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MiniReview

The inward struggle for life: a case of yeast

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Abstract

Apoptosis, a particular type of programmed cell death, is but one of many ways by which organisms safeguard their onticity and permanence. Any living system is facing aggressive factors in its surroundings, living and nonliving, with which it is engaged in the Darwinian struggle for life. But is also continuously subjected to the tendency towards inner destruction, due to the second law of thermodynamics. Consequently, it wages an additional, no less fierce, inward struggle for life. A number of processes have evolved to defend the inner integrity of living systems, which comprise monitoring, policing, filtering, sorting, partitioning, repairing, replacing, discarding, and also asymmetric distribution of components. The cell division itself may be conceived as a specific protective mechanism in service of maintaining ontic self. Active self-maintenance does not occur in the non-living world and should thus be considered as an essential mark of life. As in other cases, yeast appears to be an organism of choice to disclose the universal armaments used in the inward struggle for life.

Keywords: Aging; Apoptosis; Asymmetry; Biological policing; Inward struggle for life; Yeast

1. Introduction

Ever since Darwin, the expressions “selection” and “struggle for life” have belonged to the most frequent terms in biology, accounting for existence (onticity), permanence, diversity, evolution and adaptation of living entities. As Darwin pointed out, he adopted the two expressions from human affairs: the former from the practice of breeding, the latter from Malthus’s analysis of the dynamics of human populations. It should be kept in mind that, when applied to the world of simple organisms and of “living” molecules, the expressions are mere metaphors. They provide a vivid, but also seductively anthropomorphic, insight into the

course of evolution. In the world populated by entities of diverse stability those entities persist that are the most stable. ”The universe is populated by stable things” ([1], p. 13), and “selection” and “struggle for life” are the means that enable outlasting those organisms which are relatively more stable.

In Darwin’s conception, the main actor of the struggle for life was the living individual, which was competing with other individuals of the same or different species for space, resources and the number of offspring. The “enemy” was external, so one can speak of the “outward struggle for life”. In principle, in case that no adversaries were present and resources were inexhaustible, the individual might last for an unlimited time. Either by itself, or as a sequence of its descendants. Yet, Darwin was already aware that some unknown cause(s) brings about small deviations in the succession of generations and that this variability is, at the same time, a generator of changes and hence, inevitably, of ever new competitors. What Darwin could not know in his time was the fact that the very cause of this variability is the all-pervading physical principle, the second law of thermodynamics.

Yet, the consequence of the second law are not only mutations but also the fact that every organism is continually exposed to a disorganising “force”, a tendency towards the loss of order and correlations within the organism. Stability is not jeopardised only by external competitors, but equally by internal processes. To maintain internal order and structuring and to oppose the disorganising “force”, an organism has to incessantly perform a work that can be dubbed the “inner ontic work”. It comprises continual repair and replacement of damaged parts, discarding irreparable components, waste disposal, sorting and partitioning. Since keeping a perfect order and preventing a loss of even a single correlation would require infinite amount of energy, the organism has to trade-off, with the consequences of asymmetric assortment of components between distinct compartments, aging, rejuvenation through reproduction and investment into progeny, apoptosis, necrosis. The ontic work is complemented with the “inner epistemic work”: it is necessary to recognise the damaged components, to distinguish them from normal ones, to specify those that should be discarded. And again, perfect recognition would need infinite amount of energy or perfect cognitive devices. Instead of perfection, partial recognition should do, in many times only slightly biased when compared with random search and random selection [2]. In fact, kinetic competition of normal and damaged components for surveillance devices may be the predominant form of quality control, accompanied with unavoidable wastage of time and resources, including energy (e. g. [3]).

It should be recognised that these processes of quality control are running at all levels of biological organisation: at the level of genome, cell, individual organism up to the level of community (and, in the human case, society) and ecosystem. It may be useful to bring together the diverse internal processes representing the inner ontic and epistemic work under a single notion, the “inward struggle for life”. It complements the outward, Darwinian struggle for life. The principle of minimal complexity, also called Delbrück’s principle, may be applied to examine these processes [4]. The principle stipulates that the most efficient way to study a concrete biological phenomenon is by studying it on the simplest organism in which this phenomenon can be found – here it is experimentally best accessible and, because of its evolutionary simplicity, theoretically the most comprehensible. In the present paper, arguments are being presented of why yeast may be a suitable organism for studying the inward struggle for life. The paper does not intend to present a comprehensive review of literature, which, particularly in case of repair, aging and apoptosis, is already immense. Reference is being made to relevant review articles, some papers are cited as providing instructive examples, and the main attention is being devoted to a specific phenomenon, exciting but little known so far, the asymmetry of sorting and partitioning in yeast.

