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Principles of Gene Manipulation

An Introduction to Genetic Engineering

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AN INTRODUCTION TO GENETIC ENGINEERING

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Abbreviations and Conversion Scale

amber (mutation) = *am*
dihydrofolate reductase = DHFR
gene for DNA ligase = *lig*
kilobases = Kb
megadaltons = Mdal.
molecular weight = mol.wt.
plaque-forming unit = pfu
temperature-sensitive (mutation) = *ts*

Scale for conversion between Kilobase pairs of duplex DNA and molecular weight.

Preface

Advances in biology continue to be made at a striking and ever increasing rate. One of the pace-setters is the subject matter of this book, *gene manipulation*, often popularly referred to as *genetic engineering*. A consequence of the phenomenal rate of progress in this subject has been that many biologists have found it impossible to keep pace with current developments, a situation exacerbated by the free use of jargon, and as with all rapidly growing fields it will be some time before comprehensive texts catch up. We have written this book to fill the resultant vacuum.

In the present state of the art, basic techniques are at the point of becoming well established and the trend is towards applying them to solve particular problems. Thus we have endeavoured to give readers enough details of these basic techniques to enable them to follow the current literature and future developments. In using this approach we hope that the content of the book does not date too quickly, but that the principles explained herein will provide an introduction to gene manipulation for some time to come.

The book is based on a series of twenty lectures on gene manipulation given at the University of Warwick to students on biology, microbiology and biochemistry degree courses. It is intended as an introduction to the subject for advanced undergraduates or people already in biological research, and consequently we have assumed that the reader has some prior knowledge of basic molecular biology. The literature has been surveyed up to the end of June 1979. The references cited are intended to point the reader towards the mainstream of the subject and to attribute original results to researchers. However, in a book of this size it is impossible to detail every paper. We have chosen examples from the literature which we feel best illustrate particular topics and hope that we have not offended colleagues whose experiments have not been mentioned.

Finally, it is a pleasure to acknowledge the skilled assistance of Mrs Debbie Bowns and Miss Dianne Simpson who had to interpret our sometimes impenetrable handwriting in producing the typescript; and Malcolm Davies for compiling and checking all the references.

R. W. Old
S. B. Primrose

August, 1979

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Chapter 1. Introduction

INTRODUCTION

The phrase 'gene manipulation' means different things to different people. Some, for example, would consider the sophisticated genetic manipulations undertaken daily by *Escherichia coli* geneticists as genetic manipulation. Most people, however, consider gene manipulation in a much broader context. In fact, in most Western countries there is a precise *legal* definition of gene manipulation as a result of Government legislation to control it (see Chapter 10). In the United Kingdom gene manipulation is defined as 'the formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation'.

The definitions adopted by other countries are similar and all adequately describe the subject matter of this book. However, in order to fully understand this definition it is necessary to consider the early development of the subject.

The Early Experiments

Some bacterial species can take up exogenous DNA by a process known as *transformation*.† Most transformable strains do not discriminate between uptake of DNA from a similar species and DNA from a completely different organism. Thus it should be relatively easy to introduce foreign DNA into bacteria. Probably the first recorded experiment of this kind is that of Abel & Trautner (1964). They reported that competent *Bacillus subtilis* could be transformed successfully with poxvirus DNA to yield infectious virions. However, current knowledge of poxvirus multiplication suggests that this result was an artefact.

Numerous groups of workers have reported the uptake of exogenous DNA by plant and animal cells and most of these experiments follow a

† The sudden change of an animal cell possessing normal growth properties into one with many of the growth properties of the cancer cell is called *cell transformation*. Cell transformation is mentioned in chapter 8 and should not be confused with bacterial transformation which is described here.

similar pattern. The plant or animal cell is supplied with radioactive bacterial or viral DNA of a buoyant density different from that of the host. In general, the persistence of DNA of donor buoyant density over a period of time in host tissues has been taken as evidence for the continued presence of donor DNA, at least partially intact, within the plant or animal cell. The appearance of radioactivity at the buoyant density of the host DNA is sometimes observed. This could represent integration of small amounts of donor DNA into the host chromosome but more likely represents donor DNA which has been degraded and whose breakdown products have been reincorporated.

