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Review Article

Programmed Cell Death in *Neurospora crassa*

A. Pedro Gonçalves[1,2] and Arnaldo Videira[1,2]

[1] *Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal*
[2] *Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal*

Correspondence should be addressed to A. Pedro Gonçalves; apgoncalves@ibmc.up.pt and Arnaldo Videira; avideira@ibmc.up.pt

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Programmed cell death has been studied for decades in mammalian cells, but simpler organisms, including prokaryotes, plants, and fungi, also undergo regulated forms of cell death. We highlight the usefulness of the filamentous fungus *Neurospora crassa* as a model organism for the study of programmed cell death. In *N. crassa*, cell death can be triggered genetically due to hyphal fusion between individuals with different allelic specificities at *het* loci, in a process called "heterokaryon incompatibility." Chemical induction of cell death can also be achieved upon exposure to death-inducing agents like staurosporine, phytosphingosine, or hydrogen peroxide. A summary of the recent advances made by our and other groups on the discovery of the mechanisms and mediators underlying the process of cell death in *N. crassa* is presented.

1. *Neurospora crassa* as a Model Organism

Neurospora crassa is a nonpathogenic filamentous fungus, very easy to maintain, grow, and manipulate. *N. crassa* enjoys modest nutritional requirements: the common minimal medium (Vogel's minimal medium) includes a sugar, a nitrogen source (ammonium and nitrate), phosphate, sulfate, potassium, magnesium, calcium, trace metals, and a small amount of the vitamin biotin [1]. Moreover, *N. crassa* is one of the fastest growing filamentous fungi (approximately 10 cm per day under optimal conditions), justifying its appearance among the first colonizers of recently burned vegetation [2]. It is prone to genetic experiments like the induction of mutations, genes, and mutants isolation, microscopic analysis, biochemical testing, and so on. Thus, *Neurospora* presents some features that turn it into a very attractive option to be used in the laboratory.

N. crassa is a multicellular ascomycete. It was initially documented in 1843, when several Parisian bakeries were infested by cultures of an orange sporulating mould [3]. A century later, mycologists Cornelius Shear and Bernard Dodge moved it to the *Neurospora* genus, based on the discovery that this fungus possesses a sexual morphological structure called perithecia [4]. Literally translated, "Neurospora" means "nerve" plus "spore" and the explanation for this name resides in the fact that the fungal spores display longitudinal striations resembling animal axons which belong to the nervous system. In its natural habitat, *Neurospora* is found essentially in tropical and subtropical regions but also in temperate climates [2]. Figure 1 shows spots of *N. crassa* colonization that can be easily observed following a forest fire. During the 20th century, this fungus was the basis of some breakthrough discoveries in the molecular genetics field. The Nobel Prize in Physiology and Medicine was awarded to George Wells Beadle and Edward Lawrie Tatum in 1958, because of their "one gene-one enzyme" pioneering hypothesis. The theory, which conceived the idea that particular portions of genetic material lead to the synthesis of specific proteins, was described in 1941 [5] and allowed the comprehension of one of the most basic aspects of Biology. In another work using *N. crassa* during the 1940s, Srb and Horowitz showed that metabolic pathways comprise a series of steps each of them catalysed by an enzyme [6].

The aforementioned works of renowned geneticists represent only a few examples of successful applications of *N. crassa* in the study of the molecular basis of biological processes. The fungus has also been used to study circadian

Figure 1: *N. crassa* in a natural habitat. In nature, *Neurospora* is commonly found as one of the first colonizers of burned vegetation. The picture depicts growth of *N. crassa* on a burned tree in Portugal (note the presence of the orange mould throughout the trunk, indicated with arrows).

rhythms, gene silencing, DNA repair, cell differentiation, and mitochondrial biology [7]. More recently, in 2003, the genome of *N. crassa* was fully sequenced [8]. Access to this information, together with the availability of valuable genetic tools such as a large collection of deletion strains and a rich assortment of plasmids for protein expression, provided by the Fungal Genetics Stock Center [9], makes *N. crassa* a great model organism to work with. Our group has focused on the mechanisms employed by the mitochondrial respiratory chain to produce energy in *N. crassa* for several years [10–12] and, more recently, became interested in the process of programmed cell death [13–22].

