A MODEL FOR ULTRAVIOLET AND PHOTOREACTIVATING LIGHT EFFECTS IN *EUGLENA*

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ABSTRACT A unified mathematical model is presented of the reversible effects of ultraviolet (UV) and photoreactivating (PR) light on the chloroplast-forming ability of dark-grown *Euglena gracilis* (var. *bacillaris*). This model is an extension of several aspects of target theory and also of a model for the decay of photoreactivability in *Euglena* proposed by Schiff et al. The data presented in several earlier papers are compared with the predictions of the proposed unified model and reasonably good agreement is found.

INTRODUCTION

Euglena gracilis is particularly suitable for the study of ultraviolet (UV) radiation effects since its chloroplast-forming system is considerably more sensitive to UV than is its colony-forming ability. Furthermore, UV damage to the chloroplast-forming system is completely reversible by photoreactivating (PR) light over a wide range of doses (references 1, 2).

In this paper we describe a unified model which furnishes predictions about both UV and PR effects in *Euglena*. The model predictions are compared with data presented in several earlier papers (2, 3, 4). Target theory has been assumed to apply to the UV inactivation of chloroplast formation (5). A preliminary model for the decay of photoreactivability was described by Schiff, et al. (1) and by Hill, et al. (4).

METHODS

Dark-grown *Euglena gracilis* (var. *bacillaris*) was used in all experiments described here. Chloroplast-forming ability of cells was estimated by the fraction of green colonies that appeared during incubation. A germicidal lamp was used as a UV radiation source (90% of the output at 2537 A) and a black light, as a PR source (peak output at 3500 A). Detailed descriptions of the materials and methods are given in the papers by Lyman, et al. (5); Schiff, et al. (1); and Hill, et al. (2, 3, 4).

MODEL

In this paper we consider only those PR effects in *Euglena* which can be reversed by UV and only those UV effects which are photoreactivable or which affect photoreactivation. The model consists of the following postulates:

(I) A *Euglena* cell contains n centers, each of which is composed of m sites. Sites interact with UV and PR radiation; individual sites interact independently of one another.

(II) If a site has interacted with a photon of UV, and thereby undergone a photochemical change, it is said to be occupied. Interaction of an occupied site with a photon of PR renders it unoccupied, i.e., subject to interaction with a photon of UV. Occupied sites will not interact with UV, nor will unoccupied sites interact with PR.

(III) The chance that an occupied site will interact with a photon of PR (i.e. the cross-section for photoreactivation) decreases hyperbolically as the dose of the antecedent UV increases.

(IV) The chance that an occupied site will interact with PR decreases exponentially with the duration of incubation in nutrient medium.

(V) In order for a cell to form a green colony it must have at least one center free of occupied sites.

(VI) A center with one or more occupied sites will not replicate; during mitosis centers are randomly distributed among daughter cells. Decay of photoreactivability is unaffected by mitosis.

Some quantitative relationships will now be presented which are consequences of applying the model postulates to various experimental situations. Derivation of these relationships will be found in Appendix I.

UV-Inactivation

The expected fraction of green colonies after exposure of *Euglena* to UV light,

$$G = 1 - (1 - e^{-m\alpha\theta})^n \qquad (1)$$

where n is the number of centers per cell, m, the number of sites per center, α, the cross-section (mm^2/ergs) for photoinactivation of a site, and θ, the dose of UV in ergs/mm^2 which is proportional to the mean number of UV photons hitting a unit area. As θ becomes large $\log_e G$ tends toward $\log_e n - m\alpha\theta$. (In reference 3 $m\alpha\theta$ is expressed as D/D_0, where D is the UV dose in ergs/mm^2 and D_0, the dose required for a "single inactivation event".)

Photoreactivation

The expected fraction of green colonies after exposure of *Euglena* to UV and then to PR light,

$$G = 1 - (1 - [1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})]^m)^n \qquad (2)$$

where Φ is the dose of PR in minutes and is proportional to the mean number of PR photons hitting a unit area. $\beta/(\theta - \theta_0)$ is the cross-section for reactivation and depends on β, the cross-section for reactivation in the absence of UV, θ, the dose of UV, and θ_0, a constant. Equation 2 holds only for values of $\theta \geqq \theta_0 + 1$.