The majority of experiments involving DNA uptake by eukaryotes has emanated from Ledoux's laboratory at Moll (Ledoux & Huart 1968, Ledoux *et al.* 1971). Their early experiments involved barley grains which were dehusked and surface sterilized. Each seed was then sectioned 1 mm from the end distal to the embryo and immersed in radioactive DNA from *Micrococcus lysodeikticus*. After 72 h radioactive DNA could be extracted and this had a buoyant density of 1.712 g/cm^3 compared with 1.702 g/cm^3 for barley DNA and 1.731 g/cm^3 for *M. lysodeikticus* DNA. Following sonication, DNA species with the densities of barley and micrococcal DNA were observed suggesting that this hybrid DNA consisted of covalently linked donor and recipient DNA. In similar experiments with *Arabidopsis thaliana* (a small weed) seedlings as host tissue, treatment of the F1 progeny, which had DNA of intermediate density, with more *M. lysodeikticus* DNA resulted in a new intermediate peak of greater buoyant density. This could be repeated over several generations producing peaks of greater density

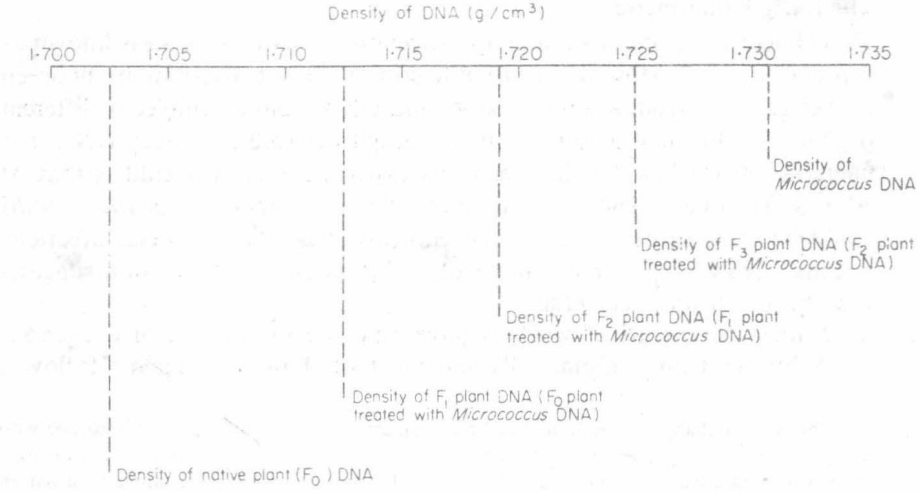

Fig. 1.1 Density of DNA from 'hybrids' of *Arabidopsis thaliana* and *Micrococcus lysodeikticus*. See text for details.

each time (Fig. 1.1). Ultrasonication again led to the production of DNA of host and donor buoyant densities.

There have been many attempts to repeat the experiments of Ledoux *et al.* but most have met with no success. Kleinhofs *et al.* (1975), for example, failed to observe such intermediate peaks when using axenic plants. However, when precautions against bacterial contamination failed, prominent intermediate peaks were observed and these were ascribed to bacteria growing on the roots. It would thus appear that foreign DNA is not integrated into the plant cell genome. The biggest problem with experiments like those of Ledoux and his colleagues is the determination of the fate of the exogenous DNA. It is apparent that density gradient centrifugation does not give unequivocal results. Much more sensitive techniques are available now (e.g. the 'Southern Blot' technique, page 7) and their development has greatly facilitated progress.

