INTRASPECIFIC PLASTICITY IN CIRCADIAN RHYTHMS IN PHOTOSYNTHETIC ORGANISMS: EVIDENCE FROM *Euglena gracilis* Z

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Introduction

Circadian rhythms are ubiquitous occurrences in the natural world. Most of the physiological processes and behavioral functions in many diverse organisms are expressed rhythmically according to the day and night cycles. These circadian rhythms are controlled and maintained by self-sustaining biological oscillators, and provide the organisms with survival advantages by optimizing the organisms' responses to its environment and enhancing its fitness [1].

Photosynthetic unicellular algal flagellate *Euglena gracilis* Z (Klebs) has widely been used in chronobiology for decades [2]. This strain (Z) is certainly the most studied laboratory strain among all the *Euglenoids* [3]. Although various circadian rhythms in *E. gracilis* have been elucidated to date, the nature of the cellular machinery responsible for those overt and mostly robust rhythms is yet one of the central puzzles. Apparently, none of the studies have dealt with plasticity of circadian rhythms within the same species of a photosynthetic organism. If present, it is skeptical as to why a motile photosynthetic organism like Euglena requires plasticity in circadian rhythms.

In the present study, it was hypothesized that, within the same Z strain, remarkably different plastic responses in circadian rhythms do exist. To test this hypothesis, we obtained four Z strain collections that had been stocked independent to each other in four different locations and investigated the circadian rhythmicity of resistance to UV-C, which had widely been studied previously.

Material and Methods

Organisms and culture conditions

Four independently stocked cultures of *Euglena gracilis* strain 'Z' (tentatively named Z_A, Z_B, Z_C and Z_D) were used for the experiment. Hereinafter, we use the term "sub strains" to these four groups for clarity, although we are not yet certain about the taxonomic nomenclature of these. For all the experiments, the algae were cultured axenically at 25 °C and photo-autotrophically under continuous light (LL) with cool-white fluorescent lamps (National FL20SS-N18, Tokyo: see Bolige *et al*, 2007[4] for the lamp irradiance spectrum) at 84 µmol m^{-2} s^{-1} in modified Cramer-Meyer medium according to Bolige *et al*, 2005[2]. All four sub-strains were maintained under the same conditions for two months prior to experiments.

Circadian rhythms of resistance to UV-C

The alga was first grown in LL as described above and when the cell titer reached 5–

7×10^4 cells/mL, was transferred to continuous dark (DD). Cell suspensions (5 mL) from cultures that had been transferred to DD were withdrawn every 2 h starting from 18^{th} h in DD, and were placed in a Petri dish (3.7 mm diameter), which was then placed on a turntable automatically rotating at 15 rpm and, were exposed to UV-C at various doses from the top. UV-C irradiation was from a germicidal lamp (peak = 254 nm; GL-15, Panasonic, Tokyo, Japan) with an intensity of 2.2 W/m^2 to administer a dose of 1.1 kJ/m^2 UV-C as the median lethal dose (LD_{50}) as previously determined (unpublished data).

Immediately after exposure to UV irradiation, viable cells were counted after staining with 0.03% Neutral Red (NR). Cells with both cytoplasm and chloroplasts stained red and brown-red, respectively, were considered dead. However, cells having a clear, unstained cytoplasm and chloroplasts with few or no red particles were considered alive (see Plate 1).

Results and Discussion

In preliminary experiments, it was found that the mean LD_{50} of UV-C (average of LD_{50} values obtained at the phases of minimum and maximum resistance to UV-C) was ~1100 J/m^2 for Z_B, Z_C and Z_D whereas it was ~105 J/m^2 for Z_A. It was surprising to see that the LD_{50} of UV-C irradiation was significantly lower (ca. 10 times) in Z_A compared to that of Z_B and other two sub-strains. Based on these results, to examine the circadian rhythmicity in UV-C resistance, dark-arrested cells were exposed to a UV-C dose of 1100 J/m^2 except for Z_A where a dose of 80 J/m^2 was employed [2].

