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Developmental Biology of Prokaryotes

EDITED BY J H PARISH

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Developmental Biology of Prokaryotes

EDITED BY

J. H. PARISH
MA, DPhil
Senior Lecturer in Biochemistry
University of Leeds

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Contributing Authors

N.G. CARR Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

G.H. CHAMBLISS Department of Bacteriology, University of Wisconsin, 500 Lincoln Drive, Madison, Wisconsin 53706, U.S.A.

K.F. CHATER John Innes Research Institute, Colney Lane, Norwich NR4 7UH, U.K.

J.B. CLARK Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, Oklahoma 73069, U.S.A.

W.D. DONACHIE Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, U.K.

C.S. DOW Department of Biological Sciences, University of Warwick, Coventry, Warwickshire CV4 7AL, U.K.

R.S. HANSON Department of Bacteriology, University of Wisconsin, 500 Lincoln Drive, Madison, Wisconsin 53706, U.S.A.

M.J. MERRICK John Innes Research Institute, Colney Lane, Norwich NR4 7UH, U.K.

J.H. PARISH Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K.

S.C. RITTENBERG Department of Bacteriology, University of California, Los Angeles, California 90024, U.S.A.

M.F. THOMASHOW Department of Bacteriology, University of Washington, Seattle, Washington 98195, U.S.A.

J.B. WATERBURY Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, U.S.A.

R. WHITTENBURY Department of Biological Sciences, University of Warwick, Coventry, Warwickshire CV4 7AL, U.K.

Preface

This is a text on prokaryotic morphogenesis, differentiation and development. It is intended for advanced undergraduate and postgraduate courses in microbiology and assumes a knowledge of elementary microbiology, biochemistry and molecular biology, such as would be acquired during first-year university courses for biology students, or alternatively from a familiarity with a textbook on basic microbial physiology, such as Sutherland & Dawes (1976). The present book contains detailed references to the original literature and forms an introduction to prokaryotic developmental biology for scientists from other disciplines. The authors hope that they may widen the biological horizons of biochemists and molecular biologists by illustrating the natural history of groups of organisms, all of which are successful inhabitants of our planet and some of which have been so neglected as to be almost unknown to molecular scientists.

As editor, it is a pleasure for me to acknowledge the help and encouragement I have received from the contributors, publishers and everyone involved in the production of the book. I am particularly grateful to Margaret Parish for help in the assembly and checking of the text.

University of Leeds

John Howard Parish

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Chapter 1. Introduction

J.H. PARISH

1.1 PROKARYOTIC DIVERSITY AND UNITY

Prokaryotes are sometimes presented by textbook writers as belonging to a rather homogeneous group of similar organisms. In particular, books and lecture notes of biochemists may even include a diagram illustrative of a generalized bacterium, typically a sausage-shaped organism complete with cell wall, flagella, pili and few other discernable structures. We might enquire how a generalized animal could be drawn to illustrate features found in (for example) a sponge, a bee and an elephant. Prokaryotes actually form a very diverse group. This is to be expected of organisms that have occupied the surface of this planet for at least two-thirds of its existence and presently occupy every conceivable ecological position. Different prokaryotes will grow at temperatures between 0 and 100°C and can utilize nutrients varying from carbon dioxide and nitrogen as sole carbon and nitrogen sources through an astonishing variety of organic chemicals to the complex mixtures characteristic of the most extreme forms of parasitism and host dependence.

And yet one has the feeling that there is a certain unity among prokaryotes: the lack of a separate nuclear domain, the relatively small cell size, the absence of distinct intracellular membrane systems apart from the plasma membrane, all these have proscribed several metabolic, physiological and anatomical possibilities that are exploited by eukaryotes.

1.1.1 Some typical prokaryotic properties

Prokaryotic cells contain a cytoplasm enclosed by a plasma membrane. In the majority of prokaryotes, the membrane is surrounded by a sacculus composed of peptidoglycan and other polymers. Gram-negative prokaryotes have an outer membrane surrounding the peptidoglycan. All internal membranes are formed from invaginations of the plasma membrane and the nucleoid, the counterpart of the eukaryotic nucleus, is not bounded by a membrane. Prokaryotes are small; with the exception of cyanobacteria, the cell volume rarely exceeds 5 μm^3.

