-encoded factors for normal functioning. Interestingly, these results are in accord with the endosymbiotic theory of origin of eukaryotic cells.

Introduction

The ability to regenerate the hyphal tips continuously gives the fungi the potential for indefinite growth, corroborated by the discovery of a more than 1500-year-old fungal colony of Armillaria bulbosa spread over a large forested area in North America (Smith et al., 1992). Because of hyphal fusions and perforated septa, the fungal colony is a cytoplasmic continuum in which aged or dysfunctional organelles – the nuclei and mitochondria – can be replaced by the migration of functional copies from other cellular compartments. Additionally, their multinuclear condition enables any potentially deleterious mutant nuclear gene to be complemented by its functional allele in other nuclei. These unique features of fungi undoubtedly contribute to their immortality. Therefore, the findings of senescing strains in Neurospora spp. as well as a few other fungal genera including wild strains of the ascomycete Podospora anserina are of great interest. In these fungi, senescence is promoted by the same features that otherwise contribute to its immortality. For example, the dysfunctional mitochondria containing the senescence-determining factor spread throughout the mycelium and become dominant – a phenomenon called suppressivity. Because genetic tools are best developed in Neurospora, this fungus has considerable promise as a model for investigation of mechanisms in aging and death in higher eukaryotes.

The symbols used herein are as recommended by Perkins et al. (2001). The wild-type gene is in three-letter lowercase letters; the mutant allele in lowercase italics. Its putative protein product is in nonitalic capital letters, e.g., ND. A heterokaryon is denoted by enclosing symbols of the component nuclei, separated by a plus sign, in parenthesis; for example, the wild-type allele natural death is designated nd++; and the mutant allele nd is in lowercase italics. Its putative protein product is in nonitalic capital letters, e.g., ND. A heterokaryon is denoted by enclosing symbols of the component nuclei, separated by a plus sign, in parenthesis; as for example (sen+sen+). The mitochondrial mutants are in brackets, for example (poky). The plasmid name is in capital letters prefixed with a lower-case ‘p’, e.g., pKALILO.
abbreviated as pKAL. The free autonomously replicating pKAL is denoted as AR-kalDNA, and the pKALILO inserted into mitochondria as mtIS-kalDNA (Griffiths et al., 1990).

**Discovery**

In 1951, Sheng (1951) obtained a colony of *Neurospora crassa* from the plating of UV-irradiated conidia that ceased growth in successive transfers under all nutritional conditions, whether grown on agar surfaces or in liquid cultures. This mutant was named *natural death* (*nd*). It led to the belief that senescing strains may be isolated from nature for genetic investigations of the phenomenon of aging and death that characterizes the development of all eukaryotic organisms.

The first nuclear-gene senescing mutant from nature was obtained by an unusual method: individual nuclei from mycelium of wild *Neurospora intermedia* were extracted in the form of uninucleate conidia (Navaraj et al., 2000) and plated (see Fig. 3 in Maheshwari, 2005). Among 150 homokaryotic cultures, one showed senescence upon subculturing. The mutant gene *senescent* was introgressed into *N. crassa* through a series of nine backcrosses for gene mapping and preservation in a heterokaryon (Navaraj et al., 2000).

In 1965, E.L. Tatum’s group described mutants of *N. crassa* symptomatic of senescence that arose during routine transfers of cultures. These had fine hyphae lacking visible flow of cytoplasm, showed ‘stop-start’ growth or stopped growing altogether (Diacumakos et al., 1965; Garnjobst et al., 1965). Microinjection of mitochondrial preparation from the normal strains into the slow-growing strains restored normal growth but microinjection of DNA from nuclei had no effect, indicating that the abnormal phenotype is determined by mitochondria. The aforementioned studies showed that: (1) senescence is due to nuclear – or cytoplasmic (mitochondrial) mutations and (2) natural populations are a source for obtaining potentially senescing strains, either through sampling of conidia (Perkins & Turner, 1988) or by plating soil containing ascospores (Pandit & Maheshwari, 1996a) and (3) although initially indistinguishable from an immortal strain, a senescing strain is identified by cessation of growth in extended propagation in long tubes (race tubes) or by serial transfers in culture tubes using conidia (Griffiths & Bertrand, 1984; Maheshwari et al., 1994). Senescence usually manifests in five to 30 subcultures made at weekly intervals at room temperature. A strain that has not senesced in some 50 subcultures is commonly regarded as immortal. In *Neurospora*, senescing strains have been found in populations of *N. crassa*, *N. intermedia* and *Neurospora tetrasperma* (Griffiths & Bertrand, 1984; Maheshwari et al., 1994; Maas et al., 2005).

