Mutagenicity testing

a practical approach

Edited by
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Preface

This book is intended to be used as a practical laboratory manual for people wishing to learn some of the techniques presently used in mutagenicity testing. Strictly speaking, 'mutagenicity testing' is too narrow a term to cover all the techniques described in the following chapters. 'Genetic toxicology' is perhaps a better term, although even this does not convey the full impact which the use of these tests has already made in the study and possible prevention of cancer, a group of disorders in which mutation is now thought to play a critical role.

Mutation is a manifestation of change of, or damage to, the structure of DNA. Agents which cause DNA damage and mutation, as well as being potentially capable of causing hereditary disorders in the offspring and succeeding generations of exposed populations, are also likely to be carcinogenic. For these reasons, testing for the induction of DNA damage and for mutagenicity, using a variety of short-term tests, has become an accepted part of the toxicological evaluation of drugs, industrial intermediates, cosmetics, food and feed additives, pesticides, biocides etc. In many countries such testing is mandatory, and international standards for the conduct of genetic toxicology are now emerging.

Fundamental research in the mechanisms of induced mutation and of carcinogenesis has also benefited from the development of highly refined short-term tests for genotoxicity. Monitoring human populations for signs of exposure to genotoxic agents, and monitoring the environment itself (e.g., air, water) are other activities which use tests based on the detection of DNA damage and mutation.

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Abbreviations

8AG	8-azaguanine
BP	benzo[a]pyrene
BrdUrd	5-bromodeoxyuridine
BSS	balanced salt solution
BZD	benzidine
C.E.	cloning efficiency
CHO	Chinese hamster ovary
4-CMB	4-chloromethylbiphenyl
CP	cyclophosphamide
DLT	dominant lethal test
DMBA	7,12-dimethylbenz(a)anthracene
DMEM	Dulbecco's modified Eagle medium
DMS	dimethylsulphate
DMSO	dimethylsulphoxide
EDTA	ethylenediamine tetraacetic acid
EGTA	ethyleneglycobis(β-aminoethyl)ether tetraacetic acid
EMS	ethyl methanesulphonate
ENU	N-ethyl-N-nitrosourea
FCS	foetal calf serum
HBSS	Hank's balanced salt solution
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HPRT	hypoxanthine-guanine phosphoribosyl transferase
LD_{50}	lethal dose, median
MEM	minimal essential medium
M.F.	mutant frequency
M.I.	mitotic index
MMS	methyl methanesulphonate
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MNU	N-methyl-N-nitrosourea
M.T.D.	maximum tolerated dose
NCE	normochromatic erythrocytes
NOR	nucleolar organiser region
4NQO	4-nitroquinoline-N-oxide
OUA	ouabain
PBS	phosphate-buffered saline
PCB	polychlorinated biphenyls
PCC	premature chromosome condensation
PCE	polychromatic erythrocytes
PHA	phytohaemagglutinin
SCE	sister chromatid exchange
SDS	sodium dodecyl sulphate
TCA	trichloroacetic acid
6TG	6-thioguanine
TK	thymidine kinase
TTC	triphenyl tetrazolium chloride
UDS	unscheduled DNA synthesis

Contents

CHAPTER 1

Background to Mutagenicity Testing

S. VENITT and J.M. PARRY

1. BACKGROUND READING AND SOURCES OF INFORMATION

For this book we have selected nine different methods for detecting genotoxic effects of chemicals, the object being to provide very detailed practical advice on how to do each test. Each chapter contains an introduction to the theory and background of the test, with sufficient references to allow for further reading. However, the literature on this topic is vast, and still growing at an alarming rate, to the extent, perhaps, that the newcomer may well wonder how to cope with it all. Nevertheless, even before setting foot in the laboratory, the aspiring genetic toxicologist must already have (or must acquire) a working knowledge of the basic concepts of genetics and of genetic toxicology. To this end we include a brief and necessarily simplified account of the scientific basis of mutagenicity testing (Section 2). This must be seen as an opportunity for newcomers to paddle in the shallows before plunging into an ocean of information in which it is very easy to drown. The newcomer, suitably moistened, should then learn to swim by dipping into some of the literature listed in *Table 1*.

1.1 Books and Journals

For the neophyte, a good start would be to consult a recent and comprehensive text-book on genetics, a subject fundamental to an understanding of the toxicology of genetics. Reference (1) is an excellent source of information on genetics. Access to the specialist literature of genetic toxicology may be gained by consulting *Table 1*.

1.2 Computerised Data Bases

Assistance with literature searches on assay systems or specific chemicals can be obtained from the Environmental Mutagen Information Centre (EMIC), Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. There is a branch of EMIC in the United Kingdom: EMIC (UK), Department of Genetics, University College of Swansea, Swansea SA2 8PP, UK.
Two very comprehensive on-line systems are:

(i) DIALOG (*Main Office*: DIALOG Information Services, Inc., 3460 Hillview Avenue, Palo Alto, CA 94304, USA. *European Office*: DIALOG Information Retrieval Service, PO Box 8, Abingdon, Oxford OX13 6EG, UK).
(ii) DATASTAR (Willoughby Road, Bracknell, Berkshire RG12 4DW, UK).

Table 1. Reference Sources for Genetic Toxicology.

