ments, immunochemical studies in the frog [M M. Dewey, P. K. Davis, J. K. Blaise, L. Barr, *J. Mol. Biol.* **39**, 395 (1969)] showed that they also occur in isolated saccule membranes, in the myoid and ellipsoid of the photoreceptor inner segment, and in inclusions in the overlying retinal epithelium.

8. M. Kaitz, *Vision Res.* **16**, 151 (1976).
9. Animals had been reared in the dark since birth, with exposure to dim red illumination as necessary during changing of water bottles, and so forth. To maintain dark adaptation during surgery, a dim red light was used and, in addition, an opaque contact lens was temporarily placed over the experimental left eye. For further details of the procedures, see C. M. Cicerone and D. G. Green, *Vision Res.* **17**, 985 (1977).
10. The new congenic strain of RCS rats that is of the wild type at the retinal dystrophy genetic locus and is the best available genetic control for the pink-eyed, retinal dystrophic strain was not available to us at the time of this study. The control albino animals we used were free of the retinal dystrophy and hence are called "normal."
11. All data reported here were obtained under dark-adapted conditions. We have observed a Purkinje shift with light adaptation in the response of the normal rat ganglion cell axon (C. M. Cicerone, in preparation).
12. Certain units recorded from animals near 5 months of age were so insensitive that determining spectral sensitivity was not possible, for light was lost when narrow-band interference filters were inserted. Other units were best fit by nomogram curves with peaks different from 500 or 520 nm. Although the histology showed remaining rod nuclei (which we carefully tried to distinguish from pycnotic nuclei), none of the units encountered and classified at 5 months of age showed a rhodopsin spectral sensitivity. We are led to two alternatives. (i) The rhodopsin units may have been those too insensitive to allow a determination of spectral sensitivity. (ii) The process of light transduction by rods and cones may differ so that, despite the loss of outer segments, cones may be capable of responding to light, but rods without outer segments may be totally incapacitated.
13. Eyes were enucleated, lentectomized, and immersed in a fixative containing 2 percent formaldehyde, 3 percent gluteraldehyde, 1 percent acrolein, and 2.5 percent dimethyl sulfoxide in a 0.1*M* sodium cacodylate buffer at *p*H 7.2. The tissue was postfixed in 2 percent osmium tetroxide in the same buffer, strained in 0.5 percent uranyl acetate in maleate buffer of *p*H 5.8, dehydrated through alcohols, and embedded in Epon. Sections 1 μm thick stained with 0.1 percent methylene blue in 1 percent borax were used for all light microscopy. In normal animals, the cone nuclei were easily identified by their larger size, lighter nucleoplasm, position in the outer third of the outer nuclear layer, and characteristically clumped chromatin (*6*). Identification was more difficult in the RCS animals. Some nuclei exhibited the distinctively clumped pattern and lighter nucleoplasm of the normal animals but were somewhat smaller than normal cone nuclei. Other nuclei were microglia-like, and still others were smaller and contained a single mass of darkly staining chromatin within the nuclear membrane. This last class of nuclei resemble rod nuclei but also may be a stage in the degeneration of either rod or cone nuclei. Only the conelike chromatin patterns were counted as cone nuclei for this report. Microglia-like nuclei were not reported. The third class were counted as rodlike, but their identification is tentative. In any event, the numbers reported are the upper limit for rodlike nuclei in the RCS animals.
14. Anatomical [L. D. Carter-Dawson, M. M. LaVail, R. L. Sidman, *Invest. Ophthalmol. Visual Sci.* **17**, 489 (1978)] and physiological [U. C. Dräger and D. H. Hubel, *J. Comp. Neurol.* **180**, 85 (1978)] research on the visual system of mice with hereditary retinal degeneration has been reported. These studies are closely related to our work in the RCS rat and point to a similar pattern of visual decline in mice with hereditary retinal degeneration.
15. W. Noell has suggested that the plasma membrane of cone cells may house visual pigment and that this store of pigment is the basis for visual capacity in the aged RCS eye.
16. Supported by NIH grants EY02055 to C.M.C., EY00379 to D.G.G., and EY01281 to L.J.F.

