Minireview

Programmed death in yeast as adaptation?

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I consider that death is not a primary necessity, but that it has been secondarily acquired as an adaptation. [Weismann, A., (1889) Essays Upon Heredity and Kindred Biological Problems. Clarendon Press, Oxford].

Abstract During recent years, several pieces of indirect evidence of a programmed death in yeast have been published. Among them there are observations that some mammalian pro- or anti-apoptotic proteins induce or prevent the death of yeast; some toxic compounds kill yeast at lower concentrations if protein synthesis is operative; this death, as well as the death due to certain mutations, shows some apoptotic markers. In April 2002, the yeast programmed death concept received direct support. Madeo et al. [Madeo et al., Mol. Cell 9 (2002) 911–917] disclosed a caspase which is activated by H_2O_2 or aging and is required for the protein-synthesis-dependent death of yeast. Thus, a specific apoptosis-mediating protein was identified for the first time in *Saccharomyces cerevisiae*. Independently, Severin and Hyman [Severin, F.F., Hyman, A.A., Curr. Biol. 12 (2002) R233–R235] discovered that death of yeast, induced by a high level of a pheromone, is programmed. In particular, the death was found to be prevented by cycloheximide and cyclosporin A. It required mitochondrial DNA, cytochrome *c* and the pheromone-initiated protein kinase cascade. When haploids of opposite mating types were mixed, some cells died, the inhibitory pattern being the same as in the case of the killing by pheromone. Inhibition of mating proved to be favorable for death. Thus, pheromone not only activates mating but also eliminates yeast cells failing to mate. Such an effect should (i) stimulate switch of the yeast population from vegetative to sexual reproduction, and (ii) shorten the life span and, hence, accelerate changing of generations. As a result, the probability of appearance of new traits could be enhanced when ambient conditions turned for the worse.

Key words: Yeast; Programmed death; Mitochondria; Caspase; Pheromone

1. Introduction

More than a century ago, August Weismann suggested that death because of aging occurs due to the operation of an adaptive biological mechanism rather than to accidental disorder in a complex living system [1]. For many years, the scientific community did not accept this idea. Recently Weismann's concept was, however, revisited [2,3] since it became clear that a programmed death (apoptosis) really exists at least at the cellular level if we deal with multicellular organisms. Programmed death systems were assumed to be also operative (a) at the subcellular level (self-elimination of mitochondria during apoptosis), (b) at the supracellular level (collective apoptosis when an apoptotic cell sends death signals to cells-bystanders) and (c) at the level of entire organs (apoptotic degeneration of some organs during ontogenesis) (for review, see [3]). The problem arose whether death of an organism might be programmed. There are some indications that such a phenomenon (called 'phenoptosis') exists in unicellular organisms. In bacteria, altruistic programmed death was suggested to be used by a population (i) to prevent expansion of phage infection, (ii) to purify a population from those cells whose genome or some other key systems are damaged, and (iii) to optimize the number of bacteria in the medium, etc. [3–7].

2. Programmed death of yeast: pieces of indirect evidences

Until very recently studies on programmed death of free-living unicellular eukaryotes were at a preliminary stage. Staurosporine (a non-specific protein kinase inhibitor inducing apoptosis in cells of higher organisms) was shown to stimulate reactive oxygen species (ROS) production and to kill *Tetrahymena* [8]. Petit and his colleagues [9] identified in the slime mold *Dictyostelium discoideum* an apoptosis-inducing factor (DdAIF) structurally and functionally similar to the mammalian AIF [10]. It was found that DdAIF is involved in cell death caused by adding protoporphyrin IX or differentiation-inducing factor-1 (DIF-1). However, one should keep in mind that *D. discoideum* at some stage of its life cycle is a multicellular organism (for some other indications of the programmed death of constantly unicellular eukaryotes, see reviews [4,6]).

