Dark-Recovery in a Petite Mutant of *Saccharomyces cerevisiae* Irradiated with Ultraviolet Light

Cellular recovery from radiation injury produced under conditions of low metabolic activity (liquid holding recovery) is an enzymatic process requiring an energy supply[1–3]. It has been shown that cellular respiration supplies the energy for recovery during the time of liquid holding in yeast cells[1,3]. For example, anoxic cells held in water after irradiation do not recover from the suffered injury, the process of recovery starting only under aerobic conditions[1]. Furthermore, no liquid holding recovery (LHR) could be demonstrated in yeast strains with a defective system of cytochromes (petite colony mutants)[4,5] when kept in water after irradiation[1,6]. On the other side, according to Korogodin[1], the ability of cells to sustain fermentation seems to play a certain, although still unclear, role in recovery. Many of the yeast strains investigated by this author recovered after irradiation only when they possessed both respiratory and fermentative functions and yeast that were obligatory aerobes, were usually unable to recover[1].

To explain the above mentioned facts, we adopted the following working hypothesis: a) the required energy for cellular postirradiation recovery may be supplied by respiration and/or fermentation during the time of LH. b) Fermentation alone may be able to supply the required energy only if an adequate substrate is available and the compounds of high phosphate group transfer potential are synthesized in quantities sufficient to support the mechanisms of molecular enzymatic repair. Part b of this hypothesis can be tested by submitting petite mutants to irradiation and incubating them in solutions of low glucose concentration, so that the expected LHR might occur.

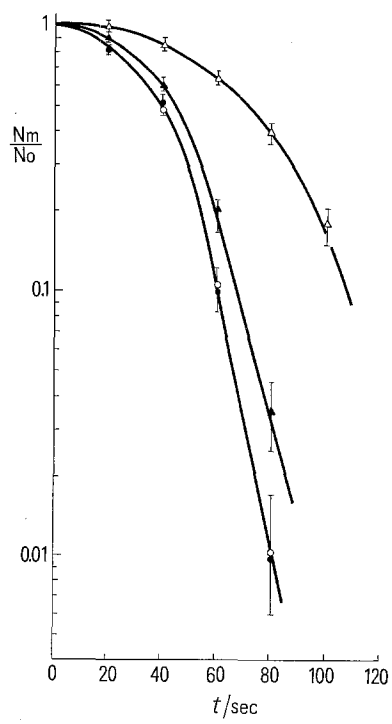

Fig. 1. Survival of petite strain of yeast (211-p) and normal diploid strain (211) after inactivation by UV-light. *t:* UV-exposure time (Dose rate = 30 ergs/mm²/sec). Survival observed on immediate plating: (●), strain 211-p; (▲), strain 211. Survival observed after 24 h of storage in distilled water at 25 °C in the dark (delayed plating): (○), Strain 211-p; (△), Strain 211.

Materials and methods. 1. Strains. Two diploid strains of *Sacharomyces cerevisiae,* 211[7] and 211-p, were used, the latter a spontaneous petite, isolated and identified as a mutant of the former[8,9]. 2. Culture and irradiation conditions. Media: a) Liquid growth medium (YED), 2% glucose and 0.5% yeast extract. b) Solid growth medium: YED plus 2% agar. c) Minimal media: solutions of different glucose concentrations (0.015–0.30%). Strains were kept on agar slants at 4 °C. Subcultures growing in YED were diluted after attaining the stationary phase to 4.10^5 cells per ml. Irradiation was performed on 5.0-ml aliquots from the cellular suspension in dishes of 5-cm diameter. The UV-source was a Philips, TUV, 15-W lamp (254-nm) and a constant flux of 30 ergs/mm²/sec was used. During irradiation cultures were stirred magnetically. Each irradiated sample and unirradiated controls were plated on nutrient agar and the remainder incubated in distilled water or in solutions containing glucose. All experiments were performed in the dark. Plating of samples incubated in glucose was delayed 2–24 h after irradiation. Surviving fractions were determined by counting visible colonies (diameter ≧ 0.1 mm), 4 days after plating. All experiments have been repeated at least 3 times. Figures correspond to one typical experiment. 95% confidence limits are shown.

