METHODOLOGY *in* BASIC GENETICS

WALTER J. BURDETTE

METHODOLOGY *in* BASIC GENETICS

Edited by

WALTER J. BURDETTE, A.B., A.M., Ph.D., M.D.

Professor and Head of the Department of Surgery and
Director of the Laboratory of Clinical Biology,
University of Utah College of Medicine;
Surgeon-in-Chief, Salt Lake County Hospital;
Chief Surgical Consultant, Veterans Administration Hospital,
Salt Lake City, Utah

HOLDEN-DAY, INC., *San Francisco*

1963

Printed in the United States of America

Library of Congress Catalog Card Number 63–15646

PREFACE

Genetic mechanisms have been clarified in the past by examining heredity in diverse groups of organisms with greater knowledge emergent from comparative scrutiny than would have been possible otherwise. This experience now provides one means for evaluating reproduction at the molecular level. The gene itself has yielded to probing, so that the functional unit is now regarded in terms of codons, cistrons, pseudo-alleles, etc.; and, since recombination has not proved always to be a reciprocal event, copy-choice and genic conversion conform to certain analytical experience. Studies on the genetics of microorganisms have been particularly useful in demonstrating episomal relationships and bringing about freedom from conceptual conformity suggested by the more stylized means of fertilization, meiosis, and mitosis in higher metazoa. A beginning has also been made in determining how genic action leads to phenotype and events in differentiation. For example, recent work on invertebrate hormones suggests that humoral mechanisms may be involved in the differential initiation of genic action and transfer of coded message to cytoplasm with enhancement of the rate of protein synthesis at the ribosomal level. As the genetic code is confirmed for individual organisms and biochemical events, mechanisms of heredity can be analyzed with a degree of precision not possible in the past. The methods for manipulating organisms, chromosomes, and biological events to the advantage of the investigator are essential ingredients for taking advantage of these opportunities for research in an era of exceedingly rapid progress in acquiring genetic information. The arrangement adopted for presentation of pertinent methodology in this volume combines a survey of the present status of knowledge and methods with discussions by qualified investigators. The material has been organized into presentations about mutation and recombination, gene-protein relationships, and cytoplasmic inheritance, with individual chapters devoted to the status of these subjects in viruses, bacteria, protozoa, fungi, and *Drosophila*. It is hoped that the collection will be of value from instructive and heuristic as well as historic viewpoints.

WALTER J. BURDETTE
Chairman,
Genetics Study Section

Salt Lake City, Utah
March, 1963

PARTICIPANTS

Edward A. Adelberg, Ph.D.
Department of Bacteriology, University of California, Berkeley, California

Thomas Anderson, Ph.D.*
The Institute for Cancer Research, Philadelphia, Pennsylvania

Edgar Altenburg, Ph.D.
Genetics Laboratory, Texas Medical Center, Houston, Texas

Louis S. Baron, Ph.D.*
Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D.C.

Walter J. Burdette, Ph.D., M.D.*
Department of Surgery, University of Utah College of Medicine, Salt Lake City, Utah

Arthur Chovnick, Ph.D.
Department of Genetics, University of Connecticut, Storrs, Connecticut

James Crow, Ph.D.*
Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin

August H. Doerman, Ph.D.
Department of Biology, Vanderbilt University, Nashville, Tennessee

Robert S. Edgar, Ph.D.
Division of Biology, California Institute of Technology, Pasadena, California

*Member, Genetics Study Section

Sterling Emerson, Ph.D.
Division of Biology, California Institute of Technology, Pasadena, California

Boris M. Ephrussi, Ph.D.
Western Reserve University, Cleveland, Ohio

Harriett Ephrussi-Taylor, Ph.D.
Western Reserve University, Cleveland, Ohio

F. C. Fraser, Ph.D., M.D.*
Department of Genetics, McGill University, Montreal, Canada

Ernst Freese, Ph.D.
Laboratory of Molecular Biology, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland

Norman H. Giles, Ph.D.*
Department of Botany, Yale University, New Haven, Connecticut

Francis B. Gordon, Ph.D., M.D.*
Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland

John W. Gowen, Ph.D.
Department of Genetics, Iowa State University, Ames, Iowa

Earl L. Green, Ph.D.*
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Melvin M. Green, Ph.D.
Department of Genetics, University of California, Davis, California

