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Review

Circadian clocks and antiaging: Do non-aging microalgae like *Euglena* reveal anything?

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ABSTRACT

Microalgae that divide symmetrically in all aspects do not age. While the evolutionary reason for this is obvious, little attention has been paid to the mechanistic explanations. A great deal of study involving many research fields would be needed to explain the mechanisms if we suppose that the immortality results from a lifelong sufficiency of defense from stress or from an essential part of counteracting age-accompanied damage accumulation. Additionally, little is known about the relationships between homeostasis and circadian clocks in antiaging, although each of these has been studied separately. Here, we present a conceptual generalization of those relationships, as suggested by evidence from non-aging microalgae, mainly *Euglena*. The circadian gating of mitosis and circadian temporal coordination may respectively reduce radiation- and disharmony-induced stress in which homeostasis cannot be involved, whereas circadian resistance rhythms may greatly help homeostatic defense from radiation- and metabolism-induced stress. We also briefly sketch mammalian aging research to compare the current status of knowledge with that of algal antiaging.

1. Introduction

Circadian rhythms, found in almost all organisms from cyanobacteria to humans, are a biological rhythm with a period of close to, but not exactly, 24 h when freed of entrainment by 24-h time cues (Moore-Ede et al., 1982; Edmunds, 1988). The free-running period is kept relatively constant against the fluctuation of environmental changes; in particular, temperature is thought to be compensated for because a difference in constant temperature of 10 °C invokes a less than 10% difference in the period. When entrained to the time cues of day/night cycles, they run exactly with a 24-h period and establish a stable phase-relationship to and serve as anticipatory mechanisms for coping with the 24-h cycles.

Aging is usually defined as physiological decline with advancing age that increases morbidity and mortality, and appears to commence at around the age of maturity due most probably to age-accompanied damages beyond a critical level that compromise cellular function. In post-reproduction, organisms do not *evolutionarily* require antiaging mechanisms, and thus, natural selection cannot discard aging (Medawar, 1952; cf. Kirkwood, 2005). Here, antiaging is defined as any biological mechanism for counteracting aging processes, including defense from stress.

Aging very often parallels declining amplitudes in circadian rhythms. Unraveling their causal relationships is very difficult and little is known about how circadian rhythms play a role in antiaging, although some evidence suggests that they may do so in mammals. Not surprisingly, knowledge of the relationships is even more limited in microbes.

Aging that occurs post-reproduction is not limited to animals, but is also observed in ciliates such as *Paramecium* and *Tetrahymena*, which undergo aging after sexual maturation as they repeat cell division. Although they have been subjected to interesting studies on circadian rhythms (Wille and Ehret, 1968; Edmunds, 1988; Miwa et al., 1996; Hasegawa et al., 1999) and aging (Sonneborn, 1954; Smith-Sonneborn, 1971, 1979, 2005), little research has been carried out regarding their relationships. The finding that circadian short-period mutants of *Paramecium bursaria* mature sexually (and thus, based on Section 2.4, perhaps may age) early (Miwa and Yajima, 1995) may be relevant, this, however, may result from a pleiotropic effect of circadian-clock genes because the circadian clock and developmental clock are not related in such a way that one is an integral part of the other.

Another kind of aging in microbes appears to be related not to their sexual reproduction, but rather to asymmetric division. Budding yeast is a model organism for aging research (Laun et al., 2007), and the mother cell lineages undergo aging through

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morphologically asymmetric division; aging here may not be related to the yeast having sex, but to their asymmetry. Similarly, *Escherichia coli* has been shown to undergo aging (Stewart et al., 2005); although its division is morphologically symmetric, it is not strictly or biologically symmetric because a distinction exists between mother and daughter lineages in which only the former reveals replicative senescence; while the distinction is not morphological as in budding yeast, the functional or biological meaning of the distinction may be common to both systems. Unfortunately, little is known about circadian rhythms in yeast (Edmunds, 1988), and no reports of circadian rhythms in *E. coli* have been published.

Asexual microbes that divide with "strict" symmetry do not age, and otherwise would not exist. The flagellated microalga *Euglena gracilis* is such an asexual microbe. First, no evidence of sex, meiosis, conjugation, or autogamy has been found in over a century of research history. Second, while they have no chance to rejuvenate through sexual reproduction in cultures as ciliates, they show no indication of clonal death or replicative senescence (cell division life span). Third, although aging associated with asymmetric division should inevitably lead to a gradual decrease in the cell population growth rate in cultures, the alga shows no such indication; *e.g.*, the pattern of DNA by flow cytometry is constant at any point in the exponential growth phase, and the cell population growth rate can be maintained in semicontinuous cultures, indicating that individual cells do not exhibit replicative senescence, generation by generation.

Therefore, in the symmetrically dividing microbes, the advancement of age is completed within a cell cycle, and age-associated (or metabolically derived) accumulation of damages, if any, should be eliminated within that cycle through defense against stress, which should theoretically be strong enough to prevent damage from occurring and/or accumulating. Note that in the ciliates and asymmetrically dividing microbes, the advancement of age occurs and the age-associated damage accumulates not only within a cell cycle, but also beyond generations.