Decay of Photoreactivability

In Absence of Cell Division. If *Euglena* is exposed to UV, then incubated in the dark in nutrient medium for a period of time, t, short enough so that virtually no cell division occurs, and then exposed to PR light the expected fraction of green colonies,

$$G = 1 - (1 - [e^{-\alpha\theta} + (1 - e^{-\alpha\theta})(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0})^{-At}}]^m)^n \qquad (3a)$$

where A is the photoreactivability decay rate.

In Presence of Cell Division. If *Euglena* is exposed to UV, then incubated in the dark in growth medium for a period of time, t, and then exposed to a saturating dose of PR the expected fraction of green colonies,

$$G = 1 - (1 - 2^{-\frac{t}{\tau}}[e^{-\alpha\theta} + (1 - e^{-\alpha\theta})e^{-At}]^m - (1 - 2^{-\frac{t}{\tau}})e^{-m\alpha\theta})^n \qquad (3b)$$

where τ is the generation time. When θ is sufficiently large and $2^{-\frac{t}{\tau}}e^{-mAt}$ is small we have

$$G \doteq 1 - \exp(-2^{-\frac{t}{\tau}} n e^{-mAt})$$

or

$$\log_e[-2^{\frac{t}{\tau}} \log_e(1 - G)] \doteq \log_e n - mAt. \qquad (3b')$$

UV-PR-UV

After UV, then PR, and then a second dose of UV the expected fraction of green colonies,

$$G = 1 - (1 - [e^{-\alpha^*\theta^*}(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta}))]^m)^n \qquad (4)$$

where α^* is the cross-section for inactivation of a reactivated site and θ^* is the second dose of UV in ergs/mm^2. This equation can be expressed as

$$\frac{1}{m}\log_e(1 - (1 - G)^{\frac{1}{n}}) = -\alpha^*\theta^* + \log_e(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})), \qquad (4')$$

a form which is convenient for verifying the functional relationship of the various parameters on the right-hand side.

RESULTS

Estimation of Parameters and Comparison of Predictions with Observations

UV-Inactivation. In earlier papers (3, 5) it was shown that UV-inactivation of green colony formation obeys multi-target kinetics and hence that equation 1 may be presumed to hold for appropriate values of the parameters. Using this relation the number of centers (n) was estimated by the method of least squares from 28 sets of inactivation data and found to be 43.5 ± 10.5 (SE). The calculations were carried out on a digital computer using the SAAM program (references 6, 7) with equal weight assigned to each datum. For further details of these calculations see reference 3.

Photoreactivation. Using equation 2 the parameters, n, m, α, β, and θ_0 were estimated by the method of least squares from each set of photoreactivation data shown in Fig. 1 except the set for which $\theta = 960$ ergs/mm^2. These estimates were then averaged and the mean values found to be $n = 42.6 \pm 7.8$, $m = 3.10 \pm 0.11$, $\alpha = 0.0281 \pm 0.0016$, $\beta = 4.22 \pm 0.43$, and $\theta_0 = 24.5 \pm 2.3$. Note that the estimate of n agrees closely with the previous estimate, 43.5. The family of predicted curves based on the foregoing parameter values are also shown in Fig. 1. The agreement between the predicted and observed values is seen to be very close. Of the cells exposed to 960 ergs/mm^2 UV, 15% were estimated to have undergone changes irreversible by PR; accordingly, in determining the corresponding predicted curve shown in Fig. 1, each of the numbers calculated using equation 2 and the above parameter values was multiplied by 0.85.

We will now consider the evidence for writing the exponent involving Φ in equation 2 as a hyperbolic function of θ, namely $\beta\Phi/(\theta - \theta_0)$. The argument will be presented assuming (a) that the general form of equation 2 is

$$G = 1 - (1 - (1 - e^{-f(\Phi,\theta)}(1 - e^{-\alpha\theta}))^m)^n$$

where $f(\Phi, \theta)$ is an arbitrary differentiable function of Φ and θ and (b) that $m = 3.1$, $n = 42.6$. The above equation can be rewritten as

$$-\log_e \{1 - (1 - (1 - G)^{\frac{1}{42.6}})^{\frac{1}{3.1}}\} = f(\Phi, \theta) - \log_e (1 - e^{-\alpha\theta}).$$

