PHOTOREACTIVATING ENZYME IN THE SEA URCHIN EGG*

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Photoreactivation of ultraviolet (UV) damage is a widespread biological phenomenon, having been demonstrated in many plant and animal phyla (for review, see Jagger, 1958). There is considerable evidence that the mechanism of one type of photoreactivation is the splitting of UV-induced pyrimidine dimers in DNA by a photoreactivating enzyme in the presence of light (Setlow, 1966). However, in some organisms photoreactivation is indirect and does not involve either a dimer-splitting reaction or a light-dependent enzyme which acts directly on DNA (Jagger and Stafford, 1965). Thus, the demonstration of photoreactivation in an organism does not necessarily imply the existence of a photoreactivating enzyme. Two criteria may be used to demonstrate the presence of such an enzyme that acts on DNA (Rupert, 1960): (1) the ability of an extract of an organism to photoreactivate UV-inactivated transforming DNA, and (2) the destruction of this photoreactivating ability by proteolytic enzymes.

Photoreactivation of the UV-induced delay in cleavage in Arbacia punctulata eggs has been shown by Blum et al. (1949) and by Marshak (1949). We have obtained evidence for the presence of photoreactivating enzyme in these eggs by the two criteria given above. Heretofore, such an enzyme has not been demonstrated in any metazoan tissue.

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Preparation of extract. Ovaries were removed from live animals and placed in cold homogenizing solution (0.5 M KCl containing 1 mM EDTA, 5 mM ß-mercaptoethanol, and M/15 phosphate buffer, pH 7). All further steps were carried out at 4°C. Following gentle agitation, the eggs were separated from the ovaries by filtration through gauze. They were then centrifuged at 150 x g for 30 seconds and washed in the above solution three times. The last supernatant contained no photoreactivating activity. The final suspension of 5-10 % eggs was homogenized in a glass homogenizer with teflon pestle. The homogenate was centrifuged at 2,000 x g for 15 minutes, and the supernatant fluid was retained. The pellet contained no photoreactivating activity. The supernatant, containing between 3 and 11 mg protein/ml by the Biuret assay (Layne, 1957), was diluted in M/15 phosphate buffer, pH 7, with 1 mM EDTA for the photoreactivation experiments. Since no photoreactivating activity was found either in the pellet or in the supernatant from the last wash before homogenization, it is very improbable that contaminating microorganisms contribute to the effects observed.

Photoreactivation of transforming DNA. Hemophilus influenzae DNA bearing a streptomycin resistance marker was inactivated by a germicidal lamp to about 1 % survival of transforming activity. Samples in 0.1 ml volumes, at a concentration of 0.25 µg DNA/ml, were placed in white porcelain spot plates. Various dilutions of the egg extract were added to the DNA in 0.1 ml aliquots. In some experiments, 0.05 ml calf thymus DNA at a concentration of 1 mg/ml was added to the spot plate to protect the transforming DNA from degradation by nucleases in the crude egg extract. For photoreactivation (at 37°C), these samples were exposed to illumination from blacklights filtered to eliminate all wavelengths below 3250 A. The incident intensity was about 7,000 ergs/mm^2/min. Control samples were incubated in the dark. Following illumination or dark incubation, 0.1 ml aliquots were withdrawn and assayed for their ability to transform streptomycin-sensitive H. influenzae to streptomycin resistance (Goodgal and Herriott, 1961).

Crystalline bovine pancreatic trypsin (Calbiochem) was dissolved in M/15 phosphate buffer prior to use.

Results. The results of a photoreactivation experiment are shown in Fig. 1. It is apparent that there is a considerable increase in the number of transformants with time

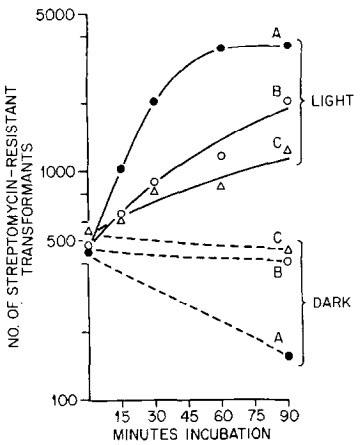

Fig. 1. Number of transformants resulting from irradiated transforming DNA (1% survival) incubated with an Arbacia extract at concentrations of A: 1.1; B: 0.2; and C: 0.1 mg/ml protein.

of exposure of the reaction mixture to blacklight illumination, and also that the rate of this increase is roughly proportional to the concentration of the extract. In addition, there is a decrease in the number of transformants after dark incubation, and the extent of this decrease is larger with greater extract concentration. The decrease in the dark is presumably due to nucleases in the extract, since the addition of calf thymus DNA protects against this loss of activity (see Table II). The ratio of the maximum number of transformants after illumination to the number after dark incubation is approximately 20; a similar ratio has been obtained in this laboratory after maximum photoreactivation of the same DNA with a photoreactivating enzyme extracted from yeast.

Evidence that the increase in transformation is the result of enzyme activity is shown in Tables I and II. Incubation of the extract at 37°C for 30 minutes prior to assay destroys its activity (Table I). Hence, either (1) the active component is very heat

TABLE I
Inactivation by heat of photoreactivating capacity of Arbacia extracts for H. influenzae transforming DNA.

Treatment of material before assay	Light conditions during PR assay	No. of transformants/ml
Extract; 30 minutes at 0°C	L	2167
Extract; 30 minutes at 0°C	D	448
Extract; 30 minutes at 37°C	L	891
Extract; 40 minutes at 60°C	L	946
Extract; 40 minutes at 60°C	D	863
Buffer (no extract)	D	940

Diluted extract contained 1 mg protein/ml. Incubation 90 minutes in light (L) or dark (D).

TABLE II
Inactivation by trypsin of photoreactivating capacity of Arbacia extracts for H. influenzae transforming DNA.

Material assayed	Light conditions during PR assay	No. of transformants/ml
Extract	L	502
Extract	D	63
Extract + trypsin	L	121
Extract + trypsin	D	48
Buffer + trypsin	D	70

Diluted extract contained 0.3 mg protein/ml. Incubation 90 minutes. 60 μg/ml trypsin. Calf thymus DNA added before incubation.

labile, or (2) the active component is inactivated by other components of the crude extract. In either case, the active component is presumably stabilized in the presence of its substrate (irradiated DNA), since its activity is readily assayed at 37°C. If the extract is incubated with trypsin during blacklight illumination, its photoreactivating capacity is greatly reduced (Table II).

Discussion and conclusions. The heat lability and trypsin sensitivity of the Arbacia extract indicate that its photoreactivating capacity is due to an enzyme.

Photoreactivation of H. influenzae transforming DNA by yeast enzyme repairs around 90% of the damage from 2537 A radiation (Setlow, 1963). Since the Arbacia extract photoreactivates this DNA to approximately the same extent as the yeast enzyme, there must be a large, if not complete, overlap in the types of lesions repaired by the yeast and Arbacia extracts. Photoreactivation of transforming ability in UV-irradiated H. influenzae DNA by yeast extract is the result of monomerization of pyrimidine dimers (Setlow, 1966), and it may be inferred that the Arbacia extract probably acts by the same mechanism. It is therefore likely that most or all of the photoreactivable lesions which cause cleavage delay in Arbacia punctulata are pyrimidine dimers.

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