MiniReview

Nuclear behavior in fungal hyphae

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Abstract

A characteristic feature of fungal hypha is the presence of large number of nuclei in a common cytoplasmic environment. Where it has been examined, the coenocytic mycelium is commonly heterokaryotic. The nuclei cooperate, compete or combat. It is proposed that in addition to their classical role in heredity, supernumerary nuclei in filamentous fungi serve as store house for nitrogen and phosphorus in the form of DNA which is degraded by regulated autophagy. The breakdown products recycled, giving hyphal tips the capability of persistent extension and foraging in new areas.

1. Introduction

Filamentous fungi have multinuclear hyphal compartments in cytoplasmic continuity due to perforated septa (Fig. 1). The multinuclear condition arises because nuclear divisions are not obligately coupled to cytokinesis. Consequently, even if formed from a single uninucleate spore, a mycelium contains roughly millions of haploid nuclei. The behavior of nuclei in mycelium is unpredictable because of mutations [1], variations [2,3], competition [4] and selection [5]. This led to the remark: "fungi are a mutable and treacherous tribe" [6].

2. Sheltering of lethal mutations

Heterokaryosis, arising due to spontaneous mutations, is widespread in fungi [7].

In fungi which form uninucleate conidia, the nuclear population can be examined through phenotypes of homokaryotic cultures – 2–3% of homokaryotic cultures from a laboratory wild-type strain of *Neurospora crassa* were morphs (Fig. 2), which reappeared in the purified 'wild-type' culture after a passage of time [2]. It is difficult to maintain a mycelium genetically pure.

In coenocytic cells, recessive lethal nuclear gene mutations are masked by their wild-type alleles in other nuclei; and therefore, these pass undetected until the mutant nuclei segregate as viable uninucleate spores. Analysis of a wild-collected, phenotypically normal strain of *Neurospora* using microconidia led to the discovery of a single nuclear-gene mutant, *senescent* (*sen*) (Fig. 3), dying in 2–4 subcultures [8]. The *sen* nuclei were preserved in association with wild-type nuclei in a heterokaryon, from which the *sen* nuclei could be re-extracted, avoiding permanent loss of genotype. RFLP analysis of the mitochondrial genome in *sen* mycelia showed deletions and gross nucleotide sequence rearrangements [9]. DNA sequence of *sen*-specific fragments

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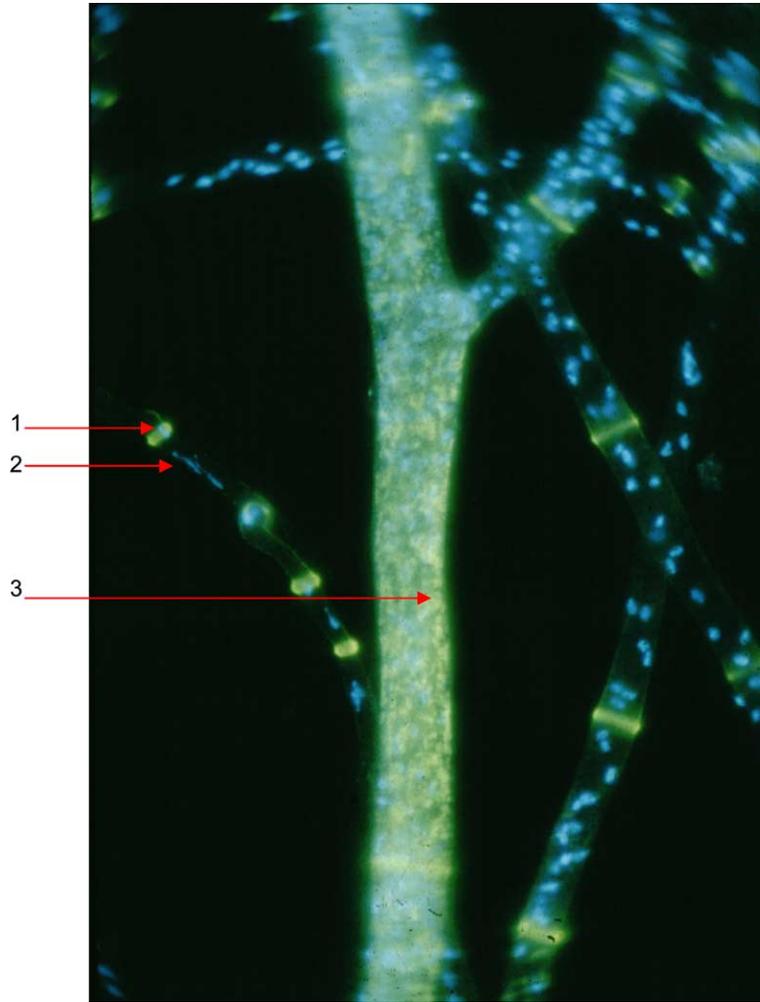 *E-mail address:* fungi@biochem.iisc.ernet.in.