Transgenosis

Transgenosis is a term introduced by Doy *et al.* (1973) to describe the artificial transfer of genetic information from bacterial cells to eukaryotic cells by means of transducing phages. Four groups of workers have reported such experiments. Merril *et al.* (1971) took human fibroblasts from a patient with galactosaemia due to lack of galactose-1-phosphate uridyl transferase. The cells were infected with either λ *gal* T$^+$ or λ*gal* T$^-$ (where T specifies the transferase). Infected cells were assayed for phage-specific RNA and galactose-1-phosphate uridyl transferase. By four to five days after infection with phage as much as 0.2% of the total labelled RNA in the cells hybridized with λ DNA compared with less than 0.005% of the RNA from uninfected cells. Horst *et al.* (1975) used as recipient cells cultured skin fibroblasts from a patient with generalized gangliosidosis and characterized by a severe deficiency of β-galactosidase. The deficient human cells were incubated with the phage λ *plac or* λ *plac* DNA. The expression of the phage genome in the deficient fibroblasts could be demonstrated by detection of higher β-galactosidase activity after incubation with phage λ *plac* in 3 out of 19 experiments, and in 4 out of 16 experiments after treatment with λ *plac* DNA. λ *plac* DNA induced much higher enzyme activities than phage particles. The β-galactosidase activity in the infected fibroblasts was indistinguishable immunochemically and physicochemically from *E. coli* β-galactosidase. Doy *et al.* (1973) used φ80 *lac*$^+$ and λ *gal*$^+$ bacteriophage to treat haploid callus cultures of *Lycopersicon esculentum* (tomato) and *Arabidopsis thaliana*. These cultures grow on a defined medium containing glucose or sucrose but die when lactose or galactose is the sole carbon source. Treatment with the appropriate phage resulted in cultures which were able to grow on lactose or galactose, albeit more slowly than comparable calluses grown on glucose. The ability of these cultures to survive persisted over many subculturings and an immunological

test confirmed the presence of the bacterial enzyme in the treated plant cells but not in the controls. Conceptually similar experiments to those of Doy *et al.* were performed at the same time by Johnson *et al.* (1973) with λ *lac* and cell suspension cultures of *Acer pseudoplatanus* (sycamore). After treatment with phage the cells were able to grow slowly on lactose whereas in the absence of bacteriophage or with λ$^+$ the cells stopped growing and usually died. They were, however, unable to detect the bacterial enzyme either by assay or by electrophoresis.

Interesting as these experiments may be, considerably more evidence is needed to prove that *transgenosis* is a genuine phenomenon. There may be trivial reasons for the observed effects. Even so, transgenosis is purely of historical interest because it does not have the potential for large scale genetic manipulation afforded by other systems (see Chapters 3 and 4). The reason for this is set out below.

The Basic Problem

Although many people have attempted to transform pro- and eukaryotic cells with foreign DNA, transgenosis apart, their experiments have met with little success. Assuming that the exogenous DNA is taken up by the cells there are two basic reasons for the observed failures. Firstly, where detection of uptake is dependent on gene expression then failure could be due to lack of accurate transcription or translation. Secondly, and more importantly, the exogenous DNA may not be maintained in the transformed cells. If the exogenous DNA is integrated into the host genome then there is no problem. However, there are only two well-documented examples of this: the integration of plasmid DNA into the yeast genome (Hinnen *et al.* 1978, Struhl *et al.* 1979, see pp. 45-6) and the co-transformation of mouse cells with plasmid and phage DNA (Wigler *et al.* 1979, see page 100). The exact mechanism whereby this integration occurs is not clear. If the exogenous DNA fails to be integrated then it will probably be lost during subsequent multiplication of the host cells. The reason for this is simple. In order to be replicated DNA molecules must contain an *origin of replication* and in bacteria and viruses there is usually only one per genome. Such molecules are called *replicons.* Fragments of DNA are not replicons and in the absence of replication will be diluted out of their host cells. It should be noted that even if a DNA molecule contains an origin of replication this may not function in a foreign host cell.