During the last decade, the above problem was intensively studied in yeast. The majority of these studies dealt with expression of mammalian pro- and anti-apoptotic proteins in these microorganisms. Kane et al. [11] reported that anti-apoptotic mammalian protein Bcl-2 partially rescued a yeast mutant lacking superoxide dismutase. Manon and co-workers [12] found that pro-apoptotic mammalian protein Bax caused growth arrest of yeast cells, which was accompanied by cyto-

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Abbreviations: AIF, apoptosis-inducing factor; Apaf-1, apoptotic protease-activating factor 1; DdAIF, *Dictyostelium discoideum* apoptosis-inducing factor; ROS, reactive oxygen species

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chrome *c* release from mitochondria to cytosol. Coexpression of anti-apoptotic Bcl-x_L prevented the effects of Bax. In the same laboratory, it was later found that neither cytochrome *c* nor ATP/ADP antiporter are required for the Bax-induced death of yeast [13]. Thus, cytochrome *c* release might be a side effect of Bax. For example, it is known that Bax, if added together with mitochondrial porin, induces permeabilization of an artificial phospholipid membrane to cytochrome *c* [14]. It was found that porin is required for killing of yeast by Bax [15]. However, it was also shown [16] that some proteins other than porin are necessary for the Bax-induced yeast death. No cytochrome *c* release was found in this study. The Bax-induced death of yeast seemed to be a consequence of an oxidative stress since some plant proteins preventing the Bax-induced ROS burst rescued the Bax-expressing yeast when co-expressed with Bax [17,18].

Some other studies have demonstrated that certain mutations in the yeast genome result in death showing features of apoptosis [19,20]. In the latter paper, the authors claimed that they observed a cytochrome *c* release and mitochondrial depolarization, but the data presented are hardly sufficient to make such a conclusion.

Three cases were described which point to programmed death of yeast treated by a lethal dose of some toxic compounds. Madeo et al. [21] and Corte-Real et al. [22] reported that H_2O_2 and acetate respectively kill yeast, the death being accompanied by some typical apoptotic changes. In both cases, higher concentrations of the poison were required to kill the cells if translation was inhibited by cycloheximide. (cf. Christensen et al. [8] who reported that killing of *Tetrahymena* by staurosporine was prevented by actinomycin D). These facts suggested that the programmed death mechanism in yeast is inducible. However, complications in interpretation of these data may arise due to pleiotropic effects of such agents as H_2O_2. It was found [23] that H_2O_2 stimulates synthesis of at least 115 proteins and represses synthesis of 52 other proteins in the yeast cell. Therefore it is difficult to exclude that decrease in the H_2O_2 sensitivity of yeast by cycloheximide is a side effect.

Narasimhan et al. [24] showed that the plant antibiotic osmotin kills yeast. Several features of apoptosis, including ROS overproduction, were revealed. The anti-oxidant *N*-acetyl-L-cysteine prolonged the life of the osmotin-treated yeast. Some components of the pheromone cascade were shown to be involved in the osmotin effect[1].

3. Discovery of yeast caspase

In 2001, Laun et al. [25] reported that apoptotic markers are inherent in death of yeast because of aging. Quite recently the same group [26] succeeded in finding in *S. cerevisiae* a caspase, a key enzyme of the animal apoptotic cascade (this discovery was, in fact, predicted by Koonin, Dixit and their bioinformaticist colleagues analyzing the yeast genome [27]). Madeo et al. [26] studied a 52 kDa protein, the sequence of which resembled that of mammalian caspases. It was found that overexpression of the enzyme resulted in removal of a 12 kDa fragment of this protein and appearance of the caspase-like proteolytic activity. If potentially catalytic cysteine 297 of the protein was mutated, no formation of the 12 kDa product was observed. Overexpression of the 52 kDa protein reduced viability of the prolonged yeast culture, the effect being prevented by a caspase-specific inhibitor. Addition of H_2O_2 to the yeast overexpressing p52 made the cell extract able to hydrolyze typical caspase substrates. Deletion of the corresponding gene increased the lethal dose of H_2O_2 by a factor of 3 and entailed appearance of 6–8% 'immortal' cells surviving more than 28 days under nutrient-depleted conditions whereas all the wild-type cells died within 10 days. The authors called the 52 kDa protein yeast caspase 1 (YCA1).

Madeo et al. [26] identified one more component of the YCA1-mediated yeast programmed death. It was shown that the absence of a protein encoded by the *Ygl129c* gene completely prevented the death caused by the YCA1 overexpression. As indicated by Berger et al. [28], this protein is an ortholog of the animal mitochondrial protein DAP-3, which is involved in apoptosis induced by some cytokines [29,30].