2. From repair, replacement and elimination through aging to death

Every organism, even under the resting state of no growth and no replication, exhibits a continuous turnover of its cellular components. This statement is now a commonplace in biology, but was received as a surprise when first presented by Rudolf Schoenheimer in 1942 in his book Dynamic states of body constituents [5]. Usually, the permanent flux of matter is considered as a necessity because life as an open thermodynamic system out of equilibrium need do work and dissipate energy in order to maintain the distance from equilibrium. In addition, life must be able to accommodate to the permanently changing surroundings, and turnover is being viewed as part of regulatory processes. However, it would remain unexplained why even DNA, which serves as a material carrier of heredity and, in the conventional view, is still considered as a "blueprint", should also exhibit turnover [6]. Bridges [7] pointed out that the problem of DNA turnover has been remarkably neglected by investigators. Even in resting and starving bacteria there is much more turnover of chromosomal DNA than has been previously thought.

The turnover of DNA may be a result of repair processes. As many as 3×10^5 lesions in DNA may occur every day in every cell of human body (spontaneous oxidation, hydrolysis, alkylation, strand brakes etc.) [8]. When DNA replicates the probability of errors increases [9]. The same may apply to DNA transcription. Without repair, a cell or an organism would break down in a short while. These observation from DNA may be generalised: Turnover of cell constituents is a consequence of the incessant inward struggle for life, of tenacious resistance to the second law of thermodynamics. In analogy with macroscopic machines we can also assume that working molecular machines are being worn-out more quickly than those out of operation, but even the latter are undergoing unavoidable, even if slower, deterioration.

A literature on repair and generally on quality control in living systems is enormous. To get a quick review of recent state of the art, an issue of the magazine Science from 1999 may be consulted. In addition to a paper on DNA repair [10], there is a paper dealing with proof-reading and editing during translation [11], and another paper on posttranslational control of folding, refolding and degradation of proteins [12]. A more complex cellular process of the secretory pathway in endoplasmic reticulum has also been reviewed [13]. However, to get a full picture of the quality control at the level of molecules, papers on regulatory pathways for nuclear pre-mRNA turnover [14], on mRNA quality control [15,16] and t-RNA monitoring [17] should also be taken into account. In all the studies mentioned here yeast has been the main organism on which quality control studies have been accomplished. Eliminatory checking in animals, which starts at the level of conception and goes through embryogenesis to brain control mechanisms, are outside the scope of this paper.

Of particular importance is the control of DNA during cell division. The cell cycle includes safety mechanisms, checkpoint arrests, in which cells are given the chance to stop dividing long enough to detect DNA damage and to repair it before continuing with DNA replication or mitosis [18-20]. There are extrinsic checkpoints, which are activated upon external damage of DNA due to UV light, chemicals, oxygen by-products, but also intrinsic ones that function in each cell cycle under "ordinary" conditions to ensure the proper temporal order of events [9]. A "guardian complex" may constantly monitor the integrity of the genome so that an intrinsic checkpoint may exist during unperturbed DNA replication [9]. Most of the checkpoints mechanisms have been identified in studies with budding and fission yeasts, but they are evolutionary conserved and so can potentially be exploited to search for therapeutic agents against cancer.

It is remarkable that the processes of quality control, which may be optimally tuned to maintain a cell or an organism in a "healthy" state under familiar, ordinary conditions, are activated under stress. For instance, synthesis of chaperones and proteases, which are

involved in protein surveillance under ordinary non-stress conditions, are induced as part of the heat shock response. The latter had been the first example discovered of the response to environmental stress. The accumulation of unfolded or misfolded proteins can be expected under heat shock, but this need not be so in other cases of environmental stress. Still, the alarm reactions to stress are similar under all conditions. Such apparently exaggerated activities may be suggestive of an excessive reaction of humans exposed to a sudden danger. The resemblance may not be superficial – it will be shown in another publication that both cellular stress response and human individual's alarm behaviour under presumed deadly threat are adaptive and are examples of "biological rationality". Although originally discovered in Drosophila, the stress response has been found in all organisms examined, and studies with yeast have provided particularly rich data of universal validity [21,22].