The Basic Techniques

If fragments of DNA are not replicated then the obvious solution is to attach them to a suitable replicon. Such replicons are known as *vectors* or *cloning vehicles.* Small plasmids and bacteriophages are the most suitable vectors for they are replicons in their own right, their maintenance does not necessarily require integration into the host genome and their DNA can be

isolated readily in an intact form. The different plasmids and phages which are used as vectors are described in detail in Chapters 3 and 4. Suffice it to say at this point that initially plasmids and phages suitable as vectors were only found in *Escherichia coli*.

Composite molecules in which foreign DNA has been inserted into a vector molecule are sometimes called DNA *chimaeras* because of their analogy with the Chimaera of mythology—a creature with the head of a lion, body of a goat and the tail of a serpent. The construction of such composite or *artificial recombinant* molecules has also been termed *genetic engineering* or *gene manipulation* because of the potential for creating novel genetic combinations by biochemical means. The process has also been termed *molecular cloning* or *gene cloning* because a line of genetically identical organisms, all of which contain the composite molecule, can be propagated and grown in bulk hence *amplifying* the composite molecule and any gene product whose synthesis it directs.

Although conceptually very simple, the insertion of a piece of foreign DNA into a vector demands that certain techniques be available. These are:

(1) mechanisms for cutting and joining DNA molecules from different sources,
(2) a method for monitoring the cutting and joining reactions, and
(3) a means of transforming *E. coli,* since the first vectors used functioned as replicons in this organism.

It is interesting to note that all three techniques were developed about the same time and quickly led to the first cloning experiments which were reported in 1972 (Jackson *et al.* 1972, Lobban & Kaiser 1973). The methods for cutting and joining DNA molecules are now so sophisticated that they warrant a chapter of their own (see Chapter 2). Further details on the transformation of *E. coli,* and the use of gel electrophoresis for monitoring the cutting and joining of DNA molecules are given in the next section.

Agarose Gel Electrophoresis

The progress of first experiments on the cutting and joining of DNA molecules was monitored by velocity sedimentation in sucrose gradients. However, velocity sedimentation through sucrose gradients has two disadvantages. Firstly, the DNA molecules have to be labelled and their position can only be detected by fractionating the gradient and measuring the radioactivity in each fraction. Secondly, DNA molecules of similar size are not resolved. Both these problems were overcome with the introduction of agarose gel electrophoresis.

As early as 1966, Thorne (1966, 1967) had demonstrated that agarose gel electrophoresis could be used to separate the different molecular configurations of polyoma viral DNA, e.g. covalently closed circular molecules,

nicked circles and linear molecules. However, a few years elapsed before Aaij & Borst (1972) showed that electrophoresis in agarose gels could be used to separate not only molecules of the same mol. wt. but different configuration, but also molecules of different mol. wt. (Fig. 1.2). They were also able to show that the migration rates of the molecules were inversely proportional to the logarithms of their molecular weights thus permitting accurate sizing of DNA molecules. Another advantage of agarose gel electrophoresis is that the migration of DNA can readily be detected without recourse to radio-labelling. The bands of DNA in the gel are stained with the intercalating dye ethidium bromide and as little as 0.05 μg of DNA can be detected by direct examination of the gels in ultraviolet light.

