MOLECULAR AND CELLULAR MECHANISMS OF MUTAGENESIS

Edited by J. F. Lemontt
and W. M. Generoso

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FOREWORD

It has been nearly 35 years since the peacetime Biology Division of Oak Ridge National Laboratory was started, born of rather inauspicious conditions. Virtually no facilities were available and most of the wartime scientists had left. So, when we started out, it was obvious to me that something had to be done to reestablish research. Even more, because Oak Ridge was not known at that time for its biological work but rather for the separation of Uranium 235, nuclear reactor development, and radioisotope production, a new tradition had to be promoted. Although good biological work had been done at Oak Ridge during the war to protect the workers and the results of this work were quite excellent, very few installations remained.

When we started the work of the Biology Division, it became essential to make it part of the flow of modern biology all over the world. As Director, I had to do more than just attract promising scientists. We created an atmosphere conducive to creative research and nurtured all of the other aspects of a productive laboratory. Of course, we carefully prepared the results of our work in publishable form. We made a sincere effort to invite seminar speakers and lecturers to come to Oak Ridge despite the sacrifices this presented to our early budget. We also had to do something more, and here I "cashed in" on my experience of the previous 15 years.

In the early 1930s, I was in close contact with our colleagues at Cold Spring Harbor Laboratory. Having spent a few summers in Woods Hole, I worked with a number of physiologists, geneticists, and others. My interest in always knowing what was going on at Cold Spring Harbor served me very well as I began to develop the Biology Division at Oak Ridge. I had actually attended one of the first symposia that was held under Dr. Reginald Harris, and my close contact with Cold Spring Harbor increased through my friendship with Milislav Demerec, who later became the director of the biology work there. I came to know Dr. Berwind Kaufmann, a cytologist on the staff; we became very close and exchanged assistants with our laboratory at the National Institutes of Health in Bethesda. I was especially interested in the way the programs of their annual sym-

posia were organized. Not only did the Laboratory organize the proceedings, but they also published most conferences. I saw that this was a very good way to break the isolation of a not-so-accessible laboratory. My close interest in the developments at Cold Spring Harbor has not diminished over the years and I am still a Trustee Emeritus.

In those early days at Oak Ridge following the war, we met for our first symposium in a wooden structure in a section of Oak Ridge that was a shopping center situated near the only restaurant in town. I, of course, concentrated my efforts on the areas of genetics, biochemistry, and biophysics, in which I was most interested, and steered the laboratory along those lines.

Our first symposium was held in 1948, and I had learned how to organize these symposia since I had worked in close cooperation with Demerec and had attended many of the symposia at Cold Spring Harbor. I don't think I missed many of these lectures. I chose the topic "Radiation Genetics," in which I had a strong interest and I knew many of the outstanding working scientists.

How then did we get from Oak Ridge to Gatlinburg? Very simple. We planned a symposium on photosynthesis and we wanted to invite Linus Pauling, who was at that time persona non grata to the Atomic Energy Commission. No objection was made to our inviting him, but some officials did not want him around the Oak Ridge area because it was still under heavy security at that time. In order to accommodate our guest speaker, our workshop was held in nearby Gatlinburg at the Mountain View Hotel. With no formal meeting room, the proprietors simply screened across the entrance hall and we sat around the fireplace to begin our first workshop there. In fact, I was told this was the first time that anyone had held a scientific meeting in Gatlinburg!

In the early 1950s, we moved to Gatlinburg, and it became our headquarters for symposia since housing facilities, except for a rather rustic guest house, were nonexistent in Oak Ridge. Thus the tradition was set to conduct scientific meetings in Gatlinburg. It took the hotel owners a few years to catch on that this could be an important source of income to them before the start of the tourist season. Gatlinburg was at that time a rather sleepy town without the number of tourists that now pass through its borders, so we continued to have our meetings away from the Laboratory.

As I observed earlier, it is most important to publish the proceedings of these organized symposia, and for this Cold Spring Harbor had developed its own method of publication. To establish a similar mechanism, I approached a number of publishers – Academic Press and a few others – and they said they didn't want to print

symposia proceedings. As you know, the whole picture has changed in the last 10 to 15 years.

I talked to Detlev Bronk, who originally organized the Johnson Foundation for Biophysics of the University of Pennsylvania and who had some connection with Wistar Press. He suggested that the Journal of Cellular and Comparative Physiology might publish our proceedings. The symposium proceedings were first issued as supplements to this Journal. As our publications grew in time beyond the capability of Wistar, we switched to other means of publication. Several volumes were translated into the Russian language.

In retrospect, I realize the benefit of consistently bringing out all of our proceedings through one publishing house so that the work is more readily recognized as a distinct series that reflects the interests of and connection with the Biology Division and affords greater distinction than these proceedings now have. Looking over the various titles of the thirty or so symposia that have been organized by the ORNL Biology Division, one can see that they truly demonstrate the broad interest of those who organized them and cooperated toward their successful presentation. It also indicates, by sequence and diversity, the changes that took place over the years. Published from a single source, a kind of history of the Division would have been more clearly evident.