2. Programmed Cell Death-Controlled "Suicide" of Cells

Balance between cell division and cell death is of supreme importance for the development and maintenance of multicellular organisms. Deregulation of this equilibrium can lead to pathological conditions, namely, cancer and neurodegenerative disorders. Therefore, the balance between life and death is tightly controlled and abnormal elements can be effectively eliminated by a process called "programmed cell death" [23]. Decades ago, programmed cell death was held synonymous with apoptosis, and the concepts of apoptosis and necrosis were the only used to explain the death of cells. However, in recent years, it has become evident that this is an oversimplification of the highly sophisticated mechanisms guarding the organism against potentially harmful situations. Many reports have been published and many terms have been proposed to define dissimilar pathways of cell death. However, some of these distinct ways of dying might not be really different, because there are many overlapping features and the precise biochemical mechanisms are often unclear. To overcome this issue, the Nomenclature Committee on Cell Death has recently proposed unified criteria for the definition of cell death and its different morphologies and molecular signals [24]. Despite the advances made in the comprehension of the cell death subject, several mechanisms are still a matter of debate and new approaches might unravel new pathways and mediators.

Cell death studies have been carried out for decades using mammalian models. However, it has become clear that lower eukaryotes and even prokaryotes undergo programmed cell death when insulted with chemical agents and other stress signals. Humans, the nematode *Caenorhabditis elegans*, the fly *Drosophila melanogaster,* and the yeast *Saccharomyces cerevisiae* represent major organisms used to investigate programmed cell death. Because of the aforementioned advantages of using *N. crassa* as a model organism and because it was shown that it presents additional proteins with homology to cell death-related molecules of mammalian cells when compared with other models such as yeasts [25], we anticipated that it would be a good prototype to study the fundamentals of cell death. More specifically, *in silico* searches predict dozens of cell death-associated genes in filamentous species that seem to be absent in *S. cerevisiae* with a part of them being fungal-specific and related to heterokaryon incompatibility (please see the next section). Moreover, the similarity between mediators of cell death (like BIR1, AMID, CulA, and HtrA) in humans and filamentous fungi is higher than the similarity of the same proteins between yeasts and filamentous species [25].

3. Advances on the Understanding of Cell Death in *N. crassa*

To our knowledge, the first report of cell death in *N. crassa* was published back in the 1950s when Strauss described that unstable attempts of auxotrophic strains to grow in the absence of the required nutrient result in cell death [26]. Later, it was observed that other stimuli lead to cell death in *N. crassa*, particularly a combined stress of moderate heat shock (45°C) and carbohydrate deprivation [27] or the incubation with the polymer chitosan [28] or the small antifungal peptide PAF26 [29]. Interestingly, the two latter stimuli disturb intracellular calcium (Ca^{2+}) homeostasis during the process of cell death induction.

Filamentous ascomycetes, namely, *N. crassa*, possess a defense system for non-self-recognition that leads to programmed cell death and functions as a barrier to viral transfer between fungal individuals and to prevent resource plundering [30, 31]. This process occurs upon hyphal fusion between individuals that are genetically dissimilar at *het* loci (11 *het* loci have been identified so far). A cell death program on the fusion compartment and surrounding cells is triggered, leading to the rejection of heterokaryon formation. This was therefore termed "heterokaryon incompatibility." At the molecular level, heterokaryon incompatibility seems to be controlled by the transcriptional regulator VIB-1 [32], which is downstream of a negative regulation by the IME-2 kinase

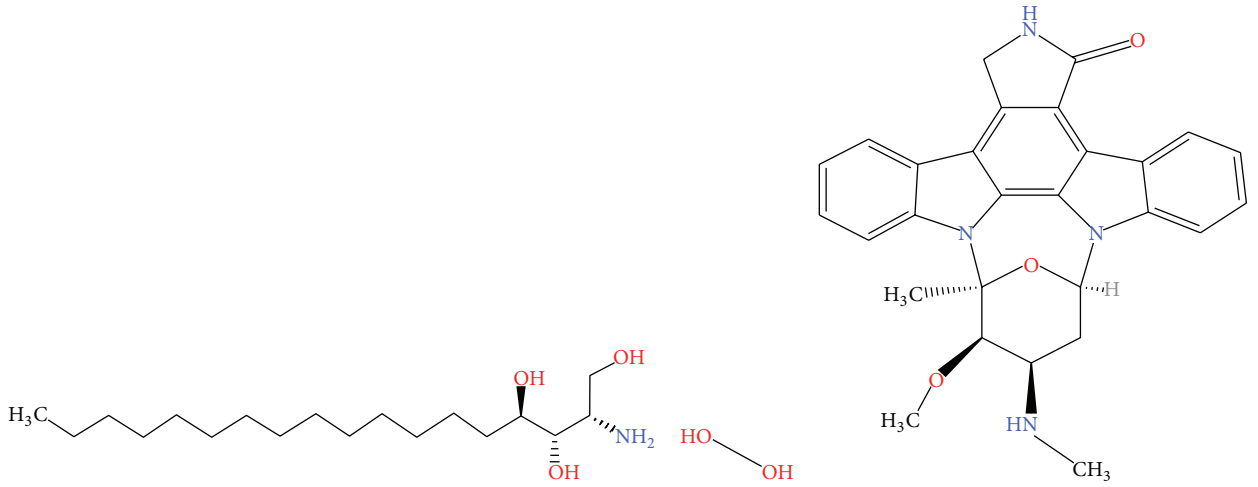

FIGURE 2: Chemical structures of cell death-inducing agents: phytosphingosine (a), hydrogen peroxide (b), and staurosporine (c). Chemical structures were obtained from http://www.chemspider.com/.