Prokaryotes can respond to changes in their environmental milieu by

metabolic responses, movement, change in growth rate and by a variety of changes in cell shape, structure and function.

Metabolic responses

A characteristic feature of prokaryotes is their method of metabolic response. They have developed extraordinarily sophisticated mechanisms for the regulation of metabolism by controlling enzyme biosynthesis. As the study of this process has led to an understanding of much of the molecular biology of prokaryotes and has become the basic language used for diagnosing the molecular basis of the processes discussed in this book, some of the main features are summarized in the later part of this chapter (section 1.2).

Movement

Not all prokaryotes are motile. Those that are use one of three basic processes: swimming by rotation of one or more flagella, rotation of a helical cell around an axial filament and gliding over a solid surface. The mechanisms of the first two processes are understood in detail. Moreover the second process (typical of spirochetes) is essentially a variant of the first. The filament is a flagellum that rotates and, because of its restricted movement inside the outer sheath of the organism, generates gyratory movement of the cell; see Berg, Bromley & Charon (1978) for an excellent review of flagellate and spirochete motility.

Prokaryotic motility is not random but is directed to certain ends. In the case of *E. coli* (a flagellate) a detailed model of the flow of information from chemotaxis receptors to the motility apparatus accounts for the finding that the organism continues swimming up a gradient of attractant (or down a gradient of repellent) but stops and reorientates in an unfavourable gradient (Simon, Silverman, Ridgway, Komeda & Hilmen, 1978).

Growth rates and changes in cell type

Prokaryotes adjust their growth rate in response to their environment and are also able to change the size, shape, structure, chemical composition and function of their cells in a variety of ways. These properties are unique to prokaryotes and serve to highlight the enormous diversity of the group. In the following sub-section (1.1.2) the scope of these changes is introduced with particular reference to the groups of organisms discussed in the book.

1.1.2 Morphogenesis, differentiation and development

In prokaryotic biology these terms have specific meanings (Whittenbury & Dow, 1977).

Morphogenesis is a process that leads to the formation of a cell of defined size and shape. The most straightforward example is the reproduction of a cell by binary fission. The process is understood in most detail in *Escherichia coli* (Chapter 2). If we were to design the simplest possible bacterium, a num-

ber of very difficult molecular problems would be posed in ensuring that the organism knew where and when to septate and that DNA replication was in phase with cell division. A real organism not only solves these problems but also is able to adjust its growth rate in different environments.

Differentiation refers to the change of one cell type to another. It is thus a specialized type of morphogenesis. Development (or pattern formation) refers to the morphogenetic response of a cell to its intracellular environment. In other words, development is the communication of information for a cell to differentiate because of its position in relation to other cells. Several purposes may be served by differentiation and development: the differentiated cell may be specialized for survival in a hostile environment; differentiation may be an integral part of a specialized life cycle; a differentiated cell may be required for a specialized metabolic task.

Endospores are the resistant forms of many bacteria; they are non-dividing cells, more resistant to desiccation, heat and other adverse conditions than the vegetative organisms (Chapter 3). Endospore formation (or 'sporulation') is an example of differentiation and has been studied in most detail in *Bacillus subtilis* (Chapter 4). Like many cases of differentiation, the morphological changes are accompanied by a carefully timed programme of biochemical change.

The Actinomycetales represent a very large and widely distributed group of bacteria that form rounded cells from rods. The simplest organisms of this type are probably the arthrobacters (Chapter 5) in which rods and spheres can interconvert in a morphogenetic cycle. In more advanced members of the group the vegetative cells become more elongated and the life cycles become more complex through many genera including *Mycobacterium*, *Nocardia*, *Actinomyces* and *Streptomyces*. The next chapter (6) covers *Streptomyces*, which was chosen because the organism introduces several new features. The vegetative rods have become mycelial filaments and the spheres (*Streptomyces* spores) are resting forms and are produced in a complex structure that represents an elegant example of development. This very specialized development or 'colonial morphogenesis' is a response to a fairly common nutritional problem. Streptomycetes rely on extracellular enzymes for production of their nutrients. A web of mycelium achieves a concentration of bacteria so that loss of enzymes by diffusion is less critical than it would be for one small cell producing its own small amount of enzyme.

