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REVIEW

Physiological Scenarios of Programmed Loss of Mitochondrial DNA Function and Death of Yeast

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Abstract—Recently it was convincingly shown that the yeast *Saccharomyces cerevisiae* does possess the basic modules of programmed cell death machinery. As programmed cell death is suicide for a unicellular organism, it is reasonable to assume that they trigger the program when the death is beneficial for the rest of the population. Not surprisingly, most of the scenarios of physiological death of *S. cerevisiae*, i.e. cell death in stationary culture, during meiosis, during mating, and driven by viruses are dependent on quorum sensing, meaning that they depend on the cell density. Here we also discuss possible mechanisms that govern fitness decline during replicative aging of *S. cerevisiae* cells. We argue that loss of mitochondrial DNA function that occurs during replicative aging is programmed and adaptive. Indeed, yeast cells with nonfunctional mitochondrial DNA are known to be extremely stress-resistant, and also the presence of a subpopulation of such cells might protect the culture from degeneration by preventing the fixation of opportunistic mutations.

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Programmed cell death (PCD) is an essential physiological process vital for development, maintenance of tissue homeostasis, and the immune defense of multicellular organisms. However, several elements of the PCD mechanism have been shown for unicellular organisms. As PCD equals suicide for a unicellular organism, it is generally not clear why the mechanism has been preserved during their evolution. Recently we have argued that the reason for the preservation is that in certain cases death of an organism could be beneficial for the surviving individuals [1, 2].

We speculated that an altruistic suicide of an organism makes sense only if it "feels" the near presence of the individuals of the same species. *Saccharomyces cerevisiae* produces at least two quorum sensing factors – phenylethanol and tryptophol. These factors trigger pseudohyphal growth under conditions of nitrogen starvation [3]. While there is no established role for these alcohols in yeast PCD, in many cases the death of yeast depends on the culture density. It has been shown for another yeast species, *Candida albicans*, that the quorum sensing alcohol farnesol does trigger PCD [4]. Cases of *S. cerevisiae* quorum sensing-dependent PCD are reviewed in [2, 5]. The deaths during chronological aging, mating, meiosis, and the one initiated by viral infection fall into this category. Here we summarize novel data on quorum sensing-dependent PCD. In this review we also speculate that the viability decline during replicative aging is also adaptive.

QUORUM SENSING-DEPENDENT TYPES OF YEAST PCD

Chronological aging is a viability decline during extended incubation in stationary culture. It was shown that yeast cell death in this case is accompanied by PCD markers. It is assumed that the physiological role of such PCD is that the cells which die in the stationary culture provide nutrients for the surviving ones [6]. While chronological aging happens on both liquid and solid media, the mechanisms of PCD activation differ. For

Abbreviations: ER, endoplasmic reticulum; PCD, programmed cell death; ROS, reactive oxygen species.

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cells grown on solid media the key factor triggering PCD is ammonia concentration. Ammonia is secreted by the cells and initiates their death in the middle of the colony, thus supporting growth at the periphery [7]. It was suggested that such signaling supports the spatial expansion of the colony [8, 9].

Chronological aging in liquid media is used as a model to study aging of higher organisms. Indeed, yeast chronological aging and aging of mammals have much in common: caloric restriction increases the life spans. Moreover, the effect is mediated by the same group of genes: *TOR1*, *SCH9*, *RAS2*, and *SIR2* in yeast and their homologs in higher organisms [10]. Acetic acid plays a major role during massive death of yeast in stationary cultures [11]. It accumulates in the media and triggers death that is accompanied by accumulation of reactive oxygen species (ROS) in the cells, nuclear DNA fragmentation, and also dependence on yeast caspase homolog Mca1 [12, 13]. Apparently, acetic acid causes acidification of the cytoplasm and sharply raises the concentration of protonated superoxide – a highly reactive form of ROS [14].