[33]. The production of reactive oxygen species (ROS) and the induction of genes involved in phosphatidylinositol and (Ca^{2+}) signaling pathways are also implicated in the phenomenon [34]. Heterokaryon incompatibility was also shown to be induced by the ectopic expression of the bacterial HET-C homologue from *Pseudomonas syringae*, phcA [35]. Cell death associated with heterokaryon incompatibility features several hallmarks of apoptosis such as DNA condensation and fragmentation, plasma membrane shrinkage, vesicle formation, and internalization of vital dyes [31, 34, 36].

Altogether, accumulating evidence shows that programmed cell death in *N. crassa* can be achieved chemically or genetically. During the last years, our group has focused on the study of the molecular basis of cell death using a chemical induction approach. The process has been mainly induced with either phytosphingosine, hydrogen peroxide, or staurosporine (Figure 2). Below we summarize the main findings from the work with these compounds.

3.1. Phytosphingosine (PHS) and Hydrogen Peroxide (H_2O_2). Phytosphingosine (PHS) is a natural long-chain sphingoid base [37]. The evidence that this sphingolipid has potent antifungal activity against *Aspergillus nidulans* with mitochondrial involvement [38] prompted us to investigate the effects of the drug in *N. crassa*. Treatment of conidia with PHS results in reduced viability, impairment of asexual spore germination, production of ROS, YO-PRO1 staining, and DNA condensation and fragmentation, suggesting the induction of an apoptosis-like cellular death [16, 17]. Analysis of gene expression during PHS-induced cell death by DNA microarrays revealed that most of the alterations at the transcriptional level correspond to upregulation of genes. However, there is a very strong enrichment of genes encoding mitochondrial proteins in the set of genes that are downregulated by the drug that likely explains its effects in the fungus [22]. This may be correlated with the fact that deletion of genes encoding subunits of the mitochondrial complex I, like NUO9.8, NUO14, NUO21, NUO21.3c, NUO30.4, NUO51, and NUO78 (but not the deletion of components of the other complexes of the respiratory chain) confers increased resistance to PHS. The same resistance profile is paralleled by the treatment of complex I mutants with H_2O_2, indicating shared intracellular mechanisms after the treatment with PHS and H_2O_2.

We observed that complex I mutant strains generate less ROS than wild type when exposed to PHS [16]. Transcriptional analyses of H_2O_2-treated wild type versus Δ*nuo14* cells showed that genes encoding mitochondrial proteins are the most enriched category among those with higher expression in the mutant in the presence of the insult [22]. Thus, absence of a functional complex I results in lowered production of ROS upon treatment with PHS and confers increased tolerance to some drug-induced transcriptional alterations and this may explain why these cells cope better with the growth insult elicited by PHS and H_2O_2. The involvement of the mitochondria during PHS-induced cell death is further stressed by the evidence that deletion mutants for subunit 4 of mitochondrial ATP synthase, for a mitochondrial aldehyde dehydrogenase, and for the homologue of the mammalian apoptosis-inducing factor (AIF) are more resistant to the drug than wild type. On the other hand, Δ*amid* cells, lacking a homologue of the mammalian apoptosis-inducing factor-homologous mitochondrion-associated inducer of death (AMID) are more sensitive to PHS than wild type [16]. Another group showed that deletion of the tRNA processing molecules TRANSLIN and TRAX confers increased resistance to PHS [39]. More recently, we observed that treatment with PHS, as well as staurosporine, causes the export of reduced glutathione (GSH) [17], although both drugs induce cell death through very distinct mechanisms (see below). Addition of exogenous GSH does not revert the effects of PHS, neither in *N. crassa* [17] nor in *A. nidulans* [38].

In *S. cerevisiae* there is evidence showing that response to distinct cellular stresses such as heat [40] and

nitrogen starvation [41] is correlated with the accumulation of phytosphingosine species. Additionally, yeast mutant cells defective in the addition of inositol phosphate to ceramide are particularly sensitive to treatment with PHS [42]. Exposure of *S. cerevisiae* to PHS also reduces the uptake of some amino acids by specific transporters, namely, tryptophan, leucine, histidine, and proline, leading to amino acid starvation [43].