Bdellovibrio (Chapter 7) has solved the same nutritional problem by a different mechanism. The organism grows in the periplasmic space (the domain defined by the outer and plasma membranes of a Gram-negative bacterium) of a substrate cell (such as an *E. coli* or a *Pseudomonas*). The bdellovibrio's extracellular enzymes are contained within the periplasm. As the substrate cell cannot survive this invasion, the bdellovibrios require a specialized type of differentiation: a free-swimming flagellate bdellovibrio attacks its substrate cell, penetrates, grows into a spiral cell and then frag-

ments to produce more flagellates.

The prosthecate bacteria (Chapter 8) demonstrate a different type of differentiation. In many of these organisms, more than one type of cell is obligately required because of an inherent asymmetry of the cell cycle. If two such types are required, the organism is said to be dimorphic.

Chapters 9 and 10 cover different groups of cyanobacteria (the archaic phrase, blue-green algae, is not used in this book). Filamentous cyanobacteria demonstrate two types of differentiated cell: akinetes that are the resting forms and heterocysts that are specialized cells capable of fixing nitrogen. Heterocysts, unlike akinetes and all other examples of resting cells, are incapable of germination; they are doomed to a life of self sacrifice in the interest of the nutrition of their fellows. The positioning of heterocysts and akinetes in the filament is under strict control and is an example of pattern formation. Pleurocapsalean cyanobacteria have a complex life cycle in which a variety of specialized division processes yield a population of relatively large numbers of daughter cells from the fragmentation of a large organism.

Finally, myxobacteria (Chapter 11) form fruiting bodies. The unique type of cell-cell interaction and colonial morphogenesis in this group is a different solution to the problem of relying on extracellular enzymes for growth. Differentiation in the myxobacteria consists of a rounding up and encystment of the cells to form myxospores.

Several groups of organisms have been omitted from the book. In particular, encystment is not unique to myxobacteria; another important group of cyst-forming bacteria is *Azotobacter* (see Sadoff, 1975 for review). Also the very specialized life cycles of certain groups of obligate parasites of eukaryotic cells, for example *Chlamydia* (Becker, 1978), have not been included, partly owing to lack of space and partly because they can only be seen in the context of the biology of extreme parasitism.

1.2 PROKARYOTIC GENE EXPRESSION AND CONTROL

Many of the chapters describe evidence for mechanisms of regulation of morphogenesis, differentiation and development that rely on a background understanding of the regulation of gene expression that has been worked out largely from studies on *E. coli* and its phages. The processes are summarized here; see Watson (1976) and Lewin (1974, 1977) for details.

1.2.1 Gene expression

Genome organization

Prokaryotes are haploid organisms and have a single chromosome in the form of a covalently continuous loop of double-stranded DNA with a size corresponding to between 1 and 10×10^6 base pairs. The prokaryotic chromosome is an example of a replicon, that is a piece of DNA capable of being replicated