The left-hand side, say $h(G)$, can readily be evaluated for $0 < G < 1$ with the help of the following approximation

$$1 - (1 - G)^{\frac{1}{42.6}} \doteq -\frac{\log_e (1 - G)}{42.6} - \frac{1}{2}\left(\frac{\log_e (1 - G)}{42.6}\right)^2.$$

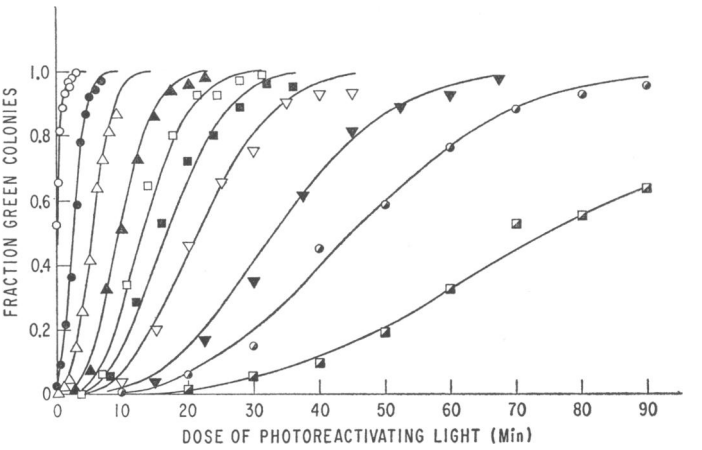

FIGURE 1 PR of green colony-forming ability in dark-grown *Euglena*. ○ UV 48, ● 80, △ 110, ▲ 160, □ 208, ■ 255, ▽ 320, ▼ 480, ◑ 640, ◪ 960 ergs/mm². Predicted curves are based on equation 2 using the least squares estimates $n = 42.6 \pm 7.8$, $m = 3.10 \pm 0.11$, $\alpha = 0.0281 \pm 0.0016$, $\beta = 4.22 \pm 0.43$, $\theta_0 = 24.5 \pm 2.3$. The curve for $\theta = 960$ ergs/mm² was determined by multiplying each of the values calculated using equation 2 by 0.85 (see text).

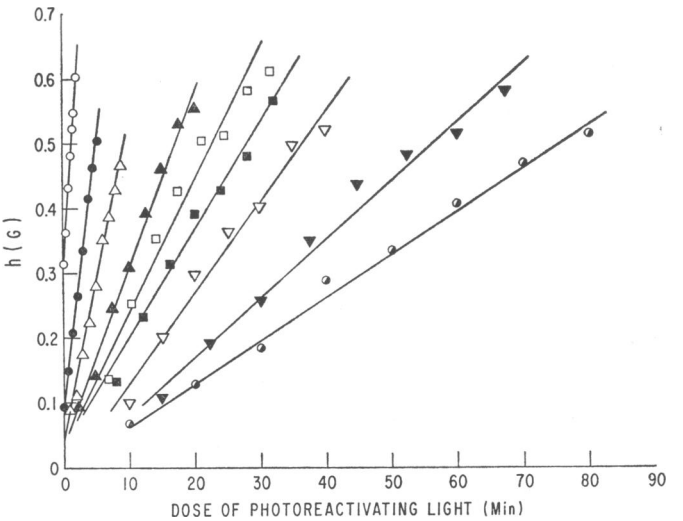

FIGURE 2 Data shown in Fig. 1 (except the set for which $\theta = 960$ ergs/mm²) transformed to obtain straight line plots. $h(G) = -\log_e \{1 - (1 - (1 - G)^{\frac{1}{42.6}})^{\frac{1}{3.1}}\}$ where G is the fraction of green colonies (see text). For explanation of symbols see legend of Fig. 1.