3.2. Staurosporine (STS). Staurosporine (STS) is a bacterial alkaloid initially isolated from *Streptomyces staurosporeus* during a screening for protein kinase C inhibitors [44], which was later shown to display a broad kinase inhibitory activity [45]. The protein kinase C homologue Pkc1 of *S. cerevisiae* was validated as an essential target of STS [46]. This drug displays strong anticancer and antimicrobial activities and is widely used by the scientific community as a prototypical cell death-inducing agent. Importantly, some STS analogues displaying better selectivity profiles, such as UCN-01, CGP41251, or PKC412, are currently under evaluation in clinical trials for the treatment of different forms of cancer [47]. In *N. crassa*, STS induces loss of cell viability, marked impairment of conidial germination, chromatin fragmentation, YO-PRO1 staining, uptake of vital dyes, and early ROS production [15, 17, 18]. In contrast to the observations with PHS, deletion of some subunits of mitochondrial complex I such as NUO9.8, NUO14, NUO30.4, and NUO51 (but not others like NUO78) results in hypersensitivity to STS. Interestingly, complex I assembly status of these mutant strains cannot explain the increased susceptibility to STS because cells with similar assembly phenotypes display different sensitivity to the drug. Thus, it seems that some of the proteins play a specific role during intracellular cell death signaling or execution. This in line with observation that mammalian complex I subunits execute particular programmed cell death programs: GRIM-19 (NUO14 homologue) regulates cell death by binding a cytomegalovirus RNA [48] and is also involved in β-interferon- and retinoic acid-induced cancer cell death [49]; cleavage of NDUFS1 (NUO78 homologue) [50] by a caspase and cleavage of NDUFS3 (NUO30.4 homologue) by granzyme A [51] mediate cell death; downregulation of NDUFA6 (NUO14.8 homologue) induces apoptosis in HIV-1-infected cells [52]. STS and PHS definitively act by different mechanisms, but mitochondria and respiration are central for the cell death process induced by both drugs.

Because of the involvement of mitochondrial complex I during the fungal response to STS, we decided to combine STS with the classical complex I inhibitor rotenone. It was observed that the combination of the drugs displays synergistic activity against the growth of *N. crassa* and the clinically relevant fungi *Aspergillus fumigatus* and *Candida albicans* [15]. Surprisingly, this synergistic behavior is also observed in complex I mutant strains (in which the enzyme is already nonfunctional), suggesting a complex I-independent for the action of rotenone. Indeed, other complex I inhibitors (piericidin A and diphenyleneiodonium) do not act like rotenone in combination with staurosporine and the combination STS plus rotenone is synergistic even against *S. cerevisiae* cells which are devoid of complex I. This led us to study the mechanisms of rotenone activity. Using thyroid cancer cells as the model system, we observed that the drug acts as an antimitotic agent, causing cell death following cell cycle arrest and mitotic catastrophe with p53 being a pivotal player in the process [19]. Importantly, the combination of STS with rotenone is also synergistic in thyroid cancer cells [19, 20], validating *N. crassa* as a good model to study broad mechanisms of programmed cell death.

The exogenous addition of GSH or its precursor N-acetyl-cysteine (NAC) effectively blocks STS-induced cell death, pointing to the importance of ROS generation during the fungal response to STS [15]. We observed recently, for the first time in fungi, that the export of GSH is a crucial event during the cell death program driven by STS [17]. It seems that GSH efflux following treatment with STS (or PHS, with even faster kinetics) is an early and specific event of cell death rather than a secondary effect such as a detoxification mechanism. Thus, *N. crassa* exports GSH when exposed to STS causing a change in the intracellular environment to a more oxidative redox state. The consequent decrease of the internal GSH/GSSG ratio modulates intracellular redox signaling and may facilitate the oxidation of proteins or lipids. Antioxidants like β-carotene and ascorbic acid are ineffective in the modulation of the effects of STS and a combined treatment with STS and rotenone results in increased depletion of GSH [15].

Analysis of transcriptional alterations associated with treatment with STS by DNA microarrays revealed that the drug strongly induces high levels of expression of a gene encoding a member of the ABC (ATP-binding cassette)-transporter family, *abc3* [18]. This result was confirmed at the gene level by qRT-PCR and at the protein level by western blotting with a house-made specific antibody. This antibody allowed the localization of ABC3 at the cell surface. Interestingly, the deletion of *abc3* results in extreme sensitivity to STS. Because of the significant homology between ABC3 and the human P-glycoprotein, shown to mediate multidrug resistance in cancer cells [53], we measured the levels of intracellular and extracellular STS after treatment of *N. crassa* cells. To achieve this, a method that took advantage of the fact that STS fluoresces when excited with UV light was devised. We showed that ABC3 performs drug efflux to the extracellular space, describing for the first time a transporter of the broadly used STS [18]. In agreement with this, a combined treatment of STS and the P-glycoprotein inhibitors verapamil and sodium orthovanadate results in synergistic inhibition of growth in *N. crassa* as well as in the pathogenic *A. fumigatus* and *C. albicans*, likely due to blockage of STS efflux. Sodium orthovanadate is not selective and, in cancer cells, the drug causes dose-dependent and caspase-mediated cell death by interfering with the PI3K/Akt/mTOR signaling cascade [21].