Felix L. Haas, Ph.D.
M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas

Philip E. Hartman, Ph.D.
Department of Biology, The Johns Hopkins University, Baltimore, Maryland

William Hayes, Sc.D.
Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, England

Walter E. Heston, Ph.D.*
National Cancer Institute, National Institutes of Health, Bethesda, Maryland

F. Jacob, M.D., D.Sc.
Pasteur Institute, Paris, France

John L. Jinks, Ph.D.
Department of Genetics, University of Birmingham, Birmingham, England

Burke H. Judd, Ph.D.
Department of Zoology, University of Texas, Austin, Texas

David R. Krieg, Ph.D.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Joshua Lederberg, Ph.D.*
Department of Genetics, Stanford University, Palo Alto, California

Leonard Lerman, Ph.D.
Department of Biophysics, University of Colorado, Denver, Colorado

Edward B. Lewis, Ph.D.
Division of Biology, California Institute of Technology, Pasadena, California

D. L. Lindsley, Ph.D.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Salvador E. Luria, M.D.
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

H. J. Muller, Ph.D.
Department of Zoology, Indiana University, Bloomington, Indiana

David L. Nanney, Ph.D.
Department of Zoology, University of Illinois, Urbana, Illinois

Edward Novitski, Ph.D.
Department of Biology, University of Oregon, Eugene, Oregon

Clarence P. Oliver, Ph.D.
Department of Zoology, University of Texas, Austin, Texas

Irwin I. Oster, Ph.D.
Division of Chemotherapy, The Institute for Cancer Research, Philadelphia, Pennsylvania

Ray D. Owen, Ph.D.*
Division of Biology, California Institute of Technology, Pasadena, California

T. S. Painter, Ph.D.
Department of Zoology, University of Texas, Austin, Texas

Haig P. Papazian, Ph.D.
Department of Botany, Yale University, New Haven, Connecticut

David D. Perkins, Ph.D.
Department of Biological Sciences, Stanford University, Palo Alto, California

Donald F. Poulson, Ph.D.
Department of Biology, Yale University, New Haven, Connecticut

John R. Preer, Jr., Ph.D.
Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania

R. H. Pritchard, Ph.D.
Medical Research Council, Microbial Research Unit, Hammersmith Hospital, London, England

Charles M. Rick, Jr., Ph.D.*
Department of Vegetable Crops, University of California College of Agriculture, Davis, California

Herschel L. Roman, Ph.D.
Department of Genetics, University of Washington, Seattle, Washington

Francis J. Ryan, Ph.D.
Department of Zoology, Columbia University, New York, New York

L. Sandler, Ph.D.
Department of Genetics, University of Washington, Seattle, Washington

Werner Schmid, Ph.D.
Department of Zoology, University of Texas, Austin, Texas

William J. Schull, Ph.D.*
Department of Human Genetics, University of Michigan, Ann Arbor, Michigan

Albert Siegel, Ph.D.
Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona

Gunther S. Stent, Ph.D.*
Virus Laboratory, University of California, Berkeley, California

Curt Stern, Ph.D.
Department of Zoology, University of California, Berkeley, California

Wilson S. Stone, Ph.D.*
The Genetics Foundation, University of Texas, Austin, Texas

George Streisinger, Ph.D.
Institute of Molecular Biology, University of Oregon, Eugene, Oregon

R. P. Wagner, Ph.D.
Department of Zoology, University of Texas, Austin, Texas

Katherine S. Wilson, Ph.D.*
Genetics Study Section, Division of Research Grants, National Institutes of Health, Bethesda, Maryland

E. L. Wollman, M.D., D.Sc.
Pasteur Institute, Paris, France

Charles Yanofsky, Ph.D.
Department of Biological Sciences, Stanford University, Palo Alto, California

Norton D. Zinder, Ph.D.
Rockefeller Institute, New York, New York

CONTENTS

Mutation and Recombination in Drosophila

Gene-Protein Relationships

Cytoplasmic Inheritance

Appendices

MUTATION AND RECOMBINATION IN BACTERIOPHAGE

Ernst Freese, Ph.D.

