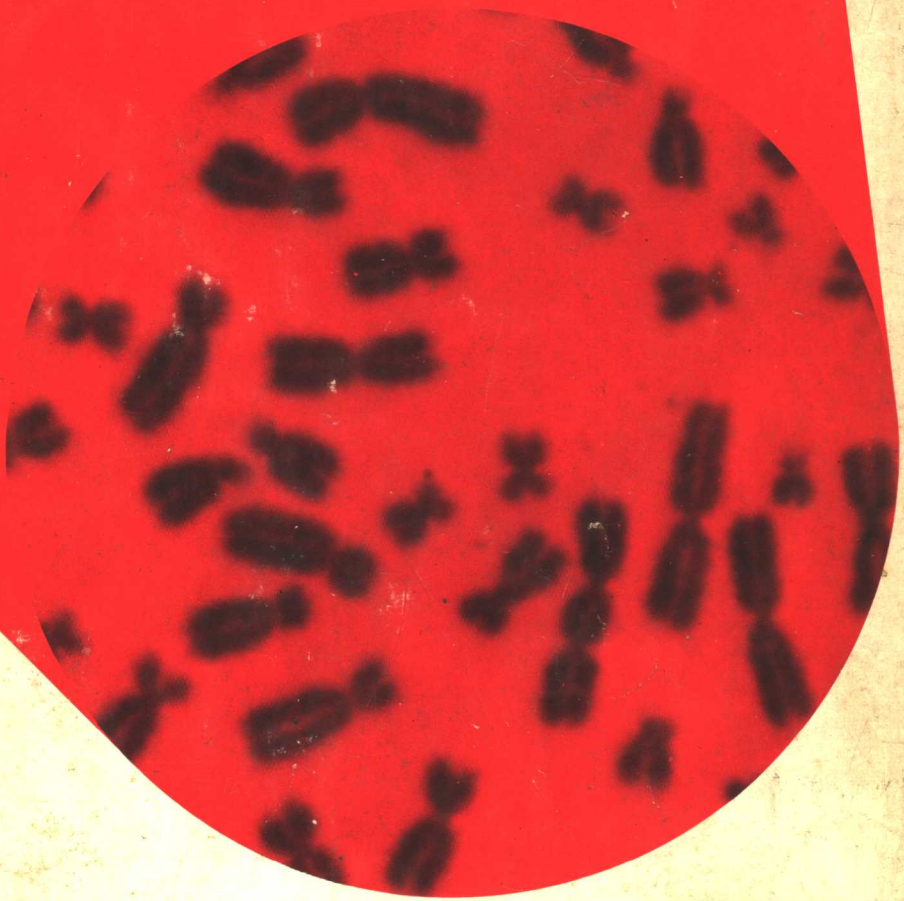
CONCEPTS IN RADIATION CELL BIOLOGY

EDITED BY GARY L. WHITSON



CELL BIOLOGY
A SERIES OF MONOGRAPHS

ACADEMIC PRESS

CONCEPTS IN RADIATION CELL BIOLOGY

Edited by GARY L. WHITSON

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Knoxville, Tennessee

1972

ACADEMIC PRESS New York and London

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DD

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 79-182619

PRINTED IN THE UNITED STATES OF AMERICA

Preface

One of the most difficult tasks in compiling and editing a technical book such as this is to present, without loss of continuity, a central theme that encompasses and unifies the current concepts of the field. The field of cell biology itself is very broad, and cellular phenomena are often as numerous and varied as the kinds of cells that exist either as unicellular organisms or as tissue elements of plants and animals. Therefore, any special subject area within the realm of cell biology, such as the effects of radiations on cells, could be large and diverse; and it is.

It has not been possible to review the entire field of radiation cell biology. Rather, in this book, current concepts of various cellular radiobiological phenomena in selected cell types are surveyed. Since most books on this subject are of a review nature, the decision was made to present some important techniques that offer students and investigators the necessary information for further experimentation in radiation cell biology.