Gene expression data obtained with microarrays was also used to identify other putative mediators of STS-induced cell death. We showed that two STS highly induced genes, NCU09141 and NCU02887, present homology with β-subunits of voltage-gated potassium channels. In line with a role for this proteins during the action of STS, an inhibitor of these potassium channels, 4-aminopyridine, enhances cell death elicited by STS in *N. crassa*, *A. fumigatus,* and *C.*

albicans [18]. In addition, microarray data also unraveled a novel transcription factor with a role on STS-induced cell death that we are currently characterizing.

The literature on the effects of STS in fungi other than *N. crassa* is scarce. In *S. cerevisiae*, a group of genes termed *stt* (for "staurosporine- and temperature-sensitive") were isolated. This set of genes whose respective deletion strains are particularly susceptible to STS includes protein kinases such as Pkc1, Pi4k, and Bck1, mediators of Golgi to vacuole protein sorting (Vps18, Vps34, Vps11, Vps45, and Vps33), a protein involved in glycophosphatidylinositol anchor synthesis (Gpi1), the acetoacetyl-CoA thiolase involved in ergosterol biosynthesis Erg10, vacuolar H^+-ATPase mutants (Vma1, Vma2, Vma3, Vma4, Vma11, Vma12, and Vma13), and a subunit of oligosaccharyltransferase [46, 54–56].

4. Concluding Remarks

The understanding of the molecular mechanisms of programmed cell death has benefited from the intensive research carried out in the last years, but it is still open to new approaches and discoveries. Ongoing projects in our group include, for example, the determination of the role of Ca^{2+} during STS-induced cell death. We observed that incubation with this drug (but not PHS) promotes a well-defined profile of alterations in cytosolic Ca^{2+} levels, similarly to what is observed when fungal cells are exposed to other cell death stimuli [57]. On the other hand, we are taking advantage of the recent developments on the transcriptomics field and employing high-throughput RNA sequencing (RNA-seq) to study the transcriptional profile of *N. crassa* cells submitted to different cell death conditions and identify novel cell death intervenients.

It is inevitable to stress that there are several important differences between filamentous fungi and yeast programmed cell death. Due to their multicellular nature, filamentous species require cell death to accomplish different developmental and defense processes, such as the aforementioned heterokaryon incompatibility. Another discrepant aspect resides in the characteristics of the electron respiratory chain in both types of fungi. Whereas complex I is undoubtedly involved in programmed cell death in *N. crassa* [15, 16, 22], it is absent in *S. cerevisiae*. However, in the latter, under certain nutritional conditions, the overexpression of the alternative NADH dehydrogenase NDI1, the first component of the yeast electron transport chain, results in programmed cell death [58]. Our group is also interested in unraveling links between cell death, oxidative stress, mitochondrial bioenergetics, and specific enzymes, such as NAD(P)H dehydrogenases [13, 14].

In summary, evidence points to the usefulness of using *N. crassa* as a model for the study of the mechanisms of programmed cell death although the available data is still limited. We anticipate that this fungus will be an invaluable tool for future investigations on the cell death field.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] H. J. Vogel, "A convenient growth medium for *Neurospora* (medium N)," *Microbial Genetics Bulletin*, vol. 13, pp. 42–43, 1956.

[2] D. J. Jacobson, J. R. Dettman, R. I. Adams et al., "New findings of *Neurospora* in Europe and comparisons of diversity in temperate climates on continental scales," *Mycologia*, vol. 98, no. 4, pp. 550–559, 2006.

[3] A. Payen, "Extrain d'un rapport addressé à M. Le Maréchal Duc de Dalmatie, Ministre de la Guerre, Président du Conseil, sur une altération extraordinaire du pain de munition," *Annales de Chimie et de Physique*, vol. 9, pp. 5–21, 1843.

[4] C. L. Shear and B. O. Dodge, "Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group," *Journal of Agricultural Research*, vol. 34, pp. 1019–1042, 1927.

[5] G. W. Beadle and E. L. Tatum, "Genetic control of biochemical reactions in *Neurospora*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 27, no. 11, pp. 499–506, 1941.