If the data points in Fig. 1 are plotted as values of $h(G)$ corresponding to various lengths of exposure to PR then, as shown in Fig. 2, the set of points for each length of exposure to UV tend to fall in a straight line. (The set of data for which $\theta = 960$ ergs/mm² is omitted from this graph as some of the cells exposed to this dose of

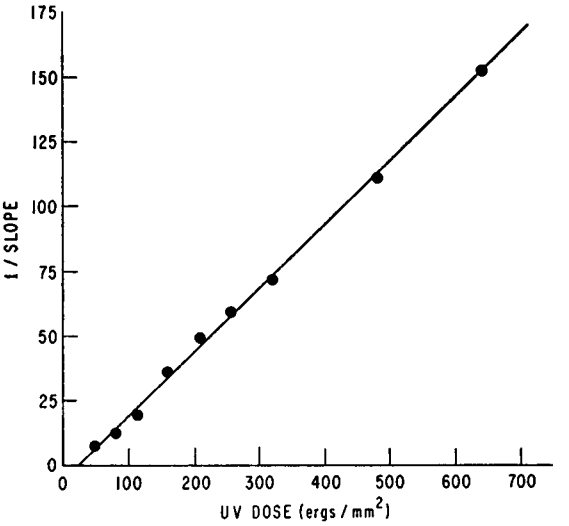

FIGURE 3 The reciprocal of the slopes of the fitted straight lines in Fig. 2 plotted as a function of UV dose (ergs/mm²).

UV underwent changes irreversible by PR.) The slopes of these lines are the respective rates of response to PR and as such are proportional to the corresponding cross-sections for reactivation. In Fig. 3 are shown the reciprocals of the least squares estimates of the slopes in question plotted against the length of UV exposure; a linear relationship is evident.

As the slopes are the derivatives of $f(\Phi, \theta)$ with respect to Φ we have that

$$\frac{1}{\text{derivative of } f(\Phi, \theta)} = a\theta + b$$

where a and b are constants. The least squares estimates of a and b are 0.245 ± 0.004 and -5.2 ± 1.3, respectively. The chance that the true value of the intercept, b, is positive is somewhat less than 0.01.

Rearrangement and integration of equation 5 yields

$$f(\Phi, \theta) = \frac{\Phi}{a\theta + b}, \quad \text{since} \quad f(0, \theta) = 0.$$

It is convenient to write $\Phi/(a\theta + b)$ as $\beta\Phi/(\theta - \theta_0)$ where $\beta = 1/a$ and $\theta_0 = -b/a \geqq 0$. Since the coefficient of Φ in equations 2, 3a, and 4 is necessarily non-negative and since β is defined as the cross-section for reactivation in the absence of any antecedent UV, these equations are meaningful only if $\theta \geqq \theta_0 + 1$. Calculation of β and θ_0 from the estimates of a and b yields the values 4.08 and 21.1, respectively, which agree quite well with the previous estimates, $\beta = 4.22$ and $\theta_0 = 24.5$.

The finding that the cross-section for reactivation decreases hyperbolically as the

dose of antecedent UV increases is unexpected, nevertheless, such a decrease would be a natural consequence if UV produced an inhibitor of a photoreactivating enzyme. There is considerable evidence that such an enzyme is present in other photoreactivable systems (references 12, 13) as well as suggestive evidence that a similar enzyme occurs in *Euglena* (reference 2). The kinetics of the assumed inhibition can readily be deduced from the law of mass action and the principle of conserva- of enzyme. A detailed derivation of the required form of the cross-section for reactivation will be found in Appendix II.

Alternatively, if the cross-section for reactivation is assumed to be an exponential function of θ, say $e^{-a'\theta-b'}$, where a' and b' are suitable constants, then the plot in Fig. 3 would be of the function $e^{a'\theta+b'}$ which is clearly inappropriate. A similar argument applies if the cross-section for reactivation is assumed to be the average of several exponential terms.

Decay of Photoreactivability (UV-Decay-PR)

In absence of cell division. In Fig. 4 are shown the observed proportions of green colonies as a function of PR dose. The theoretical curves are based on equation 3a; n, θ, and θ_0 being taken as 42.6, 96, and 24.5, respectively, and m, α, β, and A estimated from the data as 2.5, 0.04, 1.9, and 0.023. It is seen that the data fit the theoretical curves fairly well.