**Distinction between senescence and other cell death phenomena**

Senescence is the progressive loss of growth potential of mycelium culminating in total cessation of growth when the culture is considered to be as dead. The respiration of mycelial suspensions or conidia of all senescing strains ('Cytoplasmic mutants symptomatic of senescence', 'Senescence caused by mitochondrial plasmids', 'Senescence caused by nuclear mutations') measured as oxygen uptake switches from cyanide-sensitive cytochrome-mediated to a KCN-insensitive, salicylhdroxamic acid (SHAM)-sensitive, alternate pathway mediated by alternative oxidase and is associated with deficiencies of cytochrome *a* and *b*. Because the hyphal cells are interconnected through the septal pores, dysfunctional mitochondria accumulate throughout the mycelium, resulting in displacement of normal mitochondria, and deficiency of ATP (Pall, 1990). A single study on the ultrastructure of plasmid-associated senescing hyphae showed vacuolization, breakdown of nuclear and mitochondrial membranes, loss of cristae and accumulation of dense material in the mitochondrial matrix (Bok et al., 2003). Senescence in fungi is distinct from apoptosis as there is neither typical DNA fragmentation nor the release of cytochrome *c*. Senescence is not equivalent to autolysis because there is no hyphal fragmentation or lysis of the cell wall. Senescence is perhaps not analogous to necrosis, because there is no documented microscopic evidence of loss of membrane integrity or release of cellular constituents. Senescing mycelium of strains bearing pKALILO showed large vacuoles (Fig. 1), as in the incompatible cell fusions due to nonallelic *het* loci (Glass & Kaneko, 2003). An expected alteration in senescing mycelium is extensive depolymerization of actin-cytoskeleton.

The life span of strains collected from the same place may vary (Table 1). However, the replicate subcultures from the same parent stock die in about the same subculture. Interestingly, the death of duplicate cultures after these had been stored at −15°C for 12 months occurred in fewer subcultures, indicating that degenerative changes continue even in the frozen state (Maheshwari et al., 1994). One apparently normal strain did not resume growth after storage. Subsequently, it was found that the strains tested contained a circular mitochondrial plasmid (D’Souza et al., 2005a). The results of this experiment suggest that senescence in fungi is an expression of a preprogrammed pathway that is kept in check in the long-living (‘immortal’) strains by mechanisms that are not understood.

**Distinguishing between nuclear and cytoplasmic senescence**

Whether the senescence-determining factor resides in the nucleus or in the mitochondria can be determined from genetic methodology.
Genetic cross

When two Neurospora strains of complementary mating types, mat A and mat a, are crossed, the progeny inherit the mitochondria only from the protoperithecia-forming (female) parent. In reciprocal crosses: (i) long-living female X short-living male, progeny is long living whereas in (ii) short-living female X long-living male, progeny is short living.

Heterokaryon test

A mycelium containing nuclei from a senescing and a nonsenescing strain is constructed by mixing germinating conidia of a long-living strain with that of a senescing strain. The neighboring cells of two genetically related strains interconnect, and the heterokaryon containing mixed cytoplasm grows indefinitely on minimal medium due to the complementation of nuclei from the original strains. Color and auxotrophic markers are incorporated into the fusing strains by prior genetic crossing for detection of the heterokaryon (Fig. 2). The short-living or long-living nuclear types are extracted by plating conidia formed by heterokaryotic mycelium on appropriate media.