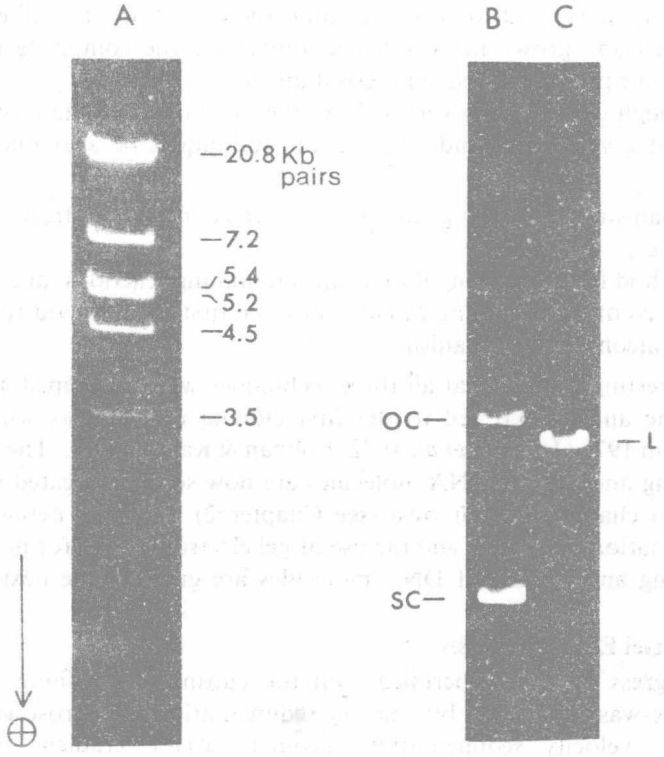

Fig. 1.2 Electrophoresis of DNA in agarose gels. The direction of migration is indicated by the arrow. DNA bands have been visualized by soaking the gel in a solution of ethidium bromide (which complexes with DNA by intercalating between stacked base pairs) and photographing the orange fluorescence which results upon ultraviolet irradiation. (A) Phage λ DNA restricted with *Eco* RI and then electrophoresed in a 1% agarose gel. The λ restriction map is given in Fig. 4.4. (B) Open circular (OC) and super-coiled (SC) forms of a plasmid of 6.4 Kb pairs. Note that the compact super-coils migrate considerably faster than open circles. (C) Linear plasmid (L) DNA produced by treatment of the preparation shown in lane B with *Eco* RI for which there is a single target site. Under the conditions of electrophoresis employed here, the linear form migrates just ahead of the open-circular form.

The electrophoresis in lanes B and C was performed in a 0.7% agarose gel.

Readers wishing to know more about the factors affecting the electrophoretic mobility in agarose gels of the different conformational isomers of DNA should consult the paper of Johnson & Grossman (1977).

Frequently it is necessary to know what sequences in a DNA fragment are transcribed into RNA and, clearly, it would be helpful to have a method of detecting fragments in an agarose gel that are complementary to a given RNA. This can be done by slicing the gel, eluting the DNA, and hybridizing to DNA or RNA either in solution, or after binding the DNA to filters. This method, which is time consuming and inevitably leads to some loss in the resolving power of gel electrophoresis, has now been replaced by a neat method described by Southern (1975). This method, often referred to as *Southern blotting* is shown in Fig. 1.3. DNA in the gel is denatured by alkali treatment and the gel is then laid on top of buffer-saturated filter

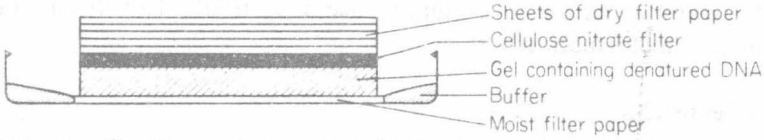

Fig. 1.3 The 'Southern blot' technique. See text for details.

paper. The top surface of the gel is covered with a cellulose nitrate filter and overlaid with dry filter paper. Buffer passes through the gel drawn by the dry filter paper and carries the DNA to the cellulose nitrate for which it has a high affinity. The DNA fragments bound to the cellulose nitrate can then be hybridized to radioactive DNA or RNA and hybrids detected by autoradiography.