It is my tendency to discuss not only the background and history of our activities in research but also to look ahead to what lies before us. It is, of course, not possible to predict what might come up in the future. Who would have thought ten years ago that monoclonal antibodies would be on the horizon? However, even in the late 1930s it was obvious to some of us that the nucleic acids would become the central theme for the future development of genetics. We surmised that radiation biology might serve as a sort of model for an understanding of certain approaches to the detection of damage created by adverse chemical agents on living cells and their subsequent repair processes.

In regard to the process of repair, which is being discussed with its very latest developments at this symposium, it is well to point out that it had been recognized years ago that a number of the steps in biochemical and biophysical reactions could not proceed without following an established pattern. In the multitude of reactions that are the basis for the function and growth of living systems, some steps could go wrong by the nature of biological activity or through outside interference. If the living system did not have the capability of repair or activation of alternate pathways to accomplish the same thing, we might not be here on this earth today! Many of the above points were discussed as an advanced development some 40 years ago, and they were not very popular when expressed at that time.

I believe we are really at the threshold of an entirely new development of the interplay between genetics, biochemistry, and biophysics that will have a tremendous impact on medical sciences, as well as the industrial development in the area of chemical production. Our new generation of investigators may have different ideas, and I am anxious to see our young colleagues develop their own thinking. They have done a very good job thus far; and I am certain that by improving upon our earlier efforts in these areas, many important new developments in the basic approaches to biological problems will continue to unfold. Some have not yet reached the stage of application and, traditionally, there has always existed a certain lag time between experimental technique developments and their application.

I already mentioned the important developments in repair techniques that have become a most beautiful tool for the understanding of basic radiation effects and now also for the effects caused by chemicals on living cells. What I am especially referring to here are the new advances in genetic engineering, hybridization, plasmid transfer, chromosome exchanges, and the effects of shifting genes. There are probably many others that we haven't yet discovered or that have not yet been reported. I can see that with the development of these new tools, very significant progress will be made leading to a more comprehensive understanding of the basic mechanisms, how the cell functions, and what controls the different steps in development. I am looking forward to the new array of practical discoveries.

The topics of our present symposium include the most important active areas of research in fundamental mutagenesis to date and should be of general interest to all workers in genetics, mutagenesis, carcinogenesis, molecular biology, etc. It is, therefore, a great pleasure for me to see that, through this symposium, the ORNL Biology Division has once again taken the initiative in bringing together these various research areas in an attempt to stimulate more progress in molecular mutagenesis.

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PREFACE

Mutagenesis is a fundamental cellular process that contributes to genetic variation, one that is observed to occur both "spontaneously" and as a result of exposure to DNA-damaging agents. Over the past two decades tremendous progress has been made in understanding cellular mechanisms of mutagenesis. Most of these basic studies have been focused on two areas: one involves a molecular description of DNA base-sequence changes in mutants long after the original mutational event, and the other is concerned with genetically controlled cellular processes that convert damaged DNA to an altered damage-free base sequence. This latter approach, largely dependent on use of mutant strains exhibiting aberrant DNA metabolism (repair, replication, recombination, or mutagenesis), has been particularly fruitful in leading to useful working hypotheses and concepts such as error-free and error-prone repair. Unfortunately, precise molecular mechanisms of mutagenesis have remained elusive, partly because adequate tools have not been available to elucidate specific gene action or to determine the mutational consequences of specific DNA lesions.

Now that recombinant DNA technology has become a powerful tool of molecular biology, we can look forward to a new era of progress when genes controlling mutagenesis and DNA repair can be isolated and used as probes for direct studies of their own expression and regulation. The ability to clone, sequence, and manipulate specific genes has made it possible to enlarge our view of how a cell mutates its genome. For example, transposable elements now appear to be responsible for a significant fraction of spontaneous mutation events involving large insertions, deletions, or other gross DNA rearrangements. Point mutations involving single-base changes seem more likely to arise from damaged DNA, e.g., alkylated bases, pyrimidine dimers, apurinic/apyrimidinic sites, or other lesions with ambiguous base-pairing properties. How cells convert such noncoding lesions into transition, transversion, or frameshift mutations is still a difficult and challenging central problem in mutagenesis.

There now exists a large body of evidence suggesting that induced mutations result from copying errors introduced into DNA during repair and/or replication. Elucidation of error-prone repair or replicative error mechanisms for any organism will depend on biochemical studies of the interaction between cloned genes and purified gene products.