4. A pheromone induces programmed death of yeast

In another paper published quite recently, Severin and Hyman [31] reported for the first time that the programmed death of a unicellular eukaryotic organism could be induced by a natural signal substance released by these organisms. Effects of α-factor (a pheromone peptide produced by the α-type haploid cells of *S. cerevisiae*) were studied. It was already known that α-factor at concentrations below 1 μM forced yeast of the opposite mating type (*a*) to conjugate with the α-type yeast whereas its higher concentrations caused arrest of the *a* cell cycle and then death (for review, see [32]). The question was whether this death is programmed. The data obtained by Severin and Hyman clearly demonstrate that the answer should be 'yes'.

1. The pheromone-induced death was shown to be accompanied by appearance of a series of typical features characteristic of apoptosis in animals: (a) ROS were produced at an early stage of the suicide program; (b) typical changes in arrangement of chromatin and nuclear DNA fragmentation occurred at a late stage; (c) both ROS production and death required mitochondrial DNA; (d) cytochrome *c* was necessary for death but not for ROS production.
2. Like in yeast phenoptoses induced by toxic compounds [21,22,24], the α-factor-induced ROS production and death required protein synthesis.
3. Both the mating and the death mechanisms induced by the pheromone shared some common steps: (a) only cells of the opposite sex (*a*-type yeast) showed the α-factor-caused ROS production and death, whereas α-type yeast was resistant (diploid cells, like α-cells, are known to be insensitive to the death signal of α-factor); (b) a mutation in the pheromone protein kinase cascade (*ste 20* gene [33]) prevented both death and appearance of apoptotic markers; (c) mutation in the gene coding for calmodulin enhanced

[1] When this manuscript was already submitted to FEBS Letters, one more paper by Corte-Real and her colleagues appeared. The authors succeeded in demonstrating cytochrome *c* release during the acetate-induced programmed death of *S. cerevisiae*. This was shown by Western blots as well as spectrophotometrically. Moreover, it was found that mutants lacking apocytochrome *c* heme liase, mitochondrial DNA or an H^+-ATP-synthase subunit did not show this programmed death. The H^+-ATP-synthase inhibitor oligomycin was without effect. The death was accompanied by a lowering of the level of the cytochrome *c* oxidase subunit II whereas the complex III activity remained constant [40].

the ROS-producing response and death caused by α-factor; this effect being expected since calmodulin is known to attenuate the pheromone mate signal [34].

Involvement of mitochondria and cytochrome *c* in yeast programmed death phenomena is noteworthy. On the face of it, this observation is surprising since in the *S. cerevisiae* genome there are no genes encoding Apaf-1 (apoptotic protease-activating factor 1), as well as Smac and AIF, i.e. proteins involved in the mitochondrion-mediated apoptosis of animal cells [35]. This means that yeast possesses a suicide mechanism requiring mitochondrial DNA product(s), cytochrome *c* and caspase but independent of the animal-type Apaf-1, etc.

Another observation by Severin and Hyman [31] was that cyclosporin A, inhibitor of many types of apoptosis in animal cells [10], is also inhibitory in yeast. In animal systems, this agent is used to arrest opening of the permeability transition pore in the inner mitochondrial membrane (for review, see [36]). In yeast, the pore was reported to be cyclosporin A-resistant [37,38] although cyclophilin, a cyclosporin A target, was found in the yeast mitochondria [39]. Perhaps, sensitization of the yeast mitochondrial pore to cyclosporin A requires also some other protein(s) induced by pheromone.

Novel observations concerning the yeast programmed death mechanism are summarized in Fig. 1. It is assumed that at high concentrations, pheromone saturates a receptor on the surface of the yeast cell, sending a signal to a putative transcription factor via the ste20-mediated protein kinase cascade controlled by the Ca^{2+}–calmodulin–calcineurin system. As a result, synthesis of some programmed death protein(s) is induced that stimulate ROS formation and/or cyclosporin A-sensitive permeability transition pore in the inner mitochondrial membrane. This, in turn, entails swelling of the mitochondrial matrix and, as a consequence, disruption of the outer mitochondrial membrane. Cytochrome *c* is released to the cytosol and, via an Apaf-1 functional analogue X, activates a caspase (YCA1?), which switches on terminal events of the programmed death.

