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On the Role of Herbicide Binding B Protein as Regulator of UV-Sensitivity of PSII: A Model Explaining Dark-survival Characteristics of Cyanobacteria

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Our studies indicate that damage to PSII causes lethality under conditions which are known to promote excision-repair of damage to DNA. Herbicides, DCMU and Artrazine, which specifically bind to B protein with high affinity have been found to modify the UV-sensitivity in a concentration dependent manner implicating a direct involvement of B protein. Based on our work with *A. nidulans* a model is presented in which reaction centre of PSII is assumed to be the target and B protein to be a regulator of repair of damage by far-UV light. The significance of damage to DNA and the use of viability as a parameter for studying *in vivo* regulation of photosynthetic electron transport as well as the development of functional PSII particles are discussed in light of this model.

Key Words: Cyanobacteria, Lethality, UV, PSII, Photoreactivation

Introduction

It is known that short wave UV-light inactivates both DNA as well as the photosynthetic electron transport system in cyanobacteria (O' Brien & Houghton 1982, Van Baalen 1968). Presumably these organisms survived the higher stress of solar radiation during the precambrian era because of mechanisms which could either protect against or repair the photochemical damage induced by such radiation. Both dark and light dependent repair of damage to DNA have been shown to exist in cyanobacteria. These processes seem to be similar to those found in other prokaryotes and eukaryotes (Saito & Werbin 1970, Castellani et al. 1964, Patrick & Haynes 1964). However, the nature of damage to the photosynthetic electron transport system and its contribution to lethality has largely remained unexplored.

Interestingly, however, cyanobacteria show gradual increase in the loss of viability following UV-irradiation in the dark although dark-repair of damage to DNA is functional (Asato A 1972, Bhattacharjee et al. 1987). In this communication we suggest that this lethality during dark incubation may be explained as a consequence of damage to functional photosystem II (PSII). We also present a model based on two states of the PSII regulated by the herbicide binding protein B, which differ in their relative sensitivity to UV light to explain the dark-survival characteristics of *Anacystis nidulans*.

Materials and Methods

Strain: Strain BD-1 of *A. nidulans* was used in all the experiments.

Growth Medium: BG 11 medium was used both for liquid and agar plates as described by Allen (1968).

Growth condition: Cultures were grown at 36°C in a water bath shaker in light from tungsten lamps at an intensity of 12W m^{-2}. Cells from exponentially growing cultures were used for all experiments. Generation time under these conditions of growth was 10 hr.

UV-irradiation: Philips germicidal TUV 15W lamp was used. Fluence rate for irradiation was 13J m^{-2} as measured by IL 700 radiometer International Light INC. Detector used was PI171 C# 1027. Cells were exposed in a sterile petriplate in liquid BG 11 medium in 10ml volumes and constantly stirred during irradiation.

Photoreactivation: 400W Philips fluorescent mercury vapour lamp was used as described earlier (Chatterjee & Bhattacharjee 1975). Samples of cell suspensions with and without UV-irradiation were transferred to petriplates (9 cm diam.) and exposed to the white light from this source for 15min. The cells suspensions were then transferred to flasks covered with aluminium foil for dark incubation as described in post-UV incubation procedure given below. Temperature during photoreactivation was controlled at 30°C.

Post-UV incubation: Irradiated cell suspensions with or without photoreactivation were incubated in 100 or 150 ml conical flasks covered with aluminium foil and transferred to water bath shaker at 36°C for 24hr. After this dark incubation of 24hr the aluminium foils were removed from the flasks and all the suspensions were incubated in light to grow as described above in Growth

Abbreviations: DCMU: 2/3-4, Dichlorophenyl/-1; 1-dimethylurea; PSII: Photosystem II; RC: Reaction Centre of PSII

conditions, for 7hr and then PSII activities and viability of the cells were measured.

Measurement of colony-forming unit: Cell suspension with serial dilutions in BG11 medium were plated on freshly prepared agar plates and incubated as described earlier (Bhattacharjee & David 1977).

Chemicals: 2/3–4, Dichlorophenyl/-1, 1-dimethylurea (DCMU) was obtained from K K Laboratories Inc. Cleveland, Ohio.

