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Review

Natural causes of programmed death of yeast *Saccharomyces cerevisiae*

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Abstract

The existence of cell death program in unicellular organisms has been reported for a number of species. Nevertheless, the question why the ability to commit suicide has been maintained throughout evolution is far from being solved. While it is believed that altruistic death of individual yeast cells could be beneficial for the population, it is generally not known (i) what is wrong with the individuals destined for elimination, (ii) what is the critical value of the parameter that makes a cell unfit and (iii) how the cell monitors this parameter. Studies performed on yeast *Saccharomyces cerevisiae* allow us to hypothesize on ways of possible solutions of these problems. Here we argue that (a) the main parameter for life-or-death decision measured by the cell is the degree of damage to the genetic material, (b) its critical value is dictated by quorum sensing machinery, and (c) it is measured by monitoring delays in cell division.

Keywords: Yeast; Programmed death; yca1; Altruistic; Group selection

Apoptosis – a form of active cell death – is widely used by multicellular organisms, e.g. during development or as a mechanism to remove damaged and/or potentially cancerous cells. Apoptotic machinery has been also reported for yeast *Saccharomyces cerevisiae*. In particular a number of harsh treatments induce cytochrome *c* release [1–3], mitochondrial thread-to-grain transition [3,4], and nuclear DNA fragmentation ([5,6], for review see [7]). There are homologs of mammalian pro-apoptotic proteins found in yeast, including AIF [8], a homolog of HtrA [9] and a caspase homolog named Yca1 [10,11].

It has been argued that the active death machinery of a unicellular organism *S. cerevisiae* has been preserved throughout evolution because it allows the populations of the yeast to eliminate unwanted individuals thus improving the fitness of the group. In favor of this argument it should be mentioned that in nature yeast cells often exist as clonal groups; therefore, the evolutional rules of "kin selection" (selection of traits favorable for close relatives of the mutated individual, reviewed in [12]) can be applied for *S. cerevisiae* populations. Regarding the significance of individual yeast cell suicide for population, a number of questions arise:

1. When cell groups choose to eliminate unwanted individuals, which parameter of the cell physiology is being monitored by the cells?
2. Which factors determine the value of this parameter critical for such a decision?
3. What are the cellular mechanisms for monitoring this parameter?

The answer to the first question can be speculated on theoretical grounds.

Which parameter is being detected by the cells as natural suicide signal? On one hand, it has been argued that there are several physiological stresses which make yeast cells trigger the death cascade. These situations include the already mentioned ones (death during mating, chronological aging, colony growth) as well as death of stressed cells during meiosis (reviewed in [7]). On the other hand, stress is known to induce mutations in a number of unicellular species including yeast (reviewed in [13]). Even though some of these mutations could be adaptive

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(see [14] for review), the vast majority of spontaneous mutations are neutral or deleterious [15]. Moreover, accumulation of individuals with high numbers of slightly deleterious mutations can lead to fixation of a portion of these mutations. The fixation followed by degeneration is most likely in populations experiencing regular bottlenecks (an abrupt and severe reductions in the number of individuals at certain stages of the history of population [15]), which is probably applicable to natural populations of yeast. Thus, active death of highly mutated individuals could be useful for the population. A set of models known as kin selection shows that the benefit of a population can outweigh the individual selective benefit if the individuals are highly related (see [16]). Apparently, these models are applicable to clonal yeast populations.

Therefore, for yeasts the degree of damage to the genetic material is possibly the best parameter to measure for making life-or-death decision.

The second question concerns the critical value of the parameter. Whatever the parameter is, its critical value is expected to depend on cell density, i.e. to be governed by quorum sensing. Indeed, cellular suicide is evolutionarily justifiable only if it occurs within a population; further, the expected benefit of this act (in terms of inclusive fitness, i. e. fitness of an individual as measured in terms of the survival and reproductive success of his close relatives) depends on the size of the population. Is this line of reasoning being supported by experimental data? A number of substances have been shown to be involved in intercellular communication in yeast. The most established ways of quorum sensing in yeast are sexual pheromones. Haploid *S. cerevisiae* can exist in *a* or α form. α-Cells secrete α-factor, while *a*-cells secrete *a*-factor. When cells of *a* type detect the presence of α-factor, they activate mating mechanism preparing themselves for fusion with α-type cells. Treatment of *a*-type cells with α-factor is a commonly used method of synchronizing them in G1 stage of the cell cycle. It was known for decades from laboratory practice that high concentrations of α-factor in the synchronization assay are toxic for cells. Recently, we tested the hypothesis that the toxicity is mediated by the induction of programmed death. It indeed appeared that high concentrations of α-factor kill yeast, and the death shows typical markers of apoptosis [17].