This book is concerned with several of the issues discussed above and is based on the proceedings of the recent "Symposium on Molecular and Cellular Mechanisms of Mutagenesis," held April 5-9, 1981, in Gatlinburg, Tennessee. Organized by the Biology Division of Oak Ridge National Laboratory with support from the U.S. Department of Energy, National Institute of Environmental Health Sciences (with assistance from Drs. F. J. de Serres and M. D. Shelby), and the University of Tennessee - Oak Ridge Graduate School of Biomedical Sciences, the conference had two main objectives: (1) to present a more expanded view of those biological phenomena that contribute to or affect mutagenesis, and (2) to promote greater interaction between investigators working on different problems with different organisms. The symposium was largely successful and included presentations on mismatch repair, DNA damage-inducible repair, the "adaptive-response" repair, site-specific mutagenesis, DNA replication errors in vitro and in vivo, mutagenesis by transposition, repair and mutagenesis of yeast mitochonfrial DNA, and DNA repair in human chromatin.

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CONTENTS

CHAPTER 1

MUTAGENESIS FROM A CHEMICAL PERSPECTIVE: NUCLEIC ACID REACTIONS, REPAIR, TRANSLATION, AND TRANSCRIPTION

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SUMMARY

Simple directly acting alkylating agents can be classified in terms of their mutagenic efficiency and their chemical reactivity. The most mutagenic are the N-nitroso compounds and these have a preference for reacting with nucleic acid oxygens in vitro and in vivo. In contrast, the alkyl sulfates are generally poor mutagens and react almost exclusively with base nitrogens. Other classes of alkylating agents also show correlations between oxygen reaction and mutagenicity. Ethylating agents are more oxygen-specific than the analogous methylating agent and, in a substantial number of cases, also more mutagenic at lower levels of treatment.

Sites of substitution by ethyl nitroso compounds (e.g., N-ethyl-N-nitrosourea, N-ethyl-N'-nitro-N-nitrosoguanidine) in double-stranded nucleic acids are as follows: phosphate >> N7-G > O^2-T, O^6-G > N3-A > O^4-T, O^2-C > other N. In single-stranded nucleic acids the reactivity of the O^2 of C, N1 of A, and N3 of U, T, or C is considerably greater. Certain of these derivatives have been shown in in vitro transcription or ribosome binding studies to mispair; namely, O^2-alkyl T, O^4-alkyl T, O^6-alkyl G, O^2-alkyl C, 3-alkyl C, 3-alkyl U or T and 1-alkyl A. In all cases, nonspecific mispairing occurs with high frequency. During in vivo replication such errors are probably relatively rare but nevertheless postulated to occur.

There is evidence that various types of repair enzymes exist in bacteria and mammalian cells that can remove, to varying extents, N-3 and N-7 alkyl purines, O^6-alkyl G, O^2-alkyl T, O^4-alkyl T, and O^2-alkyl C. Phosphotriesters in DNA appear to be very stable.

When substitution occurs on a site necessary for basepairing or in a site causing steric hindrance or electronic shielding of Watson-Crick sites, the result is ambiguity in transcription rather than termination. It is hypothesized that any or all unrepaired promutagenic lesions can be expressed as errors during replication.

Other mutagens described in terms of their chemical reactions and repair include simple nonalkylating agents, most of which change basepairing due to deamination or tautomeric shifts and the metabolic products of aromatic amines and polyaromatic hydrocarbons.

INTRODUCTION

Mutagenesis is defined as a heritable change that can occur through indirect, as well as direct, change in the genetic message. This paper, however, will focus on the direct effects of chemical modification on DNA or viral RNAs that are both genome and messenger. Since man is the species we are most concerned about, the effect of modification on mammalian cells or whole animals will be stressed whenever possible.

Many of the types of chemicals which have been assessed in terms of their mutagenicity in mammalian systems (Bartsch et al., 1980; Maher and McCormick, 1978; Montesano and Bartsch, 1976; Pienta, 1980) and for which there are data on the mechanism of chemical modification of nucleic acids are shown in Figs. 1 and 6.

CHEMICAL REACTIONS OF SIMPLE ALKYLATING AGENTS

A large group of mutagens are simple alkylating agents (Fig. 1), although these differ greatly in their mutagenicity. For this reason, we have been working for a number of years to elucidate the chemical reactions of nucleic acids with the "good" mutagens (e.g., N-nitroso compounds), as compared to the "poor" mutagens (e.g., alkyl sulfates).

Single-stranded (ss) homopolyribonucleotides were first used as models for RNA in most of these studies. Significant differences were found between methylating agents and the analogous ethylating agents, suggesting that the greater mutagenicity of, for example, ethyl methanesulfonate (EtMS) compared to methyl methanesulfonate (MeMS) could be due to reaction with oxygens or exocyclic amino groups. However, in RNA the exocyclic amino groups were not measurably reactive, while all oxygens, including phosphodiesters and ribose, could be modified with ethylnitrosourea (EtNU) (Table 1) (Singer, 1976, 1977). Both EtNU and ethyl nitrosoguanidine (ENNG) ethylated oxygens predominately. Two new derivatives of cytosine (C)