Measurement of PSII Activities

DCMU-sensitive fluorescence of chlorophyll a: Cell suspensions were used directly to monitor fluorescence at 680 nm when excited at 610nm in a spectrophotofluorometer (AMINCO). Hamamatsu R446 photomultiplier tube (multialkali wide range 185–870 nm) was used as detector. The samples were kept in dark for 1min before excitation and the fluorescence signals were recorded in a time base chart recorder. DCMU-sensitive fluorescence. f_{dc}, was calculated as fractional increase in fluorescence yield by addition of 10^{-5}M DCMU (final concentration) in the sample. Fluorescence yield was measured at 22 sec from the start of the excitation as shown in figure 1.

O_2 evolution: O_2 evolution was measured by an oxygraph using Clark type electrode. The amplifier was designed and developed in our laboratory by S S Rane. Projector lamp was used with a red filter (cut off filter CORNING 3–66)) for irradiation of the cell suspension. Signal was recorded in a time base chart recorder. Rate of O_2 evolution was obtained from the slope of the trace as shown in figure 2.

$$f_{dc}\,\% = \frac{\Delta F \times 100}{F_{-dcmu}}$$

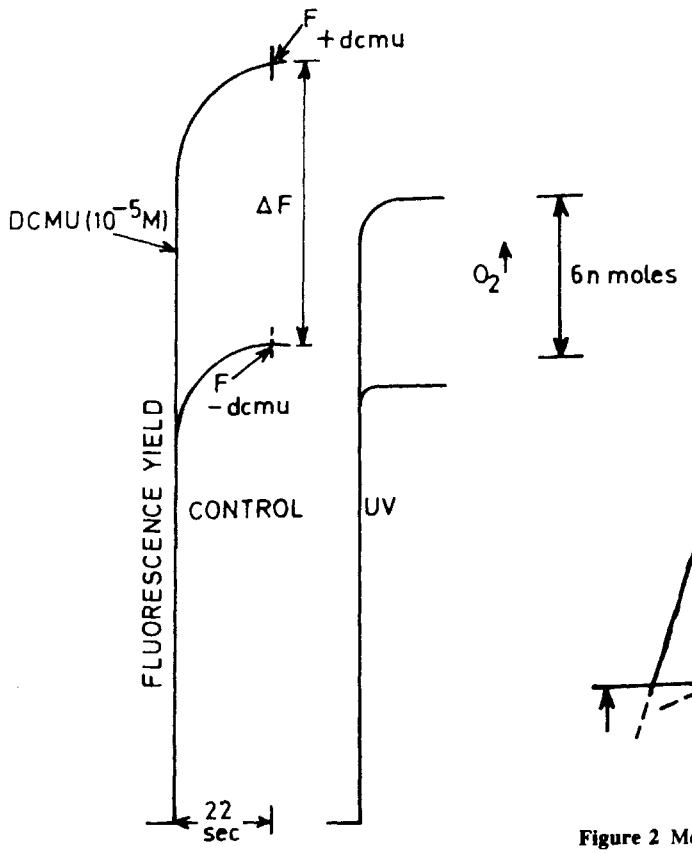


Figure 1 Measurement of inactivation of variable chlorophyll-*a* fluorescence by UV

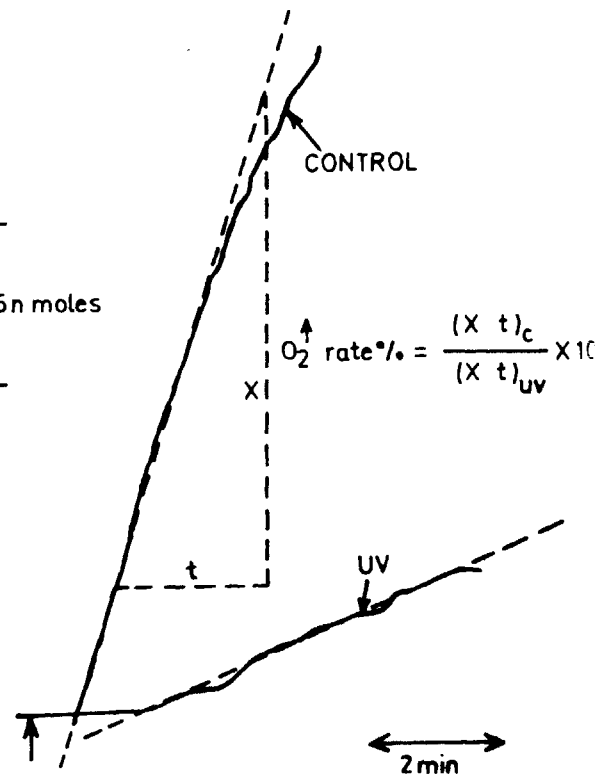


Figure 2 Measurement of inactivation of oxygen evolution rate by UV-light. Scale of O_2 concentration shown in the trace was determined by using Sodium dithionite in distilled water

