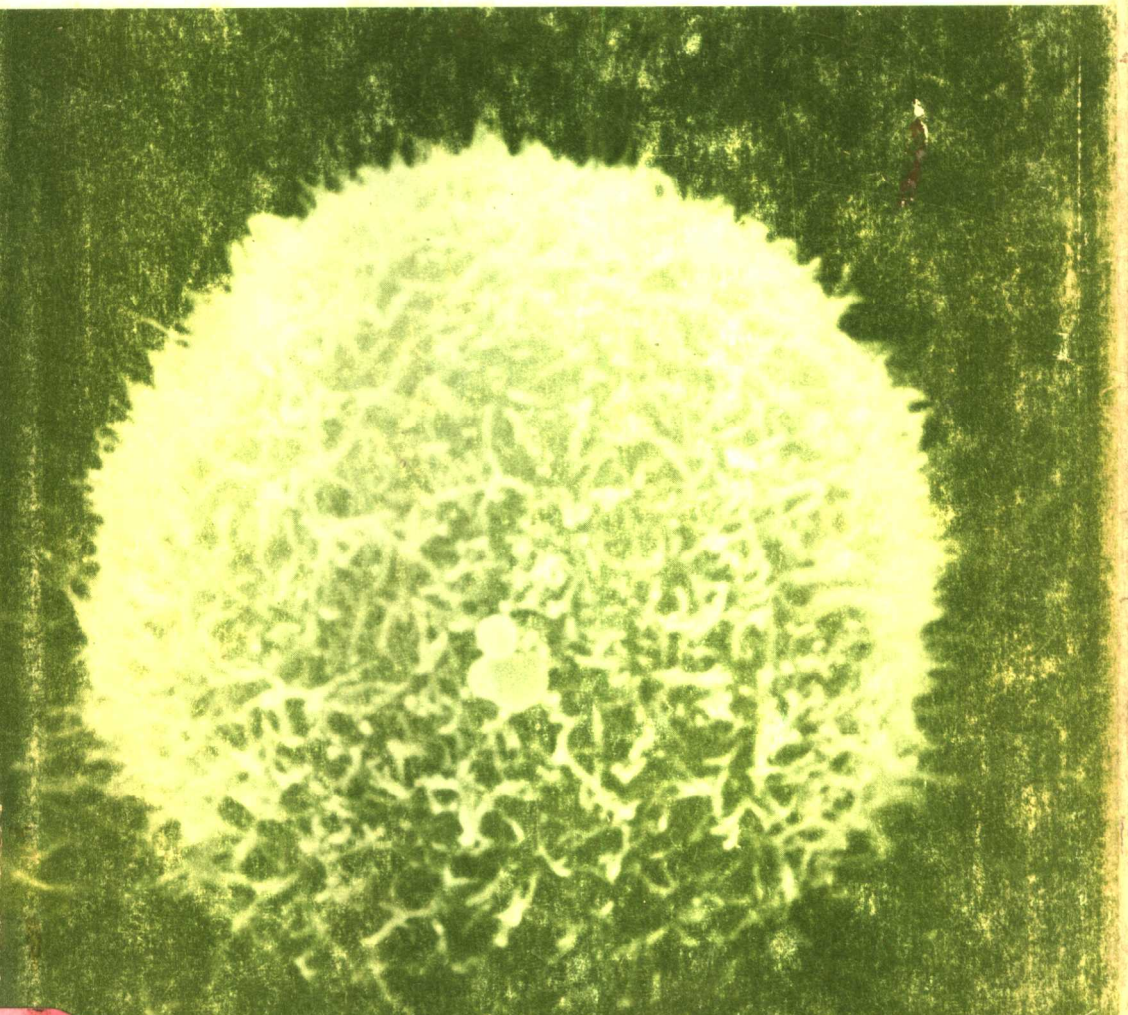
# Control of Gene Expression

N. Maclean



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# Control of Gene Expression

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# *Preface*

Almost all animal and plant cells contain at least one and often two copies of each gene possessed by the whole organism. Realization of this astonishing fact, and of its consequences for genetics, has developed gradually over the last century. One implication of this broad gene distribution is that those few genes which are actively expressed in a cell at any one biological place and time must be preferentially selected for activity: meanwhile the vastly greater number of genes within that cell which are biologically irrelevant to its structure and function remain silent and unexpressed. Although we now have some inkling as to how such differential gene expression is accomplished in bacteria, we are largely ignorant of the process in eukaryotic organisms. What is clear, however, is that the process in higher cells is not itself simple, and that many complex mechanisms are likely to be involved.