In presence of cell division. In Fig. 5 are shown semi-log plots of $-2^{t/\tau} \log_e (1-G)$ as a function of time (see equation 3b'). The generation number, t/τ, was estimated by $\log_2 (N_t/N_0)$ where N_t denotes the observed number of cells at time t. The col-

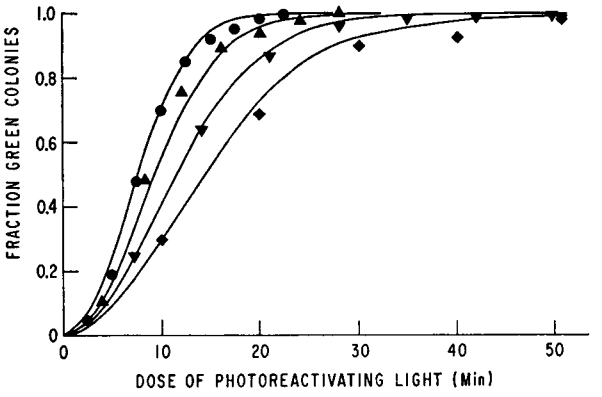

FIGURE 4 The kinetics of PR during the decay of photoreactivability. Dark-grown *Euglena* was treated with 96 ergs/mm² of UV and then inoculated into growth medium. Aliquots were withdrawn ● immediately afterwards, ▲ 8 hr, ▼ 16 hr, ◆ 24 hr later and exposed to increments of PR light. Predicted curves are based on equation 3a with $n = 42.6$, $\theta = 96$, and $\theta_0 = 24.5$ and the least squares estimates $m = 2.51 \pm 0.88$, $\alpha = 0.04 \pm 0.03$, $\beta = 1.89 \pm 0.76$, and $A = 0.023 \pm 0.002$.

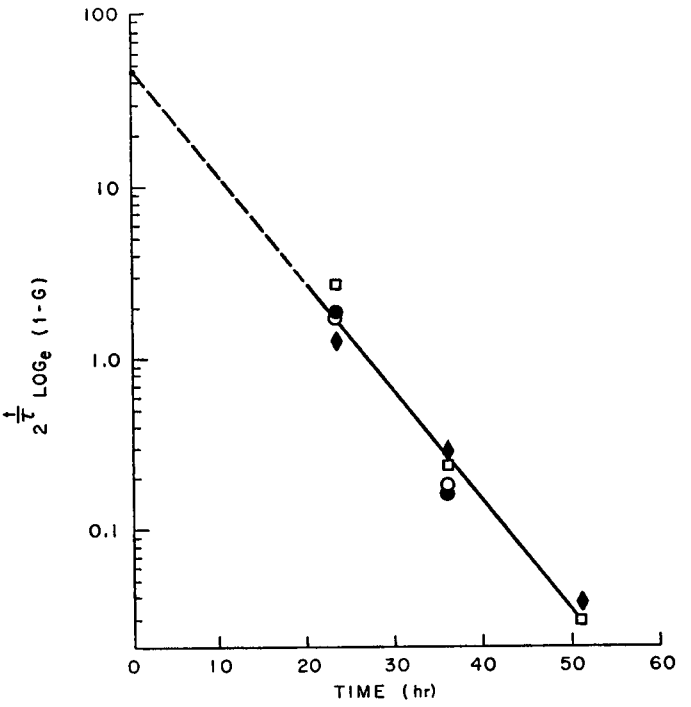

FIGURE 5 The decay of photoreactivability during growth. Suspensions of dark-grown cells were treated with UV, then inoculated into growth medium. Aliquots were withdrawn at 23, 36, and 51 hr and plated in the light. ○ UV 288, ● 384, □ 576, ◆ 768 ergs/mm². The predicted straight line is based on equation 3b′ with the least squares estimates $n = 47.3 \pm 0.10$ and $mA = 0.138 \pm 0.042$.

linearity of the plots is consistent with the right-hand side of equation 3b′; that is, since high doses of UV are being used, there is no dependence of the slope on the UV dose; moreover, the number of centers prior to the onset of decay (the antilogarithm of the intercept) was estimated to be 47.3 ± 0.10, which is in reasonable agreement with 42.6, the estimate made earlier from other data.

