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Circadian rhythms of resistance to UV-C and UV-B radiation in *Euglena* as related to 'escape from light' and 'resistance to light'

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Abstract

Radiation-induced stress, either from visible or UV light, is strongest at midday. We found that, in the absence of stress or time cues, *Euglena gracilis* Z was the most resistant to UV-C and UV-B at subjective midday, whether judged from immediate or reproductive survival. The circadian UV-resistance rhythms were free-running in stationary cultures under 1-h light/1-h dark cycles or continuous darkness, indicating that cell-cycle dependent DNA susceptibility to UV was not involved. We moreover examined what was the primary cause of the circadian UV resistance, estimated as the immediate cell survival. The half-maximal lethal dose (LD_{50}) of UV-C at subjective midday (the most resistant phase) was 156 J/m^2, which is ~3-fold that at subjective midnight. The same was true for UV-B, except the LD_{50} was ~13-fold that of UV-C. Temperature during UV irradiation had little effect, indicating that survival was not mediated via enzymatic reactions. Non-enzymatic antioxidants were added 5 min before UV irradiation. Dimethylsulfoxide (a hydroxyl radical scavenger) increased survival after UV-B, but had little effect after UV-C; conversely, sodium ascorbate increased survival after UV-C, but not after UV-B. These findings suggest that circadian rhythms of resistance to UVs involve a common mechanism for maximizing non-enzymatic antioxidative capacity at subjective midday, but the specific antioxidants differ.

Keywords: Antioxidant; L-ascorbic acid; Circadian rhythms; Dimethylsulfoxide; *Euglena gracilis*; Photo-oxidation; UV-sensitivity

1. Introduction

Photosynthetic organisms cannot escape solar radiation because they use solar energy for photosynthetic electron transport (PET). Major cellular compounds, such as nucleic acids, proteins, and lipids, absorb, and are thereby damaged by, solar UV radiation, which is most intense at midday when plants must photosynthesize. Even visible light can bring about photosensitization, the process whereby a molecule excited by light excites another molecule that does not absorb light, that results in photo-oxidative damage [1–4], which is also greatest at midday.

Despite these dangers, many photosynthetic organisms, including the algal flagellate *Euglena gracilis*, display a circadian rhythm of PET capacity with a maximum at subjective midday [5–8]. This endogenous state occurs naturally at midday and recurs with a circadian period, which is ~26 h in this alga, in an environment without external time cues. The circadian enhancement of PET capacity would amplify the photo-oxidative stress at midday because PET activity itself generates reactive oxygen species (ROS) [1–4].

Midday is the most challenging time for photosynthetic organisms; radiation-induced stress is greatest at midday, whether the radiation is visible or UV and whether it attacks cellular constituents directly or indirectly through photosensitization. Therefore, we propose the hypothesis we call 'resistance to light'.

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Photosynthetic organisms may display a circadian rhythm that maximizes protection from the radiation-induced stress at subjective midday, so that their opportunity to live and reproduce may increase.

This hypothesis complements and does not contradict Pittendrigh's 'escape from light' hypothesis [9–11], which is the prevailing opinion on the evolutionary origins of circadian rhythm. Without resistance to light-induced damage, cells would have been killed by photo-oxidation; only cells that had endogenously allocated light-sensitive reactions to night intervals would have survived. Undoubtedly, the allocation would be minimally essential, at least for organisms that thrive in the euphotic zone. However, we think it would have been difficult for photosynthetic organisms to survive the damaging effects of sunlight by that mechanism alone. Therefore, the 'resistance to light' hypothesis predicts that mechanisms to defend against radiation-induced stress might be allocated to day intervals, becoming maximal at subjective midday.

This study tested this prediction in *E. gracilis*, which has been studied previously with respect to protection from oxidative damage [12–14]. Moreover, numerous aspects of the circadian rhythm in this alga have been studied [6,7], including the biochemical mechanisms for autonomous oscillation [15], physiological mechanisms for 'gated' cell population growth [16] and photoperiodic induction of cell reproduction [17].