Results and Discussion

Lethal effect of far-UV light is very efficiently photoreactivated in cyanobacteria (Van Baalen & O'Donnell, 1972). However, if actively growing cells after UV-exposure were incubated in dark before transfer to light for growth there was gradual increase in lethality concomitant with loss of photoreversibility with increasing dark incubation period (Asato 1972). In cyanobacteria *Synechocystis*, the damage to DNA was repaired by dark-excision repair system like those in heterotrophs (O'Brien & Houghton 1982). It was therefore surprising that cyanobacteria lost viability whereas heterotrophs like *E. coli* and yeast showed enhanced recovery during post-UV dark incubation attributed to DNA dark excition repair activity (Castellani et al. 1964, Patrick & Haynes 1964). Interestingly, cells of *A. nidulans* when incubated in dark prior to UV-exposure, became highly resistant to lethal effect of far-UV light (Bhattacharjee & David 1977). Similarly, incubation in light with herbicides DCMU and atrazine which bind specifically to high turnover B protein of PSII also increased the UV-resistance, like cells transferred to dark when a 24hr post-UV dark incubation was allowed (figure 3) (Bhattacharjee et al. 1987). DCMU in light is known to inhibit protein synthesis in *A. nidulans* (Smith 1977). However, if protein synthesis was blocked by chloramphenicol instead of DCMU, the cells became increasingly more sensitive to UV (Bhattacharjee 1977). This indicated, that dark-survival was related to the effect of UV light on PSII. Direct involvement of PSII as lethal target was evident when its inactivation was measured by monitoring the changes in DCMU sensitive fluorescence of cholophyll-*a*. A clear correlation with loss of lethality during post-UV dark incubation was observed (Bhattacharjee et al. 1987). Moreover, consistent with high UV-resistance by dark-incubation prior to UV-exposure, no significant difference in the fraction of DCMU-sensitive fluorescence between control and UV-irradiated cells was observed (Bhattacharjee et al. 1987). This led us to a model in which the reaction centre (RC) of PSII was considered a lethal target and B protein a regulator of its UV-sensitivity (Bhattacharjee & David 1987). Since loss of viability, imply loss of colony forming ability, it was predicted by the model that inactivation of RC by UV measured prior to cell division should correlate with loss of viability. Figure 4 shows the scheme of measurement of two functions of PSII, (i) fraction of DCMU sensitive fluorescence f_{dc}, and (ii) O_2 evolution rate as well as lethality by counting colony-forming units in the case of cells actively gowing in light prior to UV-exposure. Transfer to light after a prolonged dark incubation synchronized cell division of *A. nidulans* (Asato 1979). We also observed a similar synchrony as shown in figure 4. The inactivation of PSII functions were measured at 0.7 generation time following transfer of control and UV-exposed cells from dark to light. Figure 5 shows the inactivation of both the PSII activities as functions of UV fluence. These activities were also simultaneously photoreactivated by 15min exposure to white light immediately following UV-exposure and prior to 24hr dark incubation (figure 5). This clearly correlated with photorecovery of viability (Bhattacharjee et al. 1987.)