[6] A. M. Srb and N. H. Horowitz, "The ornithine cycle in *Neurospora* and its genetic control," *The Journal of Biological Chemistry*, vol. 154, no. 1, pp. 129–139, 1944.

[7] R. H. Davis and D. D. Perkins, "*Neurospora*: a model of model microbes," *Nature Reviews Genetics*, vol. 3, no. 5, pp. 397–403, 2002.

[8] J. E. Galagan, S. E. Calvo, K. A. Borkovich et al., "The genome sequence of the filamentous fungus *Neurospora crassa*," *Nature*, vol. 422, no. 6934, pp. 859–868, 2003.

[9] K. McCluskey, A. Wiest, and M. Plamann, "The Fungal Genetics Stock Center: a repository for 50 years of fungal genetics research," *Journal of Biosciences*, vol. 35, no. 1, pp. 119–126, 2010.

[10] A. Videira, "Complex I from the fungus *Neurospora crassa*," *Biochimica et Biophysica Acta*, vol. 1364, no. 2, pp. 89–100, 1998.

[11] A. Videira and M. Duarte, "On complex I and other NADH: ubiquinone reductases of *Neurospora crassa* mitochondria," *Journal of Bioenergetics and Biomembranes*, vol. 33, no. 3, pp. 197–203, 2001.

[12] A. Videira and M. Duarte, "From NADH to ubiquinone in *Neurospora* mitochondria," *Biochimica et Biophysica Acta*, vol. 1555, no. 1–3, pp. 187–191, 2002.

[13] P. Carneiro, M. Duarte, and A. Videira, "Characterization of apoptosis-related oxidoreductases from *Neurospora crassa*," *PLoS ONE*, vol. 7, no. 3, Article ID e34270, 2012.

[14] P. Carneiro, M. Duarte, and A. Videira, "Disruption of alternative NAD(P)H dehydrogenases leads to decreased mitochondrial ROS in *Neurospora crassa*," *Free Radical Biology and Medicine*, vol. 52, no. 2, pp. 402–409, 2012.

[15] A. Castro, C. Lemos, A. Falcão, A. S. Fernandes, N. Louise Glass, and A. Videira, "Rotenone enhances the antifungal properties of staurosporine," *Eukaryotic Cell*, vol. 9, no. 6, pp. 906–914, 2010.

[16] A. Castro, C. Lemos, A. Falcão, N. L. Glass, and A. Videira, "Increased resistance of complex I mutants to phytosphingosine-induced programmed cell death," *The Journal of Biological Chemistry*, vol. 283, no. 28, pp. 19314–19321, 2008.

[17] A. S. Fernandes, A. Castro, and A. Videira, "Reduced glutathione export during programmed cell death of *Neurospora crassa*," *Apoptosis*, vol. 18, no. 8, pp. 940–948, 2013.

[18] A. S. Fernandes, A. P. Gonçalves, A. Castro et al., "Modulation of fungal sensitivity to staurosporine by targeting proteins identified by transcriptional profiling," *Fungal Genetics and Biology*, vol. 48, no. 12, pp. 1130–1138, 2011.

[19] A. P. Gonçalves, V. Máximo, J. Lima, K. K. Singh, P. Soares, and A. Videira, "Involvement of p53 in cell death following cell cycle arrest and mitotic catastrophe induced by rotenone," *Biochimica et Biophysica Acta*, vol. 1813, no. 3, pp. 492–499, 2011.

[20] A. P. Gonçalves, A. Videira, V. Máximo, and P. Soares, "Synergistic growth inhibition of cancer cells harboring the *RET/PTC1* oncogene by staurosporine and rotenone involves enhanced cell death," *Journal of Biosciences*, vol. 36, no. 4, pp. 639–648, 2011.

[21] A. P. Gonçalves, A. Videira, P. Soares, and V. Máximo, "Orthovanadate-induced cell death in *RET/PTC1*-harboring cancer cells involves the activation of caspases and altered signaling through PI3K/Akt/mTOR," *Life Sciences*, vol. 89, no. 11-12, pp. 371–377, 2011.

[22] A. Videira, T. Kasuga, C. Tian, C. Lemos, A. Castro, and N. L. Glass, "Transcriptional analysis of the response of *Neurospora crassa* to phytosphingosine reveals links to mitochondrial function," *Microbiology*, vol. 155, no. 9, pp. 3134–3141, 2009.

[23] Y. Fuchs and H. Steller, "Programmed cell death in animal development and disease," *Cell*, vol. 147, no. 4, pp. 742–758, 2011.