However, recently it was doubted whether chronological aging of yeast is relevant to model aging of multicellular organisms. Burtner and coauthors [11] showed that by increasing the osmolarity of the media or by deleting *RAS2* and *SCH9* one can not only increase the chronological lifespan but also increase the resistance to acetic acid treatment. They concluded that acetic acid accumulation in the media is the primary reason for the death, and therefore it is not obvious what aspects of mammalian aging can be studied using this model [11]. Burhans and Weinberger [15] agree that acetate is the primary reason for the death, but they argue with the conclusion. Indeed, acetic acid accumulating in the culture causes the same phenomena that are noted during senescence of cultured mammalian cells, i.e. ROS accumulation, replicative stress, and arrest in the S-phase of the cell cycle (instead of G1), which in turn leads to activation of PCD [16, 17].

Yeast cells can undergo not only chronological, but also replicative aging: mother cells produces a limited number of buds (typically 15-20) after which their viability starts to decline and the markers typical for senescent mammalian cells begin to accumulate [13, 18]. Importantly, certain gene deletions (e.g. *SCH9*, *TOR1*) increase both chronological and replicative life spans [19]. There is also accumulating evidence that the same gene affects aging of *Caenorhabditis elegans*, *Drosophila melanogaster*, and mammals [10]. In agreement with that, the processes of yeast chronological and replicative aging appear to be interdependent. Indeed, it was shown that in nutrient-depleted chronological cultures the cells that have already produced at least one daughter cell are more likely to enter S-phase than the virgin daughters, which tend to accumulate in starvation-resistant G0 phase [20]. At the same time, it is known that during yeast cell division most of the markers of oxidative damage [21] and extra-chromosomal DNA [22] are actively transported to the mother cell. Therefore, it is likely that preservation of the daughter (replicatively young) cells is adaptive and is aimed to maximize the percentage of undamaged cells capable of surviving the stress of chronological aging.

To summarize this part, it appears that while chronological aging of yeast fully depends on such a simple molecule as acetate, it has much in common (is regulated by similar machinery) with other experimental models of aging.

Another example of yeast quorum sensing-dependent PCD is the one induced by unsuccessful mating. Haploid *S. cerevisiae* cells are of *a* or α mating type. The cells secrete *a* or α mating factor, correspondingly. When the *a* cells detect α factor, they start to prepare themselves for the fusion with the cells of the opposite mating type. It was known for decades from laboratory practice that high concentrations of α factor could be toxic. We have shown that high concentrations of α factor kill yeast, and that the death shows markers of apoptosis [23]. Apparently, high concentration of α factor in the culture can be achieved at high cell density. α-Factor at high concentration induces a sharp increase in $[Ca^{2+}]$ in the cytosol, which triggers elevation of the mitochondrial membrane potential and ROS production leading to mitochondrial fragmentation and cell death [23, 24].

Death of yeast during meiosis presents another case of cell density-dependent type of death [25, 26]. As in the case of pheromones, one can say that meiosis-associated death strongly depends on mitochondria. Indeed, most of the mutations compromising mitochondrial function inhibit entry into meiosis. Apart from their direct role, mitochondria promote meiosis by inducing alkalinization of the media: it was shown that high pH values favor initiation of the meiotic program [27]. Therefore, entry into meiosis and, in this way, the accompanied PCD is regulated by cell density.

Saccharomyces cerevisiae cells when infected by viruses start to secrete proteins toxic for the surrounding cells. Low doses of these proteins kill the cells with markers of apoptosis, while higher ones induce necrosis [28]. Interestingly, not only these proteins but viruses *per se* are capable of killing the cells in these ways [29]. While it is not clear whether this type of death is physiological or pathophysiological, it obviously depends on the cell density. The very phenomenon of virus-induced PCD offers an explanation for one of the very conservative features of apoptosis. It is known that apoptotic mammalian cells transport calreticulin from the endoplasmic reticulum (ER) to the plasma membrane. Once at the cell surface, calreticulin initiates cell engulfment by macrophages, thus preventing inflammation [30]. Recently it was shown that yeast calreticulin also re-localizes in a similar way during cell death, although the authors have not suggested any