INDUCED *and* SPONTANEOUS MUTATIONS *in* BACTERIOPHAGE

The aim of mutagenic work with phages is twofold: to determine with which relative frequency the different types of mutagenic base-pair changes in DNA occur spontaneously, which of them can be induced artificially, and how these changes come about in detail; to analyze for such mutants the detailed structural alteration of other molecules (RNA, proteins) in relation to the various base-pair changes. This paper will be concerned only with the first approach since work on the second aspect is just beginning.

The following base-pair changes in DNA are conceivable: replacements of one or more base pairs by other base pairs, deletions, insertions (additions of one or more base pairs), and inversions. Single base-pair replacements can be subdivided into transitions, the exchange of one purine by the other purine and one pyrimidine by the other pyrimidine, and transversions, the exchange of a purine by a pyrimidine and *vice versa*.[288]

Since the spontaneous mutagenic mechanisms are unknown, it is mandatory to proceed indirectly as follows: (1) use mutagens with chemical action which is either known or determinable by present chemical means; (2) surmise from this chemical effect and the mode of DNA duplication which base-pair changes a particular agent can induce; (3) show that genetic experiments employing this mutagen agree with the chemical expectation; (4) compare the genetic properties of spontaneous mutations, or mutations induced by an agent whose chemical effect is not well understood, with the genetic properties of those mutations whose base-pair changes have been determined previously. If these properties differ for mutations of different origin, the mutagenic mechanism must be different; otherwise it may be similar or identical. Understanding

of spontaneous mutations increases with the number of mutagenic chemicals of different specificity with known mutagenic mechanisms.

THE INDUCTION OF MUTATIONS

A survey of literature on mutations in bacteriophage shows that almost every conceivable agent can be found mutagenic under some conditions. Of interest, however, is only a large mutagenic effect; with respect to a given system of testing, the frequency of mutants per viable phage should be, after the mutagenic treatment, at least twice that of the spontaneous rate in the control. Smaller effects are usually regarded with distrust. The following will serve to illustrate this statement.

Background of spontaneous mutations

When several stocks of the same standard or mutant phage type are prepared, there is a different frequency of spontaneous mutants in each of them. The reason is that, in some cultures, the first mutation arises early and, since it multiplies together with the nonmutant phages, gives rise to a large background of mutant phages in the final stock; in other cultures, the first mutant phage occurs late and hence a small frequency of spontaneous mutants is found in the stock. In many cases, it is important to work with a stock having as small a background of spontaneous mutants as possible. This can be accomplished by growing several stocks, each with a phage inoculum that is as high as possible, but such that the probability of adding a mutant phage with the inoculum is, say, one-fifth. In this way, at least some of the stocks will have a relatively small background. For example, with phage *T*4, standard stocks with a frequency of $1\text{–}2 \times 10^{-4}$ *r* mutants per viable phage can be obtained in this way; the smallest frequency of revertants in an *r*II-mutant stock depends on the mutant and varies from 10^{-9} to 10^{-2}.

Detection of mutation induction

Mutations can be detected as forward or as reverse mutations. To explain this the example of the *r*II-type mutants of phage *T*4 will be used. The system for the plating properties of standard and *r*II-type mutants is indicated in table 1.

Table 1

PLAQUE TYPE OF STANDARD TYPE (*s*) AND *r*II-TYPE (*r*) MUTANTS ON BACTERIA *E. coli* B and K12 (λ).

	s	*r*II
B	w	*r*
K	w	—

*r*II phages do not plate on K, providing a selective method for the detection of revertants and recombinants.

The method of measuring the induction of forward mutations, for example, changes from standard-type phages to *r*-type mutants, has the advantage of being less specific but it is also less sensitive. It is less specific because the change of any one of many base pairs, within the *r*II region, can give rise to a mutation, and it is therefore irrelevant whether the mutagenic agent predominantly attacks A-T or G-C pairs or whether it induces only transitions, transversions, or other changes. However, this detection system is also rather insensitive since the mutagenic effect of any, even a very specific, mutagen can be detected only if it causes a significant increase in the total frequency of mutants above the spontaneous background (against which one usually cannot select).