Subject	*References*
Effects of mutagens and carcinogens on DNA structure and function, and how these effects relate to carcinogenic activity.	2,3,4
Discussion of the use and validation of short-term tests for genotoxicity, with special reference to the prediction of carcinogenicity.	5–9
Mechanisms of chemical mutagenesis.	1,10
Application of current knowledge of genetic toxicology to the study of mutation and carcinogenesis in human populations (e.g., population monitoring, dose-response effects, risk estimates, relationship between mutagenicity and carcinogenicity): series of articles commissioned and published under the auspices of the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC). *Note*: Earlier articles may be found in *Mutation Research, Reviews in Genetic Toxicology*.	11–28
Comprehensive reviews of the performance of individual assay systems as judged by the available literature: Gene-Tox Reports commissioned by the U.S. Environmental Protection Agency Gen-Tox Program. Of particular relevance to the subjects covered in this book are the following: *E. coli* reverse mutation test (Chapter 3, ref. 30), sister-chromatid exchange (Chapter 7, ref. 31), mutation in cultured mammalian cells (Chapter 8, refs. 32,33,34), *in vitro* and *in vivo* cytogenetics and the micronucleus test (Chapters 7 and 9, refs. 35,36), DNA repair tests (Chapter 4, refs. 37,38), mutation in yeasts (Chapter 5, refs. 39,40), the host-mediated and body fluids assay (Chapter 3, ref. 41) and mutation in *Drosophila melanogaster* (Chapter 6, refs. 42,43). *Note*: There are also other Gene-Tox Reports on assay systems which we have not included in the present volume [see *Mutation Research, Reviews in Genetic Toxicology*].	29–43
Evidence for the carcinogenicity of individual chemicals, mixtures or processes, with an evaluation of their genotoxic properties: see IARC Monographs.	44,45
Review of the role of chromosomes and chromosomal damage in the aetiology of cancer.	46
Guidelines on minimum criteria for conducting short-term tests, professional standards, interpretation, supplementary assays, mutagens in food, body fluids and excreta, nitrosation products.	47,48,59,60
The role of metabolic activation of foreign compounds in mutagenicity and carcinogenicity.	52,53

SPECIALIST JOURNALS:

Mutation Research, Carcinogenesis, Environmental Mutagenesis, Teratogenesis, Carcinogenesis and Mutagenesis, Journal of Toxicology and Environmental Health, Cancer Research, Journal of the National Cancer Institute, Food and Chemical Toxicology, Chemico-Biological Interactions.

2. A BEGINNER'S GUIDE TO THE BACKGROUND OF MUTAGENICITY TESTING

2.1 **The Genetic Material**

2.1.1 *Structure of DNA*

To live and to multiply, organisms depend on the information encoded in their genes (the units of inheritance). With the exception of certain viruses, this information is carried by deoxyribonucleic acid (DNA), a giant linear macromolecule

whose structure allows the economical coding and mass storage of an enormous amount of information, and its accurate copying and translation. The genetic code is remarkably simple, bearing in mind that the messages it conveys can lead to such diverse products as a herpes virus, a Blue Whale and J.S. Bach.

The genetic code employs just four 'letters' which are arranged in *codons* (*Figure 1*). Each codon consists of three 'letters', therefore 4^3 (64) different codons are possible. Proteins are composed of only 20 different amino acids, and it was predicted and subsequently shown that the genetic code is *degenerate*, i.e., one amino acid can be coded for by more than one codon. A linear sequence of codons specifies a linear sequence of amino acids (*Figure 1*).

The linear arrangement of different amino acids in a protein determines the way in which it folds up into a three-dimensional molecule. This in turn determines the properties of the protein (e.g., whether it has catalytic properties or structural properties; whether it can form ordered associations with other proteins or with other macromolecules; whether it can affect the enzymic or structural properties of other proteins). In general, proteins are assembled from smaller subunits known as *polypeptides*.

The letters in the genetic code consist of four different nitrogenous bases. Two are purines: *adenine* (A), *guanine* (G) and two are pyrimidines: *thymine* (T) and *cytosine* (C). A DNA molecule is a linear duplex: each strand of the duplex consists of a sugar-phosphate 'backbone' carrying a linear array of bases, the structural unit of sugar (deoxyribose), phosphate and base being a *monodeoxyribonucleotide*, a chain of monodeoxyribonucleotides being a *polynucleotide*. The array of bases constitutes the coding sequence of the DNA, the codon being the lowest order of genetic information. A *structural gene* is a polydeoxyribonucleotide, i.e., a linear sequence of codons, which codes for a polypeptide. The opposite strands of DNA are linked together by hydrogen bonds (which are relatively weak) between specific atoms in each base, the physical and chemical constraints being such that A can bond only with T, and G only with C. The AT and GC *base pairs* therefore enforce *complementarity* between opposite strands of the duplex (*Figure 1*).

Although the informational content of DNA is encoded in a linear form, the three-dimensional structure of the duplex molecule is that of a double helix, the sugar-phosphate backbone of each strand forming a spiral, with the flat nitrogenous base pairs stacked one upon the other in a staggered array, the whole structure being reminiscent of two intertwined spiral staircases.

2.1.2 *Replication of DNA*

In order to make identical copies of itself for eventual distribution to daughter cells, the DNA duplex unzips (each hydrogen bond breaking in turn) and each base pair separates: the replicating region now comprises two single strands (*Figure 1*). Each single strand now has an array of unpaired bases available for pairing with incoming complementary monodeoxyribonucleotides. The assembly of monodeoxyribonucleotides in the correct sequence into the new 'daughter' strand is thus strictly determined by the base sequence of the parental strand