Cytoplasmic mutants symptomatic of senescence

A class of cytoplasmic mutants exhibit alternate rapid growth, followed by slow or no growth on agar media (Bertrand et al., 1980; de Vries et al., 1986; Almasan & Mishra, 1988, 1990). The mtDNA of these mutants, for example (stopper), have deletions and exhibit severe deficiencies of cytochromes b and aa3 (Almasan & Mishra, 1991). The defective mitochondria are maintained in a heteroplasmic state with normal mtDNA, as in several human diseases (Wallace, 1999). In Fig. 2, has been illustrated a technique for preserving lethal nuclear mutants in heterokaryons. As long as nd or sen is associated with a normal allele in other nuclei of the heterokaryon, it is indefinitely stable. However, its mtDNA undergoes rapid deletions when removed. Cloning and sequence analysis of deleted fragments in nd and comparison with wild-type mtDNA revealed high frequencies of deletions of intervening sequences of the mitochondrial genome due to unequal crossovers between mispaired copies of mtDNA sequences.

The nucleotide sequence of sen-specific EcoRI-5 fragments suggested that intramolecular recombination between stretches of 18 nucleotide palindromic sequences (5'-CCCTGCAGTACTGCAGGG-3') in mtDNA that potentially form hairpin structures could promote preferential intramolecular recombination (Dieckmann & Gandy, 1987; Almasan & Mishra, 1991) between homologous repeats, resulting in deletions (Fig. 3). The studies of growth mutants of fungi including the yeasts (Contamine-Picard & Picard, 2000) have revealed the innate instability of mtDNA, and the requirement of nuclear gene-encoded proteins for maintaining the integrity of mtDNA.

Table 1. The number of passages (transfers) of agar-grown stock cultures of four strains of Neurospora intermedia before death*

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<td>Before storage</td>
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<td>Maddur 1991–59</td>
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<tr>
<td>Maddur 1991–60</td>
<td>15</td>
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<tr>
<td>Maddur 1992–18</td>
<td>29</td>
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*Subculturing by asexual spores (conidia) or vegetative hyphae on slants was at intervals of 5–10 days. From Maheshwari et al. (1994).
Senescence caused by mitochondrial plasmids

The determination whether growth-impaired mutants will arise upon prolonged cultivation from the ‘normal’ strains, as the spontaneously occurring petite mutants in yeast, led to isolation of maternally inherited (poky) (Mannella et al., 1997) and stopper (stp-B1) mutants. Neurospora intermedia cultures isolated from nature were subjected to extended culturing either by continuous vegetative growth in race tubes or sequential conidial transfers in slants (Griffiths & Bertrand, 1984). Approximately 30% of the strains from Kauai were senescent in prolonged culturing and were named ‘kalilo’, Hawaiian for ‘dying’. Senescing strains of N. crassa were found in the state of Maharashtra in India, and were named ‘maranhar’ meaning ‘prone-to-death’ in the Hindi language (Court et al., 1991). Both kalilo and maranhar strains contain linear mitochondrial plasmids that have no nucleotide sequence homology.

The circular plasmids pVARKUD (3675 bp) and pMAURICEVILLE (3581 bp) contain an ORF encoding a reverse transcriptase. From the sequence data of mtDNA and plasmid DNA, it was deduced that the retroplasmid inserts into the mtDNA. The insertion is affected by prior formation of a variant plasmid form having a cDNA copy of a tRNA-like cloverleaf structure at the 3' end (Akins et al., 1989). The variant plasmid DNA integrates by homologous recombination into mtDNA, close to or within the genes encoding the mitochondrial rRNAs (Myers et al., 1989; Chiang et al., 1994). For unknown reasons, the mitochondria containing the variant plasmids divide faster than the wild type; the presence of nonfunctional mtDNA molecules exceeding a threshold concentration in obligate aerobes is expected to cause drastically lowered ATP production and to result in death (Myers et al., 1989). Although Akins et al. (1986) found that impaired growth and cytochrome deficiencies correlated with the time of insertion of variant plasmid into mtDNA, Fox & Kennell (2001) found no correlation between the time of insertion of plasmid sequence and its suppressive accumulation (Stevenson et al., 2000). Insertion of pKAL (8642 bp) and pMAR (7052 bp) into mtDNA was correlated with disruption of the nuclear membrane and vacuolization in the mitochondrial matrix (Bok & Griffiths, 2000).
Spread of plasmids