Transformation of *E. coli*

Early attempts to achieve transformation of *E. coli* were unsuccessful and it was generally believed that *E. coli* was refractory to transformation. However, Mandel & Higa (1970) found that treatment with $CaCl_2$ allowed *E. coli* cells to take up DNA from bacteriophage λ. A few years later Cohen *et al.* (1972) showed that $CaCl_2$-treated *E. coli* cells are also effective recipients for plasmid DNA. The $CaCl_2$ probably causes changes in the structure of the cell wall that are necessary for uptake of DNA. Whereas almost any strain of *E. coli* can be transformed with plasmid DNA, albeit with varying efficiency, only *rec*BC$^-$ mutants can be transformed with linear bacterial DNA (Cosley & Oishi 1973). RecBC$^-$ mutants lack a nuclease which would otherwise degrade the DNA before it was integrated. Whereas linear bacterial DNA completely fails to transform RecBC$^+$ cells, linear λ DNA transfects† them with 30% efficiency compared to RecBC$^-$ cells.

† Transformation of a cell with DNA from a virus is sometimes referred to as *transfection*. This term is used to indicate that the DNA is infectious, i.e. can give rise to progeny virus particles.

Why is there such a difference between linear bacterial and λDNA? Presumably the answer lies in the ability of λ DNA to circularize following uptake—a strategy which prevents it from attack by the RecBC exonuclease (Benzinger *et al.* 1975).

The efficiency of transformation of *E. coli* is not high. Although efficiencies of 10^7 transformants/μg vector DNA can be achieved this represents uptake of only 1 DNA molecule/10^3 molecules added. With such low transformation efficiencies some cloning experiments are not feasible and currently much effort is being devoted to improving the efficiency of the process. As will be seen from the next chapter, many bacteria contain restriction systems which can influence the efficiency of transformation. Although the complete function of these restriction systems is not known yet, one role they do play is the recognition and degradation of foreign DNA. For this reason it is usual to use a 'restrictionless' (r^-) mutant of *E. coli* as transformation host.

DNA Sequencing

This chapter would not be complete without giving at least passing mention to the techniques of DNA sequencing. Although the availability of such techniques is not *essential* to the success of a cloning experiment they do provide much useful information about the product formed. The principles behind these techniques are too complex to be discussed here but the interested reader is recommended to read the review article by Air (1979).

Chapter 2. Cutting and Joining DNA Molecules

CUTTING DNA MOLECULES

It is worth recalling that prior to 1970 there was simply no method available for cutting a duplex DNA molecule into discrete fragments. DNA biochemistry was circumscribed by this impasse. It became apparent that the related phenomena of host-controlled restriction and modification might lead towards a solution to the problem when it was discovered that restriction involves specific endonucleases. The favourite organism of molecular biologists, *E. coli* K12, was the first to be studied in this regard, but turned out to be an unfortunate choice. Its endonuclease is perverse in the complexity of its behaviour. The breakthrough in 1970 came with the discovery in *Haemophilus influenzae* of an enzyme that behaves more simply. Present-day DNA technology is totally dependent upon our ability to cut DNA molecules at specific sites with restriction endonucleases. An account of host-controlled restriction and modification therefore forms the first part of this chapter.

Host-controlled Restriction and Modification

Host-controlled restriction and modification are most readily observed when bacteriophages are transferred from one bacterial host strain to another. If a stock preparation of phage λ, for example, is made by growth upon *E. coli* strain C and this stock is then titred upon *E. coli* C and *E. coli* K, the titres observed on these two strains will differ by several orders of magnitude, the titre on *E. coli* K being the lower. The phage are said to be *restricted* by the second host strain (*E. coli* K). When those phage that do result from the infection of *E. coli* K are now replated on *E. coli* K they are no longer restricted; but if they are first cycled through *E. coli* C they are once again restricted when plated upon *E. coli* K (Fig. 2.1). Thus the efficiency with which phage plates upon a particular host strain depends upon the strain on which it was last propagated. This non-heritable change conferred upon the phage by the second host strain (*E. coli* K) that allows it to be replated on that strain without further restriction is called modification.