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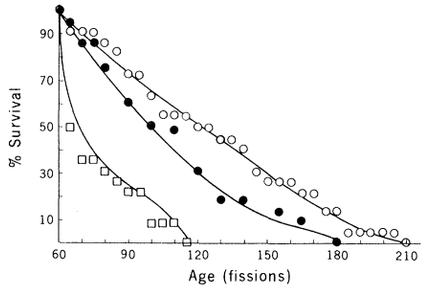
DNA Repair and Longevity Assurance in *Paramecium tetraurelia*

Abstract. *At given doses and clonal ages, ultraviolet irradiation-induced DNA damage reduced clonal life-span, but when followed by photoreactivation, extension of clonal life-span was observed. If photoreactivation preceded the ultraviolet treatment, no significant beneficial effect was detected. Because studies of others have shown that photoreactivation repair monomerizes the ultraviolet-induced cyclobutane dimers in DNA, but does not affect the other photoproducts, these results indicate that DNA damage can influence the duration of clonal life-span unless that damage is repaired. Repeated treatment with ultraviolet and photoreactivation resulted in significant mean and maximal clonal life-span extension when compared with untreated controls, and it is assumed that the rejuvenation effect was due to the correction or prevention of some age damage.*

Paramecia were used to study the biological effect of ultraviolet-induced DNA damage versus photoreactivation (PR)-repaired damage on clonal senescence. These cells exhibit cellular aging (*1*), show age-correlated sensitivity to ultraviolet reversible by PR (*2*), have been shown to monomerize induced dimers by PR in their nuclear DNA (*3*), express age-induced mutations (*4, 5*) suggesting loss of repair with increased age, and have many parallels with human cells in culture (*4, 6*). Clonal senescence can be characterized by a decreased probability that a given cell will give rise to a viable cell at the next cell division (*1, 7*). As in multicellular organisms, fertilization marks the origin of a new generation, and predictable changes occur in the phenotypes of cells (*1, 2*). Death of the clone occurs some 150 to 200 cell divisions, or fissions, later—in about 40 days, when the procedures described below are used.

Aging cells were maintained in daily isolation lines (*8*). Replicate samples of the fertilized cells were carried as sublines. Each day, one cell of a subline was passed seriatim to a new depression; its products were counted on the following day, and the daily fission rate was determined. A subline is considered dead when an isolated cell disappears. The fission age of the cell is the number of fissions since fertilization. The mean clonal life-span is the average fission age at death of all sublines. Maximal life-span is the largest fission age observed for any subline of a clone at death. Isolation lines provide the source of cells for the controls, treatment with ultraviolet only, ultraviolet plus PR, PR plus ultraviolet, and PR only (*9, 10*).

As the fission age of the clone increased, the ultraviolet dose required to reduce the mean clonal life-span decreased; 5400 ergs at cells 40 fissions old versus 2700 ergs at cells 140 fissions old (Table 1). At critical doses and ages, a negative shift in the survival curve was



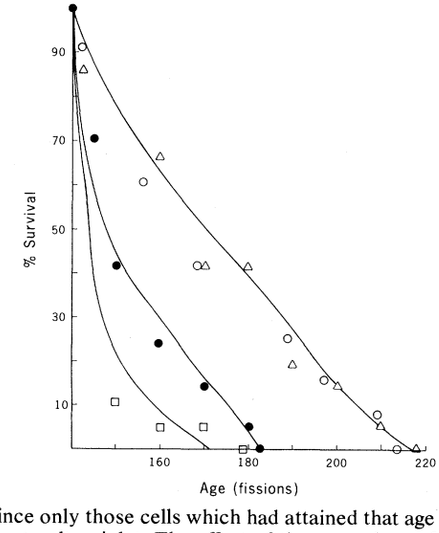


Fig. 1 (left). Effect of ultraviolet only and ultraviolet plus photoreactivation on clonal life-span. At 60 fissions, one member of a dividing cell was given ultraviolet only at 5400 erg/mm^2 (squares), the other cell member was given ultraviolet plus photoreactivation (open circles). Control cells (closed circles) were taken from the same population. The ultraviolet treatment was given 1^1/$_2$ hours after cell division. The experiment starts at 100 percent survival since only those cells which had attained that age were used. Fig. 2 (right). Induced resistance to ultraviolet. The effect of the same dose of ultraviolet (2700 erg/mm^2) on clonal life-span varied when cells 140 fissions old were previously untreated (open squares) or had received ultraviolet plus photoreactivation when 80 fissions old (open triangles). The respective untreated controls (closed circles) and control cells which received ultraviolet plus photoreactivation when 80 fissions old (open circles) are included.

