Tertiary Level Biology

Genetics of Microbes

Second Edition

Brian W Bainbridge

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Preface

Writing a textbook on microbial genetics in about 200 pages was undoubtedly a difficult task, but I have been encouraged by the response from both students and lecturers to the first edition. The requirement for a second edition is also a measure of the need for such a book. My experience as a lecturer has shown that what is needed first is an intelligible framework which can be read in a reasonable period of time. Armed with these principles, a student can then go to reviews and the original literature with a reasonable chance of understanding the jargon and the details. Molecular genetics is now so well advanced that it is easy to lose track of the purpose of a set of experiments in the wealth of sequence data and complex interactions. I have therefore kept the same format for this edition with a well-illustrated text giving original papers, popular reviews, monographs and detailed reviews to enable the student to take the subject further as required.

I have altered the sequence of the chapters by moving a considerably revised chapter on recombinant DNA to earlier in the book. This is because the new techniques are so fundamental to our understanding of how genes are constructed, mutated, expressed, regulated and recombined. Bacterial and phage genetics, on which the new technologies depend, are also dealt with earlier and an attempt has been made to introduce the concept of reverse genetics. The major change in emphasis is the importance of molecular genetics within microbial genetics. It should not be forgotten that these techniques have applications throughout biology, medicine and agriculture and it is hoped that this edition will help people in these areas to appreciate the beauty of the systems which are being exploited so successfully.

The chapters on fungal genetics have also been expanded to include the spectacular advances in the construction of yeast plasmids and artificial chromosomes. In addition similar techniques are now being extended to the filamentous fungi with important implications for the molecular biology of eukaryotic microbes and also for the industrial manipulations of fungi. Antibiotic production by the Streptomycetes is

also profiting from cloning techniques and one such advance is described in the last chapter.

I should like to thank a number of people who have made the revision of this book possible. I am very grateful to Ms Susan Elliott who has made an excellent job of new and revised diagrams and also to my daughter Judith for one of the diagrams. I am also grateful for the comments on the first edition, made over the last five years, by our own undergraduates. They clearly expressed a view when my own clarity was not of the best. I would also like to thank my daughter Ruth for allowing me to have unreasonable access to the home computer during the preparation of the manuscript. Finally I must thank my wife Margaret for her help and support during the preparation of this edition.

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CHAPTER ONE

BASIC PRINCIPLES OF MICROBIAL GENETICS

1.1 Introduction

The genetic study of microbes has played a highly significant role in the recent developments in molecular biology, recombinant DNA technology and the preparation of useful products such as insulin, human growth hormone and blood clotting factors. It was no coincidence that the first artificially-produced hybrid DNA was constructed using bacterial plasmids, and many of the spectacular advances and discoveries have been dependent on microbial systems or on microbial models. This success can be traced back to the first experiments on the molecular genetics of DNA in the genetic transformation of bacteria, as well as to the first isolation of metabolic mutants in fungi. Microbes are ideally suited to the combined biochemical and genetic approach which had early successes in the solution of the genetic code and the regulation of gene activity. The discovery and analysis of plasmid and bacteriophage systems laid the foundation for the exploitation of recombinant DNA techniques, which in their turn were dependent on the discovery of highly specific enzymes, also in bacteria. These techniques have revealed details of genetic organization which traditional genetic methods could not have brought to light. However, this should not be allowed to overshadow the contribution which microbial genetics has made to our understanding of natural variation, in studies on the origin of antibiotic resistance in pathogenic bacteria and the control of antibiotic synthesis in the streptomycetes and the fungi. Later chapters will review the recent extension of modern techniques to the yeasts, filamentous fungi and streptomycetes.

Recombinant DNA techniques now influence all areas of genetics, from gene structure to gene–protein interactions, from the

development of the fruit fly to theories of evolution based on gene and protein structure homologies, particularly genetic counselling, and even forensic medicine. A thorough grasp of microbial genetics is of enormous help in understanding how this progress has been made and how similar systems have been exploited, using animal and plant viruses, for studying and improving higher organisms. The principles of microbial genetic techniques have also been extended to the analysis and manipulation of higher plant, animal and human somatic cells. This chapter will review the basic procedures of mutant isolation and identification, biochemical analysis of gene function and the construction of gene maps, as a preparation for later chapters which deal with recent progress in our understanding of genetic processes.

1.2 Basic procedures and terminology

Genetics is concerned with the ways in which organisms vary and how this variation is passed on to the next generation. A certain amount of information can be gained by observing the differences and similarities between parents and offspring, but more information can be obtained if an experimental procedure is adopted. This procedure can be summarized as follows:

1. Isolation of genetically-pure strains
2. Isolation of strains showing variation for a particular character
3. Crossing of two genetically different strains in a controlled manner
4. Quantitative analysis of the progeny from the cross.