Results. Figure 1 shows the survival to UV-light of strains 211 and 211-p. Cells of the wild type strain (211) are more UV-resistant than the petite mutant yeast and

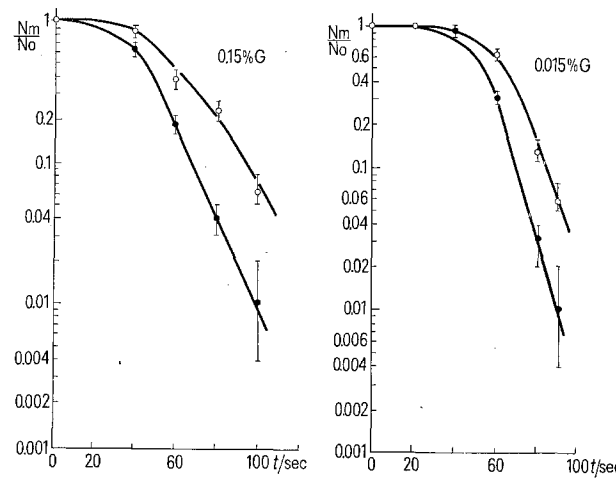

Fig. 2. Recovery of petite strain of yeast after exposure to UV-light and storage in solutions containing 0.15% glucose (left) and 0.015% glucose (right). (●), Survival observed on immediate plating; (○), survival observed on delayed plating.

[1] V. I. Korogodin, M. Meissel and T. Remesova, in *Radiation Research 1966* (Ed. G. Silini; North Holland publ. Co., Amsterdam 1967), p. 538.
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[3] M. H. Patrick and R. Haynes, Radiation Res. *23*, 564 (1964).
[4] G. Bernardi, M. Faures, G. Piperno and P. Slonimski, J. molec. Biol. *48*, 23 (1970).
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[6] J. T. Lyman, UCRL-16030 (1965).
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the storage in distilled water after irradiation increases their survival. The dose reduction factor, that is the ratio between doses giving the same survival is DRF = 1.6. The petite strain do not recover from radiation injury, even under conditions favoring recovery of normal strains. Figure 2 shows survival curves obtained under similar conditions to those for Figure 1, but in this case the petite population was incubated in 0.15 and 0.015% glucose, respectively for 24 h before plating on nutrient agar. The effect of LH on UV-irradiated petite cells is positiv. For 0.15% glucose, DRF = 1.4; for 0.015% glucose DRF = 1.2. To investigate the dynamic of this process, surviving fractions for different LH periods and for different doses of radiation were determined (Figure 3). The rates of recovery (slopes) depend on the dose and on the glucose concentration of the holding media. Unirradiated petite cells held in glucose solutions (0.015–0.30%) do not exhibit increase in colony forming cells over the 24-h holding period. However, bud initiation, which corresponds with DNA synthesis in yeast[10], takes place 10–14 h after incubation in solutions containing 0.30% glucose, each budding cell still giving one colony, as verified by microscopic observations. In irradiated cells, bud initiation occurs 13–17 h after incubation in 0.3% glucose, depending on the absorbed dose. The ratio of survival at $t = 24$ h and $t = 0$ h[11] is defined as the recovery index: FR = $(N_m/N_0)_{t=24\,h}$: $(N_m/N_0)_{t=0h}$. FR increases with the dose (Figure 4). Maximum recovery is attained by a glucose concentration of 0.15%.

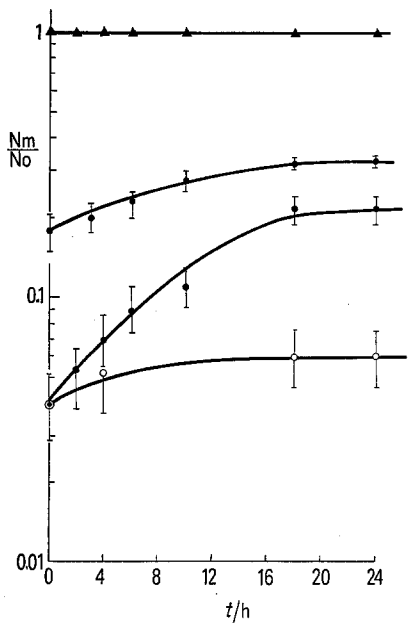

Fig. 3. Surviving fractions after storage for different periods in various glucose concentrations: (▲), unirradiated cells, 0.15% ≦ (G) ≦ 0.30%; irradiated cells: (●), t = 80 sec, (G) = 0.15%; (●), t = 60 sec, (G) = 0.15%; (○), t = 80 sec, (G) = 0.30%. (t/h: time of LH in h).