Fig. 1. Fluorescence image of surface-grown, fixed hyphae of *Neurospora crassa* stained for DNA (Hoechst 33258) and chitin (Calcofluor white). Nuclei in young hyphae are spaced and stained brightly, and were presumably migrating in the direction of the spindle pole body. In hypha towards the left, a nucleus (1) is passing through the septal pore, to be followed by another spindle-shaped nucleus (2). In comparison nuclei in mature hyphal compartment (3) have stained poorly, hinting to their breakdown.
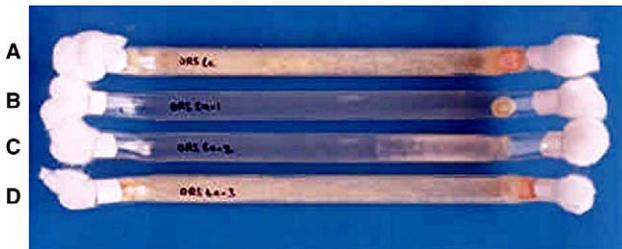

Fig. 2. Phenotypes of microconidia-derived cultures from a standard laboratory strain of *Neurospora crassa*. Cultures were inoculated at the right end of race tubes and grown for same time period. (A) ORS-6a (standard laboratory wild type strain), (B) button morphology, (C) carpet morphology, (D) wild-type. Courtesy Keyur K. Adhvaryu.

revealed deletions close to potential hairpin structures, implying that the wild-type *sen*[+] gene encodes a factor for protecting the mitochondrial genome from recombination and deletion events. Several fungi harbor transposable elements which survive in coenocytic cells, although harmful to uninuclear cells due to induced chromosome rearrangements [10].

3. Nuclear divisions

Microinjection into frog eggs of cytoplasm from dividing and non-dividing cells [11]; and intra- and inter-specific fusions of cultured animal cells in different stages of the cell cycle established that initiation of nuclear division is controlled by factors in the cytoplasm [12]. Therefore, the division of nuclei in the common cytoplasmic milieu of hypha should be synchronous. However, in *Aspergillus nidulans* fairly synchronous nuclear divisions occurred only at the fastest doubling time [13]. According to one study, nuclei divide only in the apical cell [14], followed by their migration into distal compartments; although the opposite is reported [15]. In *N. crassa*, nuclear divisions in germinating vegetative
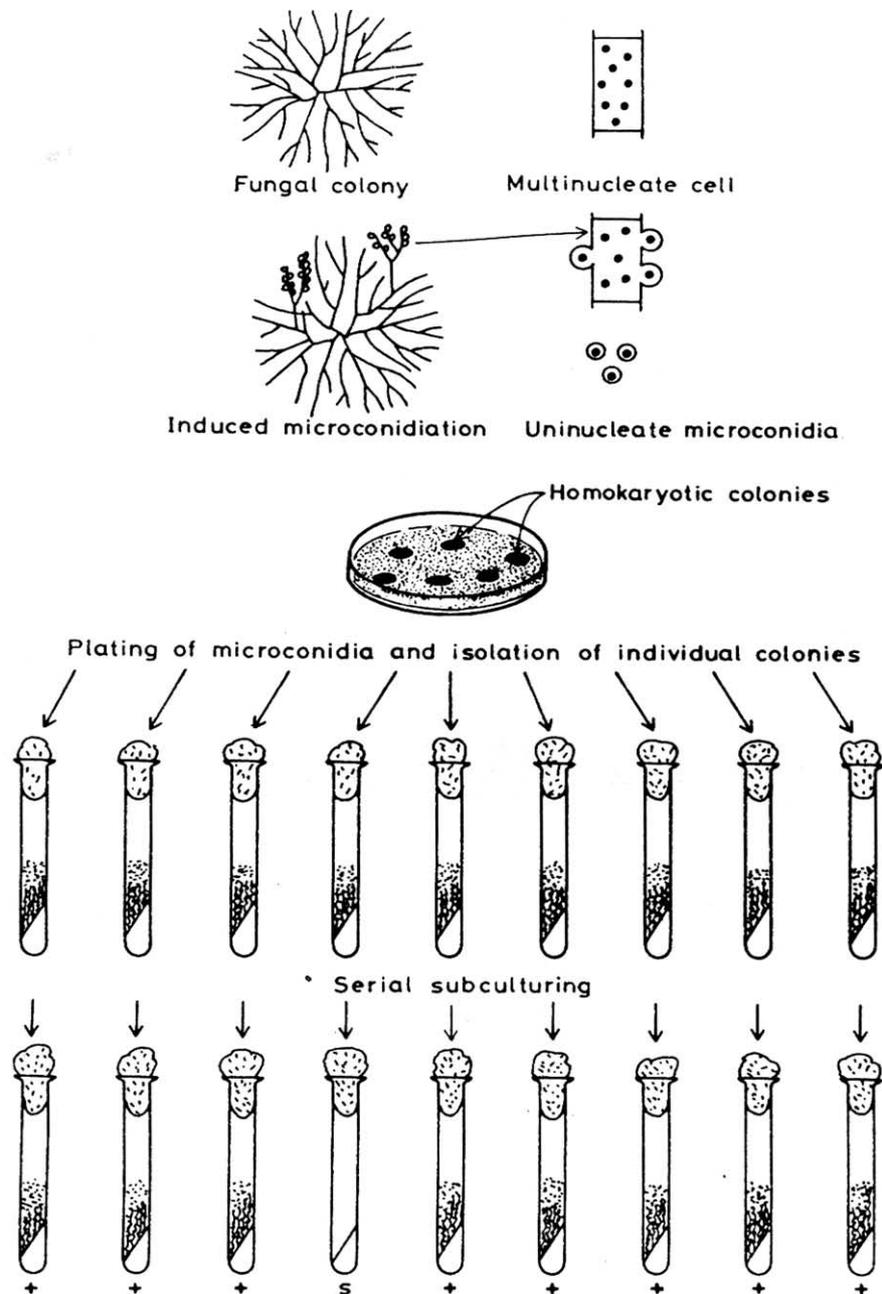
Fig. 3. Diagram to illustrate masking of lethal nuclear mutation in multinuclear hypha and method of extraction as a homokaryon. Derivation of *senescent* by plating of microconidia from a phenotypically normal strain of *Neurospora intermedia* collected from nature. +, normal phenotype; s, death phenotype. Reprinted from Maheshwari, R., 2005. *Fungi: Experimental Methods in Biology*, by courtesy of Taylor and Francis Group, LLC.