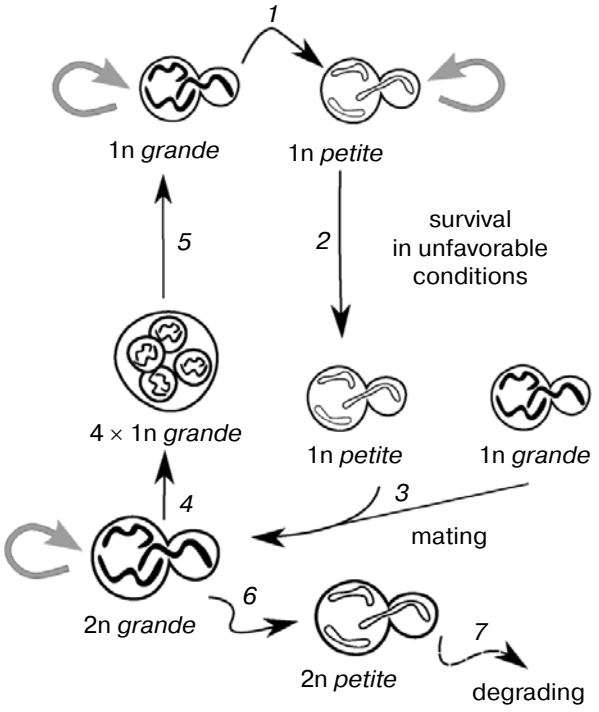
function for this phenomenon [30]. Importantly, plants possess homologs of calreticulin, and they localize to the ER and to the plasmalemma. Moreover, hyperexpression of plant calreticulin inhibits cell-to-cell transport [31]. Thus, one can speculate that in yeast cells the re-localization of calreticulin prevents exit of the viral particles.

QUORUM SENSING-INDEPENDENT PCD OF YEAST

The only physiological scenario of yeast cell death that has not been shown to depend on the cell density is the one caused by replicative aging. Although there is an indirect connection between replicative and chronological aging [20] (see above), there are no indications for a signal coming from outside the cells and triggering replicative aging. However, there is evidence that this type of death is physiological and programmed. What causes replicative aging? Lai and coauthors discovered that a single amino acid substitution in Atp2 (a subunit of F_0F_1-ATP synthase) abrogates the mother–daughter asymmetry of distribution of oxidatively damaged material. As a result, these cells when grown at 37°C undergo clonal senescence, i.e. all cells die after a certain number of cell divisions [32]. In this case clonal senescence was apparently due to the fact that the daughters were "born old", i.e. they had the same share of damaged material as their replicatively old mothers. Thus one can assume that mother–daughter asymmetry of damage distribution is a key factor contributing to replicative aging of yeast. Replicatively old mother cells accumulate markers of cell senescence (oxidative damage, extrachromosomal DNA and DNA breaks, etc.) and this is regulated by the TOR pathway and via Sir2 [13]. Recently it was shown that aged diploid cells lose their ability to undergo meiosis [33]. Most likely this is due to the fact that the cells lose their mitochondrial DNA, and this happens approximately in the middle of their replicative life span [34]. On one hand, this does not seem surprising: it is known that as a result of mitochondrial DNA loss, yeast cells can lose their ability to survive on non-fermentable substrates (*grande–petite* transformation [35]). On the other hand, one can speculate that this transformation is programmed. Indeed, *petite* cells acquire elevated stress resistance, and this is in part due to activation of a retrograde pathway of mitochondrial signaling [36, 37]. Moreover, under normal conditions after each round of cell division 1-10% of haploid cells become *petite*. This mutation level is approximately five orders of magnitude higher than the rate of a spontaneous chromosome loss [38].