Although not readily apparent in each chapter, the general theme resides in the underlying macromolecular basis for cellular changes in irradiated cells. The first few chapters deal mainly with the effects of nonionizing radiations ranging from ultraviolet to visible light. The remaining chapters, except the last one, which presents the use of laser light in cellular studies, deal mainly with ionizing radiations. The evidence thus far obtained from ultraviolet studies implicate DNA as the main target macromolecule responsible for such radiation injury as division

delays, delayed DNA replication, and lethality. On the other hand, photodynamic effects also favor proteins as important target molecules for radiation injury in cells. Recent evidence on ionizing radiation also favors DNA as the main target molecule responsible for cellular radiation injury, but less is known about specific biochemical changes and repair processes in cells exposed to this type of radiation.

This book is aimed primarily at the level of advanced students in radiation biology. It begins with physical–chemical studies on ultraviolet-irradiated DNA. The remaining chapters, beginning with viruses, are organized in an increasing order of complexity of cell types, including cells of higher plants.

GARY L. WHITSON

This photograph of Dr. Hollaender was taken at Dartmouth College in August, 1968, at the Fifth International Congress on Photobiology where he was awarded the Finsen Medal, which he is holding in his left hand. The medal was awarded to Dr. Hollaender "for fundamental contributions in the early development of photobiology, in particular radiation genetics." Permission to reproduce this photograph was granted through the courtesy of Per Hjortdahl of the Dartmouth Medical School, Hanover, New Hampshire.

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Chapter 1
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I. Introduction

A principal aim in modern radiation biology is to interpret the effects of radiation on living systems in terms of the changes observed in cellular components and functions. A typical experiment consists of irradiating a cell under well-defined conditions and then measuring the effect of the radiation on one of several biological factors, such as the ability to form colonies, the rate of cell growth and division, and the rate of mutation. One is interested in how these gross biological effects are correlated with changes in the rate of cellular functions such as the synthesis of DNA, RNA, or protein. Such correlations are often difficult to make, partly because of the myriad of metabolic control mechanisms that may be in various stages of breakdown and partly because of enzymatic repair of the damaged DNA. In recent years a strong effort has been made to approach this problem at the molecular level and ask: What are the chemical changes, particularly in DNA, that occur during and following the irradiation of a cell and how can these events affect the various cell functions?

The various events that follow the interaction of a cell with radiation are diagramed in Fig. 1. Designated in the figure are three broad areas of research in radiation biology in which one can carry out experiments and organize data: They are the physicochemical, biochemical, and biological aspects of the interaction between radiation and the cell. Linking these areas is a challenging problem, and thus far only a limited degree of success has been achieved.

In this chapter we shall concern ourselves with the contents of the first box in Fig. 1. In particular, we shall deal only with the effects of UV* on

* Abbreviations and symbols: BrUra, 5-bromouracil; PO-T, deamination product of the cytosine–thymine adduct, 6-4′-[pyrimidin-2′-one]-thymine; UV, ultraviolet radiation; $\widehat{TT}_1$, *cis-syn* thymine–thymine cyclobutane dimer; $\widehat{TT}_2$, *trans-syn* thymine–thymine cyclobutane dimer; $\widehat{CC}$, cytosine–cytosine cyclobutane dimer; $\widehat{CT}$, cytosine–thymine cyclobutane dimer; Y, unspecified pyrimidine nucleoside; N, unspecified