spores [16], or in chemically swollen macroconidia, or in the wall-less *slime* mutant [17] and in the *ropy* mutant defective in nuclear distribution were asynchronous [18]. Fungi have closed mitosis, however, the nuclear membrane may become partially permeable at certain stage of nuclear division cycle [19].

Although the proximate trigger for nuclear division originates from the cytoplasm, the ultimate trigger is external. In the germ-tube of biotrophic rust fungi, a thigmotropic stimulus is necessary for inducing nuclear divisions and concomitant infection structure differentiation

[20]. Thigmotropic or/and chemical stimuli also induce nuclear divisions in mycorrhizal fungi, which have per spore ~ 2000 [21] or even 20,000 nuclei [22]. Surprisingly, the nuclei in a single spore may vary significantly in the types of sequences of ribosomal DNA [3]. How genetically divergent nuclei arise in mycorrhizal fungi, where sexual reproduction is not reported, is an enigma [23].

In vegetative hypha nuclear divisions are commonly asynchronous; however, synchronized nuclear divisions are necessary for construction of spore-bearing ascocarps and basidiocarps. In Basidiomycotina,

simultaneous division of nuclei in the dikaryotic hypha, the formation of the hook cell and its fusion with the penultimate cell (clamp connection) ensures each hyphal compartment with two nuclei differing in their mating-type factors.

4. Nuclear movement and morphological changes

Fungal mutants have provided evidence that nuclear movement requires a motor protein to move the nucleus through the cytoplasm, a track for the nucleus to move on, and a coupling mechanism to link the motor to the nucleus [24–26]. A new technique is to label nuclei with histone – GFP (green fluorescent protein) and study nuclear movement in living hypha by video-microscopy. Movement of nuclei from opposite directions to reach a branch initial within a hyphal compartment suggests individual regulation of nuclear movement. Velocities ranging from 0.1 to 40 μm [27], to over 600 μm min^{-1} [28], imply existence of different motor proteins. Nuclei are evenly distributed in hyphae of wild-type strains. However, in the *nud* (*nuclear distribution*) mutants of *A. nidulans* [27] and the *ro* (*ropy*) mutants of *N. crassa* [29] are clustered; implicating involvement of motor proteins in regulation of nuclear distribution.

Morphology of fungal nuclei is quite variable: moving nuclei may be spindle-shaped (Fig. 1), those migrating through septum dumbbell-shaped, and those entering into a branch maybe stretched thread-like. Phase-specific shapes are reported in *N. crassa*. G1 nuclei are compact and globular, S and G2 phase nuclei are ring-shaped [30].