2. Materials and methods

2.1. Organisms and chemicals

The algal flagellate *E. gracilis* Klebs (Z) was cultured photoautotrophically and axenically at 25 °C. Cultures were irradiated unilaterally by an array of daylight-type white fluorescent lamps (Toshiba Mellow-White, Tokyo) at a light intensity of 84 μmol/m^2/s (6 klx), unless otherwise stated [17,18]. In order to reduce opportunities for contamination, Cramer's medium [19] was slightly modified, such that the metal solution containing sodium citrate was replaced with Hutner's metal solution [20]. This made it much easier to handle the cultures. The alga was first grown under continuous light (LL) and was then transferred to either the cycles of 1-h light and 1-h dark (LD: 1, 1) or continuous dark (DD). Approximately 7 ml of *Euglena* culture were withdrawn automatically and fixed with 0.5 ml of 20% neutral formalin containing 20% NaCl every 2 h. The cell number was counted with a Coulter Electronic Particle Counter.

All the chemicals were the highest grade commercially available. Dimethylsulfoxide (DMSO), sodium ascorbate (Asc) and trypan blue (TB) were purchased from Wako Chemicals Inc. (Tokyo) and neutral red (NR) from Merck Japan (Tokyo).

2.2. Definition of CT in non-dividing cultures

The time of the transfer from LL to DD is defined as CT12 [6,7]. Our previous study indicated that the period of the circadian rhythm of the photo-induction of the commitment to cell division is approximately 26 h under DD [17]. Therefore, CT12 was considered to occur at the 0th, 26th, 52nd, 78th, and 104th h in DD. When the culture was transferred from LL to LD: 1, 1, the onset of cell population growth, or CT12, occurs on average at the 22nd, 48th, 74th, and 100th h [16]. We assumed that CT12 in non-dividing cultures also occurs at these times in LD: 1, 1.

2.3. Survival of UV irradiation

The effects of UV-C and UV-B radiation on cell survival were examined in stationary (non-dividing) cultures. The stationary cultures were obtained either by inducing dark-arrest of cell cycle progression by transferring the cultures to DD [18] or by bringing about population-induced cell cycle arrest by culturing to the highest possible cell density at $\sim 2.0 \times 10^5$ cells/ml in LL; the overcrowded cultures were transferred to LD: 1, 1 to be UV-irradiated.

UV-C from a germicidal lamp (peak at 254 nm; Hitachi, GL-15 W, Tokyo) was used at an intensity of 1.3 W/m^2 for 30 s (giving 40 J/m^2), unless otherwise stated. UV-B radiation from a Philips UV-B lamp (peak at 315 nm with λ between 300 and 320 nm; Philips, TL20W/01, Tokyo) was used at 10 W/m^2 for 180 s (giving 1.8 kJ/m^2), unless otherwise stated. A different Philips UV-B lamp (TL20W/12RS) was used in one experiment only.

The fluence rate was measured with UV Meter model UVC-254 (Custom, Tokyo) for UV-C with UV Light Meter model UV-340 (Lutron, Tokyo) for UV-B. The spectral properties of these three lamps was measured with EPP2000C (StellarNet Inc., USA) and shown in Fig. 1. UV irradiation was carried out against a dark background at 25 °C, except for one experiment carried out at 0 °C. Cell survival was evaluated as the percentage of cells that excluded the dyes trypan blue (TB) or neutral red (NR); surviving cells capable of reproducing were estimated by colony-forming activity.

2.4. Colony forming activity

About 300 cells (0.1 ml) were spread on each triplicate plate (9 cm diameter) of agar (1.5%) containing the medium above; the plates were immediately placed on a turntable that rotated 60 cm below a germicidal lamp to give an even exposure to the UV-C light. Immediately, they were then transferred to LL at ~42–84 μmol/m^2/s; another triplicate set of plates was treated similarly but UV-irradiation as controls.