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Research article

Enhanced excision repair and lack of PSII activity contribute to higher UV survival of *Chlamydomonas reinhardtii* cells in dark

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ABSTRACT

Plant cells are known to differentiate their responses to stress depending up on the light conditions. We observed that UVC sensitive phenotype of light grown asynchronous *Chlamydomonas reinhardtii* culture (Light culture: LC) can be converted to relatively resistant form by transfer to dark condition (Dark culture: DC) before UVC exposure. The absence of photosystem II (PSII) function, by either atrazine treatment in wild type or in D1 (psbA) null mutant, conferred UV protection even in LC. We provide an indirect support for involvement of reactive oxygen species (ROS) signalling by showing higher UV survival on exposures to mild dose of H_2O_2 or Methyl Viologen. Circadian trained culture also showed a rhythmic variation in UV sensitivity in response to alternating light–dark (12 h:12 h) entrainment, with maximum UV survival at the end of 12 h dark and minimum at the end of 12 h light. This rhythm failed to maintain in "free running" conditions, making it a non-circadian phenotype. Moreover, atrazine strongly inhibited rhythmic UV sensitivity and conferred a constitutively high resistance, without affecting internal circadian rhythm marker expression. Dampening of UV sensitivity rhythm in Thymine-dimer excision repair mutant (cc-888) suggested the involvement of DNA repair in this phenomenon. DNA excision repair (ER) assays in cell-free extracts revealed that dark incubated cells exhibit higher ER compared to those growing in light, underscoring the role of ER in conferring differential UV sensitivity in dark versus light incubation. We suggest that multiple factors such as ROS changes triggered by differences in PSII activity, concomitant with differential ER efficiency collectively contribute to light–dark (12 h: 12 h) rhythmicity in *C. reinhardtii* UV sensitivity.

1. Introduction

Photosynthetic organismal physiology is highly tuned to the light conditions as it is not only the sole source of energy but also of stress that challenges the very survival of the organism under high light conditions. The biology of these organisms have therefore evolved to survive under this abiotic stressor. Nearly a quarter of the genes of cyanobacterial genome are dedicated to respond optimally to the presence of varying light intensities (Gill et al., 2002; Montgomery, 2007). However, this doesn't include circadian rhythm genes although they constitute a higher order regulation that imparts fitness advantage to the organism in periodically changing external conditions involving light and temperature (Dodd et al., 2005; Yerushalmi and Green, 2009). Simulations using genome scale metabolome of cyanobacteria have revealed major transitions in metabolic state, especially involving energy metabolism, in non-rhythmically changing light conditions (Montagud et al., 2010). Even in higher plants, processes from metabolism to development are intricately regulated by phytochromes, the light sensors of the photosynthetic organisms (Chen and Chory, 2011; Franklin and Quail, 2010). Recently the effect of light conditions in spatial repositioning of chromosome domain containing CAB genes has also been shown in *Arabidopsis thaliana* (Feng et al., 2014).

It is but natural to expect influence of light or the resultant metabolic state of the photosynthetic organism on stress response. Outcome of various biotic and abiotic stresses are known to be

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influenced by light condition. Samuilov et al., demonstrated that cell death in pea plant cells in response to CN^- stress was greatly increased in the presence of light when compared to dark conditions (Samuilov et al., 2003, 2008). This phenomenon was specifically observed in guard cells which are photosynthetically active. Epidermal cells which were derived from same progenitor as guard cells but lacked chloroplast showed no effect of light, invoking the role of photosynthetic machinery in determining Programmed Cell Death (PCD). Similarly, high PCD in response to UVC (henceforth UV) stress in *A. thaliana* was observed on post-UV exposure to light (Danon et al., 2004). Biotic and abiotic lesion mimicking mutants, such as acd11 in *Arabidopsis* or lls1 in maize, have also shown their requirement of light for PCD like responses (Brodersen et al., 2002; Gray et al., 2002; Doyle et al., 2010). Plant pathogen interaction and gaining a Systemic Acquired Resistance (SAR) are among other significant physiological processes known to be affected by light conditions (Zeier et al., 2004).

Reactive Oxygen Species (ROS) signalling is known to play an important role in plant functions and stress responses. ROS and redox cues are essential for maintaining vital functions of plants like energy and metabolic fluxes, resistance, developmental programs and acclimatisation (Suzuki et al., 2012; Choudhury et al., 2013). Recently many studies have suggested that chloroplast also plays a significant role in PCD in plants in a manner akin to mitochondria in animal system (Samuilov et al., 2003). Chloroplast remains a major source of ROS in plants and the production of ROS is tightly linked with metabolic state and light intensity (Karuppanapandian et al., 2011). Studies by Doyle et al. with *Arabidopsis* suspension cultures showed that apoptosis-like PCD in response to heat stress was affected by Chloroplast inhibitors (norflurazon, spectinomycin and lincomycin) and antioxidants (ascorbate, reduced glutathione, etc) (Doyle et al., 2010). Similarly Samuilov et al. showed that CN^- induced PCD is affected by chloroplast ROS. Addition of ROS from external sources waived the requirement of light for enhanced ROS in this system (Samuilov et al., 2008), raising the possibility of ROS mediated signalling.