5. Spatial relations

A unique mechanism of gene regulation in a basidiomycete fungus is based on internuclear spacing [31]. The type of hydrophobin – a class of fungal proteins rich in non-polar amino acids – secreted by the dikaryotic hyphae of *Schizophyllum commune* (Basidiomycotina) was a function of the internuclear distance. The homokaryotic hyphae secreted hydrophobin SC3 (which coats aerial hyphae and hyphae at the surface of fruit bodies) as determined using immunochemical staining methods, but not hydrophobin SC4 (which coats the air channels within a fruit body) and SC7. Internuclear distance could be modulated by growing the fungus on a hydrophobic or a hydrophilic surface. On a hydrophilic surface, the nuclei were adjacent (1.6 μm + 1.5 μm); the hypha secreted hydrophobins SC4 and SC7, but not SC3. On a hydrophobic surface the nuclei were separated (13–16 μm); the hyphae secreted hydrophobin SC3, but not hydrophobins SC4 and SC7.

6. Complementation

Heterokaryons have provided the most insights on nuclear interactions in hypha. Germ tubes of related strains fuse and grow into a mycelium wherein nuclear types mix, constituting the basis of a complementation test, i.e., the production of a wild-type mycelium when different haploid, mutant genomes in nuclei cooperate in a common cytoplasm. If nuclei carrying nonallelic mutations coexist, the phenotype of mycelium is normal.

A type of complementation in dikaryotic hypha crucial for fruiting body development in Basidiomycotina involves heterodimerization of polypeptides encoded by the idiomorphic mating type loci: heterodimer functions as a transcription factor for genes involved in morphogenesis and pathogenecity [32].

7. Nuclear interactions

In heterokaryons the proportion of nuclear types (nuclear ratio) is estimated from the ratio between corresponding homokaryotic colonies obtained by plating conidia, based on the assumption that nuclei mix freely in the cytoplasm. The observed changes in nuclear ratios in heterokaryons involving biochemical mutant genes have been of the nonadaptive type, i.e., the mutant nuclei is favored over the wild-type when the culture is grown with nutritional supplement. Ryan and Lederberg [4] reported that in a heterokaryon of *N. crassa* containing mutant *leucine* (*leu*) nuclei and back-mutated prototrophic (*leu*$^+$) nuclei when grown with leucine supplement; the mycelium became pure *leu* after only a *single subculture*, suggesting that the *leu*$^+$ nuclei were "inactivated", or "eliminated" – example of nuclear combat in hypha.

Davis [5] studied competition between nuclei of a pantothenate-requiring (*pan*-1) strain of *N. crassa* and a spontaneous mutant which he symbolized as *pan-1 m*. The uptake of pantothenate by *pan-1 m* occurred at much lower concentrations than the *pan* strain, demonstrating a selective advantage when grown on limiting pantothenate concentration. At unlimiting pantothenate concentrations the ratio of the two types of nuclei (*pan-1 m*$^+$/*pan m*) remained constant; at low concentrations of pantothenate the ratios fluctuated widely, manifested by slow and rapid growth associated with cyclical changes in component nuclei.

In certain genotypes, extreme changes in frequency of nuclear types occur. Pitchaimani and Maheshwari [33] generated a prototrophic (*his*-3 + *his*-3$^+$ (*EC*)) heterokaryon of *N. crassa* by transformation of a histidine auxotrophic strain (*his*-3) with *his*-3$^+$ plasmid. (The appended symbol '(*EC*)' refers to a *gene* that has been integrated ectopically by transformation.) A gradual but drastic reduction in prototrophic *his*-3$^+$ (*EC*) nuclei

occurred when this heterokaryon was subcultured only in the presence of histidine. Construction and analyses of three-component heterokaryons containing a mutant *his-3* gene, a wild type *his-3*[+] and *his-3*[+] (*EC*) alleles in separate nuclei revealed specific nuclear interactions: change in nuclear ratio resulted from interaction of auxotrophic nuclei with prototrophic nuclei containing a *his-3*[+] gene at the normal chromosomal location. Unexpectedly, despite a 400-fold reduction in *his-3*[+] (*EC*) nuclei, the activity of histidinol dehydrogenase in mycelia grown in the two conditions was similar – i.e, the dosage of wild-type nuclei did not correlate to enzyme activity.

Similar experiments with other fungi and mutant marker genes of catabolic pathway are needed to assess whether changes in concentration of a gene product (enzyme) can occur through alteration in the number of nuclei.