This book does not, therefore, set out primarily to explain how gene expression is controlled in eukaryotes, but rather to discuss and compare the experimental systems currently being used to investigate this problem. I have attempted to sketch fairly briefly the present state of knowledge of gene expression in bacteria in Chapter 2 and the probable roles of the nucleic acids in the process in eukaryotes within the concluding chapters. However, the greater part of the book is devoted to a discussion of some of the more important or promising experimental systems currently being exploited in this area by biologists and biochemists. I hope the book will prove of use to students and scholars whether they are on the "inside" or the "outside" of these systems. Not only does the book indicate the "state of play" within the experimental systems at the moment, but it also attempts to compare present results and future potential. Here I tread on dangerous ground. Not only am I seriously limited by my own partial knowledge of some of these systems—with only one, the synthesis of haemoglobins, am I intimately concerned—but my own judgement about which systems deserve mention and which are the more promising is bound to be a very arbitrary one.

Writing the book has confirmed in my own mind however, a suspicion which I entertained at the outset. It is that many of those engaged in

exploiting these systems in laboratories throughout the world are often poorly informed about the advances and advantages of other systems aiming at the same intellectual goals. When experiments are reported and discussed in the literature, the authors often fail to mention similar or contrasting evidence gleaned from another system. Such an omission may sometimes be intentional, but more often, in my view, it is accidental and simply reflects the near impossibility of constantly keeping abreast of the vast relevant literature. Perhaps this volume will help to partially alleviate that problem.

Because much of the book involves a discussion of experimental systems with which I am not in day to day contact, I have lent heavily on others with more intimate knowledge and "inside" understanding than myself. These people have generously given their time to read and criticize and I am very greatly indebted to them. They include:

Dr. Ruth M. Clayton
Prof. J. M. Mitchison
Prof. G. T. Stevenson
Dame Honor Fell
Dr. D. J. Cove
Dr. V. A. Hilder
Dr. W. T. Drabble
Dr. A. E. Wild
Prof. M. A. Sleigh
Prof. Sir W. B. Wigglesworth
Dr. J. R. Tata
Dr. J. B. Gurdon
Dr. W. R. Branch
Prof. F. C. Kafatos
Dr. G. Goldspink
Dr. D. A. Morris
Dr. J. M. Barry
Dr. D. R. Garrod

Of course I have not invariably taken their advice and in any event the opinions expressed in the book are my own unless I have indicated otherwise.

Many others have read and criticized small sections or provided invaluable discussion and contact. Most of the plates have been generously provided by other biologists both here and in the United States. These have been acknowledged within the body of the book. My own colleagues in the Department at Southampton have provided a friendly and stimulating environment for my own efforts, both scientific and literary, and the typing skills of Mrs. Anne Wharmby, Mrs. Mavis Lovell and Mrs. Monica Meek are gratefully acknowledged.

N. Maclean

Southampton, 1975

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# 1. The Control of Gene Expression and Its Levels of Action

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In the broadest terms an animal or plant is the result of an interaction between its genetic material and the environment. Studies on gene expression may therefore, in the widest sense, include work on the yields of vegetable crops, the behaviour of octopuses or the development of fish. This book interprets gene expression in a more limited but fundamental way. We will here concentrate on the synthesis of particular proteins as the expression of individual genes, only occasionally discussing the later modification of the protein molecules and rarely emphasizing the function of the proteins as the determinants of the ultimate animal or plant form.

Even if gene expression is accepted in the limited sense, as being the production of particular protein molecules, this production line still involves a number of different levels of control. Control at the level of transcription of the gene sequence into RNA is exerted by the gene sequence itself, sometimes also by neighbouring sequences, by the state of the chromatin in which the gene is located, by the availability and specificity of the RNA polymerase enzymes and by the availability of the appropriate nucleotides for synthesis of that RNA. For those genes whose expression involves synthesis of protein by translation of the RNA intermediate (omitting, that is, genes coding for RNA and not for protein, e.g. ribosomal RNA genes) a whole spectrum of post transcriptional control mechanisms exists, involving modification of the transcribed RNA precursor, availability of functional ribosomes and the regulated destruction of the messenger RNA molecule.