If postulate IV is dropped and instead it is assumed that the sensitivity of the sites to PR does not decay, then the right-hand side of equation 3b′ becomes $\log_e n$, i.e. a horizontal line, which is clearly inconsistent with the data shown in Fig. 5. On the other hand, if postulate VI is dropped, then equation 3b′ becomes

$$\log_e(-\log_e(1 - G)) = \log_e n - mAt.$$

In this case the number of centers prior to the onset of decay is estimated to be about 100 which exceeds considerably the over-all estimate (42.6) derived from the UV inactivation data.

UV-PR-UV. In Fig. 6 are shown plots of $\frac{1}{m}\log_e(1 - (1 - G)^{1/n})$ as functions of the second dose of UV (θ^*) or, in the case of the control data, of the first

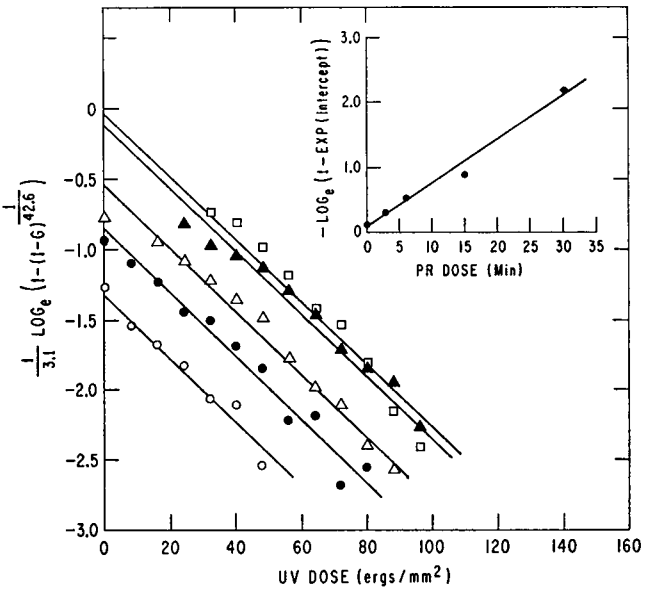

FIGURE 6 UV-PR-UV. Suspensions of dark-grown cells were treated first with UV 96 ergs/mm² and then with different amounts of PR light. ○ 3, ● 6, △ 15, ▲ 30 min. Following PR, each suspension was exposed to a range of UV doses. □ control, no pretreatment with UV or PR light. If equation 4 holds then $\frac{1}{3.1}\log_e(1-(1-G)^{\frac{1}{42.6}}) = -\alpha^*\theta^* + \log_e(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1-e^{-\alpha\theta})) = u(\theta^*, \Phi)$, say. Predicted curves are based on $u(\theta^*, \Phi)$ with $\theta = 96$, $\theta_0 = 24.5$ and the least squares estimates $\alpha^* = \alpha = 0.0221 \pm 0.0019$ and $\beta = 4.82 \pm 0.46$. *Inset figure.* Intercepts of main figure transformed to obtain a plot linear in Φ. If equation 4 holds then $-\log_e(1 - \exp(\text{intercept})) = \frac{\beta\Phi}{\theta - \theta_0} - \log_e(1 - e^{-\alpha\theta})$.

dose of UV (θ); m was assumed to be 3.1 and n, 42.6. The plots tend to be linear as required by equation 4′; moreover, since the slope of the control data agrees closely with the slopes of the remainder of the plots, it is inferred that $\alpha = \alpha^*$ (see Appendix).

The inset figure shows plots of $-\log_e(1 - \exp(\text{intercept}))$ as a function of Φ. The plot is seen to be nearly linear which is consistent with equation 4′.

INTERPRETATION

In earlier papers (3, 5) it was proposed that the entity we have here denoted a "center" be identified as a proplastid nucleus. Since plastids of *Euglena* are known to contain DNA (references 8, 9, 10) and since there is considerable evidence that DNA is a UV sensitive target (reference 11), we suggest that a "site" is a UV sensitive chemical bond in plastid DNA, three such bonds being present in each plastid. If this suggestion is correct, then the occupancy of a site can be taken to be the

state of the chemical bond, two such states being possible, one corresponding to "occupied", the other, to "unoccupied". It is thus feasible to reinterpret in physico-chemical terms postulates I–VI and hence the relationships presented in equations 1–4.

APPENDIX I

The paragraphs below are proofs of the main results stated in the corresponding paragraphs in the Model section. The numbered equations appearing at the end of each proof correspond to the main results embodied in the respective numbered equations of the Model section.