8. Diffusible trans-acting molecules

8.1. Reversible and non-infectious gene silencing

Despite a degree of autonomy in nuclear behavior, neighboring nuclei chemically communicate with one another. In *N. crassa*, synthesis of carotenoids is controlled by three genes, named *al(bino)-1*, *al-2* and *al-3*. Romano and Macino [34] introduced extra copies of carotenoid genes by transforming a wild-type strain with the *al-1*[+], *al-2*[+] or *al-3*[+] gene (Fig. 4): the transformants with high copy numbers of transgene were white or pale yellow, demonstrating that nuclei containing both the endogenous (resident) gene and the transgene can be silenced. The silencing of the duplicated *al* genes in the vegetative phase was termed ''quelling''. The amount of primary transcript (precursor mRNA) in quelled transformants was unchanged, but the level of specific mRNA for the duplicated gene was reduced, suggesting that quelling involves posttranscriptional gene silencing. In heterokaryons containing a mixture of transgenic and non-transgenic nuclei, silencing was dominant [35]. Quelling was reversible; the silenced transformants reverted to orange or pale yellow phenotypes in progressive subcultures.

To determine the mechanism of quelling, a quelled (albino) strain was mutagenized and *q*uelling *de*ficient (*qde*) orange-colored mutants were obtained. Heterokaryons containing quelled and wild-type nuclei were white: a diffusible molecule is produced which transfers the ''silenced state'' from nucleus to nucleus. A heterokaryon composed of the *qde* mutant producing no transgenic sense RNA, and a wild-type was orange, demonstrating that transgenic sense RNA is essential for silencing in the heterokaryon: the *qde* products and transgenic RNA interact to form a complex for degradation of endogenous mRNA and sequence-specific gene silencing results [36].

8.2. Irreversible and infectious gene silencing

The potato late-blight 'fungus' *Phytophthora infestans* (Straminipila), secretes a hydrophobin protein, called elicitin, which induces necrosis (a hyper-sensitive response) in the plant. By transforming *P. infestans* with
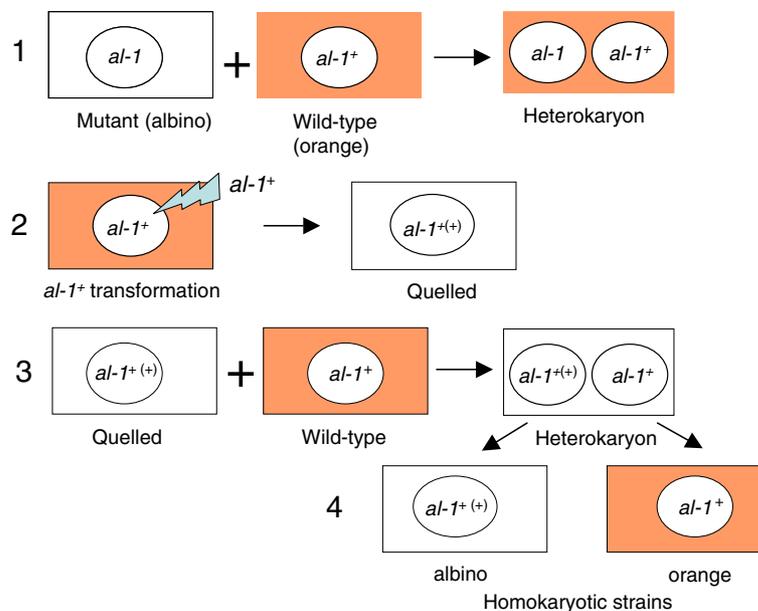

Fig. 4. Diagram of quelling in heterokaryon of *Neurospora crassa*. Rectangle represents hyphal compartment and oval represents nucleus. In genetic nomenclature for *Neurospora*, *al-1* is albino mutant (white); *al-1*[+] is wild-type (orange). Duplicated gene is symbolized in parenthesis. Only one nucleus of each type is shown.
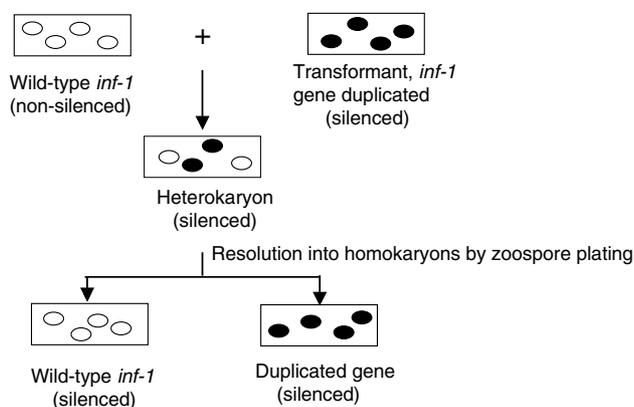
Fig. 5. Diagram of infectious gene silencing in *Phytophthora infestans*. Hyphae are represented as rectangles. Wild-type and transformed nuclei are denoted as open and filled ovals, respectively.

inf-1 gene (which encodes elicitin), mutants with duplicated *inf-1* genes were produced that were silenced for elicitin [37]. The protoplasts of silenced and non-silenced strains were fused and the silenced heterokaryotic strain was resolved by plating uninucleate zoospores. The homokaryotic strains were silenced, i.e., once gene silencing was induced, it was maintained in the homokaryotic strain even in the absence of the transgene (Fig. 5). Both internuclear gene silencing (IGS) and quelling are dominant but IGS differs from quelling in being transmitted among the diploid nuclei, i.e., in being infectious, whereas quelling is not. Moreover, IGS is irreversible; quelling is unstable. Transcriptional *inf-1* silencing was not due to methylation. The diffusible silencing factor awaits characterization.