The use of genetically-pure strains is essential if we are to understand the mode of inheritance of the variation present. To understand what this entails, it is essential to define a few genetical terms. The nucleus of each cell contains one or more densely-staining structures called *chromosomes*, arranged along which are units of inheritance known as *genes*. These are linear stretches of deoxyribonucleic acid (DNA) containing a code which controls a gene product which may be either a ribonucleic acid (RNA) molecule or a protein. Changes can occur in the DNA of a gene such that the gene product is altered. These changes may be inherited; the new strain is then known as a *mutant* strain and the process by which it occurred is called *mutation*. The mutant strain can usually be detected by changes in one or more characteristics of the organism. The mutant gene is said to be an *allele* of the original

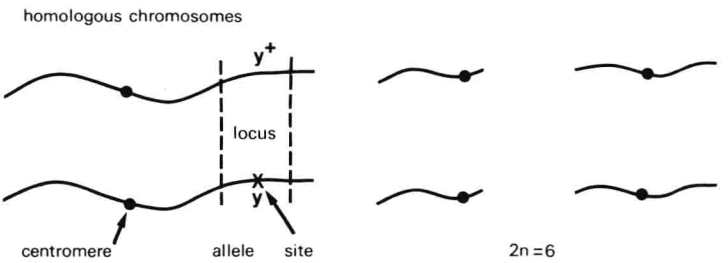

Figure 1.1 Diagram to show the use of key words in eukaryotic microbial genetics. y^+ and y are alleles located on homologous chromosomes. The region of the chromosome which they occupy is the *locus*. The cell is diploid and has three separate pairs of homologous chromosomes. The position of the mutation in y is a *site*.

or *wild-type* gene. A simple example of this is the colour of asexual spores in the filamentous fungus *Aspergillus nidulans*. The spores of the wild-type fungus are normally green, but mutation can occur to produce strains which have yellow spores. Gene symbols are given to these strains, the symbol being taken from the mutant character. The + superscript signifies the wild type allele,

$$y^+ \xrightarrow{\text{mutation}} y$$

green-spored strain — yellow-spored strain

A. nidulans is normally haploid, which means that the nucleus contains only one copy of each of the eight different chromosomes. During the development of fruiting structures, two haploid nuclei fuse to produce a diploid nucleus which has eight pairs of chromosomes, making a total of sixteen. Each pair of chromosomes is genetically and structurally different from the other pairs. Chromosomes which are genetically alike are said to be *homologous* (Figure 1.1). The y^+ and y alleles are located at identical positions on homologous chromosomes, and this position is known as the *locus* for this particular gene.

The sum total of genes in a particular strain is known as its *genotype*, and the appearance of the strain is its *phenotype*. As a diploid strain has two homologous chromosomes, it follows that there will be two copies of each gene at a particular locus. When these genes are identical, the strain is said to be a *homozygote* and when the genes are different, but still allelic, the strain is said to be a *heterozygote*.

Luckily many strains of *A. nidulans* are haploid, so the complications of diploid genetics do not apply.

1.3 Crosses involving spore colour in *A. nidulans*

Crosses between haploid strains are made by growing the strains together and allowing them to produce fruiting bodies. As the strains are haploid, there is only one allele in each strain, so the concept of genetic purity does not apply in the same way as it would to a diploid strain, which might have two different alleles in the same heterozygous strain. However, it is possible to have an impure strain of *A. nidulans* which is a mixture of yellow- and green-spored types. It is then necessary to purify the strain to produce a genetically pure *clone*. The asexual spores of this fungus have only one nucleus, so that a strain derived from a single spore can be assumed to be genetically pure unless any further mutation has occurred. The process is called *single-colony isolation* or *cloning*, and this is a basic step in eliminating unwanted variation in genetic experiments involving a wide range of microbes.

When the strains have been purified, they can then be crossed. There is a sexual stage in *Aspergillus*, and in the fruiting body a diploid nucleus is formed which immediately undergoes a division process called *meiosis*. The products of this division are four haploid types (Figure 1.2). The cross we have made can be seen to give rise to two green colonies for every two yellow colonies, as each sexual spore will have only one allele, either the yellow or the green alternative. This is one of the simplest crosses possible, giving a 1:1 ratio. We can see in this cross the basic procedures; first, the isolation of genetically pure clones; second, the choice of two strains which differed genetically and phenotypically; third, the crossing of the two strains so that sexual spores could be collected and allowed to develop into colonies which could then be analysed quantitatively to show the 1:1 ratio.

A further cross can be made between two strains, both of which have mutant spore colour. A second mutation can occur to produce spores of a pale-green colour called *chartreuse*. This mutation is located on a nonhomologous chromosome at a completely different locus from the original y^+/y mutation. A cross between the two strains results in four types of colonies: yellow, chartreuse, green and pale yellow (Figure 1.3). This is because the nonhomologous chromosomes segregate independently from each other during meiosis to give the original