Figure 1 clearly shows that, a circadian rhythm of survival after UV-C irradiation persisted in DD in all sub strains except in Z_D in which rhythmic variation was observed at the beginning (until ca. 36 h) and thereafter the rhythmicity was lost (Fig. 1d). Although other three sub strains displayed the circadian rhythmicity in UV survival, all three rhythms were different from each other in many aspects. First, while both Z_A and Z_B exhibited unimodal rhythms (one peak/trough per cycle), Z_C exhibited the bimodality (peaking twice in one cycle). Second, all three rhythms ran out of phase to each other; the maximum resistance (peak position) in Z_A (Fig. 1a) was observed at 44^{th} h (near Circadian Time - CT 6), whereas it was at 50^{th} h (near CT 12) in Z_B (Fig. 4b), and at 36^{th} h (CT 0) and 48^{th} h (CT 12) in Z_C (Fig. 4c). Similarly, the circadian minima were observed at 32^{nd} h (near CT 18) in Z_A, at 44^{th} h (near CT 6) in Z_B and at 30^{th} (CT 18) and 42^{nd} (CT 6) h in Z_C. Third, the circadian waveform in both Z_A and Z_C was sinusoidal whereas it was non-sinusoidal, but saw-tooth type (relaxation oscillator) in Z_B. Fourth, the circadian amplitudes were also different among Z_B, Z_C and Z_D where the same dose was administered. Nevertheless, the period of the rhythms (τ) was approximately 24 h in all the three sub strains that displayed the circadian rhythmicity.

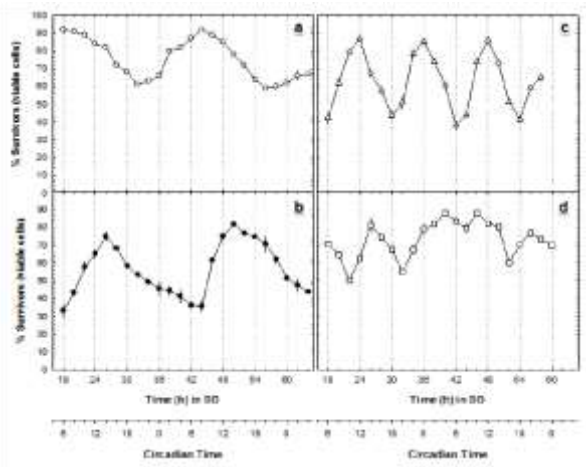

Figure 1. Circadian rhythms of resistance to UV-C irradiation in four sub strains of *Euglena gracilis* Z in DD. a) Z_A, b) Z_B, c) Z_C, d) Z_D. Exponentially growing cells in LL (84 µmol m^{-2} s^{-1}) were transferred to DD. At the times indicated on the abscissa, cell suspensions were withdrawn and subjected to a dose of 1100 J/m^2 of UV-C irradiation (2.2 W/m^2 lamp) and only for Z_A, a dose of 80 J/m^2 of UV-C irradiation (1.3 W/m^2 lamp) has been used[2]. Viability test was carried out immediately after UV irradiation using NR. The vertical bar crossing each symbol represents the SEM (n=3).

Plate 1. Identification of dead and live cells using NR staining after UV-C radiation.

This study revealed that, although the same strain of *E. gracilis* Z was used, the characteristic features in relation to circadian rhythmicity of four different "sub strains" were remarkably different. Consequently, we found that the circadian rhythmicity is plastic even within a species, if not within a strain.

Plasticity is defined in eco-physiology as the ability of a genotype (a single set of genes) to generate a range of different phenotypes, depending on the environment that the organism must endure [5]. It has been shown that these 'plastic' responses are either due to the environment or to the genotype, or sometimes due to random developmental irregularities, thus plasticity seems not always inherently adaptive. Although plasticity in responses to the environment is said to increase the organisms' ecological fitness, in some cases it might represent inevitable responses of the organisms that do not imply enhanced fitness [6]. Although plasticity plays a crucial role in heterogeneous and variable environments for sessile organisms, motile organisms like Euglena also might benefit with the plasticity, particularly when they form water-blooms. However, we are yet uncertain as to why all the four sub strains displayed the plasticity in rhythms in the same environmental conditions employed in the present study.

Conclusions and Recommendations

The circadian rhythmicity in UV-C resistance was highly plastic among sub strains used. Additionally, findings of this study suggest that there could be different sub strains or ecotypes present within the same Z strain, thus one has to be particular of the strain is to be used.

Although these four sub strains are morphologically similar, biochemical analysis might help to identify some differences among those. Moreover, until we perform a detailed genetic analysis of these sub strains, we will not be able to draw a concrete conclusion about the reasons for the observed differences among sub strains.

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