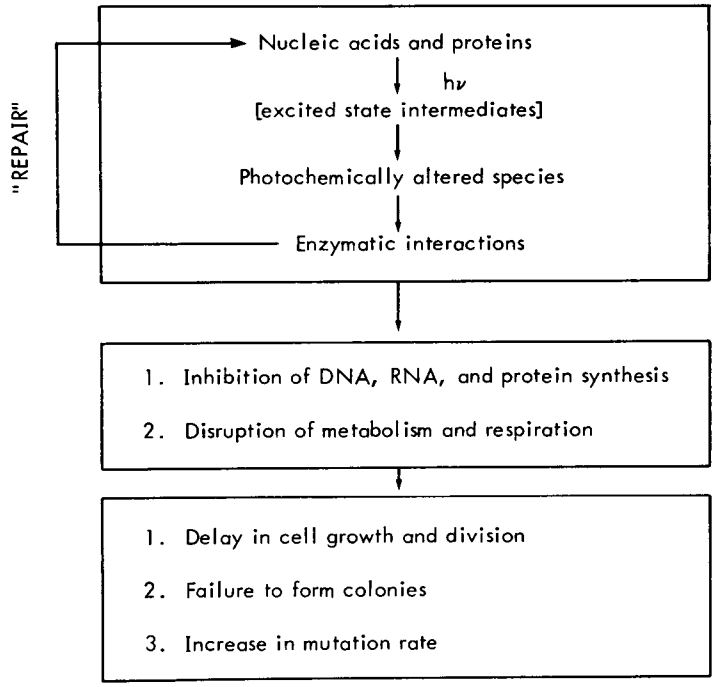

Fig. 1. Schematic representation of events following the ultraviolet irradiation of a cell.

the properties of isolated DNA molecules. We shall try to answer the question: What kinds of damage are produced in DNA by UV-irradiation, and how does this damage affect the interactions between DNA and enzymes? The remaining chapters will deal with the effects of radiation on intact biological systems.

The reason for focusing on the photochemistry of DNA and not on that of RNA or protein is that DNA occupies the most important position in the cell's machinery, as judged by the order of synthesis:

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{protein}$$

Hence, alteration of DNA by UV affects those processes that are dependent upon the integrity of the DNA template and can thus lead to lethal or mutagenic effects. Alteration of the RNA or protein component of a cell would not have as devastating an effect on the functions of a cell because extra copies of these molecules are available.

We will pay particular attention to environmental factors such as

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nucleoside; poly(dA-dT), alternating copolymer of A and T; poly(dA)·poly(dT), association of poly(dA) with poly(dT); hyphens within base sequences indicate a phosphoric diester in 3′-5′ linkage.

temperature and relative humidity, which have been found to strongly influence the rate of formation of DNA photoproducts. An understanding of these factors should help the researcher design experiments in which the physical environment of the DNA in a cell is altered and the resulting change in the photobiological effect correlated with changes in the DNA photochemistry. For example, cells are normally irradiated at room temperature in neutral buffer solutions. By varying these physical conditions, one may be able to observe a new dependence of the biological effect on dose and may be able to correlate this change with a concomitant change in the number and nature of photoproducts formed. When irradiating cells, the freedom to vary the irradiation conditions is somewhat limited because the cells may lose their viability if subjected to extreme changes in their chemical and physical environment. However, photobiological investigations have been carried out with bacterial spores (Donnelan *et al.*, 1968), vegetative cells (K. Kaplan, 1955; Smith and O'Leary, 1967; Webb, 1965), and bacterial viruses (Fluke, 1956; Hill and Rossi, 1954; Levine and Cox, 1963) subjected to extremes in temperature and relative humidity during irradiation. In some of these experiments rather great changes in the production of photoproducts were observed and these were correlated with the observed biological inactivation curves. The biological activity of transforming DNA has also been measured after irradiation over a wide range of temperatures (Rahn *et al.*, 1969).

This chapter is not intended to be a review of nucleic acid photochemistry and photobiology. For recent reviews of this subject, see J. K. Setlow (1966a) and R. B. Setlow (1968a). One of the early attempts to review nucleic acid photochemistry was made by McLaren and Shugar (1964); their book still contains a wealth of information and ideas not presented elsewhere. A recent book on molecular photobiology has been written by Smith and Hanawalt (1969). A review of the photochemistry of nucleic acid derivatives from the viewpoint of the chemist has been presented by Burr (1968).