Ammonia represents another way of cell-to-cell communication in yeast populations. Ammonia was shown to affect the fate of yeast cells grown on solid media. Ammonia concentration gradient in the aged colony ensures that the cells close to the centre of the colony die, while the ones at the periphery proliferate, allowing spatial expansion of the colony [18]. Recently, it has been shown that aromatic alcohols, tryptophol and phenylethanol, are secreted and sensed by yeast when nitrogen sources are low [19]. These compounds were shown to regulate the invasive growth of yeast on solid media [19]. At the same time, it is known that yeast cells of the same mating type incubated for several days in stationary cultures die with apoptotic markers (chronological aging, [20,21]). We found that the addition of phenylethanol (0.05%) suppressed the survival of yeast under these conditions. Importantly, the concentration of phenylethanol used does not affect growth rates of the logarithmic cultures (Severin et al., in preparation). We speculate that the addition of phenylethanol forces yeasts to overestimate the cell density and thus intensifies suicide program. Thus, cell culture density seems to be the major factor determining sensitivity of yeast cells to programmed death inducers.

Assuming that the key parameter for life-or-death decision is the degree of DNA damage and its critical value depends on cell density, the third question arises.

How do cells detect DNA damage? Such a damage causes delays of the cell duplication process (stalling of the replication fork, pausing at metaphase, etc). Importantly, the same delays are known to induce apoptosis in mammalian cells (reviewed in [22,23]). Moreover, it has been shown that Rad9-dependent delays in DNA replication can play not only pro-survival, but also pro-death role in *S. cerevisiae* [24]. What could be the mechanism of the cell cycle delay-activated death? We speculate that yeast metacaspase Yca1 plays the key role in this mechanism. Indeed, YCA1 deletion improves cell survival after a variety of stresses. *yca1Δ* cells show elevated resistance to hydrogen peroxide, chronological aging, hyperosmotic stress and some other stressful conditions (reviewed in [25]). In all of these cases, the improvement in survival is due to suppression of the development of apoptotic markers (reviewed in [25]). In the context of the aforementioned hypothesis, these data make Yca1p a perfect candidate for the role of the "guardian" of the pool of genomes in yeast populations. We hypothesized that, in a dense cell population, Yca1p is activated in the cells with the most damaged genetic materials. This predicts that stressed cultures of *yca1Δ* cells will accumulate more mutations than the control ones.

We tried to test this experimentally. To measure the levels of mutagenesis, we compared the rates of inactivation of URA3 gene in the control and *yca1Δ* cells. Preliminary experiments support the hypothesis. Indeed, URA3 *yca1Δ* cultures aged on solid media accumulate significantly higher percentage of cells carrying the inactivated gene than the control ones (Fig. 1A, see figure legend for experimental details).

While consistent with the hypothesis, these data do not exclude a direct anti-mutagenic role of Yca1p. To address that, we performed a number of experiments asking whether delays during cell division trigger Yca1p-dependent death of yeast.

We chose nocodazole, a microtubule-depolymerizing agent, as a tool to delay mitosis. To monitor the levels of chromosomal aberrations, we used the same cells as in Fig. 1A carrying URA3 marker on yeast artificial chromosome. After an overnight incubation with nocodazole, *yca1Δ* culture contained approximately 3 times less of dead cells (Fig. 1B) and 3 times more of cells which have lost the marker (Fig. 1C).

These results are consistent with the idea of "altruistic" role of Yca1p. Several earlier observations support this hypothesis. The delays in DNA replication cause Yca1-dependent cell death [26]. Chronological aging is delayed in *yca1Δ* cultures [21] and replication stress is the main determinant of death during chronological aging [27]. Moreover, in aging cultures, the cells which decided to divide are much more likely to develop