A fundamental consideration in any research is that no single experimental system can be expected to ideally or even usefully

reveal information on all aspects of a problem. When the problem is as broad as the control of gene expression this limitation is all the more evident. It follows that a wide variety of different experimental systems must be exploited in order to illuminate different aspects, the choice of experimental system being of paramount importance since it, more perhaps than the methodology or the insight of the investigator, determines what can and cannot be achieved. Although there is a tendency for the investigator to favour his system as a useful model for all aspects of the problem, an objective view soon betrays the limitations of most systems and the particular advantages of the best.

Any comparison of different experimental approaches to the same problem or to related problems exposes some other important observations. One is that, not infrequently, workers in one experimental area do not refer in their publications to equally or more informative results arising from a different but related experimental system. Sometimes this omission may be deliberate, but, often it seems to be accidental and to follow from a real ignorance of what has been achieved in the other system. Another is that semantic barriers often seem to prevent cross-fertilization and understanding between two different but fundamentally related fields. One man's protein is another man's antigen. A "determined" cell to the embryologist may be a "committed" cell to the immunologist. A third observation is that, although words such as "control" and "regulation" are used (all too often to give a modern ring to an old idea) with increasing frequency, very little of the work involves controlled degradation. Synthesis is regarded as being more fundamental and precise than degradation, and few people cite the breakdown of intermediates or products as fundamental limiting mechanisms. But, in fact, any study of control of populations size and character, which emphasized birth and ignored death, would be plainly stupid. Most studies on molecular aspects of gene expression are essentially similar to population studies on animal or plant species, the effective population is a result of a temporary balance between birth rate and death rate, and between synthesis and degradation.

In this chapter we will list the most important (but by no means all) stages and mechanisms of control, moving sequentially from the gene sequence on the DNA to the functional protein. In some cases the protein product cannot be directly assayed but some indication of its presence or activity is monitored, as, for example, in cell surface properties. These will be included, but the general

area of utilization and breakdown of the proteins themselves will be largely ignored, since this involves the broader areas of gene expression which ultimately combine to form the phenotype of the individual organism. In addition to noting the experimental systems which have contributed, or are likely to contribute, most to our knowledge of particular steps in the control sequence, some discussion of the available techniques and possible approaches to the various problems will be included.

## A. Control at the level of transcription

### 1. Elimination or destruction of the gene

Very few organisms resort to these processes as a means of controlling gene transcription. Gall midges, *Cecidomyidae*, and the nematode worm, *Parascaris equorum*, both eliminate part of the total genome from the somatic cells (discussed in Gurdon and Woodland, 1968) and tetraploid cells tend to lose chromosomes as cell divisions proceed (Migeon and Miller, 1968). It is arguable, however, whether these devices are really aimed at silencing the expression of the genes on these eliminated chromosomes, or more simply at reducing the overall DNA content of the cell.

### 2. DNA sequences which are normally incapable of transcription

Some DNA, particularly the centromeric chromatin of most chromosomes, consists of simple sequence DNA (highly repeated short sequences), as discussed in Section 7A. Such DNA is often termed consititutive heterochromatin. Although such DNA is obviously amenable to replication, it is not normally transcribed, i.e. it is not transcribed by the endogenous polymerases of that cell, nor is it necessarily transcribed by bacterial polymerases *in vitro*, i.e. in some *in vitro* systems it is transcribed, in others it is not.

There are other tracts of DNA which are not normally transcribed *in vivo* by endogenous polymerase, but which can be readily transcribed by bacterial polymerase. These include the spacer DNA which links the ribosomal gene sequences and also the L strand of the DNA double helix—only one of the two strands of the DNA duplex is normally transcribed and acts as functional genetic coding material. This sense strand is termed the H-strand. Evidence for transcription of spacer and L strand

DNA comes from the work of Reeder and Roeder (1972) and Honjo and Reeder (1973). Presumably, the *E. coli* polymerase used in these experiments is not sufficiently restricted in its activity and commences transcription at sequences which are not true initiator sequences. It also seems possible that the interchromomeric DNA of the polytene and lampbrush (and perhaps other) chromosomes falls into this transcriptional category of spacer DNA (see page 267).

### 3. Control by large scale chromatin condensation

As discussed more fully in Section 7A, large tracts of chromatin, and often entire chromosomes, are inactivated in particular cells by being rendered heterochromatic—faculative heterochromatin in this case. Thus, the transcriptional inactivity of the heterochromatic X chromosome in the human female, the Barr body of somatic female nuclei. Many other examples are known, including the heterochromatization of the entire parental set of chromosomes in the male scale insect (Brown and Nur, 1964). The condensation and heterochromatic character of the Y chromosome in the male mammal is a slightly different phenomenon and may be termed semifacultative heterochromatin (see Section 7A). Much, but not all, of the chromatin of this chromosome consists of simple sequence DNA.