Despite all the quality control mechanisms, irreversible, irreparable changes are inevitable. In the case of DNA, they manifest themselves as mutations, in the case of proteins as various chemical modifications [23]. Protein aggregation is also an appreciably irreversible process [12]. The result is an unavoidable process of biological aging. It seems mandatory to make a clear conceptual distinction between aging and senescence. In languages that do not possess two different words for describing a process of growing old, a noun with adjectives should be used. But also in common English the two words are used interchangeably; the distinction should be adopted by definition. Aging corresponds to growing old because of imperfection of quality control processes, of impossibility to prevent irreversible changes in the direction of deterioration; it is essentially a stochastic process and is measured by the chronological life span. It can be conceived as manifestation of the individual's cumulative defeats in the inner struggle for life. On the other hand, senescence is a programmed process and is evolutionary adaptive. It is associated with cell division and is determined by the replicative life span. Longevity, which also seems to be mainly pre-programmed and determined by genes, may also be linked with senescence only, and not with aging [24].

Senescence can be easily accounted for by the "disposable soma" theory of Kirkwood [25]. The theory posits that, from a finite energy budget, maximum fitness is achieved if the balance of energy invested in cellular maintenance and reproduction is tipped in favour of more reproduction at the expense of maintenance. In addition to the fact that individual cell immortality is impossible on thermodynamic grounds, there is also no need to invest too much energy for too long time for expensive maintenance of a cell or of an individual. Instead, the permanence, onticity of the biological entity is more economically secured by carrying over its "blueprint", or "recipe", from generation to generation.

In mammals, including humans, a clear-cut distinction between aging and senescence is not possible. Some organs are growing old without appreciable cell division, as for instance the brain, while others may be undergoing replicative senescence [26]. The two processes can be uncoupled in yeast and this again makes yeast a suitable organism for studying aging and senescence as distinct processes. As will be shown in the next section, replicative senescence is closely linked to asymmetry of cell division. On the other hand, aging of yeast cells can be observed in the stationary phase of growth and has already been amply studied [27].

The mark of "biological aging is the progressive decline in the ability of the organism to resist stress, damage, and disease" [28]. Obviously, the decline cannot go forever and the organism must eventually die. Dying may be a mixture of heterogeneous processes. The single word "death" may appropriately describe the ultimate state, but is utterly inadequate when applied to the processes. Here again a distinction should be made between death as a final outcome of largely stochastic damages and between "programmed cell death". The latter has often been equated to apoptosis. However, Ameisen [29] has warned that "although apoptosis is the most typical and frequent phenotype of self-destruction, it is not the sole one. For this reason, the term 'apoptosis' should not be considered as a synonym of the term

'programmed cell death', 'cell suicide' or 'self-destruction'." But neither unprogrammed cell death appears to be a single phenomenon. Necrosis, an apparently chaotic process, running downhill with no need of energy, has often be considered as an antithesis to apoptosis, which is a uniform, organised, and energy-dependent cell demise. However, necrosis may be just one, even if major, example of unprogrammed cell dying, being itself under partial cellular control [30]. It addition, it appears that the dividing line between the two major forms may be blurred. A case in point has been the observation that in the yeast *S. cerevisiae* low concentration of acetic acid induced apoptosis and higher concentrations induced necrosis [31]. The same was found in another yeast *Z. bailii* [32]. Autolysis, which was a frequent subject in yeast research in the past and is important in biotechnological applications of yeast (e.g. [33]), may be approached anew as a specific type of cell dying. The application of the expression "death" to apoptosis may be taken rather as a metaphor, since apoptosis does not mean the termination of a living entity but it is rather assisting the entity in its striving for permanence. It is a form of asymmetry that will be dealt with in the next section.

Living systems expend a substantial amount of free energy in the form of ATP for preserving their onticity and the "disposable soma" arrangement may have been selected by evolution as an optimal solution. The proportion of energy invested into quality control, when compared with that invested into growth and reproduction, has probably not been yet determined. From many studies of growth yield, done in the past, a paper of Oura [34] deserves a particular attention (see also [35]). According to Oura's calculation, about 80% of the biologically utilisable free energy available as ATP are not used by yeast for biosynthesis at the chemical level. He surmised that it is used "for the formation of cell structure". Since, however, most of the cell structures arise probably by spontaneous self-organisation, it is conceivable that a substantial part of this unaccounted-for free energy is used for repair, replacement, cleaning and all the other processes mentioned in this section.

