synthesis occurred was obtained by using cells grown in nonradioactive media and incubating them in the media containing [^{14}C]glutamine and no amino acid; no significant amount of glutamine was incorporated into the cellular protein.

Freeze-fracture of cells incubated under these conditions for 2, 4, and 6 hours showed that there was a "dilution" of the number of particles in the membranes similar to that observed with puromycin. Membranes incubated under similar conditions in the presence of amino acids and tryptose resembled Fig. 2a, in which no dilution is observed. In addition, centrifugation in sucrose density gradients showed that the membranes from cells incubated in the absence of amino acids were less dense than those from cells incubated in the presence of amino acids. When cells incubated for 4 hours in a medium without amino acids, where extensive dilution of particles was observed, were transferred to a medium containing amino acids and tryptose, there was a reappearance of particles after 3 hours resembling that of Fig. 2a. These experiments support the hypothesis that these particles are protein.

The demonstration that particles observed in freeze-fractured membranes are most probably protein raises the question of the function of these protein particles. There is evidence (*7*) that deoxyglucose transport is mediated by proteins, probably consisting of subunits, which permanently traverse the membrane lipid bilayer; this suggests that at least some of the protein particles may be involved in substrate transport.

In support of this, active transport of the nonmetabolizable sugar [^{14}C]-deoxyglucose in cells incubated in the presence of puromycin was reduced by 50 percent at 4 hours and 85 percent at 6 hours, compared to controls. These transport results were not due to "leakiness" caused by the puromycin; there were no differences in the amounts of glycerol retention by puromycin-treated cells and untreated controls that were loaded with [^{14}C]glycerol, which accumulates in *A. laidlawii* by simple diffusion (*9*), and washed with cold buffer.

Since other membrane proteins, in addition to particles, are also affected under the conditions of our experiments, it cannot be stated with certainty that the particles are transport proteins; further studies are needed. However, from the evidence (*7*) and the demonstration of a reduced rate of deoxyglucose transport in puromycin-treated cells, it is tempting to suggest that at least some of these protein particles in *A. laidlawii* may be involved in substrate translocation across the cell membrane.

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Photoreactivation of Ultraviolet-Induced Chromosomal Aberrations

Abstract. *Ultraviolet induces only chromatid-type aberrations in synchronized G_1 V-79 Chinese hamster and A8W243* Xenopus *tissue culture cells. Posttreatment with white light prevents expression of most potential aberrations in the A8 toad cell, which possesses a photoreactivation enzyme. We conclude that the major ultraviolet-induced DNA lesion leading to chromosomal aberrations is the pyrimidine dimer.*

Although the induction of chromosomal aberrations by ionizing radiation has been extensively investigated, there have been few studies of the induction of aberrations by ultraviolet light (UV), largely because of the difficulty of getting this radiation into the nuclei of cells of the classical plant materials (*1*). Study of aberration induction by UV is attractive because of the specific information available about the nature of UV-induced DNA lesions and their repair (*2*), and the possibility that it might provide additional information about eukaryote chromosome structure. Humphrey *et al.* and Chu (*3*) have investigated the production of chromosomal aberrations by UV in Chinese hamster tissue culture cells as a function of stage in the cell cycle. Both papers reported the production of aberrations by UV in the nucleic acid absorption range of 240 to 265 nm, and also stated that both the chromosome-type aberrations characteristically produced by ionizing radiation in cells in the pre-DNA-synthetic (G_1) phase and the chromatid-type aberrations produced in those in the DNA-synthetic (S) and postsynthetic (G_2) phases of the cell cycle could be induced by UV. Chu furthermore reported that production of UV-induced chromosomal aberrations could not be photoreactivated in the hamster cells by treatment with light in the 330 to 400 nm range.

It was subsequently discovered by Regan and Cook (*4*) that although cells from placental mammals lack any photoreactivating enzyme (PRE) activity, cells from nonmammalian vertebrates do possess demonstrable PRE activity capable of repairing UV-induced cyclobutane dimers in DNA. We were thus led to reinvestigate the question of whether eukaryote chromosomal aberration production by UV is photoreactivable in a nonmammalian vertebrate tissue culture cell line having demonstrable PRE activity. Our first experiments produced such unexpected and seemingly conclusive results that we report them here, even though our investigations are as yet incomplete.

Both a clonal derivative, A8W243, of a cell line from the amphibian *Xenopus laevis* and the V-79 Chinese hamster cell line were used for different experiments. The culture methods employed and the capacity of the A8W243 cells, but not the V-79 cells, to photoreactivate UV-induced loss of reproductive integrity have already been reported (*5*). The cell lines were synchronized by a modification of the mitotic harvest method of Terasima and Tolmach (*6*). Cell cycle analyses were done by pulse labeling with tritiated thymidine and autoradiography. Metaphases were collected with Colcemid (Ciba) and cytological preparations were made by the techniques widely employed in mammalian cytogenetics: hypotonic treat-

ment of suspended cells followed by fixation in methanol–acetic acid (3 : 1), air drying of the fixed cell suspension onto slides, and staining in Geimsa. Both cell lines yield preparations quite satisfactory for aberration analysis. The A8W243 cell line has a relatively stable karyotype with chromosome numbers of 36 to 38 in virtually all cells. The V-79 line is characterized by a stable "quasi-diploid" karyotype with 22 chromosomes. All aberration scoring was done by one observer employing standard techniques (*1*).