Twenty-five years after the initial discovery, a resampling of strains from Hawaii by Southern hybridization using plasmid fragments as probes showed that nonsenescant and senescent fungal strains coexist. pKAL was present at the same frequency, i.e., c. 30% (Maas et al., 2005). pKAL has also been found in *N. tetrasperma*, although at lower frequencies than in *N. intermedia*. Senescent isolates have also been identified in populations of *Neurospora* collected in Madur in southern India (Maheshwari et al., 1994; D’Souza et al., 2005a). Time-course analysis revealed integration of pMAD into mtDNA. All cultures collected from Madur were senescent and contained pMAD that was 98% homologous to pMAU first found in the Mauriceville strain of *N. crassa* collected from Texas. This raises the question as to how plasmids have become distributed in geographically separated regions. Because mitochondrial leakage during paternal transmission is thought to be rare, horizontal transmission of senescence plasmids through hyphal fusion has been suggested for the widespread distribution of senescence plasmids, despite the presence of polymorphic *het* loci that minimize, if not preclude, hyphal fusion. To test transmission of the senescence plasmid into a nonsenescant colony, a senescing kalilo strain having the nuclear marker genes *nic-1 al-2* was fused to a long-living strain having the nuclear marker genes *ad-3B cyh-1* (Griffiths et al., 1990). The resulting heterokaryon was senescent in which mtIS-kalDNA had associated with the nuclei. Evidence has been provided indicating that hypha can fuse and form heterokaryotic mycelium when growing inside a natural substrate (Pandit & Maheshwari, 1996b). The spread of plasmids by vegetative fusion of hyphae is therefore a real possibility.

To determine whether plasmids confer some advantages to the host strain, a kalilo plasmid from a nonsenescant *N. tetrasperma* was introgressed into wild-type *N. crassa*. The growth rate at 41 °C (*T* max) and fertility (perithecia production) at 30 °C (≥*T* opt) improved (Bok & Griffiths, 2000), suggesting that plasmids influence house-keeping genes in the host fungus, hastening its life cycle. Presumably, a plasmid-bearing strain initially starts out as more fertile than the strain lacking plasmids. According to the selfish gene theory of Dawkins (1989), the host fungus is a vehicle for propagating parasitic DNA; the plasmid is not only selfish DNA but true parasitic DNA, spreading by contact transmission like a molecular disease.

Temporary increase in life span

Not every culture that carries pKALISO senesces; for example 10–20% pKAL carrying isolates of *N. intermedia*, and a similar percentage in *N. tetrasperma* isolates, did not senesce (Maas et al., 2005). Crossing of these strains to nonsenescing strains showed that the ability to tolerate a potentially lethal plasmid is maternally inherited. When short-lived strains were intercrossed prior to the terminal stage, the sexual progeny became temporarily rejuvenated (Griffiths & Bertrand, 1984; Maheshwari et al., 1994). A plasmid trapping approach demonstrated that a cryptic mitochondrial retro-plasmid in the strain interacts with a senescence-inducing plasmid (e.g. pKAL) to form hybrid plasmid, suppressing the detrimental effect of the senescence-causing plasmid (Yang & Griffiths, 1993; Maas et al., 2007). Although horizontal transmission of senescence-causing plasmid can occur, the fact that *Neurospora* populations are sexual (Pandit & Maheshwari, 1996a) suggests that mechanisms operating during the sexual phase suppress the plasmid spread.

**Senescence caused by nuclear mutations**

A recessive lethal nuclear gene mutation can be maintained in multinuclear fungi—a masked in its expression by its wild-type allele in other nuclei—until segregated at the time of asexual spore formation. The homokaryotic mutant spore may produce an exceptional unstable mycelium. In this context, the isolation of a single nuclear-gene, mutant *senescent*, isolated from single uninucleate microconidia formed by a phenotypically normal wild-collected strain of *Neurospora* using microconidia assumes importance (Maheshwari, 2005). Mutant *senescent* is similar to mutant *nd* described earlier. Death occurs in six to nine subcultures at 26 °C, but in only two subcultures at 34 °C (Navaraj et al., 2000). To avoid permanent loss of genotype, *nd* and *sen* are maintained as heterokaryons from which they are extracted by conidial plating when desired.