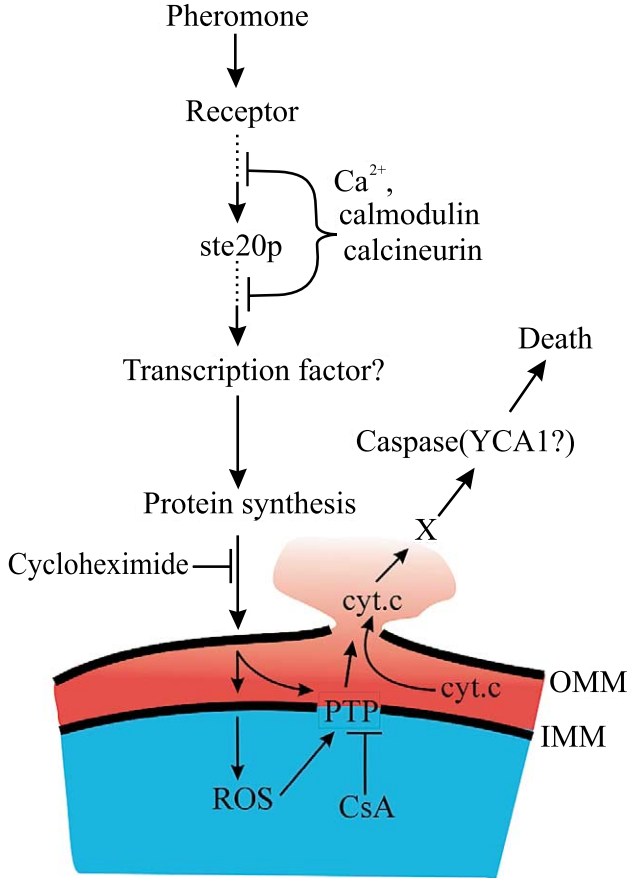

Fig. 1. Tentative scheme of the pheromone-induced programmed cell death of yeast (on the basis of data of [31] and [26]). Ste20p, component of the pheromone protein kinase cascade; PTP, permeability transition pore; CsA, cyclosporin A; OMM and IMM, the outer and inner mitochondrial membranes, respectively.

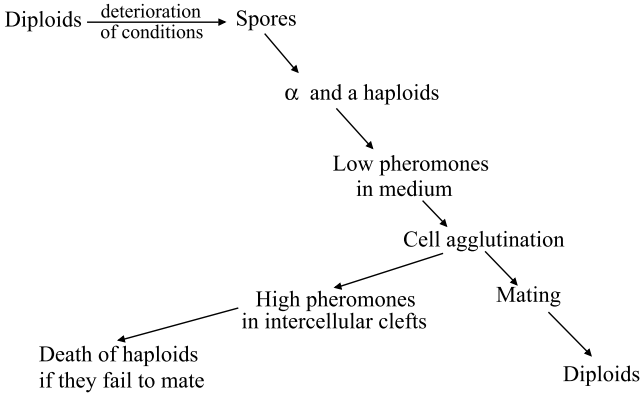

Fig. 2. The life cycle of yeast: oscillation between long-lived and short-lived modes. Deterioration of ambient conditions induces the vegetative-to-sexual reproduction switch mediated by production of pheromones that are excreted into the medium. Low concentrations of pheromones cause cell agglutination and mating of α and *a* haploid cells. Those cells that were agglutinated but failed to mate are killed by high concentrations of pheromone appearing in narrow clefts between agglutinated cells. This mechanism purifies the yeast population from such cells and accelerates change of generations in the population.

However, the most interesting observation made by Severin and Hyman [31] consists in that the killing of yeast by α-factor is a *physiological* effect of this signal peptide. To initiate ROS production and death, it was sufficient, instead of adding of a high pheromone concentration, to mix α- and *a*-type yeast cells. Both ROS production and death were found to be inhibited by mutation in the α-factor-induced protein kinase cascade.