In our first experiments A8W243 cells were collected in mitosis and seeded into plastic petri dishes (Falcon). The mitotic index for these samples ranged from 0.93 to 0.97. Two fractions were exposed to about 115 erg/mm^2 2 hours after they were planted, and one of these was kept in the dark while the other was immediately given 2 hours of photoreactivation. The exposures were made as has previously been described (*5*) except that two Sylvania daylight tubes were used instead of one General Electric BLB "black light" tube and one Sylvania daylight tube. Mitotic index was monitored by periodic fixation of test dishes. In the dishes treated with UV alone, the mitotic index remained below 0.001 until about 35 hours after irradiation. It then increased slowly to a maximum of about 0.012 at 55 to 60 hours, before falling again. As the control interdivision interval is approximately 24 hours, this represents a severe G_1-induced mitotic delay. In the photoreactivated samples the mitotic index remained below 0.001 for about 24 hours before rising to a maximum of 0.035 at about 34 hours and then falling again, thus indicating a marked photoreactivation of the UV-induced division delay. Suspensions of cells from the groups treated with UV alone were treated with Colcemid at intervals and then fixed periodically during the duration of their peak of mitotic activity. Control and photoreactivated cultures were treated with Colcemid over the duration of their peaks of mitotic activity and then fixed at 36 and at 38 hours, respectively. The results from a typical experiment are shown in Table 1. Since the aberration yields do not appear different for samples fixed at different times during the mitotic index peak for cells treated with UV alone, and since similar results were obtained in several other experiments, the individual samples for the experiment shown in Table 1 are pooled.

It will be seen that while there is no suggestion that *chromosome*-type aberrations were induced by the UV irradiation, *chromatid*-type aberrations were plentiful. It should be noted, however, that the isochromatid deletion class includes some with no sister strand reunion; on strictly morphological grounds these could equally well be chromosome-type deletions. We have arbitrarily placed them in the isochromatid class because of the large yields of chromatid deletions and chromatid exchange aberrations and the lack of any chromosome-type exchange aberrations.

Table 1 also shows that posttreatment with white light is able to eliminate most of the aberrations that would otherwise be seen following UV irradiation. In fact, photoreactivation appears complete under these conditions for chromatid deletions and the chromatid exchange aberrations, and at least highly efficient for isochromatid deletions.

As a further check that our cells were indeed irradiated in the G_1 phase of the cell cycle, and also to be sure that the result was not peculiar to our experimental methods, a parallel experiment was done in which cells were exposed to ^{60}Co gamma rays. As is shown in Table 1, there was no suggestion of the induction of any but the expected chromosome types of aberrations (although, of course, it is possible that some of the aberrations listed as terminal deletions could have actually been non-sister-union isochromatid deletions).

To test the generality of our unexpected results with the toad cell line, parallel experiments were done with the V-79 Chinese hamster cell line. No photoreactivation was attempted, in view of the known lack of PRE activity in such cells. The results are shown in Table 2. Again, UV irradiation of early G_1 cells appears to produce chromatid-type aberrations almost exclusively, although gamma irradiation at this stage appears to produce only the expected chromosome types.

These experimental results appear to us quite compelling, in spite of the small

Table 1. Chromosomal aberrations in G_1 phase A8W243 *Xenopus laevis* cells treated with UV alone, UV followed by white light, or ^{60}Co gamma rays.

Treatment	Cells scored	Chromatid-type aberrations			Chromosome-type aberrations		
		Chromatid deletions	Isochromatid deletions	Chromatid exchanges and rings	Interstitial deletions	Terminal deletions	Rings and dicentrics
G_1 control	100	5	2	1	0	0	0
115 erg/mm^2 (pooled 56- to 59-hour-sample fixations)	79	59	39	94	0	0	0
115 erg/mm^2 followed immediately by 2 hours of white light photoreactivation	93	5	15	0	0	0	0
600 r of ^{60}Co gamma rays	100	0	0	1	47	23	107

Table 2. Chromosomal aberrations in G_1 phase V-79 Chinese hamster cells treated with UV or ^{60}Co gamma rays.