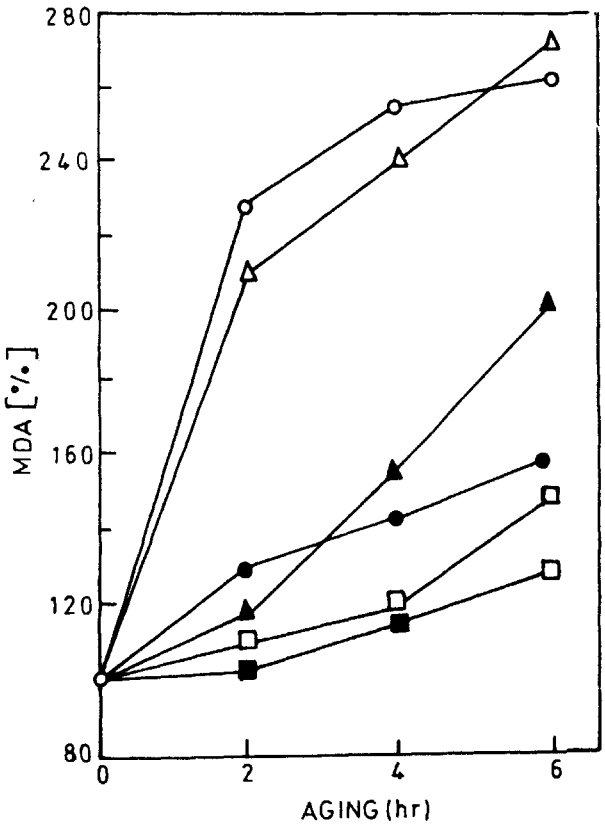
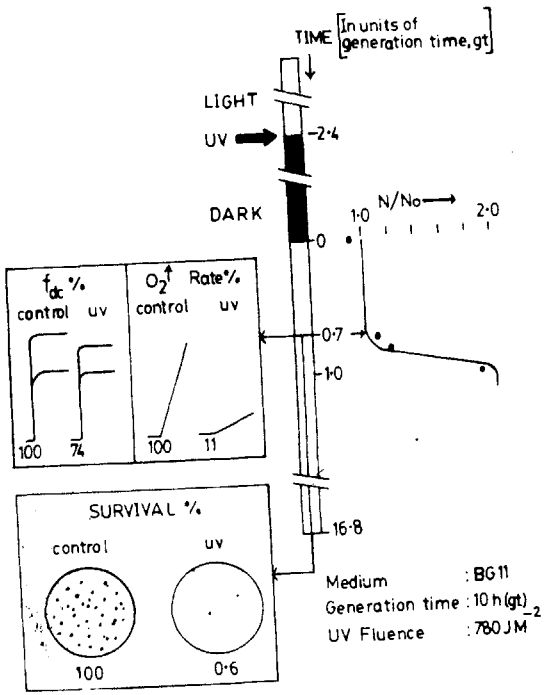


Figure 3 Development of UV-resistance as a function of incubation time in dark (●), and in the presence of 10^{-5}M DCMU in light (○), prior to exposure to 390J M^{-2} of far-UV (254nm) light. Post-UV dark incubation was for 24hr at room temperature. Plates containing control and UV-irradiated cells were then transferred to light as described in "Materials and Methods"



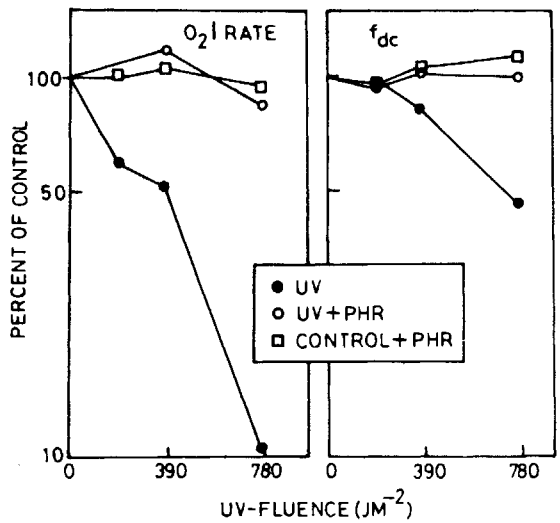


Figure 4 Scheme of the experiments to measure inactivation of PSII functions, (i) Fraction of DCMU-sensitive fluorescence of chlorophyll-*a*, f_{dc} and (ii) O_2 evolution rate, and viability with 24hr post-UV dark incubation. f_{dc} and O_2 evolution rates were measured at 0.7 generation time following transfer of the control and UV-exposed cell suspensions to light conditions used for growth. At the same time cells were plated on agar plates for determining colony-forming units as described in Materials and Methods. Cell division of the control cells were monitered by measuring colony forming units following dark to light transition. Synchronous division at about 10hr is indicated by the increase in ratio of cell number *(N/No)*. *No.* represents the number of cells at the time of transfer of cell suspension to light following the 2.4 gt dark incubation; and *N*, the cell number at indicated time in the light regime