Measurements of oxygen uptake of conidia or mycelial homogenates or germinating conidia of *nd* (Seidel-Rogol et al., 1989) or *sen* (Navaraj et al., 2000) using respiratory inhibitors, and the analyses of mitochondrial cytochromes by measuring difference spectra, revealed that in cultures grown at 34 °C, cytochromes *b* and *aa3* were present but cytochrome *c* was absent. By contrast, at 26 °C, cytochromes *b* and *c* were present but cytochrome *aa3* was diminished in the late subcultures. However, the deficiency of the respiratory chain cytochromes may not be the primary cause of death of the *sen* mutant because the cytochrome *c* and *aa3* mutants of *N. crassa* are capable of sustained growth whereas *sen* is not. A comparison of EcoRI restriction digests of dying *nd* (Seidel-Rogol et al., 1989) and *sen* with long-living wild-type *N. crassa* mtDNA showed deletions and gross sequence rearrangements (D’Souza et al., 2005b), resulting from suppressive forms of defective mtDNA molecules that are generated by defects in mtDNA maintenance due to mutations in the nuclear gene. The two gene products *ND* and *SEN* have mutually exclusive roles in the

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maintenance of mtDNA in N. crassa, presumably in protecting certain hyperactive recombination regions from cleavage by endonucleases. It is not proven, however, whether PstI palindrome is involved in the generation of deletions (Bertrand et al., 1993).

**Thermosensitivity**

The nuclear gene mutants, *nd* and *sen*, die faster at 34 °C compared with 26 °C (Fig. 4). Vigor is not regained if cultures initiated at 34 °C are shifted to 26 °C. Because respiration is critical for viability of *Neurospora*, nuclear genes – essential for cell survival – could only be identified when recovered as heat-sensitive mutants. The heat-sensitive mutants named *unknown* for unknown requirements (see Perkins et al., 2001) have restricted growth at 34–37 °C but are normal at 25 °C. These mutants may be deficient in components of multiprotein cytochrome *aa3* (Nargang et al., 1995), or of mitochondrial outer membrane and inner membrane translocase. For example, in *N. crassa*, *tom22*, *tom40* and *tom70* encode protein components of translocate of the outer mitochondrial membrane (TOM) and the growth of these mutants is severely affected at elevated temperatures (Harkness et al., 1994; Nargang et al., 1995; Grad et al., 1999). The indispensability of a nuclear-encoded component of the mitochondrial TOM could be recognized through use of a genetic trick called the sheltered-RIP, which allows a mutated indispensable allele to be maintained in a heterokaryon with a functional copy of the gene in another nucleus – the culture thus remaining viable. Modification in nucleus-encoded proteins of the mitochondrial import apparatus would be expected to have pleiotropic effects as the translocate complexes are involved in assembly of mitochondrial and enzyme proteins of the electron transport chain, mtDNA stability assembly of ribosomes, and transport of a number of small molecules (adenine nucleotides, inorganic ions, amino acids, fatty acids) across the inner membrane. In yeast, the disruption of mitochondrial carrier protein components resulted in severe growth defects (Contamine-Picard & Picard, 2000). These observations suggest that modifications of the mitochondrial import apparatus impair the amounts of nucleus-encoded proteins, affecting the stability of the mitochondrial genome and life span. In *P. anserina*, mutations in genes that encode proteins of the mitochondrial TOM complex resulted in accumulation of defective mitochondrial genomes (Jamet-Vierny et al., 1997).

How might the temperature sensitivity of *sen* be explained? SEN protein synthesized in the cytosol is an essential component of the multiprotein TOM complex, involved in recognizing proteins made on cytosolic ribosomes, and their import into mitochondria has been speculated (Neupert, 1997; Künkele et al., 1998). Depending on the growth temperature, SEN assumes two metastable structures and is inserted into the mitochondrial membrane (Fig. 5). Once the mutant form of SEN is trapped in the mitochondrial membrane, conformational interconversion is unaffected by temperature. It is likely that several of the thermo-sensitive nuclear genes, presently classified as *unknown* (Perkins et al., 2001), encode protein components of the mitochondrial translocase mediating the recognition and transfer of cytosolic-made preprotein into mitochondria. An implication of loss of cytochrome is the intracellular signaling known as a retrograde response (Jazwinski, 2000) and expression of the cyanide-resistant alternative oxidase pathway, which branches from the cytochrome pathway at ubiquinone, an expression that is insufficient to reverse the senescence. The oxidative stress could lead to production of reactive oxygen species that are postulated to attack and damage proteins, lipids or DNA – providing a genetic and biochemical link in senescence.