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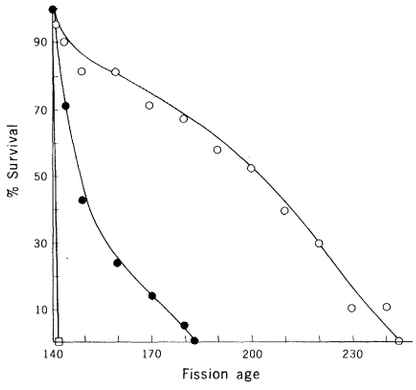

Fig. 3. Life-span extension. The normal decline in survival of untreated controls (closed circles) and their sensitivity to 3600 erg/mm² ultraviolet only (open squares) is compared with the extended clonal life-span of cells receiving their second dose of ultraviolet plus photoreactivation at 140 fissions old (open circles). The first ultraviolet plus photoreactivation treatment was given at 80 fissions.

observed (Fig. 1). When PR preceded ultraviolet, no beneficial effect was noted, but when the treatment order was ultraviolet plus PR, the clonal life-span reverted to control values or surpassed them (Table 1). In 11 of 13 trials, the mean clonal life-span of the ultraviolet plus PR groups exceeded that of controls. The probability of such an occurrence by chance selection is very small or .0113 (*11*). When cells 140 fissions old were treated with an ultraviolet dose of 2700 ergs, the percentage of survival was significantly reduced compared to controls. However, when cells of the same fission age, first treated with ultraviolet plus PR were used, no reduction in survival was detected (Fig. 2). Induced resistance to ultraviolet by ultraviolet plus PR was not found when young cells had received prior treatment with ultraviolet plus PR (Table 1).

Mean and maximal clonal life-span extension was found when the ultraviolet plus PR treatment was given a second time to old cells (Table 1; Figs. 3 and 4). The mean clonal life-span was 194 fissions compared to 153 for the treated and untreated groups, respectively. The difference is significant according to the very conservative Scheffé statistic (*11*), and represents a 296 percent increase in remaining clonal life-span or a 27 percent increase in the entire clonal life-span affected by two treatments of ultraviolet plus PR. The eventual deterioration of the clone occurred, although at a significantly advanced fission age (Figs. 3 and 4). The life-span extension of the clone was also noted in calendar days as a 50 percent increase since the longest lived subline lived 3 weeks longer than any untreated control subline.

The observation that ultraviolet-induced damage can reduce clonal life-span when unrepaired, but not when repaired by PR, demonstrates that DNA damage can contribute to clonal life-span determination. The unexpected extension of clonal life-span by ultraviolet plus PR treatment can be hypothesized to be due to repair of age-induced DNA damage since (i) prior treatment with ultraviolet plus PR conferred resistance to another ultraviolet treatment for a dose detrimental to controls of the same age and (ii) extension of life-span could only be

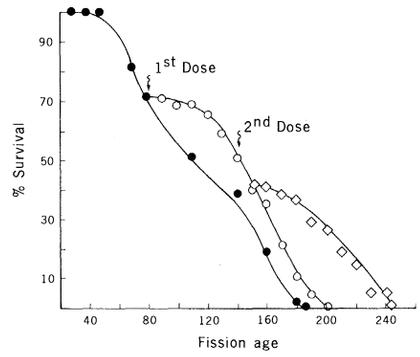

Fig. 4. Effect of ultraviolet plus photoreactivation on clonal life-span. The clonal life-span of the untreated controls (closed circles) is compared with the effect of the first ultraviolet plus photoreactivation treatment at 80 fissions (open circles) and the second ultraviolet plus photoreactivation treatment at 140 fissions (open squares) on survival and maximal life-span. The percentage of survival was corrected for the percentage of sublines within the clone still viable. For example, at 80 fissions, only 72 percent of the sublines were viable. Thus all survival values for cells at that fission age were multiplied by 0.72 to permit direct comparison of treatment effects throughout the clonal life-span.

dramatically detected in older cells. Accumulation of age damage seemed to be a prerequisite for a beneficial effect of the treatment. The hypothesis is that ultraviolet induces a repair process and PR corrects the ultraviolet-induced damage, freeing the induced repair for correction of age damage. The mechanism for this effect was not studied; the important finding is that age damage seems to be reversed or delayed by some direct or indirect mechanism. Treatment with ul-

Table 1. Effect of ultraviolet (UV) and photoreactivation (PR) on mean clonal life-span. Comparison of absolute mean values must be made only within a given age group. At an older age, the experimental group contained only cells that had achieved that age. The means for the older groups are therefore higher and show less variations. In experiments 1, 2a, and 3a untreated cells were used and in experiments 2b and 3b cells that had received prior ultraviolet plus photoreactivation treatment were used.