We must also include here the striking examples of position effect, in which genes translocated to the vicinity of a block of heterochromatin have their genetic expression modified (Section 7A) or silenced. Such heterochromatization appears to block the RNA polymerase enzymes and to involve neighbouring chromatin in this impediment. As is discussed on page 271, although constitutive heterochromatin is structurally distinct from other chromatin, the differences between facultative heterochromatin and euchromatin are not dramatic in terms of composition, and are apparently mainly explained by an altered packing arrangement.

Most inactive chromatin is not, strictly speaking, heterochromatin, however. The condensed DNA of the amphibian erythrocyte nucleus, the mitotic chromosome and the unpuffed chromomeric band of the *Drosophila* polytene chromosome do not fulfil the normal requirements of heterochromatin, but their transcriptional inactivity is surely correlated with their condensation. Whether condensed euchromatin is actually analogous to

facultative heterochromatin in its packing arrangement, or whether some crucial factor renders the one more permanent than the other, is not at present clear. But all three systems mentioned, the nucleated erythrocyte, the mitotic chromosome and the polytene chromosome chromomere, constitute valuable experimental systems for the study of transcriptional regulation.

## 4. Control of one sequence by another

This is no doubt the chief means of regulating the activity of most of the genetic activity, both in prokaryotes and eukaryotes. Whereas in the former the control gene sequences are few, and may well account for less of the entire genome than do the structural genes themselves, it seems likely that in eukaryotes most of the genome is given over to control, and each structural gene sequence is probably coupled with (and probably preceded by) a long tract of control gene sequences. Of course, the simplest type of transcriptional system is the one which involves no control and all genes are transcribed maximally all the time. With the exception of some simple viruses (see Chapter 2) this situation does not normally occur in living organisms. Instead, although some genes such as those coding for 18 and 28S ribosomal RNA may indeed be uncontrolled and except in very condensed chromatin will always be available for transcription, most genes are regulated in their activity. Such regulation is mediated either by regulatory molecules or controlled chromosome condensation, or both, and these aspects are discussed in Chapters 6 and 7.

Another possible mechanism for transcriptional regulation is linear reading, in which genes would be transcribed in the order of their arrangement on the chromosome, and their position would be tightly correlated with the time in the cell cycle when their activity was required. Provided that messenger RNA half life is not too short, such a system can presumably operate effectively and some slight evidence favouring such a system has been found in studies on the yeast cell cycle (see Chapter 4B).

The exciting growth of knowledge about bacterial operons and regulons has stimulated an intensive search for similar genetic control mechanisms in eukaryotes, but so far without success. One group of genes in the fungus *Aspergillus* is expressed coordinately in a manner suggesting a rather loose operon, but all other genes in eukaryotes, whether linked or unlinked, appear to

be controlled singly. Since both positive and negative control of bacterial operons is known, attempts have also been made to identify the type of control prevalent or universal to eukaryotes. The evidence from fungi (see Section 4D) suggests positive control and many theories of eukaryotic gene regulation are based on this model. However, as discussed in Section 7A, the negative control mechanism may well be more typical, in view of the apparent transcription of the control sequences in the formation of the Hn RNA as precursor to mRNA.

### 5. Effects of one gene on its homologue

Most eukaryotes are diploid and two copies of each gene are normally present in every cell, either as identical or non-identical alleles. Presumably the frequent phenotypic effect of gene dominance or partial dominance is not normally a result of transcriptional control but of competition between differing forms of the same protein for functional or structural priority.