The restricted phages adsorb to restrictive hosts and inject their DNA normally. When the phage are labelled with ^{32}P it is apparent that their DNA is degraded soon after injection (Dussoix & Arber 1962) and the

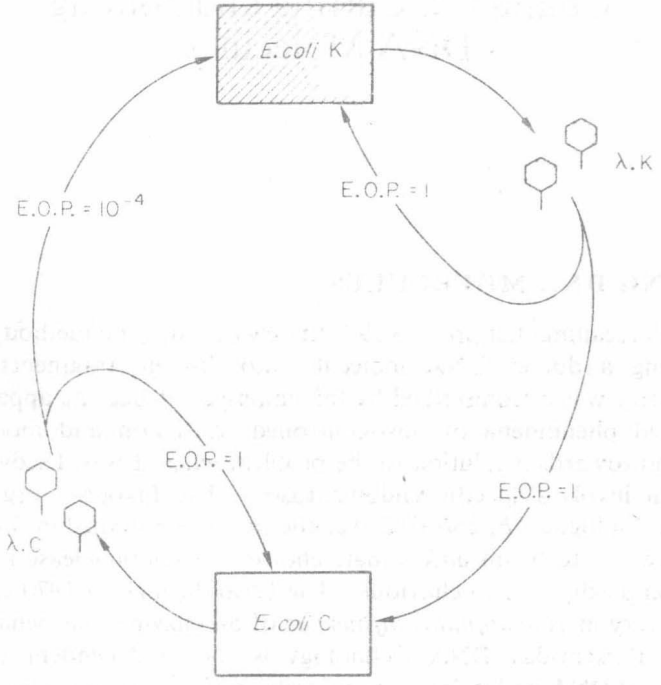

Fig. 2.1 Host-controlled restriction and modification of phage λ in *E. coli* strain K, analysed by efficiency of plating (E.O.P.). Phage propagated by growth on strains K or C (i.e. λ.K or λ.C) have E.O.P.s on the two strains as indicated by arrows. *E. coli* C has no known restriction and modification system.

endonuclease that is primarily responsible for this degradation is called a *restriction endonuclease* or restriction enzyme. The restrictive host must of course protect its own DNA from the potentially lethal effects of the restriction endonuclease and so its DNA must be appropriately modified. Modification involves methylation of certain bases at a very limited number of sequences within DNA which constitute the recognition sequences for the restriction endonuclease. This explains why phage that survive one cycle of growth upon the restrictive host can subsequently reinfect that host efficiently; their DNA has been replicated in the presence of the modifying methylase and so it, like the host DNA, becomes methylated and protected from the restriction system.

Although phage infection has been chosen as our example to illustrate restriction and modification, these processes can occur whenever DNA is transferred from one bacterial strain to another. Conjugation, transduction, transformation and transfection are all subject to the constraint of host-controlled restriction. The genes that specify host-controlled restriction and modification systems may reside upon the host chromosome itself or may be located on a plasmid or prophage such as P1.

The restriction endonuclease of *E. coli* K was the first to be isolated and studied in detail. Meselson & Yuan (1968) achieved this by devising an ingenious assay in which a fractionated cell extract was incubated with a mixture of unmodified and modified phage λ DNAs which were differentially radiolabelled—one with ^{3}H, the other with ^{32}P—so that they could be distinguished. After incubation, the DNA mixture was analysed by sedimentation through a sucrose gradient where the appearance of degraded unmodified DNA in the presence of undegraded modified DNA indicated the activity of restriction endonuclease.