An unexpected finding of Veatch and coauthors [34] was that the transition into *petite* occurs after a major (more than 400 min) delay in G1 phase of the cell cycle. These authors showed that the delay is accompanied by the loss of the mitochondrial membrane potential. They also found that during this delay the cells that lost mitochondrial DNA function acquire suppressor mutations allowing them to maintain vital levels of the membrane potential [34]. Apparently, not every newly formed *petite* cell acquires the suppressing mutation. Thus, a question arises: is it possible to consider the death of cells that did not manage to cope with *petite* transition as a physiological suicide? Here it is important to mention that while both haploid and diploid *S. cerevisiae* cells are able to exist in *petite* form, the consequences of *petite* transformation are very different between them.

Diploid cells cannot mate, and diploid *petite* cells cannot undergo meiosis. Under normal conditions the growth rate of *grande* cells is significantly higher than that of *petite* cells. Therefore, *petite* diploid cells are likely to be outcompeted by the *grande* cells (see figure). Unlike diploids, haploid *petite* cells can mate, and in theory such *petite* cells can mate with ones carrying functional mito-



Possible role of *petite* transformation of *S. cerevisiae* cells. Haploid cells can spontaneously transform into *petite* form by completely or partly losing their mitochondrial genome (*1*). Such cells are more stress resistant than *grande* cells, which gives them a selective advantage in a harsh environment (*2*). A haploid *petite* cell mates with a *grande* cell producing a *grande* diploid (*3*), which undergoes meiosis and produces four *grande* haploid cells (*4*, *5*). Diploid *grande* cells also can become *petite* (*6*), but such cells are not able to execute meiosis. As they grow slower than the original *grande* and are not able to restore functioning of the mitochondrial DNA, they are disadvantaged from the point of view of natural selection (*7*). Black worm-like structures represent mitochondria with functional DNA. Empty worm-like structures represent mitochondria, which have lost the function of their DNA. Light arrows indicate asexual reproduction; *n* is ploidy

chondria and form *grande* diploid (the figure). Possibly this sequence of events (transient passage through *petite* form) can be beneficial for surviving stressful conditions. In other words, haploid *petite* form of yeast is reminiscent of the resting (dormant, persisting) form of bacteria.

Interestingly, the frequency of mutagenesis of nuclear DNA increases several-fold upon inactivation of the mitochondrial DNA [34, 39]. Moreover, the mechanisms of multiple drug resistance (MDR) are hyperactivated in the *petite* cells [40]. Thus, for instance, the temporal existence of haploids in the *petite* form might help the cells to find mutations protecting against fungicides.

In agreement with this, the frequency of a spontaneous *petite* transformation of haploid cells is approximately 100-fold higher than that of the diploids [41].

Is *petite* transformation of the haploid cells adaptive? One can speculate that the passage through the *petite* form can protect the culture from accumulation of opportunistic mutations. As already mentioned, a certain mutation in the *ATP2* gene may lead to clonal senescence [32]. However, the mutated strain is *petite*-negative, i.e. cannot exist in the *petite* form [42]. Therefore, loss of mitochondrial DNA function is lethal for the mutant cells and thus prevents the accumulation of this mutation in the population.

Apart from *ATP2*, there are several dozens of such *petite*-negative genes. For instance, such genes are responsible for respiration-independent maintenance of mitochondrial membrane potential and transport of mitochondrial proteins encoded in the nucleus ([43], see it also for review). Thus, one can speculate that a transient passage of a haploid cell through *petite* form acts as a quality control checkpoint for a subset of its genes.

Recently it has been suggested that aging-associated decline of multicellular organisms plays a similar role, i.e. increases the selective pressure at the other, age-independent functions of the organism [44]. While the question whether aging in general is programmed or not is outside the scope of this review, we suggest that the loss of mitochondrial DNA is programmed and adaptive. It may allow survival under stressful conditions and prevent the accumulation of opportunistic mutations in the population.

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