II. Photophysics

A. Light Sources

We shall deal only briefly with the techniques used to incorporate energy from some outside source into the biological system under consideration. More detail on this problem is provided by Jagger (1967) and Johns (1968), and the reader is advised to consult these references for

additional information on experimental details. In addition, Chapter 2 in this book describes in detail some of the techniques used in irradiating viruses. These techniques are similar to those used for irradiation of other systems, such as bacteria and protozoa.

There are several different kinds of lamps available for obtaining ultraviolet radiation. In most experiments, monochromatic radiation is essential. A 15-W, low-pressure mercury lamp is widely used in photobiology. It is convenient to use and emits most of its energy (~85%) at 254 nm. Hence, no filters or monochromators are necessary to obtain radiation predominantly at this wavelength, which is close to the absorbance maximum of DNA (257 nm). However, if one wishes to irradiate at different wavelengths, as when measuring an action spectrum, then a source with a broader spectral output must be used in conjunction with a band-selecting device. Two possible sources are the high-pressure mercury lamp and the xenon lamp. The relative spectral output of these is given in Fig. 2. The output of the high-pressure mercury lamp is concentrated at certain wavelengths, the principal lines. A band-selecting device such as an interference filter or a monochromator is used to select the line of interest. An advantage of the xenon lamp is a spectral output that is continuous in the ultraviolet so that one is not restricted to using only certain wavelengths, as with the high-pressure mercury lamp. Hence, a xenon lamp is better suited for obtaining a high-resolution action spectrum.

Muel and Malpiece (1969) have described a set of solution filters that

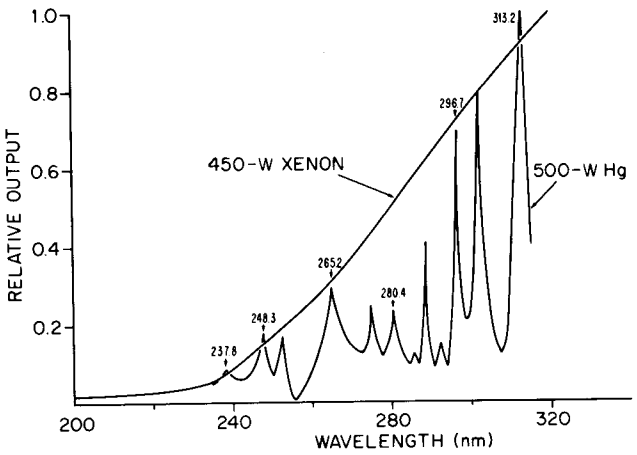

Fig. 2. Relative output in the ultraviolet region of a 450-W xenon lamp compared with that of a 500-W, high-pressure mercury lamp. The numbers indicate the wavelengths of the principal mercury lines commonly used for irradiation.

will isolate various portions of the UV spectrum. These filters are inexpensive and permit one to carry out irradiations at various wavelengths without using expensive monochromators or thin-film interference filters. One of their shortcomings, however, is their photolability, which necessitates changing the solutions constantly during any prolonged irradiation.

B. Absorption

In order for a photochemical reaction to occur, a photon must be absorbed by (or its energy transferred to) the system in which the reaction takes place. The probability that an absorbed photon will give rise to a photochemical reaction is called the quantum yield (Φ) of the reaction and is equivalent to the ratio of the number of molecules altered to the number of photons absorbed. In a cell there are two classes of macromolecules mainly responsible for the absorption of UV—proteins and nucleic acids. In the region 240 to 280 nm, the nucleic acids, in small cells, are the most important physical absorbers of light. In this wavelength region, nucleic acid absorption is 10 to 20 times greater by weight than that of protein. The absorbance (A) at some chosen wavelength of a solution of DNA in a 1-cm cell may be written as $A = c \cdot \epsilon$, where c is the concentration in moles per liter and ϵ is the molar absorptivity or extinction coefficient. Extinction coefficients for polynucleotides are usually expressed as $\epsilon(P)$, the molar extinction coefficient per nucleotide (phosphate). Generally, all DNA's, regardless of their G + C content, show values of $\epsilon(P)$ at 260 nm that vary from 6600 to 7000. The absorbance of DNA can be considered to be the sum of the absorbances of the individual nucleosides, with some modification due to hypochromicity. The hypochromicity or decrease in molar extinction upon incorporation of the nucleosides into native DNA is approximately 30% and arises from electronic interactions between the stacked bases.