3. From sorting and partitioning through senescence to apoptosis

The persistence of an entity in its changing surroundings depends on it intrinsic stability, which can be taken as an intensive variable, onticity per unit mass. But the persistence depends also on the entity's quantity, its extension. The higher is the number of copies of the entity, the more robust it is and the higher is the probability that it persists. An entity can exist as a single copy of different sizes or be dispersed, distributed in the form of many copies of an equal size. A simple asexual prokaryotic organism is an example of distributed onticity. The bacterium *E. coli* is distributed is space – it can be found anywhere on earth in huge amounts of copies, but also in time – because of the cell division, it has persisted on earth for millions of years. In a sense, seen at a certain level of graining, any unicellular asexual organism of the same species represents, together with all its contemporaries, but also with its ancestors and descendants, a clone, a single biological entity.

A multicellular sexual organism maintains its onticity in a different manner. Its onticity is dense, located in a small spot of space and in a short interval of time. It does represent a single clone neither with its kin nor with its ancestors and descendants; only the cells of an individual organism are clonal. However vigorous its proofreading, repair and replacement activities may be during its lifetime, a multicellular individual grows old and eventually passes away. To preserve the persistence of the species, the separation of germ and somatic lines evolved in more complex organisms. Only the germ line persists from generation to generation. Stem cells may occupy an intermediary position between the germ and somatic lines. In contrast to the cells of the somatic line they seem to escape irreversible and irreparable changes and preserve pluripotency and "youth". Also, in the course of a

multicellular individual's life, mechanisms of asymmetric distribution of components, sorting and partitioning, and waste disposal, fulfil the function of policing.

Yeast, although traditionally considered to be a unicellular organism, may exhibit an intermediary position between the two extreme cases, represented by a simple bacterium and a mammal. It is just for their simplicity that they may be suitable for studies of biological asymmetry. Polarity is its most elementary form. Polarity has been much researched in yeast [36]. It is a prerequisite for asymmetric sorting and partitioning and asymmetric cell division.

Budding in yeast is inherently asymmetrical. It does not mean, however, automatically that cell constituents should be distributed asymmetrically between mother and sister cells. But such asymmetric distributions have been observed in several cases in yeast. A well-known example is the lineage-specific yeast mating-type switching. Only cells that have budded previously (mother cells) exhibit the ability to undergo mating-type interconversion; newly born cells (daughter cells and spore cells) are not capable of switching mating type [37,38]. Daughter cells and spore cells become mother cells once they have gone through one cell division cycle; at that point, they acquire the ability to switch. Each cell division cycle of a daughter cell gives rise to a mother cell and to a newly born daughter cell, thereby producing two cells with different switching capabilities. Sil and Herskowitz [38] considered the lineage of daughter cells similar to stem cell lineage in animal tissues. It is a specific protein Ash1p that is asymmetrically localised in daughter cells where it prevents the mating type switching. The asymmetric partition has been found to depend on a polarisation of the cytoskeleton in the mother cell initiated by differential transcription of several genes [39], which in turn affects actomyosin-driven transport of some mRNAs to the bud. The achieved asymmetry of proteins is maintained by a septin-mediated membrane diffusion barrier at the mother-bud neck [40].

Another well-recognised asymmetry in partition has been described by Guarente and co-workers [24, 41]. DNA minicircles (ERC), excised from chromosomal rDNA but lacking centromeres, accumulate in mother cells and tend not to be passed to daughter cells. The accumulation of ERCs has been considered as a cause of replicative senescence. After about 15 generations, the number of ERCs reaches more than 1000 copies and its abundance was supposed to cause death by titrating essential transcription and replication factors.