9. Nuclear competence

In *N. crassa*, transformation of macroconidia containing heterokaryotic nuclei differing in one or two marker genes showed that at any given time, the transforming DNA integrates into one nuclear type [38–40]. The general design of experiments was as follows. A heterokaryon of strain 1 auxotrophic for marker X, and a strain 2 auxotrophic for marker Y, was transformed with a plasmid containing the Z gene. Because gene Z products complement X and Y gene products, the heterokaryon is prototrophic for the Z gene product and could be selected on a medium exclusively for heterokaryotic cell (Table 1). The heterokaryon was resolved into homokaryons by plating microconidia. However, at any given time, the transforming DNA was integrated only into an occasional competent nucleus, perhaps because nuclei in the dormant conidium are arrested at various stages of division cycle [16] with consequent differences in the structure of the nucleus and chromatin, necessary for stable integration [38].

Table 1
Transformation of a (XZ + YZ) heterokaryon with a Z^+ plasmid and its resolution to homokaryons to determine the type of nuclei transformed[a]

Class	Description	No. observed
I	Only XZ nuclear type transformed	20
II	Only YZ nuclear type transformed and XZ nuclear type untransformed	12
III	Both XZ and YX nuclear types transformed	0

[a] From [40].

10. A proposed role for multinuclear condition

Since gene products from a single nucleus can pervade the cytoplasm even of giant cell, as in the green alga *Acetabularia*; the question arises what advantage accrues to fungi from multiple nuclei in same cytoplasm, when cells in more complex forms have just one nucleus per cell? Serna and Stadler found that the rate of spore germination in *N. crassa* was not related to nuclear number [16]. Therefore, it is unlikely that multinuclear condition gives the advantage of rapid growth rate. Advantage could accrue from concomitant heterokaryosis, and through selection of the nuclear type that is best adapted to the immediate situation; however, this is without experimental support (Section 7).

In nature, fungal growth appears to be most frequently limited by phosphorus and nitrogen unavailability [41]. Therefore, a fungal strategy of growth would be to absorb and convert the limiting macronutrients into molecular forms that do not adversely affect the osmotic pressure in the cell and to recycle it for perpetual exploration of environment in conditions of nutrient non-availability. It seems likely that the sugar-phosphate backbone of DNA serves as a storage form of phosphorus and nitrogen. A regular but important observation is that whereas apical extension of colony margin continues, the nuclei in mature hyphal compartments show signs of dissolution (Fig. 1), as in incompatible heterokaryons [42]. The nuclei stain poorly with DNA binding dyes, suggesting controlled autophagy [43]. When nutrients become limiting, a signal sensed by the hyphal tip may be transmitted behind to trigger selective nuclear breakdown in mature hyphal compartments and the breakdown products mobilized for synthesis of membrane and organelles, allowing apical growth to continue. In fungi, a type of growth occurs after primary growth has ceased, for example the formation of new, intrahyphal hyphae in highly vacuolated old cells [44,45], and of short branches which secrete lignin peroxidase [46,47]. As mentioned before, the chlamydospores of mycorrhizal fungi contain an astonishing number of nuclei [22]. Nuclear DNA recycling in these spores would have the advantage of exploration by hypha until it can establish a symbiotic relationship with a host root. Indeed, mobilization of nuclei during germination of spores of mycorrhizal fungus is

reported [22].Thus, in addition to their role in heredity, supernumerary nuclei in fungi maybe store houses for phosphorus and nitrogen in the 'protected' molecular form of DNA for utilization during unavailability of macronutrients.

11. Conclusions and outlook

Multinuclear condition and concomitant heterokaryosis comprise a unique genetic system wherein the haploid nuclei have a remarkable degree of autonomy even though they are bathed by the same cytoplasm. The division rate of nuclei is affected if the natural gene order in chromosomes is interrupted by ectopic DNA sequences, resulting in extreme disproportion of nuclear ratio with phenotypic consequences. Some of the unresolved questions are: why a closed mitosis has evolved in fungi; whether homologous genes in all wild-type nuclei are transcriptionally active, contributing to the phenotype – if not, how nuclei network with one another and regulate the concentration of a gene product; what is the nature of the signal involved in transmitting selective gene silencing; how substantial genetic variations occur in different haploid nuclei within a single cell (spore) of mycorrhizal fungi in the absence of sexual reproduction? Finally, experiments are required to test whether the multinuclear state evolved for storage of macronutrients.