The method of measuring the induction of reverse mutations, for example, from *r*II-type mutants to phages giving plaques on K, is usually very specific and in some cases extremely sensitive. It is specific since many mutants can apparently revert only by changes at one or very few genetic sites, that is by a genuine back mutation to the standard genotype or occasionally by a specific suppressor mutation. The induction of these reverse mutations will succeed only if the mutagen examined is able to induce the proper base-pair change. At the same time this detection system is very sensitive if the particular reverse mutation rarely occurs spontaneously.

For many functional properties, only the induction of reverse mutations has been measured so far. The absence of reversion in such a system does not prove the non-mutagenicity of the compound examined; it even leaves the possibility that, for another system of testing, this compound would be very mutagenic.

Method of mutagenic treatment and isolation of mutants

Mutagenic agents can be arbitrarily subdivided into those acting on resting DNA and others interfering with replicating DNA.

Resting DNA.—The phages to be treated *in vitro* must be kept under proper conditions (ionic strength, divalent metals, *p*H, temperature, and so forth) that do not lead to any significant inactivation or mutagenicity as such. In some cases, molecules of the mutagen may be so large that they can enter only phage heads with pores artificially enlarged, by using either osmotic shock-resistant mutants[82] or elevated temperatures and *p*H.[483]

The frequency of viable phages usually decreases exponentially according to

$$\frac{P}{P_0} = e^{-\beta t} = e^{-n}$$

when P = viable titer of phages after the treatment (for a time t)

P_0 = viable titer of phages before the treatment

and $n = ln\frac{P_0}{P}$ = "number of lethal hits."[296, 1008]

Sometimes the concentration of the mutagen changes during the period of treat-

ment, that is, β is time dependent. In this case, the strength of the treatment is measured more adequately by the number of lethal hits n than by the time of treatment. As another possibility some mutagens may react with the phage (e.g. the DNA bases) in a nonlethal fashion, and the lethal effect may occur later by a second reaction of the altered phage. In such a case, the lethal hits would measure only the extent of the second reaction and would not be proportioned to the time of treatment.

In some cases, it is necessary to use special conditions in order to keep the mutagenic effect high and the lethal effect low. For example, hydroxylamine exerts two effects, one both lethal and mutagenic and the other only lethal.[294] The latter lethal effect can be eliminated by the use of high concentrations of sodium chloride.

The mutagenic treatment can usually be terminated in several ways: by dilution, by change of pH, by addition of a stopping compound, and so forth. The treated phages are then plated on bacteria in which the mutagenic effect can be observed. For example, for the detection of r mutants, the treated standard-type phages are plated on bacteria *E. coli* B, and the frequency of both r and mottled plaques is counted (in statistically significant numbers). Similarly the induction of reverse mutations of rII-type phages can be observed either by plating the treated phages directly on bacteria K, on which only revertants can grow, or by permitting them to go through one or a few growth cycles before plating them on bacteria K. In the latter case, even those induced mutations which cannot express themselves can show when plated directly on bacteria K. One can preadsorb treated rII phages to bacteria B that have been killed by ultraviolet light to a survival of 10^{-3} and plate these infected bacteria together with bacteria K.[296] The UV killing prevents the bacteria B from duplicating further and thus providing too much multiplication of the r-type phages; but these bacteria do retain their capacity to support one cycle of phage growth. Another possibility is to infect bateria B singly by the treated phages, let them lyse, and then examine the lysate for the ratio of mutant to nonmutant phages. The first method has the disadvantage of giving a high spontaneous background, while the second method cannot completely exclude various kinds of selection.

The frequency of mutants (of a certain phenotype) per viable phage usually increases linearly with the time of treatment, that is,

$$\frac{M}{P} = \frac{M_0}{P_0} + \gamma t = \frac{M_0}{P_0} + \frac{\gamma}{\beta} n$$

when M is the titer of mutants after the mutagenic treatment (at time t) and M_0 is that titer before the treatment. This approximate formula is correct if the number of mutagenic hits γt (for the particular phenotype regarded) is small compared to one.

If the concentration of the mutagen changes during treatment, both γ and β are time dependent but normally will change proportionally so that γ/β is nevertheless a constant. The number of lethal hits n is therefore generally a better relative measure for the number of DNA bases attacked than the time of treatment. Sometimes γ and β, that is, the mutagenic and the lethal effect, change differently when the concentration of the mutagen changes.[294] It would then be more adequate (but elaborate) to