The action spectrum of a system corresponds to the wavelength dependence of some light-induced change in the system. It is determined by measuring, at various wavelengths of irradiation, the number of incident photons needed to produce an observed change in the system. For optically dilute samples (transmission approx. 90%), the fraction of incident energy absorbed by a molecule at a given wavelength is nearly proportional to the extinction coefficient of the molecule at that wavelength. Hence, the number of incident photons needed at some wavelength to achieve a fixed percentage of change in a molecule will be inversely proportional to the extinction coefficient of the molecule at that

wavelength, provided the quantum yield is independent of wavelength. Therefore, a plot of the inverse number of photons needed at each wavelength to achieve a fixed percentage of change should resemble the absorption spectrum of the molecule. A good description of how to obtain an excitation spectrum is given by Jagger (1967).

In many biological systems, the action spectrum for UV-induced biological changes has been found to resemble the absorbance spectrum of nucleic acids. The action spectrum for the inactivation of a culture of *Escherichia coli* was obtained by Gates (1930) and is Fig. 1 in Chapter 3. This spectrum was one of the first indications that nucleic acids are the primary target for the lethal effect of UV. A discussion of UV action spectra with particular emphasis on mutation is given by Giese (1968).

C. Excited States

The absorption of a photon promotes a molecule to an excited electronic state. A molecule in such a state is often more chemically reactive than when in a ground state. As indicated in Fig. 3, a molecule in an excited state has a choice of pathways by which it can return to the original

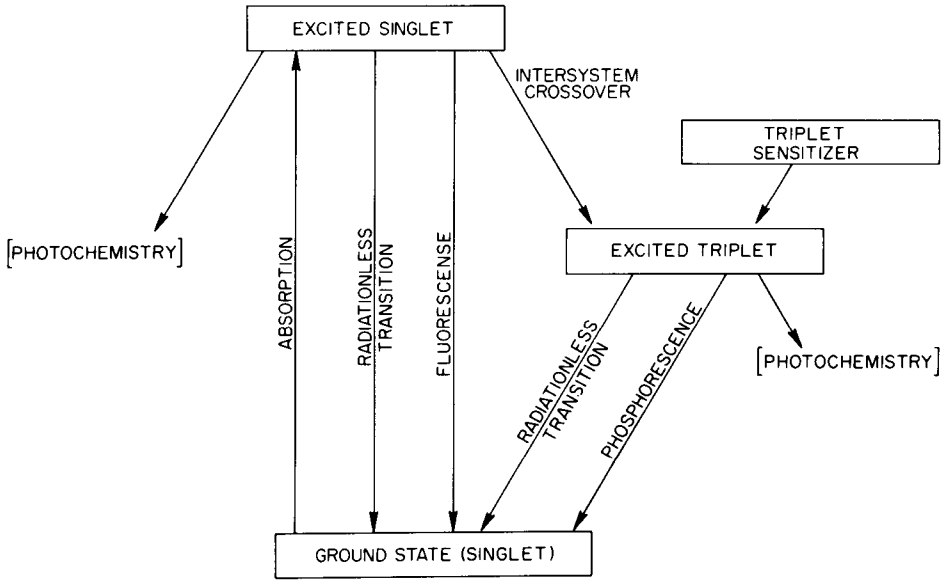

Fig. 3. Diagram of the electronic energy levels of a molecule indicating the various types of transitions between the lowest-lying excited states and the ground state.