[24] L. Galluzzi, I. Vitale, J. M. Abrams et al., "Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012," *Cell Death and Differentiation*, vol. 19, no. 1, pp. 107–120, 2012.

[25] N. D. Fedorova, J. H. Badger, G. D. Robson, J. R. Wortman, and W. C. Nierman, "Comparative analysis of programmed cell death pathways in filamentous fungi," *BMC Genomics*, vol. 6, article 177, 2005.

[26] B. S. Strauss, "Cell death and 'unbalanced growth' in *Neurospora*," *Microbiology*, vol. 18, no. 3, pp. 658–669, 1958.

[27] N. S. Plesofsky, S. B. Levery, S. A. Castle, and R. Brambl, "Stress-induced cell death is mediated by ceramide synthesis in *Neurospora crassa*," *Eukaryotic Cell*, vol. 7, no. 12, pp. 2147–2159, 2008.

[28] J. Palma-Guerrero, I.-C. Huang, H.-B. Jansson, J. Salinas, L. V. Lopez-Llorca, and N. D. Read, "Chitosan permeabilizes the plasma membrane and kills cells of *Neurospora crassa* in an energy dependent manner," *Fungal Genetics and Biology*, vol. 46, no. 8, pp. 585–594, 2009.

[29] A. Munoz, J. F. Marcos, and N. D. Read, "Concentration-dependent mechanisms of cell penetration and killing by the *de novo* designed antifungal hexapeptide PAF26," *Molecular Microbiology*, vol. 85, no. 1, pp. 89–106, 2012.

[30] N. L. Glass and I. Kaneko, "Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi," *Eukaryotic Cell*, vol. 2, no. 1, pp. 1–8, 2003.

[31] N. L. Glass and K. Dementhon, "Non-self recognition and programmed cell death in filamentous fungi," *Current Opinion in Microbiology*, vol. 9, no. 6, pp. 553–558, 2006.

[32] K. Dementhon, G. Iyer, and N. L. Glass, "VIB-1 is required for expression of genes necessary for programmed cell death in *Neurospora crassa*," *Eukaryotic Cell*, vol. 5, no. 12, pp. 2161–2173, 2006.

[33] E. A. Hutchison, J. A. Bueche, and N. L. Glass, "Diversification of a protein kinase cascade: IME-2 is involved in nonself recognition and programmed cell death in *Neurospora crassa*," *Genetics*, vol. 192, no. 2, pp. 467–482, 2012.

[34] E. Hutchison, S. Brown, C. Tian, and N. L. Glass, "Transcriptional profiling and functional analysis of heterokaryon incompatibility in *Neurospora crassa* reveals that reactive oxygen species, but not metacaspases, are associated with programmed cell death," *Microbiology*, vol. 155, no. 12, pp. 3957–3970, 2009.

[35] G. Wichmann, J. Sun, K. Dementhon, N. L. Glass, and S. E. Lindow, "A novel gene, *phcA* from *Pseudomonas syringae* induces programmed cell death in the filamentous fungus *Neurospora* crassa," *Molecular Microbiology*, vol. 68, no. 3, pp. 672–689, 2008.

[36] S. M. Marek, J. Wu, N. L. Glass, D. G. Gilchrist, and R. M. Bostock, "Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*," *Fungal Genetics and Biology*, vol. 40, no. 2, pp. 126–137, 2003.

[37] A. Morales, H. Lee, F. M. Goñi, R. Kolesnick, and J. C. Fernandez-Checa, "Sphingolipids and cell death," *Apoptosis*, vol. 12, no. 5, pp. 923–939, 2007.

[38] J. Cheng, T.-S. Park, L.-C. Chio, A. S. Fischl, and X. S. Ye, "Induction of apoptosis by sphingoid long-chain bases in *Aspergillus nidulans*," *Molecular and Cellular Biology*, vol. 23, no. 1, pp. 163–177, 2003.

[39] L. Li, W. Gu, C. Liang, Q. Liu, C. C. Mello, and Y. Liu, "The translin-TRAX complex (C3PO) is a ribonuclease in tRNA processing," *Nature Structural & Molecular Biology*, vol. 19, no. 8, pp. 824–830, 2012.

[40] G. M. Jenkins, A. Richards, T. Wahl, C. Mao, L. Obeid, and Y. Hannun, "Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*," *The Journal of Biological Chemistry*, vol. 272, no. 51, pp. 32566–32572, 1997.

[41] M. Yamagata, K. Obara, and A. Kihara, "Unperverted synthesis of complex sphingolipids is essential for cell survival under nitrogen starvation," *Genes to Cells*, vol. 18, no. 8, pp. 650–659, 2013.