The enzyme from *E. coli* K, and the similar one from *E. coli* B, were found to have unusual properties. In addition to magnesium ions, they require the cofactors ATP and S-adenosyl-methionine, and DNA degradation *in vitro* is accompanied by hydrolysis of the ATP in amounts greatly exceeding the stoichiometry of DNA breakage (Bickle *et al.* 1978). In addition, the enzymes are now known to interact with an unmodified *recognition* sequence in duplex DNA and then surprisingly, to track along the DNA molecule. The enzyme from *E. coli* B is known to track to one side only of the recognition sequence, which is asymmetric†. After travelling for a distance corresponding to between 1000 and 5000 nucleotides it cleaves one strand only of the DNA at an apparently random site, and makes a gap about 75 nucleotides in length by releasing acid-soluble oligonucleotides. There is no evidence that the enzyme is truly catalytic, and having acted once in this way, a second enzyme molecule is required to complete the double-strand break (Rosamond *et al.* 1979). Enzymes with these properties are now known as type I restriction endonucleases. Their biochemistry still presents many puzzles. For instance, the precise role of S-adenosyl-methionine remains unclear.

While these bizarre properties of type I restriction enzymes were being unravelled, a restriction endonuclease from *Haemophilus influenzae* Rd was discovered (Kelly & Smith 1970, Smith & Wilcox 1970) that was to become the prototype of a large number of restriction endonucleases—now known as type II enzymes—that have none of the unusual properties displayed by type I enzymes and which are fundamentally important in the manipulation of DNA. The type II enzymes recognize a particular target sequence in a duplex DNA molecule and break the polynucleotide chains within that sequence to give rise to discrete DNA fragments of defined length and sequence. In fact, the activity of these enzymes is often assayed and studied by gel electrophoresis of the DNA fragments which they generate (see Fig. 1.2). As expected, digests of small plasmid or viral DNAs give characteristic simple DNA band patterns.

† The recognition sequence of the restriction endonuclease of *E. coli* B is known:

5′ TGA $(N)_8$TGCT 3′

3′ ACT $(N)_8$ ACGA 5′

Very many type II restriction endonucleases have now been isolated from a wide variety of bacteria. In a recent review, Roberts (1978) lists 168 enzymes that have been at least partially characterized, and the number continues to grow as more bacterial genera are surveyed for their presence. It is worth noting that many so-called restriction endonucleases have not formally been shown to correspond with any genetically identified restriction and modification system of the bacteria from which they have been prepared: it is usually assumed that a site-specific endonuclease which is inactive upon host DNA and active upon exogenous DNA is, in fact, a restriction endonuclease.

Nomenclature

The discovery of a large number of restriction enzymes called for a uniform nomenclature. A system based upon the proposals of Smith & Nathans (1973) has been followed for the most part. The proposals were as follows:

(1) The species name of the host organism is identified by the first letter of the genus name and the first two letters of the specific epithet to form a three-letter abbreviation in italics. For example, *Escherichia coli* = *Eco* and *Haemophilus influenzae* = *Hin*.

(2) Strain or type identification is written as a subscript, e.g. Eco_{κ}. In cases where the restriction and modification system is genetically specified by a virus or plasmid, the abbreviated species name of the host is given and the extrachromosomal element is identified by a subscript, e.g. Eco_{P1}, Eco_{R1}.

(3) When a particular host strain has several different restriction and modification systems, these are identified by Roman numerals, thus the systems from *H. influenzae* strain Rd would be Hin_{d}I, Hin_{d}II, Hin_{d}III, etc.

(4) All restriction enzymes have the general name endonuclease R, but, in addition, carry the system name, e.g. endonuclease R. Hin_{d}III. Similarly, modification enzymes are named methylase M followed by the system name. The modification enzyme from *H. influenzae* Rd corresponding to endonuclease R. Hin_{d}III is designated methylase M. Hin_{d}III.

In practice this system of nomenclature has been simplified further.

(1) Subscripts are typographically inconvenient: the whole abbreviation is now usually written on the line.

(2) Where the context makes it clear that restriction enzymes only are involved, the designation endonuclease R. is omitted. This is the system used in Table 2.1, which lists some of the more commonly used restriction endonucleases.

Type II restriction endonucleases recognize and break DNA within particular sequences of tetra- , penta- , hexa- or heptanucleotides which have an axis of rotational symmetry. For example, *Eco* RI cuts at the