In the case of the facultative heterochromatization of one of the two chromosomes in the human female X or the male mealy bug, only one of the two gene copies is expressed, but the transcribed gene copy may differ from one cell to another, depending on which chromosome happened to be condensed. But two other examples are known which are not believed to be explained by condensation of one of the two sister chromosomes involved. The first is the "allelic exclusion" phenomenon demonstrated by the pattern of synthesis of immunoglobulins (see Section 3A). No mechanism is known which can account for this remarkable phenomenon, but presumably the product of one of the two alleles automatically inactivates the other copy, perhaps depending simply on which copy happened to be first transcribed. The second example is revealed by studies on the types of ribosomal RNA synthesized during the development of *Xenopus laevis* × *mulleri* hybrids (Honjo and Reeder, 1973). The ribosomal RNAs (18 + 28S) of the two species do not in fact vary, but the precursor RNA molecules can be readily distinguished by hybridization since the spacer sequences do differ considerably. Hybrids synthesize only the *laevis* type, irrespective of which species is the male and which is the female in the cross. Now it may be that this curious effect can be explained either by the RNA polymerase of the one species blocking the promoter or control sites on the homologous ribosomal RNA genes (a special form of

RNA polymerase is responsible for transcription of the ribosomal sequences) or, as suggested for allelic exclusion in immunoglobulins, that the transcriptional product of one gene (or its near neighbouring sequences) effectively represses transcription of the rival sequences in the hybrid.

### 6. Specificity and availability of RNA polymerase enzyme

As is discussed in Chapter 2, the main RNA polymerase enzyme of bacteria can be split into core and sigma fractions, the latter subunit having the ability to restrict preferentially the affinity of the enzyme for certain promoter gene sequences. No evidence has so far been obtained for a similar restriction operating in eukaryotic cells. But this is not to say that no such mechanism exists. Many genes which are transcribed seem to be much more actively transcribed at certain parts of the cell cycle than others, or in some cells than others. Work on the yeast cell cycle (Section 4B) and on the expression of the lactate dehydrogenase genes (Section 3 B) suggests that rate control is exerted over the transcriptional activity of certain genes. Probably the simplest mechanism for effecting such rate control would be via the affinity of the RNA polymerase for the promoter sequence. Of course other forms of control might easily operate, especially some form of end-product inhibition of the specific sequence transcription. Again, the best examples of such mechanisms are to be found in fungal systems (Section 4D). The existence of separate eukaryotic RNA polymerases for the ribosomal and other sequences, presumably, permits rather distinct kinds of control to affect these two types of sequence.

## B. Experimental approaches to transcriptional regulation

### 1. Antagonists of transcription

Amongst the wide range of antibiotic substances now known, several have important and specific effects on transcription and provide the investigator with extremely valuable tools. Perhaps the best and most widely used is Actinomycin D, which binds to DNA and effectively prevents transcription. Some variation in effect is noticeable in different cells and organisms, probably due to variable permeability of the cells to the drug. An excellent review of the mechanism of action of Actinomycin D and other antibiotic drugs can be found in Gale (1972). Actinomycin

will, at high doses, effectively inhibit all RNA polymerase activity, but at doses below one μg/ml it becomes relatively specific for the synthesis of ribosomal RNA and at higher doses for messenger RNA. The use of actinomycin D in the experimental work on *Acetabularia* has been particularly valuable and is discussed in the appropriate chapter.

A second antibiotic, Rifampicin, is a semisynthetic derivative of the naturally occurring rifamycin. This drug is a potent inhibitor of bacterial DNA-dependent RNA polymerase, but not of the eukaryotic RNA polymerases, except probably those of mitochondria and chloroplasts (the impermeability of mitochondria to the drug renders it ineffective *in vivo*). There is also some evidence that rifampicin will inhibit some viral polymerases and so may have value as an antiviral drug. A third substance α-Amanatin, which occurs naturally in the fungus *Amanita phalloides*, is valuable in a way complementing the use of rifampicin. γ-Amanatin inhibits only the non-nucleolar RNA polymerase of eukaryotic cells, leaving bacterial, mitochondrial and nucleolar (ribosomal) RNA synthesis mainly unaffected. Although we cannot here discuss the very numerous experiments undertaken with these inhibitors of nucleic acid synthesis, various projects discussed in later chapters in this book involve the use of these valuable drugs.

## 2. Molecular hybridization

The discovery that single stranded nucleic acids will, under appropriate conditions, hybridize with another molecule of complementary base sequence, has proved of great experimental value in this area. Not only may DNA to DNA and DNA to RNA hybrids be monitored, but the radio labelling of one of the hybrid monomers permits it to be used as a probe for the detection of complementary sequences within a cell or cell extract. In addition, with the recent availability or relatively pure bulk fractions of particular messenger RNAs and of viral reverse-transcriptase polymerases, it has become possible to synthesize radioactively labelled DNA probes which are complementary to known messenger sequences, thus permitting assay for such messenger RNA sequences in other cells and tissues. While applauding this advance we would do well to emphasize that the formation of such probes is dependent on the availability of relatively pure