If the accumulation of ERCs represents a "replicative age clock" that determines the replicative life span, another clock in budding yeast, also linked to asymmetry, was described by McMurray and Gottschling [42]. They found that as diploid yeast mothers reached the equivalent of late middle age, about 100-fold increase in genomic instability, monitored by loss of heterozygosity, occurred. Somehow unexpectedly, it was predominantly the daughters and not the mothers that tended to lose the heterozygosity. Still, there are the mother cells that were primarily affected. Old mothers are predisposed to DNA damage, and large sections of their chromosomes can break off. These fragments preferentially stay with the mother cell so that the daughter is forced to repair the damage by re-extending broken chromosome using the homologous chromosome as a template, in a process known as break-induced replication. The independence of this age-induced genomic instability from replicative senescence was proven by the observation that upon suppressing ERCs formation the timing of the genetic instability switch was not affected. The authors raised the possibility that the bias toward preserving the mother cell's genome might have implications for how the genomes of human stem cells are preserved. There is a different explanation of stem cells genome stability, raised by Cairns in his "immortal DNA strands" hypothesis [43,44]. McMurray's and Gottschling proposal that the mother cell may be viewed as a type of stem cell contrasts with the consideration mentioned above that it is the lineage of yeast daughter cells that is similar to stem cell lineage [38]. This leaving aside, the authors' observation provides an intriguing alternative to

Cairns explanations of exponential rise of genomic instability with age as a cause of increase of humans' chance of developing cancer in the final decades of life.

McMurray and Gottschling linked the genome instability of aged yeast cells with the accumulation of damaged proteins in mother cells, which, according to them, effectively eliminates the normal function of a gene product required for genome integrity. This accumulation of irreversibly oxidatively damaged carbonylated proteins was observed by Aguilaniu et al. [45] in yeast cells undergoing replicative senescence. The damaged proteins were not inherited by daughter cells during cytokinesis. The ability of mother cells to retain oxidatively damaged proteins during cell division diminished with replicative age. Actin was implicated to play role in the asymmetric distribution of damaged proteins, because inhibition of actin assembly abolished the ability of mother cells to retain oxidised proteins. Interestingly, the asymmetric distribution was also abolished in mutants lacking the silent information regulator Sir2p, a NAD dependent histone deacetylase. Since the latter regulator does also affect distribution of ERCs [46] there may be an intriguing connection between the two asymmetries. The authors suggested that this genetically determined asymmetric inheritance of oxidatively damaged proteins might contribute to free-radical defence and the fitness of newborn cells.

The latter suggestion is related to the observation of Laun et al. [47] that replicatively senescent yeast cells accumulated reactive oxygen species (ROS) in mitochondria. However, the young cells issued from buds contained little ROS. The authors suggested that replicative senescence in yeast may be, at least is part, due to damage of cellular materials by oxygen radicals, in accord with the long propounded "oxygen theory of aging" [48], and supported by the authors' previous data. To explain why the young cells, issued from buds, were "rejuvenated" the authors considered the possibility, proposed already in 1990 by Jazwinski [49] that some "death factor" must be inherited asymmetrically by the mother cell. This "death factor" could be old (pre-existing or damaged) mitochondria, which should be preferentially retained by mother cell in any cell division, while newly synthesised mitochondria should be segregated to the daughter cell.

The problem of how yeast mitochondria are distributed to daughter cells has been studied by Yaffe and collaborators and reviewed in a paper [50]. The distribution of mitochondria in the cell is not a passive process, a consequence of their random diffusion throughout the cytoplasm, and neither is passive their transmission to the daughter cells. Many studies documented colocalisation of mitochondria with microtubules. Mitochondria can move along mictotubules as a "cargo", driven by ATP powered motor proteins, kinesin and dynein. Intermediate filaments also appear to play a role in mitochondrial positioning, and actin may also have its share. Isolated yeast mitochondria are known to bind to actin filaments. Dynamin superfamily of large GTP-binding proteins has also been implicated both in determining mitochondria morphology and their distribution and partition. In addition to cytoplasmic proteins related to cytoskeleton, proteins of the outer membrane of mitochondria are also involved in the process, at least some of them functioning as an anchor point or "handle" for attachment of mitochondria to the cytoskeleton.

The intriguing problem of asymmetric distribution of mitochondria is closely linked to the "ploidy paradox" known from studies of mitochondrial inheritance in yeast: although an average yeast cell contains about 50 mitochondria, genetic and mutation analyses pointed to the existence of only a few mitochondrial genetic units. According to Piškur [51], while all copies of the mitochondrial genomes present seem to be involved in gene expression, only a few are active in transmission. In analogy with the distinction of germ line versus somatic line in ontogenesis of multicellular organism, he considers a similar division in mitochondrial DNA (mtDNA) in yeast: while the whole set of mtDNA molecules represents a kind of "soma-line", only a part of the population of mtDNA molecules represents the "germ-line".