**Outlook**

Researches on senescing strains will help to understand the origin of plasmids, their maintenance and spread in vegetative cultures and their elimination during the sexual phase. Research should further the identification of structural regions in the mitochondrial genome and the enzymes involved in recombination that can lead to deletions.
that form plasmid-like elements (Almasan & Mishra, 1990; Hausner et al., 2006).

The nuclear-gene senescing mutants are particularly valuable in the appraisal of the endosymbiotic theory. According to this theory, a eukaryotic cell evolved from the engulfment by a wall-less archaebacterium, following which an endosymbiotic relationship evolved, accompanied by the transfer of most of the bacterial genes into the host nucleus. A consequence of this evolutionary relationship is that in the present-day eukaryotic cell, the preproteins translated on the cytosolic ribosome are imported into the mitochondria via protein complexes located in the outer and inner mitochondria membranes. The natural populations of *Neurospora* are a valuable resource for obtaining both nuclear and extranuclear mutants. These mutants will be useful for identifying additional loci involved in the maintenance or replication of the mitochondrial genome and the interdependence between the nuclear and mitochondrial genetic systems. It is expected that with the availability of the genome sequence of *N. crassa* efforts will be made to identify ND and SEN.

**Fig. 5.** A hypothetical model to explain thermosensitivity of senescent. The wild-type sen$^{+}$ is assumed to encode a polypeptide component of the mitochondrial outer membrane receptor complex, and is involved in transport of several nuclear-encoded proteins including cytochrome c heme lyase (oval) and subunit polypeptides of cytochrome oxidase (triangle). The mutant SEN protein exists in two conformational states depending on the growth temperature. The conformational states of the mutant SEN protein are stabilized by insertion into the membrane. At 26°C, the conformation of SEN is more like the native state, but has reduced binding affinity for some precursor proteins destined for mitochondrial compartments. As a consequence, some mitochondria formed are deficient in some mitochondrial proteins (including cytochrome oxidase). The deficient (abnormal) mitochondria eventually dominate the normal mitochondria and cultures senesce with passages. At 34°C, the conformation of SEN is changed drastically, affecting the binding of several cytosolic peptides, and death occurs faster.
Acknowledgements

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References


populations of *Neurospora intermedia* and *Neurospora

Maas MFPM, Hoekstra RF & Debets AJM (2007) Hybrid
mitochondrial plasmids from senescence suppressor isolates of


Maheshwari R, Pandit A & Kannan B (1994) Senescence in strains
of *Neurospora* from Southern India. *Fungal Genet Newslett* **41**: 60.

Mannella CA, Collins RA, Green MR & Lambowitz AM (1997)
Defective splicing of mitochondrial rRNA in cytochrome-
deficient nuclear mutants of *Neurospora crassa*. *Proc Natl Acad
Sci USA* **76**: 2635–2639.

Myers CJ, Griffiths AJF & Bertrand H (1989) Linear kalilo DNA is
a *Neurospora* mitochondrial plasmid that integrates into the

Nargang FE, Künkele KP, Mayer A, Ritzel RG, Neupert W & Lill R
(1995) Sheltered disruption of *Neurospora crassa* MOM22, an
essential component of the mitochondrial protein major

Neurospora crassa* nuclear gene mutant derived from nature
exhibits mitochondrial abnormalities and a “death”


Pall ML (1990) Very low ATP/ADP ratios with aging of the
*natural death* senescence mutant of *Neurospora crassa*. *Mech

Pandit A & Maheshwari R (1996a) Life history of *Neurospora

Pandit A & Maheshwari R (1996b) A demonstration of the role of
*het* genes in heterokaryon formation in simulated field

Perkins DD & Turner BC (1988) *Neurospora* from natural


mitochondrial DNA in *natural-death* nuclear mutants of

Sheng TC (1951) A gene that causes natural death in *Neurospora

bulbosa* is among the largest and oldest living organisms.

Stevenson CB, Fox AN & Kennell JC (2000) Senescence associated
with the over replication of a mitochondrial retroplasmid of


Yang X & Griffiths AJF (1993) Plasmid suppressors active in the
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