Treatment	Cells scored	Chromatid-type aberrations			Chromosome-type aberrations		
		Chromatid deletions	Isochromatid deletions	Chromatid exchanges and rings	Interstitial deletions	Terminal deletions	Rings and dicentrics
G_1 control	75	2	2	0	0	0	0
150 erg/mm^2	100	13	8	57	1	0	1
500 r of ^{60}Co gamma rays	100	3	1	5	105	57	68

numbers of cells analyzed and the obvious need for more experiments. Several lines of evidence, including cell cycle analyses and the gamma ray experiments, show that at least the majority of the cells were indeed in early G_1 at the time of irradiation. Scoring of aberration yields in serial samples over the first mitotic peak following irradiation from several other experiments with the A8W243 cell line in addition to that reported in Table 1, together with extensive mitotic index determinations, rules out the possibility that any substantial number of cells bearing UV-induced chromosome-type aberrations were missed because they came to mitosis earlier or later than those with chromatid-type aberrations. Because the synchrony of our irradiated populations was not perfect, it might be argued that the chromatid aberrations were actually coming from a small percentage of cells that could have been in the S phase of the cell cycle when irradiated. However, the mitotic index in our samples was never less than 0.93, and no more than 7 percent of the cells could then have been in S. Nevertheless, 80 percent of the A8W243 cells and over 60 percent of the V-79 cells treated with UV alone contained at least one chromatid aberration, so most of them must have been induced in G_1 cells. While it is possible to argue that perhaps some of the deletions listed as isochromatid type were actually of the chromosome type, this seems a trivial consideration in light of the large yields of clear-cut chromatid types and the lack of other chromosome types. We conclude that UV irradiation in the early G_1 phase of the cell cycle produces mainly chromatid-type aberrations and that most of the lesions giving rise to these aberrations can be photoreactivated.

While these results were unexpected in light of the reports of Humphrey *et al.* and of Chu (*3*), they are easily explained. The only UV-induced DNA lesion that is known to be reversible by photoreactivation is the cyclobutane dimer occurring between adjacent pyrimidines within one chain of the DNA double helix (*2*). Since at least the majority of the lesions leading to aberrations in the UV-irradiated A8W243 cells are photoreactivable, it follows that these lesions are probably in fact dimers. Because photoreactivable dimers occur within a single one of the two parallel chains of the DNA double helix, it is not surprising that chromatid- rather than chromosome-type aberrations result. Thus, although not conclusive, we take these results both as evidence that chromosomal aberrations may result from lesions (dimers) induced directly in the DNA molecule, and as evidence in support of a single-stranded or unineme structure of the eukaryote chromosome as well (*7*).

The induction of isochromatid-type deletions and the apparent photoreactivation of the lesions giving rise to them shows that the simple dimer mechanism is not adequate to explain all aberration production by UV, however. If the lesion responsible for isochromatid deletions is a simple intrachain dimer, then it must "spread" to the other chain of the double helix in some way, possibly through abortive operation of a normal repair mechanism. Alternatively, if the isochromatid deletions arise from some other type of initial lesion, then their photoreactivability indicates a previously unknown photorepair mechanism.

If the simple dimer model for the induction of at least most chromosomal aberrations by UV light is correct, then one would expect that UV irradiation of cells in the G_2 phase of the cell cycle would yield few if any visible aberrations in the cells' first postirradiation mitosis. The lesions would affect only one of the DNA chains, and little time would be available for repair mechanisms to convert them to gross aberrations. While the results are even less complete than our results for G_1-irradiated cells, G_2 irradiation experiments we are currently conducting appear to bear out this prediction.

Finally, although additional experiments will be necessary to resolve the question completely, it seems possible that the earlier reports of chromosome-type aberration induction (*3*) could be explained in terms of mitotic delay and "derived" chromosome-type aberrations. After a successful mitosis, at least one of the two daughters of a cell in which a chromatid-type aberration was induced will contain an aberrant chromosome, which at the next mitosis will appear to be of the chromosome type; a chromatid exchange, for example, can give rise to an apparent dicentric chromosome at the second division, while a chromatid ring becomes a chromosome-type ring. Thus variable division delay might cause "generation mixing" that might well account for both the apparent induction of chromatid-type aberrations in G_2-phase cells and the chromosome-type aberrations reported by Humphrey *et al.* and by Chu (*3*).

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Membrane Recycling: Vesiculation of the Amoeba Contractile Vacuole at Systole

Abstract. *Ultrastructural data on the protozoan* Amoeba proteus *support a model of membrane recycling. At systole the amoeba contractile vacuole fuses with the cell surface and expels its contents. Observations by electron microscopy indicate that, as the vacuole empties, its bounding membrane transforms into tiny (35 nanometers in diameter) vesicles, identical to the vesicles that segregate fluid and contribute to the diastolic vacuole.*

Small membranous vesicles observed adjacent to the secretory surface in several different cell types immediately following a burst of secretory activity have suggested that the drastic increase in surface membrane due to secretory exocytosis is reversed by endocytosis of "empty" vesicles (*1*, *2*). As has been shown in a number of other situations (*3*), the processes that are carried out