Figure 5 UV-inactivation and photoreactivation of PSII activities as functions of UV fluence. 10ml cell suspensions from 15ml of actively growing cultures were irradiated with UV at indicated fluences in dark and 5ml of the irradiated sample was immediately exposed to visible light for photoreactivation along with 5ml of control which had not been exposed to UV. Photoreactivation was carried out as described in Material and Methods. All the three suspensions (UV, UV+PHR and control+PHR) were then transferred to dark for post-UV dark incubation for a period of 2.4 gt. The fraction of DCMU-sensitive fluorescence of chlorophyll-*a*, f_{dc}, and O_2 evolution rate were measured according to the schedule and procedure described in figure 4. 100% refers to the corresponding values of f_{dc} and O_2 evolution rate for cell suspension which received neither UV nor photoreactivating light. PHR in the figure indicates exposure to 3.6×10^4J M^{-2} of white light used for photoreactivation

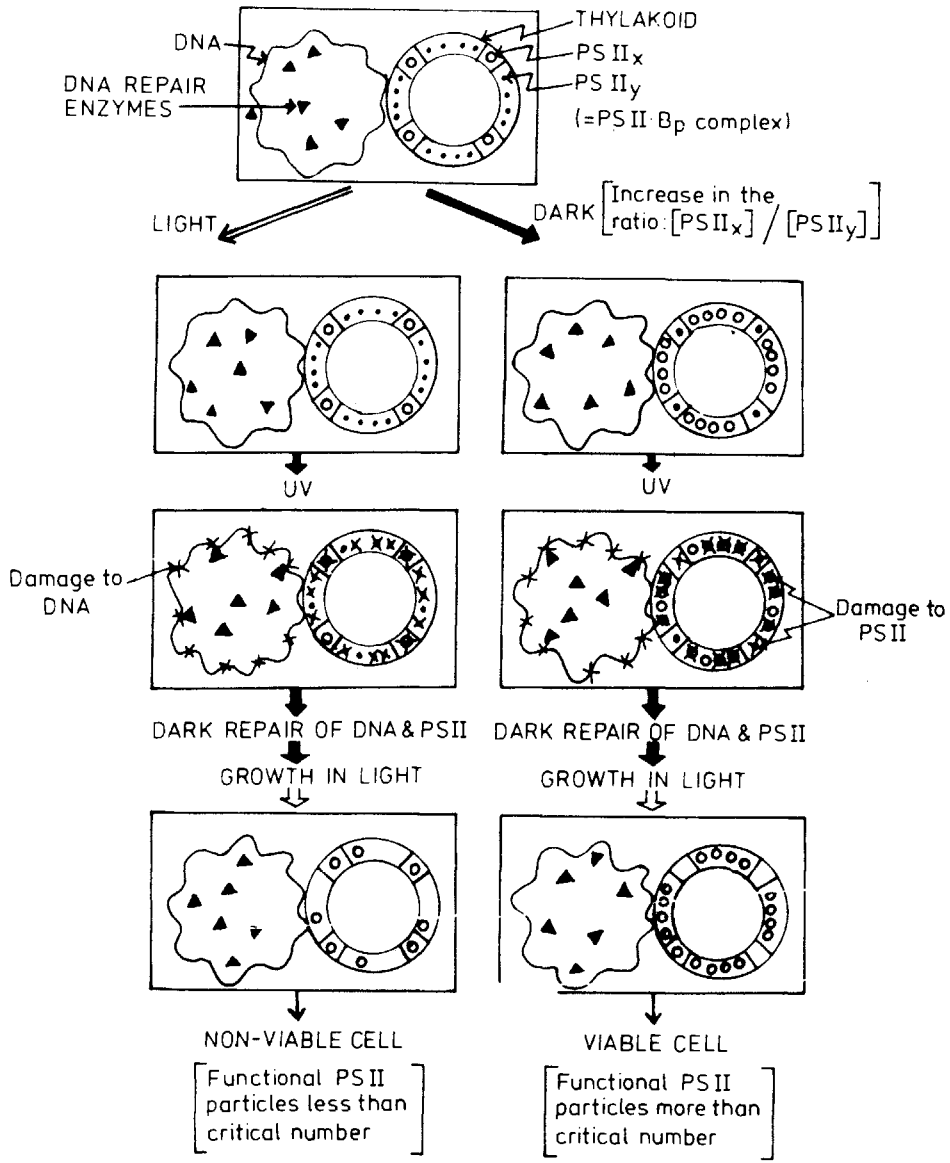


Figure 6 Independent-target (DNA and PSII) model for explainingthe differential dark-survival between cells incubated in light and dark prior to UV-irradiation. For a given UV fluence, contribution to lethality by damage to DNA is considered identical irrespective of whether the cells were in light or dark prior to UV. Differential lethality is considered to be a consequence of differential damage to PSII particles. PSII particles are assumed to exist in two states, $PSII_x$ and $PSII_y$. $PSII_y$ state represents a functional PSII particle formed by the association of the herbicide binding peripheral B protein with PSIIx (see text). PSIIy is considered to be UV-sensitive state compared to PSIIx. Dark incubation prior to UV causes an increase in the number of PSIIx because of dissociation (at least functional) of B protein from $PSII_y$. Therefore, for a given UV-fluence, there are more intact RC of PSII particles in cells which have been incubated in dark prior to UV. If this number is more than a critical number necessary to support growth of the cell under given growth conditions it divides and finally gives a colony provided the damage to DNA was repaired in such a cell. However, a corresponding cell without dark incubation prior to UV exposure has fewer PSIIx state particles and finally does not divide because the number of intact RC are less than the minimum required for cell division although the damage to DNA was repaired

The correlation of lethal effects of far UV light with inactivation of PSII functions as well as with photorepair clearly indicated PSII inactivation as a lethal event. Assuming that PSII and DNA are independent targets of UV determining cellular lethality as measured by inability of a cell to divide in dark-survival experiments the resistance of pre-UV dark incubated cells can be explained by the model presented in figure 6. The following assumptions are made:

(i) For a constant UV fluence, DNA repair during post-UV dark incubation is not significantly different in wild type cells irrespective of whether the cells were in light or dark prior to UV-exposure.