In unicellular organisms too, it has been observed that a dark pre-incubated culture was significantly more resistant to UVC compared to a light grown culture (Bhattacharjee and David, 1977). Similar observations of high resistance in dark conditions and high sensitivity in light conditions have been reported for *Chlamydomonas* and *Euglena* (Nikaido and Johnson, 2000; Bolige et al., 2005). These studies suggest that the UV sensitivity phenotype is under circadian control which is linked to replicative state of DNA that is partitioned to a specific time of the day. By using nitrate-minus conditions, it was shown that the higher sensitivity in cyanobacteria in light conditions was not due to replicating state of DNA (Bhattacharjee and David, 1977). It was also shown that a highly UV sensitive and dark repair incapable *recA*$^-$ mutant of *Synechocystis* continued to exhibit light–dark difference in UV sensitivity (Minda et al., 2005). The dark resistance could be mimicked even in light conditions if the photosynthesis process is blocked by limiting CO_2 or by using PSII blockers (David et al., 1988). Clinching evidence implicating PSII constituent was provided when the dark resistance was undone in a dosage specific manner by removing D1 (*psbA*) alleles (Minda et al., 2011). In the current study, we make an attempt to uncover the physiological basis of UV sensitivity rhythm in *Chlamydomonas reinhardtii* cells. We explored the nature of circadian rhythmicity, photosynthetic processes and ROS in the UV sensitive versus resistant conditions of *C. reinhardtii* cells. The results suggest that the cyclic metabolic changes triggered by photosynthetic drive rather than the internal circadian clock mainly contributes to light–dark (12 h:12 h) rhythmicity in UV sensitivity. In addition, we show that a combined action of high ROS and associated low DNA excision repair efficiency in light culture render the cells more sensitive to UV stress than in dark incubated cells.

2. Methods

2.1. Strains and culture conditions

All the *C. reinhardtii* strains were obtained from Chlamydomonas Resource Center, University of Minnesotta. Wild type refers to cc-125. Media used for culturing were Tris–Acetate–Phosphate (TAP), Tris–Phosphate (TP) and Sueoka's High Salt medium (HSM) as per requirement, the composition of which is according to what is described in *Chlamydomonas* Source book (Harris, 1989). Cultures were grown in temperature controlled incubators at 25 °C, under cool white fluorescent light with ~100 μmol/m^{2}sec of PAR light intensities. Liquid cultures were grown in glass flask at similar light intensities and temperatures. Media to flask volume ratio was always equal to or greater than 1:4. For dark conditions, working environment light intensities were less than 0.01 μmol/m^{2}sec. For atrazine (Cat#-45330, Sigma Aldrich) treatment, 3 μM final conc. was used in all experiments.

2.2. UV exposure

A custom UV exposure chamber was made with non-reflective black paint and had a manual shutter. It allowed simultaneous exposure of 4 plates. UV chamber housed two 15 W Sankyo Denki (G15T8) germicidal lamps, which emitted 87% of spectral energy at 253.7 nm. The fluence rate was manipulated by adjusting the distance (minimum 40 cm) and using a uniform obstruction grid. UV fluence rates were measured by International Light ILT 77 UVC monitor, after stabilising the lamp for 15 min. UV fluence rates were adjusted such that exposure time was at least 90 s. Large changes in fluence rate and time were avoided within an experiment. In most cases, where the comparison was to be made between survivals, exposures of the sets were done simultaneously to negate small errors that can originate with manual shutter or lamp fluence rate fluctuations.

2.3. L/D switch

A continuously growing culture was split into fractions at the cell density of 1×10^6 cells/ml and kept in subjective condition for 12 h. Dark incubation was provided by wrapping the flask with double layers of aluminium foil, following which an aliquot was taken and serially diluted. Multiple dilutions (10^{-1}–10^{-3}) were plated to cover a wide range of survival. Experiments were done with three biologicals (N = 3) and three technical repeats (n = 3) per each biological. Statistics was computed from such multiple sample readouts. Cells were grown in HSM media in light–dark (12 h:12 h) for circadian training. Master cultures were maintained in these conditions continuously from where a subculture was started for an experiment. The experimental culture was also kept in similar condition for at least three days before starting survival assays.

2.4. Northern analysis

Total RNA from samples of respective time points was isolated using Trisol method (Chomczynski and Sacchi, 1987). RNA (5 μg) was run on formaldehyde gel and transferred to Hybond-N+ using capillary transfer. RNA was immobilised by UV crosslinking using Stratagene UV crosslinker. PCR products (100 nt long) from TufA and PsbD gene were prepared using alpha-P^{32}-dATP and used as probe. Hybridisation was done using Ultrahyb and standard