Experiment*	Age when expanded (fissions)	UV dose (erg/mm²)	Mean life-span (fissions)†			Mean life-span	
			UV only	PR + UV	UV + PR	Control	PR only
1	40	5400	↓ 90.4 ± 18.4	99.7 ± 18.4	127.7 ± 23.9‡	114.5 ± 20.6	129.0 ± 18.4
		4500	108.7 ± 18.4	96.7 ± 21.3	130.5 ± 19.4		
		3600	125.8 ± 20.4	113.9 ± 21.3	125.6 ± 20.0		
2a	80	5400	↓ 80.0 ± 0	↓ 80.0 ± 0	131.9 ± 12.8	143.1 ± 12.6	148.4 ± 14.0
		4500	↓ 109.5 ± 10.7	↓ 100.9 ± 12.4	153.5 ± 12.3§		
		3600	140.6 ± 12.8	↓ 97.6 ± 12.4	147.6 ± 12.1		
2b‖	77	5400	↓ 77.0 ± 0	↓ 95.4 ± 16.2	137.4 ± 12.5		138.3 ± 12.8
3a	140	3600	↓ 140.0 ± 0	↓ 140.0 ± 0	158.9 ± 8.7	153.0 ± 8.5	158.0 ± 7.9
		2700	↓ 143.0 ± 0	↓ 140.0 ± 0	164.7 ± 8.5		
		1800	158.2 ± 8.3	160.2 ± 8.9	163.4 ± 7.9		
3b‖	140	3600	151.1 ± 15.6	163.8 ± 14.5	↑ ↑ 194.3 ± 12.8		169.4 ± 11.9
		2700	↑ 172.6 ± 12.8	↑ 176.2 ± 14.5	↑ 173.0 ± 18.9		
		1800	162.5 ± 12.9	163.7 ± 14.0	↑ 185.1 ± 16.3		

*The total number of sublines used was: experiment 1, 195; experiment 2a, 203; experiment 2b, 105; experiment 3a, 228; experiment 3b, 181. The lowest sample size used was 11 per experimental group. †The mean life-span values are presented with the mean 95 percent confidence interval. When sublines died within zero to a few fission posttreatment, the confidence interval was essentially zero. ↓ Indicates that the mean life-span value is significantly lower than the control mean life-span and ↑ indicates that the mean life-span value is significantly greater than the control mean life-span using analysis of variance comparison of means. The level of significance was 95 percent except the 90.4 ± 18.4 value which was significant at the 90 percent level. ↑ ↑ Indicates that this mean life-span value is significantly greater than the control mean life-span according to the conservative Scheffé procedure for simultaneous inference (*11*). ‡Cells from these sublines were used to initiate UV + PR sublines used in experiment 2b. §Cells from these sublines were used to initiate UV + PR sublines used in experiment 3b. ‖The mean life-span value for the UV + PR source cell group receiving no additional treatment was 138 ± 11.9 at 77 fissions, and 168 ± 12.5 at 140 fissions.

traviolet plus PR favored a repair process beneficial to survival. Either age damage was corrected at the time of treatment and was inherited as reduced levels of DNA damage in progeny cells, or the treatment activated a process that persisted for some cell divisions after treatment.