(ii) PSII particles exist in two states: (i) UV-resistant state ($PSII_x$) and (ii) UV-sensitive state ($PSII_y$).

(iii) Ratio of PSII particles in the two states, i.e. $[PSII_y]$ and $[PSII_x]$ decreases upon transition of the cells from light to dark because of conversion of $PSII_y$ to $PSII_x$ by step II without step I functioning in the reaction scheme (1) given below.

(iv) Light to dark transition does not alter the average number of PSII particles per cell, i.e. $[PSII_x] + [PSII_y]$ = constant for a given growth condition.

Based on the effect of post-UV dark incubation temperature on dark-survival, we postulated a regulatory role in dark-repair against potential lethal damage to RC of PSII by the peripheral high turnover herbicide (DCMU and atrazine) binding B protein (Bhattacharjee & David 1987). The relationship between $PSII_x$ and $PSII_y$ according to the regulatory model is as follows:

$$B_p + PSIIx \xrightarrow{I} PSII \xrightarrow{II} PSIIx + B_{p'} \quad \ldots (I)$$

where B_p represents herbicide binding B protein, and $B_{p'}$ altered B protein (perhaps by proteolytic cleavage in light).

It may be noted that in actively growing culture $PSII_x$ may be considered as precursor in the development of PSII particle into a photosynthetically active state, $PSII_y$, because of functional association of secondary stable electron acceptor B protein. In *in vitro* studies involving reconstitution of O_2 evolution both in cyanobacterial and spinach chloroplast PSII preparations one peripheral protein of 34KD (33KD in case of chloroplast) was found necessary per PSII particle for O_2 evolution (Koike & Inoue 1985). PSII particles in thylakoid membrane of chloroplasts were shown to be heterogenous and based on the kinetics of induction of chlorophyll-*a* fluorescence Melis postulated two states of PSII particles as steps in the development of the thylakoid membrane (Melis & Homann 1976 Melis 1985). UV-resistance of $PSII_x$ in our model has been argued to be due to the presence of a dark repair system against potential lethal damage to RC, which is inhibited by B protein in the $PSII_y$ state (Bhattacharjee & David 1987).

In the independent-target model presented here the UV-sensitivity of cells with higher ratio of $[PSII_y]/[PSII_x]$ increases because the number of potentially functional PSII particles decreases as a consequence of loss through UV-sensitive $PSII_y$ state. It is assumed that cells with less than a critical number of PSII particles required for indefinite cell division (since viability is measured by growth of a cell into a visible colony) would become non-viable (figure 6). The critical number of intact PSII particles necessary for cell division under a given set of growth condition may be estimated by application of the methods of target theory of cellular inactivation by radiation to the inactivation of O_2 evolution rate, f_{dc}, as well as loss of dark-survival by far-UV light.

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