Thus, the pheromone-induced programmed death seems to be related to a switch from the vegetative to sexual reproduction in yeast. It is well known that such a switch represents a response of the yeast cell to a change in ambient conditions for the worse. In fact, the life span of diploid yeast is about 3 days. During this period of time, a mother cell forms about 30 buds and then dies. It was recently found that certain apoptotic markers, including induction of caspase, appear in chronologically aged yeast cultures [25,26]. When ambient conditions deteriorate, the following mechanisms are actuated: (i) formation of spores, a latent form of life, from diploid cells; (ii) formation of haploid cells from spores; (iii) substitution of sexual reproduction for vegetative reproduction; and (iv) initiation of pheromone-induced programmed death resulting in shortening of the life span and, hence, in acceleration of changing of generations. The latter, like sexual reproduction, might be favorable for progressive evolution. Moreover, the pheromone-induced programmed death may be regarded as a mechanism purifying a population

from those haploid cells that, for some reason, fail to mate. Such a reason may consist, e.g. of wrong reciprocal orientation of two mating cells in a cell agglutinate. Events of this kind are quite probable since yeast possesses no mechanism for active movement. Perhaps, high pheromone concentrations appearing in clefts between adhered haploid cells kill these cells if they fail for a long time to convert to the pheromone-resistant diploid. This suggestion was confirmed by the observation that chloroquine, an inhibitor preventing zygote formation but not agglutination, strongly stimulated death when α and *a* cells were mixed [31]. The above relationships are illustrated in Fig. 2.

If diploids produced as a result of the operation of the mechanism shown in Fig. 2 are not adapted yet to new conditions, the chain of events is repeated until new trait(s) appear which help to overcome disadvantages of environmental changes that have occurred.

Thus, yeast seems to be a form of life that oscillates between short-lived and long-lived modes, the oscillation being a mechanism of adaptation to changing conditions. This seems to be in line with Weismann's paradoxical hypothesis that death can be a kind of adaptation. The next (and the most important!) problem remains whether this principle is applicable to higher organisms and, if so, to what extent.