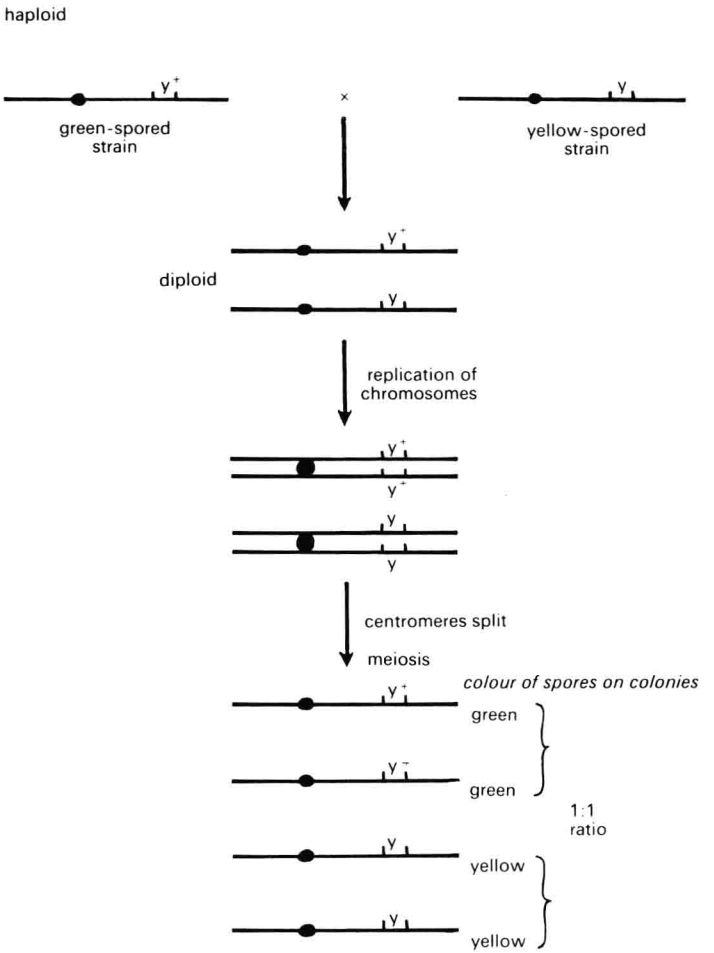

Figure 1.2 Cross between green and yellow-spored haploid strains of *Aspergillus nidulans* showing the relationships between genes and chromosomes.

parental combination of chromosomes carrying the mutant alleles of *y* or *cha*, but also producing *recombinant* combinations which are seen as the original wild-type spore colour, green, and the double mutant type, pale yellow. In other crosses the two loci may be on homologous chromosomes and may be so close that they segregate together at meiosis. In such crosses the parental types will exceed the recombinant types and give rise to the phenomenon of *linkage* which can be used

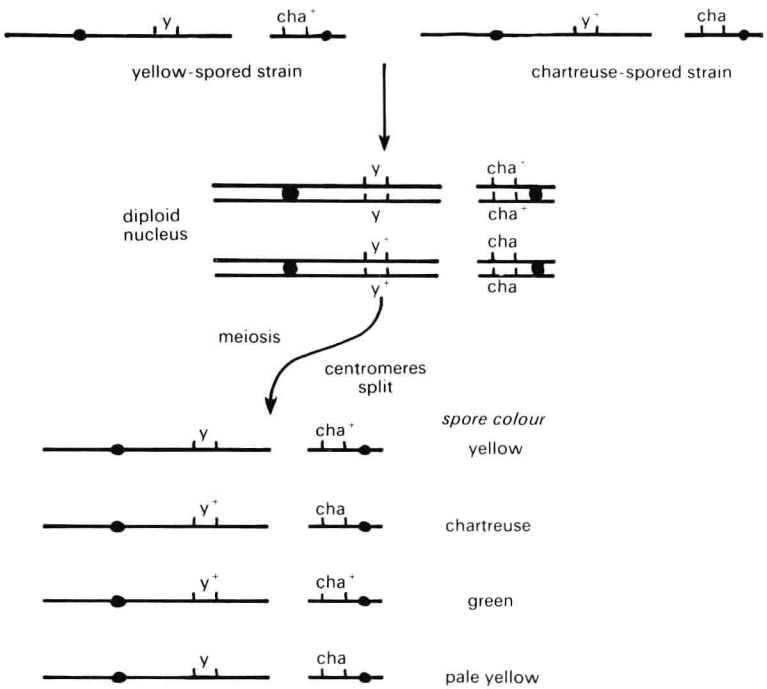

Figure 1.3 Cross between yellow and chartreuse-spored strains of *A. nidulans* showing origin of four types.

in the construction of chromosome maps. This will be referred to in section 2.2.

1.4 Crosses involving colony size in *Saccharomyces cerevisiae*

The life cycles of *A. nidulans* and the brewing/baking yeast *Saccharomyces cerevisiae* are broadly similar (Figure 1.4) but there are three major differences. Firstly, there are no filamentous mycelia or asexual spores, but only individual cells which reproduce by budding. Secondly, the diploid zygote can divide by *mitosis* to give identical diploid cells, each of which is capable of undergoing meiosis to produce sexual spores; and finally, there is a mating type in this yeast which is absent in *Aspergillus*.

The basic genetic procedure already described can also be applied to yeast. Strains are purified by separating individual cells and allowing

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