Acknowledgments

References

[1] Maheshwari, R. (1999) Microconidia of *Neurospora crassa*. Fungal Genet. Biol. 26, 1–18.

[2] Adhvaryu, K.K. and Maheshwari, R. (2000) Use of microconidia for testing genetic purity of *Neurospora* stocks. Fungal Genet. Newsl. 47, 59–60.

[3] Hijri, M., Hosny, M., van Tuinen, D. and Dulieu, H. (1999) Intraspecific ITS polymorphism in *Scutellospora castanea* (Glomales, Zygomycota) is structured within multinucleate spores. Fungal Genet. Biol. 26, 141–151.

[4] Ryan, F.J. and Lederberg, J. (1946) Reverse mutation and adaptation in leucineless *Neurospora*. Proc. Natl. Acad. Sci. USA 32, 163–173.

[5] Davis, R.H. (1960) Adaptation in pantothenate-requiring *Neurospora*. II. Nuclear competition during adaptation. Amer. J. Bot. 47, 648–654.

[6] Caten, C.E. (1996) The mutable and treacherous tribe revisited. Plant Pathology 45, 1–12.

[7] Davis, R. (1966) Heterokaryosis In: The Fungi, An Advanced Treatise (Ainsworth, G.C. and Sussman, A.S., Eds.), Vol. 2. Academic Press, New York.

[8] Navaraj, A., Pandit, A. and Maheshwari, R. (2000) *senescent*: A new *Neurospora crassa* nuclear gene mutant derived from nature exhibits mitochondrial abnormalities and a "death" phenotype. Fungal Genet. Biol. 29, 165–173.

[9] D'Souza, A.D., Bertrand, H. and Maheshwari, R. (2004) Intramolecular recombination and deletions in mitochondrial DNA of *senescent*, a nuclear gene mutant of *Neurospora crassa* exhibiting "death" phenotype. Fungal Genet. Biol. 42, 178–190.

[10] Daboussi, M.J. (1996) Fungal transposable elements: generators of diversity and genetic tools. J. Genet. 75, 325–339.

[11] Nurse, P. (1992) Universal control mechanism regulating onset of M-phase. Nature 344, 503–508.

[12] Rao, P.N. and Johnson, R.T. (1970) Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. Nature 225, 159–164.

[13] Rosenberger, R.F. and Kessel, M. (1967) Synchrony of nuclear replication in individual hyphae of *Aspergilus nidulans*. J. Bacteriol. 94, 1464–1469.

[14] King, S.B. and Alexander, L.J. (1969) Nuclear behavior, septation, and hyphal growth of *Alternaria solani*. Amer. J. Bot. 56, 249–253.

[15] Albert-Segui, C., Dietrich, F., Altmann-Jöhl, R., Hoepfner, D. and Phillippsen, P. (2001) Cytoplasmic dynein is required to oppose the force that moves nuclei towards the hyphal tip in the filamentous ascomycete *Ashbya gossypii*. J. Cell Sci. 114, 975–986.

[16] Serna, L. and Stadler, D. (1978) Nuclear division cycle in germinating conidia of *Neurospora crassa*. J. Bacteriol. 136, 341–351.

[17] Raju, N.B. (1984) Use of enlarged cells and nuclei for studying mitosis in *Neurospora*. Protoplasma 121, 87–98.

[18] Minke, P.F., Lee, I.H. and Plamann, M. (1999) Microscopic analysis of *Neurospora ropy* mutants defective in nuclear distribution. Fungal Genet. Biol. 28, 55–67.

[19] Straube, A., Weber, I. and Steinberg, G. (2005) A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. EMBO J. 24, 1674–1685.

[20] Maheshwari, R., Hildebrandt, A.C. and Allen, P.J. (1967) The cytology of infection structure development in urediospore germ tubes of *Uromyces phaseoli* var. *typica* (Pers.) Wint. Can. J. Bot. 45, 447–450.

[21] Bécard, G. and Pfeffer, P.E. (1993) Status of nuclear division in arbuscular mycorrhizal fungi during in vitro development. Protoplasma 174, 62–68.

[22] Burggraff, A.J.P. and Beringer, J.E. (1989) Absence of nuclear DNA synthesis in vesicular-arbuscular mycorrhizal fungi during in vitro development. New Phytol. 111, 25–33.

[23] Sanders, I.R. (1999) No sex please, we're fungi. Nature 399, 737–739.

[24] Fischer, R. (1999) Nuclear movement in filamentous fungi. FEMS Microbiol. Rev. 23, 39–68.