"Unfit" molecules ("soma" mtDNA) can not get access to the replication machinery in the presence of more competitive molecules. He surmises that such a mechanism provides an intrinsic control for the "quality" of the yeast mitochondrial genome.

A rich ground for speculation provides also a paper of Ling and Shibata [52] on asymmetry in mtDNA partition in budding yeast. Mammalian mtDNA exists in a circular form with a genome size, but the major species of *S. cerevisiae* mtDNA are linear head-to-tail multimers of genomic unit DNA with variable sizes (concameters). Concatemers can be formed through rolling circle replication [53]. It had been reported previously that the majority of mtDNA in *S. cerevisiae* remain in the well of the gel after pulsed-field electrophoresis, and this DNA in the well was in linear forms of concatemers [54]. In their experiments, Ling and Shibata [52] found that the majority of mtDNA from the mother cells remained in the well after pulsed-field electrophoresis, but about half of the mtDNA from the buds behaved as a monomeric form with a genome size. The authors proposed a model, in which the concatemers of mtDNA, replicating by rolling cycle mechanism, cleave into monomers (probably of a circular form) by a mechanism similar to that operating in phage packaging and there are mainly these monomers that are portioned into the bud. There may also be another reason for asymmetry in partitioning of mtDNA in yeast. Linear molecules of mtDNA in mother cells may form a small number of aggregates, linked together by recombinational junctions. The level of unresolved recombination junctions might determine the number of heritable units of mtDNA [55]. The entire problem of asymmetric partitioning of mtDNA in yeast, including its recombination and segregation after mating [56,57], seems to be unresolved and is a challenge for further investigation [58].

An innovative paper, probably opening new vistas for studies of yeast asymmetry, has been published by Jazwinski and collaborators [59]. It has a direct bearing on the recurrent theme of the present review: the ontic persistence, "immortality", of a yeast clone, despite temporariness of all its members. A yeast daughter cell is receiving cellular components from the mother cell, but it does not inherit the mother's cell age and is always younger than the mother cell. Accordingly, any substance or process responsible for the aging and eventual death of mother cells must be carefully "filtered" and not reach the daughter cells. Disruption of the "filters" should cause a loss of age asymmetry and simultaneous aging of all cells. Such a "clonal senescence" is intrinsic property of normal animal somatic lines [60]. Jazwinski decided to isolate and characterise yeast mutants with presumably broken "filters" and thus exhibiting clonal senescence. One such mutant, temperature-sensitive, was analysed and found to be affected in the gene *ATP2*, encoding the β subunit of mitochondrial ATPase. At 36°, asymmetry was apparently lost in the mutant, such that daughters were born old. At this temperature, mitochondria were deficient in membrane potential and, probably as a consequence of this defect, lacked the capacity to properly segregate into daughter cells. This ultimately resulted in the generation of cells totally lacking mitochondria. It seems rather obvious that such cells could not be viable. Although the authors suggested that the results may indicate that mitochondrial dysfunction may be a normal cause of aging (or, perhaps more, of replicative senescence), and thus this type of mutants may be useful in further studies of aging and senescence, they would be of a more general importance in the sense of the main idea of this paper: Asymmetry may be a specific strategy, among many others (including apoptosis), used by biological entities in their inward struggle for life, and yeast mutants with defects in asymmetry may help to unravel this strategy.

It should be stressed that although asymmetry of cell division in budding yeast has been known since ever, there is only recently that we have witness a revival of the old idea of Krasiľnikov [61] that fission yeast may also divide asymmetrically [62-64]. This supports the idea that asymmetry may not be a simple outcome of the mechanics of cell division but rather a mechanism of efficient sorting of functional and disabled cell constituents.

4. Prospects

Considering active self-maintenance as the main mark of life and replication as a subordinated process may broaden the traditional conception of life. New heuristic stimuli can be provided by conceiving defence against internal decay, quality control processes, and mechanisms of creation and maintenance of asymmetry, including cell division and apoptosis, jointly as manifestations of the inward struggle for life. New empirical data combined with theoretical calculations on energy and time costs of the inward struggle for life would allow the assessment of their share, apparently considerable, in the total budget of living entities. It would be rewarding to promote and extend experimental studies of asymmetrical sorting and partitioning in yeast, since they may furnish data relevant to understanding differentiation, morphogenesis and epigenetic phenomena in multicellular organisms.

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