[42] M. M. Nagiec, E. E. Nagiec, J. A. Baltisberger, G. B. Wells, R. L. Lester, and R. C. Dickson, "Sphingolipid synthesis as a target for antifungal drugs: complementation of the inositol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the *AUR1* gene," *The Journal of Biological Chemistry*, vol. 272, no. 15, pp. 9809–9817, 1997.

[43] M. S. Skrzypek, M. M. Nagiec, R. L. Lester, and R. C. Dickson, "Inhibition of amino acid transport by sphingoid long chain bases in *Saccharomyces cerevisiae*," *The Journal of Biological Chemistry*, vol. 273, no. 5, pp. 2829–2834, 1998.

[44] S. Omura, Y. Iwai, A. Hirano et al., "A new alkaloid AM-2282 of *Streptomyces* origin taxonomy, fermentation, isolation and preliminary characterization," *The Journal of Antibiotics*, vol. 30, no. 4, pp. 275–282, 1977.

[45] M. W. Karaman, S. Herrgard, D. K. Treiber et al., "A quantitative analysis of kinase inhibitor selectivity," *Nature Biotechnology*, vol. 26, no. 1, pp. 127–132, 2008.

[46] S. Yoshida, E. Ikeda, I. Uno, and H. Mitsuzawa, "Characterization of a staurosporine- and temperature-sensitive mutant, *STT1*, of Saccharomyces cerevisiae: *STT1* is allelic to *PKC1*," *Molecular and General Genetics*, vol. 231, no. 3, pp. 337–344, 1992.

[47] O. A. Gani and R. A. Engh, "Protein kinase inhibition of clinically important staurosporine analogues," *Natural Product Reports*, vol. 27, no. 4, pp. 489–498, 2010.

[48] M. B. Reeves, A. A. Davies, B. P. McSharry, G. W. Wilkinson, and J. H. Sinclair, "Complex I binding by a virally encoded RNA regulates mitochondria-induced cell death," *Science*, vol. 316, no. 5829, pp. 1345–1348, 2007.

[49] G. Huang, Y. Chen, H. Lu, and X. Cao, "Coupling mitochondrial respiratory chain to cell death: an essential role of mitochondrial complex I in the interferon-β and retinoic acid-induced cancer cell death," *Cell Death and Differentiation*, vol. 14, no. 2, pp. 327–337, 2007.

[50] J.-E. Ricci, C. Muñoz-Pinedo, P. Fitzgerald et al., "Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain," *Cell*, vol. 117, no. 6, pp. 773–786, 2004.

[51] D. Martinvalet, D. M. Dykxhoorn, R. Ferrini, and J. Lieberman, "Granzyme A cleaves a mitochondrial complex I protein to initiate caspase-independent cell death," *Cell*, vol. 133, no. 4, pp. 681–692, 2008.

[52] J. S. Ladha, M. K. Tripathy, and D. Mitra, "Mitochondrial complex I activity is impaired during HIV-1-induced T-cell apoptosis," *Cell Death and Differentiation*, vol. 12, no. 11, pp. 1417–1428, 2005.

[53] A. Breier, M. Barančík, Z. Sulová, and B. Uhrík, "P-glycoprotein—implications of metabolism of neoplastic cells and cancer therapy," *Current Cancer Drug Targets*, vol. 5, no. 6, pp. 457–468, 2005.

[54] S. Yoshida and Y. Anraku, "Characterization of staurosporine-sensitive mutants of *Saccharomyces cerevisiae*: vacuolar functions affect staurosporine sensitivity," *Molecular and General Genetics*, vol. 263, no. 5, pp. 877–888, 2000.

[55] S. Yoshida, Y. Ohya, A. Nakano, and Y. Anraku, "Genetic interactions among genes involved in the *STT4-PKC1* pathway of *Saccharomyces cerevisiae*," *Molecular and General Genetics*, vol. 242, no. 6, pp. 631–640, 1994.

[56] S. Yoshida, Y. Ohya, M. Goebl, A. Nakano, and Y. Anraku, "A novel gene, *STT4*, encodes a phosphatidylinositol 4-kinase in the *PKC1* protein kinase pathway of *Saccharomyces cerevisiae*," *The Journal of Biological Chemistry*, vol. 269, no. 2, pp. 1166–1172, 1994.

[57] Y. Zhang, S. Muend, and R. Rao, "Dysregulation of ion homeostasis by antifungal agents," *Frontiers in Microbiology*, vol. 3, article 133, 2012.

[58] W. Li, L. Sun, Q. Liang, J. Wang, W. Mo, and B. Zhou, "Yeast AMID homologue Ndi1p displays respiration-restricted apoptotic activity and is involved in chronological aging," *Molecular Biology of the Cell*, vol. 17, no. 4, pp. 1802–1811, 2006.