[25] Morris, N.R., Xiang, X. and Beckwith, S.M. (1995) Nuclear migration advances in fungi. Trends Cell Biol. 5, 278–282.

[26] Xiang, X., Beckwith, S.M. and Morris, N.R. (1994) Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. Proc. Natl. Acad. Sci. USA 91, 2100–2104.

[27] Suelmann, R., Sievers, N. and Fischer, R. (1997) Nuclear traffic in fungal hyphae: in vivo study of nuclear migration and positioning in *Aspergillus nidulans*. Mol. Microbiol. 25, 759–769.

[28] Ross, I.K. (1976) Nuclear migration rates in *Coprinus congregatus*: a new record?. Mycologia 68, 418–422.

[29] Plamann, M., Minke, P.F., Tinsley, J.H. and Bruno, K.S. (1994) Cytoplasmic dynein and actin-related Arp1 are required for normal nuclear distribution in filamentous fungi. J. Cell Biol. 127, 139–149.

[30] Martegani, E., Levi, M., Trezzi, F. and Alberghina, L. (1980) Nuclear division cycle in *Neurospora crassa* hyphae under different growth conditions. J. Bacteriol. 142, 268–275.

[31] Schuurs, T.A., Dalstra, H.J.P., Scheer, J.M.J. and Wessels, J.G.H. (1998) Positioning of nuclei in the secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet. Biol. 23, 150–161.

[32] Gillissen, B., Bergemann, J., Sandmann, C., Schroeer, B., Bölker, M. and Kahmann, R. (1992) A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell 68, 647–657.

[33] Pitchaimani, K. and Maheshwari, R. (2003) Extreme nuclears disproportion and constancy of enzyme activity in heterokaryon of *Neurospora crassa*. J. Genet. 82, 1–6.

[34] Romano, N. and Macino, G. (1992) Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. Mol. Microbiol. 6, 3343–3353.

[35] Cogoni, C., Irelan, J.T., Schumacher, M., Schmidhauser, T.J.., Selker, E.U. and Macino, G. (1996) Transgene silencing of the *al-1* gene in vegetative cells of *Neurospora* is mediated by a cytoplasmic effector and does not depend on DNA–DNA interactions and DNA methylation. EMBO J. 15, 3153–3163.

[36] Cogoni, C. and Macino, G. (1997) Isolation of quelling-defective (qde) mutants impaired in post-transcriptional transgene induced gene silencing in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA 94, 10233–10238.

[37] van West, P., Kamoun, S., van't Klooster, J.W. and Govers, F. (1999) Internuclear gene silencing in *Phytophthora infestans*. Mol. Cell 3, 339–348.

[38] Grotelueschen, J. and Metzenberg, R. (1995) Some property of the nucleus determines the competence of *Neurospora crassa* for transformation. Genetics 139, 545–1551.

[39] Pandit, N.N. and Russo, V.E.A. (1992) Reversible inactivation of a foreign gene, hph, during the asexual cycle in *Neurospora crassa* tansformant. Mol. Gen. Genet. 234, 412–422.

[40] Dev, K. and Maheshwari, R. (2002) Transformation in heterokaryons of *Neurospora crassa* is nuclear rather than cellular phenomenon. Curr. Microbiol. 44, 309–313.

[41] Pandit, A. and Maheshwari, R. (1996) Life-history of *Neurospora intermedia* in a sugar cane field. J. Biosci. 21, 57–79.

[42] Marek, S.M., Wu, J., Glass, N.J., Gilchrist, D.G. and Bostock, R.M. (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet. Biol. 40, 126–137.

[43] Klionsky, D.J. and Emr, S.D. (2000) Autophagy as a regulated pathway of cellular degradation. Science 290, 1717–1721.

[44] Miller, C.V. and Anderson, N.A. (1961) Proliferation of conidiophores and intrahyphal hyphae in *Aspergillus niger*. Mycologia 53, 433–436.

[45] Lowry, R.J. and Sussman, A.S. (1966) Intra-hyphal hyphae in "clock" mutants of *Neurospora*. Mycologia 58, 541–548.

[46] Moukha, S.M., Wösten, H.A.B., Asther, M. and Wessels, J.G.H. (1993) In situ localization of the secretion of lignin peroxidases in colonies of *Phanerochaete chrysosporium* using a sandwiched mode of culture. J. Gen. Microbiol. 139, 969–978.

[47] Moukha, S.M., Wösten, H.A., Mylius, E.-J., Asther, M. and Wessels, J.G.H. (1993) Spatial and temporal accumulation of mRNAs encoding two common lignin peroxidases in *Phanerochaete chrysosporium*. J. Bacteriol. 175, 3672–3678.