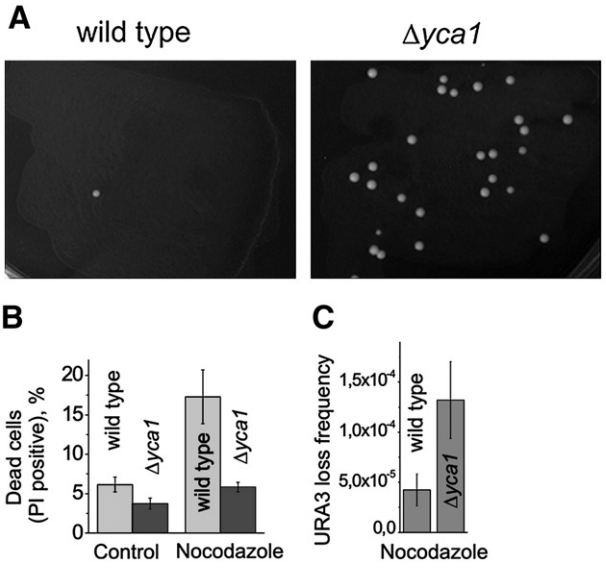

Fig. 1. Yca1 suppresses the survival of genetically damaged cells. A. Yeast artificial chromosome (YAC) carrying URA3 and TRP1 markers was introduced in the control (W303 KanMX4) and yca1 (W303 yca1::KanMX4) cells. This plasmid allows cells to grow in the absence of tryptophane and uracil in the medium. The cells were subjected to a mild stress, i.e. 10 days of aging on solid YNB media at 4°. After that, to estimate the levels of mutagenesis $5 \cdot 10^7$ cells were collected and applied on solid media without tryptophane containing 5-fluoroorotic acid (5-FOA, 10 mg/ml). Only those cells which retained the plasmid and acquired URA3-inactivating mutation grow forming colonies. The photograph (representative of the results from three experiments) shows one colony in the wild type culture and 26 colonies in *yca1Δ* cultures. B. Cells from logarithmic cultures grown on YNB-TRP liquid media were incubated in YPD containing 100 mM hydroxyurea (HU) for 2 h and then transferred to YP media with or without nocodazole (300 μg/ml). After overnight incubation, aliquots were stained with propidium iodide (PI) and counted under the microscope to estimate the percentages of dead cells or (C) plated on YNB synthetic complete media supplemented with 5-FOA (10 mg/ml). Pre-incubation with HU was used to ensure that most of the cells enter the cell cycle. At the last stage, YP was used instead of YPD to prevent proliferation of cells which acquire non-specific resistance to nocodazole during overnight incubation. The graphs represent the average of three experiments.

apoptotic markers than the resting cells [28]. Finally, cultures of peroxide-treated *yca1Δ* cells accumulate more of the oxidatively-damaged cells than the control cultures [29]. The latter result could be due to the inability of *yca1Δ* cells to trigger death after peroxide-induced DNA damage. All these data taken together provide support to the hypothesis that Yca1p is activated by damage to genetic material and thus protects the cell population from accumulation of deleterious mutations.

While this review was in preparation, an independent study was published supporting the existence of active mechanism for removal of genetically damaged yeast cells. Gomes et al. [30] provided evidence that a deletion of glutaredoxin (Grx) 2 improves the survival of cells with mutated mitochondria following cadmium stress. Together with our data, this suggests that, apart from its role in maintaining redox homeostasis, Grx2 has a second, "Yca1-like", function.

Our kin selection-based hypothesis predicts that the inactivation of Yca1 in a cell will give a short-term individual fitness advantage to this cell. Once the mutant out-competes the wild type cells, however, the population will be weakened due to accumulated deleterious mutations. While a direct experimental test of this hypothesis in yeast is a subject for future research, similar scenarios have been already observed in bacteria (reviewed in [31]). It has been shown that the stationary cultures of *E. coli* accumulate a certain percentage of dead cells. It was reported that sometimes a "selfish" mutant emerges in such cultures. Such a mutant continues to proliferate under conditions where normal cells either pause or die. After a certain time, the offspring of the mutant takes over the culture. As a result, the whole culture degenerates since it loses the resistance to stressful conditions (heat shock, peroxide, etc). Therefore, cell death appears to be necessary for the long-term survival of the culture [31].

To summarize, we suggest that most yeast programmed death scenarios follow the same path. Initially, stressful conditions induce mutagenesis in yeast. Yeast cells delayed in the division cycle "decide to commit a suicide" to preserve the genetic stability of the population. The threshold at which this decision is made is determined by the cell density via quorum sensing.

Do higher organisms possess similar "guardian of the populational genetic fund" altruistic suicide programs? While outside the scope of this review, the following point of view should be mentioned. It has been suggested that senescence-induced death of individuals could represent a result of operation of such a program coined "phenoptosis" [32].

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