UV-Inactivation

According to postulate I *Euglena* contains n centers, each of which is composed of m sites. If a dose of UV amounting to θ ergs/mm^2 is administered, the chance that a given site does not interact with a photon of UV is $e^{-\alpha\theta}$, where α is the cross-section for inactivation; $e^{-m\alpha\theta}$ is the chance that none of the m sites of a given center has interacted, i.e. is occupied; and $(1 - e^{-m\alpha\theta})^n$, that at least one site of each of n centers is occupied. Thus $1 - (1 - e^{-m\alpha\theta})^n$ is the probability that at least one center of a given cell is not occupied, i.e., by V that the cell can form a green colony. Hence the expected fraction of green colonies,

$$G = 1 - (1 - e^{-m\alpha\theta})^n. \tag{1}$$

Note that

$$G = 1 - \sum_{j=0}^{n} \binom{n}{j} e^{-jm\alpha\theta}(1 - e^{-m\alpha\theta})^{n-j} \doteq ne^{-m\alpha\theta}$$

if θ is sufficiently large.

Photoreactivation

If a dose of θ ergs/mm^2 UV is given then the chance that a given site becomes occupied is $1 - e^{-\alpha\theta}$. If now a dose of Φ min PR is administered then by III the (conditional) probability that an occupied site does not interact with a photon of PR is $e^{-\frac{\beta\Phi}{\theta-\theta_0}}$, so that the chance of the compound event is $e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})$. Thus the probability of an unoccupied site after administration of the described doses of UV and PR is $1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})$ and of an occupied center is $1 - [1 - e^{-\frac{\beta\Phi}{\theta-\theta 0}}(1 - e^{-\alpha\theta})]^m$. It follows that the expected fraction of green colonies is given by

$$G = 1 - (1 - [1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})]^m)^n. \tag{2}$$

Decay of Photoreactivability

If a lapse of time, t, occurs between successive exposures to UV and PR, then according to IV the (conditional) probability that an occupied site is capable of interacting with PR is e^{-At}, and the corresponding unconditional probability is $(1 - e^{-\alpha\theta})e^{-At}$.

(*a*) If no cell division takes place during t, then the chance that the site will interact with PR and become unoccupied is $(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}})(1 - e^{-\alpha\theta})e^{-At}$. Adding to this the chance that the site did not interact with UV, that is, $e^{-\alpha\theta}$, we get the probability of an unoccupied site. The probability of an unoccupied center is $[e^{-\alpha\theta} + (1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}})(1 - e^{-\alpha\theta})e^{-At}]^m$ from which it follows that

$$G = 1 - (1 - [e^{-\alpha\theta} + (1 - e^{-\alpha\theta})(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}})e^{-At}]^m)^n. \tag{3a}$$

(*b*) If cell division takes place during t, then the argument in (*a*) must be modified. The probability that, following UV and a lapse of time t, a site is either unoccupied or is occupied and capable of interacting with PR is $e^{-\alpha\theta} + (1 - e^{-\alpha\theta})e^{-At}$. The corresponding probability for m sites is $[e^{-\alpha\theta} + (1 - e^{-\alpha\theta})e^{-At}]^m$. If from the latter quantity we subtract the chance that a center is unoccupied, $e^{-m\alpha\theta}$, we get the probability that a center is occupied and all of its sites are capable of interacting with PR.

Now by VI an occupied center does not divide so the chance that such a center will be passed on to a specified jth generation daughter cell is 2^{-j}, where $j = t/\tau$, and τ is the generation time. Hence the probability that a given jth generation cell will receive an occupied center all of whose sites can interact with PR is $2^{-j}[e^{-\alpha\theta} + (1 - e^{-\alpha\theta})e^{-At}]^m - 2^{-j}e^{-m\alpha\theta}$. Subtracting this quantity from the chance that the center is occupied, namely $1 - e^{-m\alpha\theta}$, we get the probability that the center is occupied and at least one of its occupied sites cannot interact with PR. Denoting the latter probability by Q and the corresponding probability for n centers by Q^n, the chance that at least one of the n centers in a jth generation cell can interact with PR is $1 - Q^n$. Hence after a saturating dose of PR

$$G = 1 - (1 - 2^{-\frac{t}{\tau}}[e^{-\alpha\theta} + (1 - e^{-\alpha\theta})e^{-At}]^m - (1 - 2^{-\frac{t}{\tau}})e^{-m\alpha\theta})^n. \tag{3b}$$