Evidence is available that DNA damage accumulates in aged cells of higher organisms because of defective repair capacity (*12*). Mechanisms proposed for reduced repair in aged cells include decreased levels of DNA polymerases, loss of fidelity of DNA polymerase, or changes in the DNA-chromatin complex, reducing the accessibility of DNA lesions to repair (*13*). Ultraviolet plus PR may function to counteract such age changes in repair, or interact with some yet unknown contributor to repair capacity. Differences in repair capacity correlate with organismal life-span (*14, 15*). Cell life-span may be a reflection of the genetic needs for mutation frequencies (*16, 17*), and thus regulation of repair becomes a fundamental life maintenance and aging mechanism. Multicellular organisms can be expected to conserve the aging and life maintenance processes of eukaryote cells, since the evolution of multicellular organisms was relatively short in comparison to the long period of evolution of eukaryotes (*17*). Different cells within the same organism have different repair capacities (*13, 18*). If cell life-span determines organismal life-span (*6*), the cells with the shortest life-span (the weakest link) will determine the life-span of the whole organism, if it is assumed that the cells are essential for survival and cannot be replaced. The weakest link can be expected to differ in different species. The symptoms of aging manifested by consequences of loss of function of a particular organ should be diverse (*19*). The above results provide an explanation for the findings that organismal life-span is correlated with repair capacity of cells (*14, 15*). Exceptions would be expected because of the complexity of the repair processes, variants within species, and species whose life-span is not determined by cellular mutational deterioration. DNA damage is linked not only to clonal senescence but also to carcinogenesis (*20*). Ultraviolet-induced damage resulted in tumorigenesis in fish but not when the damage was repaired by photoreactivation (*21*).

Accordingly, age damage can be reversed or delayed. An understanding of the molecular mechanisms underlying this biological result could illuminate other means to activate the repair system. If higher organisms have maintained a reserve repair capacity, activation should lead to reduction in mutagenesis and degenerative diseases in higher organisms. The results provide a new approach for regulation and reduction of mutation frequency: the activation of a reserve repair or protection process.

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8. Daily isolation lines were initiated with a single fertilized cell in a separate depression. The next day, the number of cells was counted and one passed to a new depression. The daily fission rate was determined by the $\log_2$ of the number of cells derived from a single cell. The procedure of counting and reisolation is repeated daily and expanded to several sublines when desired. The genetic markers used to monitor aging lines for maintenance of the heterozygous phenotype in experiments 1, 2, and 3 included malate dehydrogenase codominant alleles (T. Williams and J. Smith-Sonneborn, unpublished) and trichocyst discharge ability (kindly supplied by D. Nyberg, University of Illinois). (Those sublines without the heterozygous phenotype were eliminated since they were interpreted to be lines which underwent illicit autogamy.)
9. Ultraviolet was given to synchronized cells $1^1/_2$ hours after cell division in microdrops of food diluted immediately after treatment with fresh food. Single cells alive the following day were maintained in sublines and carried in isolation lines.
10. A germicidal lamp emitting mainly at 254 nm supplied the ultraviolet at a rate of 180 to 540 J/m^2. Photoreactivation was as described in (*2*) and (*4*).
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Polyoma Virion and Capsid Crystal Structures

Abstract. *X-ray diffraction shows that complete virus particles and empty capsids crystallize isomorphously. The surface morphology of the protein coat, as revealed by electron microscopy, is the dominant structural feature determining the intensity of x-ray reflections to a resolution of approximately 30 angstroms. The structure and variability of the viral chromatin core can now be analyzed by comparison of electron density maps.*

Polyoma virus DNA, and that of the similar tumorogenic simian virus 40, is packaged inside an icosahedral capsid as a supercoiled, circular, double-stranded molecule that is complexed with cellular histones (*1*). Infected cells also produce empty, noninfectious capsids whose surface morphology is very similar to that of the complete virion (*2*). The capsid is built of 72 morphological units arranged on an icosahedral surface lattice with triangulation number T = 7 (dextro) (*3*). Polyoma virions and capsids have previously been crystallized from ammonium sulfate (*4*). Using sodium sulfate, we have now obtained large, single crystals suitable for x-ray structure analysis.

Sodium rather than ammonium sulfate was chosen for comparative electron microscopic studies of glutaraldehyde-fixed crystals to avoid reaction with ammonia. Glutaraldehyde fixation also proved convenient for neutralizing the infectivity of the virion crystals used for studies in our x-ray laboratory, which does not yet have facilities for virus containment. Purified virions and capsids (*5*) were crystallized by the hanging drop method (*6*) from half-saturated sodium sulfate. Rhombic dodecahedral crystals (the same form obtained from ammonium sulfate) grow up to 0.5 mm across in about 1 week. Large crystals ($\lesssim$1 mm) often have highly stellated vertices. Virion crystals have better-developed faces and sharper edges than capsid crystals.

X-ray precession photographs (Fig. 1, a to c) show that the particles in the crystals are arranged on a body-centered

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