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References

[1] Weismann, A. (1889) Essays Upon Heredity and Kindred Biological Problems. Clarendon Press, Oxford.
[2] Skulachev, V.P. (1997) Biochemistry (Moscow) 62, 1191–1195.
[3] Skulachev, V.P. (2001) Exp. Gerontol. 36, 995–1024.
[4] Ameisen, J.C. (1996) Science 272, 1278–1279.
[5] Raff, M. (1998) Nature 396, 119–122.
[6] Lewis, K. (2000) Microbiol. Mol. Biol. Rev. 64, 503–514.
[7] Engelberg-Kulka, H., Sat, B. and Hazan, R. (2001) ASM News 67, 617–624.
[8] Christensen, S.T., Chemnitz, J., Straarup, E.M., Kristiansen, K., Wheatle, D.N. and Rasmussen, L. (1998) Cell Biol. Int. 22, 591–598.
[9] Arnoult, D., Tatischeff, I., Estaquier, J., Girard, M., Sureau, F., Tissier, J.P., Grodet, A., Dellinger, M., Traincard, F., Kahn, A., Ameisen, J.C. and Petit, P.X. (2001) Mol. Biol. Cell 12, 3016–3030.
[10] Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Snow, B.E., Brothers, G.M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D.R., Aebersold, R., Siderovski, D.P., Penninger, J.M., Kroemer, G. and Kroemer, G. (1999) Nature 397, 441–446.
[11] Kane, D.J., Sarafian, T.A., Anton, R., Hahn, H., Gralla, E.B., Valentine, J.S., Ord, T. and Bredesen, D.E. (1993) Science 262, 1272–1277.
[12] Manon, S., Chaudhuri, B. and Guerin, M. (1997) FEBS Lett. 415, 29–32.
[13] Priault, M., Camougrand, N., Chaudhuri, B., Schaeffer, J. and Manon, S. (1999) FEBS Lett. 456, 232–238.
[14] Shimizu, S., Ide, T., Yanagida, T. and Tsujimoto, Y. (2000) J. Biol. Chem. 275, 12321–12326.
[15] Shimizu, S., Shinohara, Y. and Tsujimoto, Y. (2000) Oncogene 19, 4309–4318.
[16] Gross, A., Pilcher, K., Blachky-Dyson, E., Basso, E., Jockel, J., Bassik, M.C., Korsmeyer, S.J. and Forte, M. (2000) Mol. Cell. Biol. 20, 3125–3136.
[17] Levine, A., Belenghi, B., Damari-Weisler, H. and Granot, D. (2001) J. Biol. Chem. 276, 46284–46289.
[18] Moon, H., Baek, D., Lee, B., Trasad, D.T., Lee, S.Y., Cho, M.J., Lim, C.O., Choi, M.S., Bahk, J., Kim, M.O., Hong, J.C. and Yun, D.-J. (2002) Biochem. Biophys. Res. Commun. 290, 457–462.
[19] Madeo, F., Fröhlich, E. and Fröhlich, K.-U. (1997) J. Cell Biol. 139, 729–734.
[20] Yamaki, M., Umehara, T., Chimura, T. and Horikoshi, M. (2001) Genes Cells 6, 1043–1054.
[21] Madeo, F., Fröhlich, E., Ligr, M., Grey, M., Sigrist, S.J., Wolf, D.H. and Fröhlich, K.-U. (1999) J. Cell Biol. 145, 757–767.
[22] Ludovico, P., Sousa, M.J., Silva, M.T., Leao, C. and Corte-Real, M. (2001) Microbiology 147, 2409–2415.
[23] Godon, C., Lagniel, G., Lee, J., Buhler, J.M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M.B. and Labarre, J. (1998) J. Biol. Chem. 273, 480–489.
[24] Narasimhan, M.L., Damsz, B., Coca, M.A., Ibeas, J.I., Yun, D.J., Pardo, J.M., Hasegawa, P.M. and Bressan, R.A. (2001) Mol. Cell. 8, 921–930.
[25] Laun, P., Pichova, A., Madeo, F., Fuchs, J., Ellinger, A., Kohlwein, S., Dawes, I., Fröhlich, K.-U. and Bretenbach, M. (2001) Mol. Microbiol. 39, 1166–1173.
[26] Madeo, F., Herker, E., Maldener, C., Wissing, S., Lächelt, S., Herlan, M., Fehr, M., Lauber, K., Sigrist, S.J., Wesselborg, S. and Fröhlich, K.-U. (2002) Mol. Cell. 9, 911–917.
[27] Uren, G.A., O'Rourke, K., Aravind, L., Pisabarro, T.M., Seshagiri, S., Koonin, E.V. and Dixit, V.M. (2000) Mol. Cell 6, 961–967.
[28] Berger, T., Brigl, M., Hermann, J.M., Vielhauer, V., Luckow, B., Schlondorff, D. and Kretzler, M. (2000) J. Cell Sci. 113, 3603–3612.
[29] Kissil, J.L., Cohen, O., Raven, T. and Kimchi, A. (1999) EMBO J. 18, 353–362.
[30] Miyazaki, T. and Reed, J.C. (2001) Nat. Immunol. 2, 493–500.
[31] Severin, F.F. and Hyman, A.A. (2002) Curr. Biol. 12, R233–R235.
[32] Kurjan, J. (1993) Annu. Rev. Genet. 27, 147–179.
[33] O'Rourke, S.M. and Herskowitz, I. (1998) Genes Dev. 12, 2874–2876.
[34] Foor, F., Parent, S.A., Morin, N., Dahl, A.M., Ramadan, N., Chrebet, G., Bostian, K.A. and Nielsen, J.B. (1992) Nature 360, 682–684.
[35] Aravind, L., Dixit, V.M. and Koonin, E.V. (2001) Science 291, 1279–1284.
[36] Zoratti, M. and Szabo, I. (1995) Biochim. Biophys. Acta 1241, 139–176.
[37] Jung, D.W., Bradshaw, P.C. and Pfeiffer, D.R. (1997) J. Biol. Chem. 272, 21104–21112.
[38] Kowaltowski, A.J., Vercesi, A.E., Rhee, S.G. and Netto, L.E. (2000) FEBS Lett. 473, 177–182.
[39] Matouschek, A., Rospert, S., Schmid, K., Glick, B.S. and Schatz, G. (1995) Proc. Natl. Acad. Sci. USA 92, 6319–6323.
[40] Ludovico, P., Rodrigues, F., Almeida, A., Silva, M.T., Leao, C., Barrientos, A. and Corte-Real, M. (2002) Mol. Biol. Cell 13, 2598–2606.