If θ is large then $G \doteq 1 - (1 - 2^{-\frac{t}{\tau}}e^{-mAt})^n = 1 - \exp(n \log_e (1 - 2^{-\frac{t}{\tau}}e^{-mAt})) \doteq 1 - \exp(-n2^{-\frac{t}{\tau}}e^{-mAt})$, the last approximation holding if $2^{-\frac{t}{\tau}}e^{-mAt}$ is small.

UV-PR-UV

There are two possible ways for a site to be unoccupied after this sequence of irradiation: (*a*) It is unaffected by either dose of UV or (*b*) it interacts with the first dose of UV, then with the PR, but not with the second dose of UV. The respective probabilities are $e^{-\alpha\theta} \cdot e^{-\alpha^*\theta^*}$ and $(1 - e^{-\alpha\theta})(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}})e^{-\alpha^*\theta^*}$. The chance that a site is unoccupied after UV-PR-UV is the sum of the latter two probabilities and is $e^{-\alpha^*\theta^*}(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta}))$, hence the expected fraction of green colonies is given by

$$G = 1 - (1 - [e^{-\alpha^*\theta^*}(1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta}))]^m)^n. \tag{4}$$

Another form of equation 4 can be obtained by straightforward algebra and is

$$\frac{1}{m}\log_e (1 - (1 - G)^{1/n}) = -\alpha^*\theta^* + \log_e (1 - e^{-\frac{\beta\Phi}{\theta-\theta_0}}(1 - e^{-\alpha\theta})) \tag{4'}$$

which is linear in θ^* (the second dose of UV) and has slope $-\alpha^*$. For the control curve,

$\theta^* = \Phi = 0$ and $\frac{1}{m} \log_e (1 - (1 - G)^{1/n}) = -\alpha\theta$ which is linear in θ (the first dose of UV) and has slope $-\alpha$.

As Φ becomes large the intercept, $\log_e (1 - e^{-\frac{\beta\Phi}{\theta - \theta_0}}(1 - e^{-\alpha\theta}))$ approaches zero so that $\frac{1}{m} \log_e (1 - (1 - G)^{\frac{1}{n}})$ approaches $-\alpha^*\theta^*$, or $G \to 1 - (1 - e^{-m\alpha^*\theta^*})^n$.

It is readily found that $-\log_e (1 - \exp (\text{intercept})) = \frac{\beta\Phi}{\theta - \theta_0} - \log_e (1 - e^{-\alpha\theta})$ hence plots of this function of the intercept will tend to be linear if equation 4 holds.

APPENDIX II

It is assumed that in *Euglena*:

(1) Photoreactivation is catalyzed by an enzyme, E, and proceeds at a rate which is proportional to the concentration of E.

(2) Exposure to UV produces a substance, I, in an amount proportional to $\theta - \tilde{\theta}$, where θ is the dose of UV and $\tilde{\theta}$ is a non-negative constant.

(3) The substance I combines with E to form an inactive complex, IE; the combining reaction obeys the law of mass action.

(4) The total amount of E, active and inactive, remains constant.

The inactivation reaction can be written $I + E \rightleftarrows IE$, and from assumption 3 it follows that

$$(I)(E) = K(IE) \tag{6}$$

where ( ) denotes concentration and K is an equilibrium constant. The volume of the system is fixed hence assumption 2 implies that $(I) = C_1[\theta - \tilde{\theta}]$ and assumption 4 implies that $(E) + (IE) = C_2$, where C_1 and C_2 are constants. Using these equalities to eliminate (I) and (IE) from equation 6 we get, after some reduction

$$(E) = \frac{C_2 K}{C_1[\theta - \tilde{\theta}] + K} = \frac{C_3\beta}{\theta - \theta_0}$$

where β is the cross-section from reactivation, C_3 is a constant such that $C_3\beta = C_2K/C_1$, and $\theta_0 = \tilde{\theta} - K/C_1$.

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