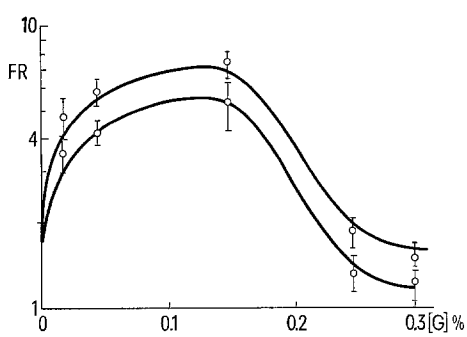

Fig. 4. Recovery index (FR) as a function of glucose concentration of the storage solution. Upper curve shows FR for a surviving fraction after exposure and immediate plating N_m/N_o = 0.010. Lower curve shows FR for N_m/N_o = 0.03.

Discussion. The repair mechanisms require the expenditure of energy. In petite yeast cells, recovery does not occur in distilled water, contrary to findings in normal cells. In these the main substrate of endogenous respiration produced during post-irradiation incubation in distilled water are the reserve polysaccharides, primarily glycogen, which supplies the energy for recovery[1]. In cells with respiratory deficiency, an energetic substrate must be added to the incubation medium in order to enable recovery, the substrate concentration being critical (Figures 2 and 4). For low glucose concentrations in the storage medium (0.015% ≦ (G) ≦ 0.15%), the synthesis of high energy compounds necessary for repair is fulfilled through fermentation. The fraction of recovering cells reaches a maximum for a glucose concentration (G) = 0.15% (Figure 4). The lower recovery verified by cells held in higher glucose concentrations could be explained by a decrease in the time available to excision repair, which takes place before DNA replication[12]. At high glucose concentrations the control of glycolysis is shifted from phosphofruktokinase to pyruvate kinase (PK). High concentrations of ATP, FDP[13] and PEP[13] can lead to inhibition of PK[14]. According to JAIN's[15] hypothesis, this could reduce the duration of G_1 phase by permitting a faster synthesis of reserve carbohydrates required as intracellular energy reservoirs for DNA replication. In fact, budding begins after 10–14 h incubation of the cells in 0.30% glucose and after 26–30 h incubation in lower glucose concentrations (0.15% ≦ (G) ≦ 0.20%). No further cell recovery is observed after bud initiation, as indicated by the plateau in the curve corresponding to (G) = 30%, UV-exposure time = 80 sec (Figure 3). The reduced substrate flow in the glycolytic pathway at higher glucose concentrations could explain the lower recovery rate by the irradiated cells (compare slopes, Figure 3), but it could also be explained in terms of glucose repression on the repair metabolic pathway(s) acting during LH. More experiments on the regulation of glucose metabolism in petite strains of yeast are in course in our laboratory in order to clarify this question. It is noteworthy that the rates of recovery vary with the UV-dose, being lower for lower doses (Figure 3), which does not occur in recovery from X-rays injury[1,16].

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[16] This work was partially supported by the International Atomic Energy Agency (Research Contract No. 785 RB) and by the Fondo de Investigacion Cientifica de la Universidad de la Republica (Uruguay).

Zusammenfassung. Erholung nach UV-Bestrahlung wurde bei diploiden atmungsdefekten Hefezellen nach Zugabe eines Energiesubstrats (Glukose) im Aufbewahrungsmedium festgestellt. Die Erholungsraten hängen von der UV-Dosis und von der Glukose-Konzentration im Aufbewahrungsmedium ab. Ein Erholungsmaximum wurde bei einer Glukose-Konzentration von 0.15% beobachtet.

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Einfluss einiger lipophiler Lösungsmittel in gasförmigem Zustand auf die CO_2-Fixierung durch Luzerne

Die seit 20 Jahren vermehrt in der Atmosphäre auftretenden chemischen Verbindungen wurden eingehend auf ihre Wirkung gegenüber Mensch[1], Tier und Pflanze[2] untersucht. Dabei wurde das Hauptaugenmerk auf solche Substanzen gerichtet, die auf Grund ihrer chemischen Reaktivität einzelne Schritte des menschlichen[3], tierischen und pflanzlichen[4] Stoffwechsels hemmen oder modifizieren und so eine mehr oder minder grosse Schädigung hervorrufen. Weniger dringlich erschien die Untersuchung solcher Verbindungsklassen, die durch physikalisch-chemische Wechselwirkung lebende Organismen beeinflussen, da hierbei die Konzentration, um eine signifikante Wirkung hervorrufen zu können, erheblich über dem «normalen» Luftverschmutzungsniveau liegen dürften.