Although little is known about the biochemical and molecular mechanisms for antiaging in *Euglena*, circadian rhythms of defense from stress have been relatively well documented in *Euglena* as compared to other microbes. In this mini-review, we have attempted to conceptualize the interactive roles played by circadian rhythms and homeostasis in the algal defense against stress, which may be an essential part of antiaging mechanisms. Of course, this attempt was not intended to contribute directly to our understanding of mammalian aging, mainly because they are very different; the alga does not show hierarchical organizations as in mammals and its cell is a whole entity whereas the mammalian cell is a part of the body. Nevertheless, since the probability exists (although little attention has been paid) that mammalian antiaging may also involve the interaction of circadian rhythms and homeostasis, our efforts might help to eventually integrate the knowledge of antiaging mechanisms in both systems.

Here, we first summarize the formal knowledge on aging, homeostasis, and circadian clocks in relation to defense against stress (Section 2). Then, mammalian aging is sketched briefly (Section 3) assuming that readers are familiar with this field; we have attempted to provide an overview from our personal perspective since an enormous body of comprehensive reviews is available. We then discuss a role for circadian clocks in cellular defense against stress in eukaryotic microalgae, mainly the flagellated alga *E. gracilis* (Section 4). Note that although *Euglena* lives either photoautotrophically, heterotrophically, or mixotrophically and that many achlorophyllous mutants have been studied, this review deals only with the photoautotrophic growth mode of green *Euglena*. We regret that we were unable to cite the enormous body of original research, without which we could not have written this review.

2. Aging, homeostasis, and circadian clocks

2.1. Aging as damage accumulation

While an erroneous view (program theory) of aging exists, which holds that aging occurs as genetically programmed "mechanisms" (or active processes) like development and programmed cell death, this theory not only contradicts evolutionary and thermodynamic thinking, but it also has no convincing evidence to support it (see Kirkwood, 2005 for a comprehensive review). An enormous body of evidence exists to support age-accompanied accumulation of cellular damages as protein oxidation, lipid peroxidation, and DNA oxidation (Yin and Chen, 2005; Chen et al., 2007; Hulbert et al., 2007; Kregel and Zhang, 2007). The damage accumulation becomes evident at around the age of maturity in most cases. Nevertheless, note that antiaging mechanisms should set in motion at birth (a fertilized egg), and at least until successfully reproducing, persist to the extent to which the accumulation does not significantly deteriorate reproductive potential. Since cases exist in which longer-lived mammalian species (*e.g.*, naked mole rats) accumulate more damage than the shorter-lived species (Buffenstein et al., 2008), tolerance to damage may be an important part of antiaging mechanisms.

Damage may result from the imperfectness of defense from stress; the most widely recognized is the oxidative stress related to reactive oxygen species (ROS). The disruption of temporal coordination within and outside the body (or cell) usually maintained by circadian organization may be another kind of stress (refer to Section 2.5). A most primitive, but greatest stress, may be starvation that leads directly to a thermodynamic equilibrium, *i.e.*, death. A stress inescapable in all organisms including non-aging microbes is spontaneous mutation that occurs during DNA duplication, resulting in information loss (and damage) despite the best efforts of the three steps of error-correcting mechanisms (Maynard-Smith, 1989); this kind of stress is purely thermodynamic and can be considered to be a thermodynamic stress.

2.2. Homeostasis and circadian rhythms

Homeostasis tends to maintain "normal" states at all levels of biological organization by pulling back (or negative feedback). Outside this state is stress, whether it is oxidative stress, ionic or nutritional unbalance, or abrupt and unpredictable changes in the environment (Fig. 1). Thus, homeostasis is a mechanism for defense from stress. Unlike circadian systems, homeostatic responses work acutely for unpredictable changes to direct the organism on what to do now (Fig. 1). Note that the normal state is by no means static, but dynamically regulated; an example is the circadian UV-resistance level rhythmically changing in day/night cycles (as discussed in Section 4.2).

Circadian rhythms are also responsible for the defense from stress mainly in two ways. First, they maintain temporal coordination both within and outside the body, whereby conflicts are lessened or removed and a circadian harmony is generated, which may not only reduce the chances and strength of stress itself (Fig. 2; Sections 2.5 and 4.2), as may be suggested by jet-lag or shift-workers syndrome (cf. Cambras et al., 2007), and may also enhance the overall efficiency of the body including homeostatic tasks. Second, circadian rhythms enhance the efficiency of the component elements of homeostatic defense that may become maximal when it is regularly most required (Fig. 2; Sections 2.5 and 4.2). In addition, circadian gating of cell division (Fig. 2; Section 4.1) affords a special type of defense from stress. However, this is not the mechanism for direct responses to stress, but rather that for preparing for the stress through anticipatorily informing the