In dieser Arbeit sollen Versuche beschrieben werden, die zeigen, dass auch – unter Normalbedingungen – chemisch inerte Verbindungen das pflanzliche Leben beeinflussen können, insbesondere den Photosyntheseapparat, dessen Funktionstüchtigkeit eine wesentliche Voraussetzung für die Entwicklung und Lebensfähigkeit der Pflanze darstellt.

Jeweils 5 Blätter von Luzerne (*Medicago sativa*) gleichen Alters und vergleichbarer Grösse wurden nach einer Präilluminierungsphase von 5 min in einer Kammer mit $^{14}CO_2$ (0.05–0.1 Vol % in der Luft, spez. Aktivität 45 mCi/m*M*) und den in Tabelle I angeführten Substanzen mit unterschiedlicher Konzentration in der Gasphase jeweils 1 min inkubiert. Die Gesamtaufnahme an $^{14}CO_2$ durch die einzelnen Blätter wurde mit einem Endfensterzählrohr bestimmt.

Die Werte in Tabelle I zeigen, dass die Photosyntheseaktivität durch die der Atmosphäre zugesetzten Substanzen gehemmt wird. Die Hemmwirkung hängt deutlich von der Wasserlöslichkeit der einzelnen Verbindungen ab. Während Äther auch in hohen Konzentrationen fast keinen Einfluss auf die CO_2-Aufnahme ausübt, reduziert das praktisch in Wasser unlösliche n-Octan schon bei einer Konzentration von 1 Vol% die CO_2-Fixierung etwa um 35%.

In einem Parallelversuch wurden Blätter nach einer Inkubationsdauer von 1 min mit heissem, wässrigem Äthanol (80% und 20%) extrahiert. Die im Stickstoffstrom eingeengten und auf 500 µl aufgefüllten Extrakte wurden papierchromatographisch zweidimensional aufgetrennt (Whatman Nr. 1, 1. Dimension Propionsäure:n-Butanol:Wasser = 142:284:200, v/v/v, 2. Dimension Äthanol : 1 m Ammoniumacetat = 7:3, v/v) und autoradiographiert. Dabei zeigte sich, dass das Verteilungsmuster der Photoysntheseprodukte bei zunehmender Konzentration der der Atmosphäre jeweils zugesetzten Verbindung gleichmässig abnimmt. Eine Ausnahme stellt Schwefelkohlenstoff dar. Hier bleibt ein markiertes Fixierungsprodukt in scheinbar unverminderter Intensität übrig, auch dann noch, wenn die CO_2-Aufnahme auf weniger als 20% des Ausgangswertes abgesunken ist, was

[1] J. R. GOLDSMITH, in *Air Pollution*, 2nd edn. (Ed. A. C. STERN; Academic Press, New York, London 1968), vol. 1, p. 547.

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Tabelle I. $^{14}CO_2$-Aufnahme durch Luzerne-Blätter während einer Minute in Abhängigkeit von der Volumenkonzentration der der Atmosphäre zugesetzten chemischen Verbindungen

Zugesetzte Verbindung (Vol %)	Diäthyläther (7.5/20 °C)[a]	Methylenchlorid (2.0/20 °C)[a]	Schwefelkohlenstoff (0.22/22 °C)[a]	Benzol (0.082/22 °C)	n-Octan (0.0015/16 °C)[a]
0	0.0602	0.0616	0.0660	0.0579	0.0688
1	0.0536	0.0594	0.0822	0.0520	0.0445
11	0.0580	0.0482	0.0340	0.0116	0.0237[c]
21	0.0516	0.0114	0.0100	0.0060[b]	—

Die CO_2-Aufnahme ist in µ*M* gemessen und bezogen auf 1 mg Blattgewicht [a] Wasserlöslichkeit in g/100 ml Wasser bei der angegebenen Temperatur (°C). [b] Für die Sättigungskonzentration von 17 Vol%. [c] Für die Sättigungskonzentration von 2.7 Vol%.