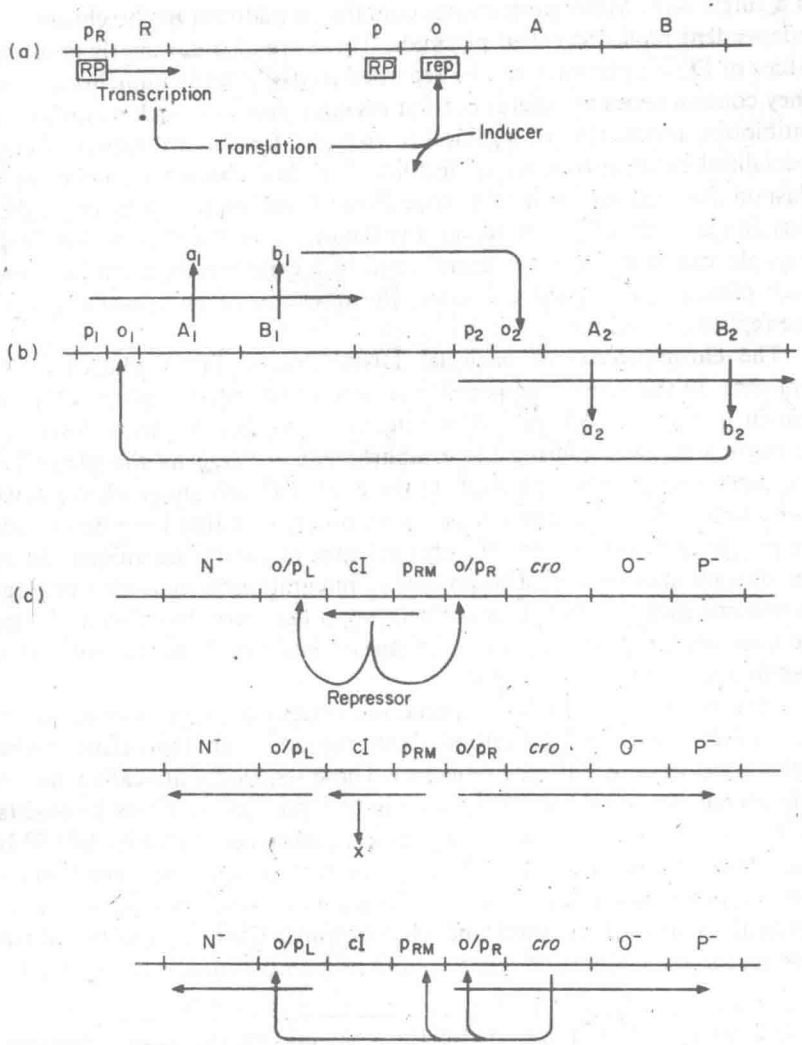

Fig. 1.1. Control of gene expression by operator-repressor interactions.
(a) Control of a single operon. p, o, A and B are promoter, operator and two structural genes respectively. R is the structural gene for the repressor (rep) with its promoter, p_R RP is RNA polymerase. The operon is only expressed in the presence of the inducer.
(b) Two linked operons that could produce a change following only transient induction. Structural genes, A_1 and A_2, code for proteins, a_1 and a_2, that define two different states or phases. In the pattern of transcription illustrated above the figure, state 1 is established. Protein a_1 is produced; a_2 is not made because b_1 is a repressor for operon 2. If an inducer which prevents b_1 binding to o_2 is present in the cell (or if b_1 is not inherited by a daughter cell), state 2 is established, as shown by the pattern of transcription below the figure. In this case, both a_2 and b_2 (the repressor for operon 1) are produced so that the change is a permanent one.

as a single unit. Many prokaryotes contain, in addition to the chromosome, independent replicons called plasmids. These are also covalently continuous pieces of DNA; plasmids can be regarded as dispensable minichromosomes. They contain genes for useful but not essential function such as resistance to antibiotics, production of antibiotics, enzymes for the utilization of certain specialized carbon sources and fertility. This last character confers upon a plasmid the capacity for it to be transferred from cell to cell by conjugation. Certain plasmids, of which the fertility factor, F, of *E. coli* is the best known example, can insert into the chromosome by a genetic recombinational event. Such plasmids are called episomes; the insertion of an episome generates one replicon from two.

The chromosomes of bacterial DNA viruses (DNA phage) are also replicons. In the case of temperate or lysogenic phages, the phage DNA may remain in the host cell in a state referred to as the prophage. Such a cell becomes a lysogen. During the establishment of lysogeny the phage DNA circularizes and forms a plasmid. In the case of *E. coli* phage Pl, the plasmid is the stable form of the prophage. In all other cases that have been studied, the prophage integrates into the chromosome at either one unique site or at one of a number of sites. The process of integration is somewhat analogous to episome insertion but does not rely upon the recombinational system of the host organism. In the case of phage λ, insertion is an example of site-specific recombination.

Prokaryotic DNA contains special sequences capable of movement, by a mechanism that is not understood, from one site to another, either within a replicon or between different replicons. These sequences are called transposable genetic elements and belong to one of three classes. Class 1 consists of the insertion sequences (IS's) which are sequences of the order of 10^3 base pairs. Several roles are played by IS's, for example the sequence homology between the F factor and a site of insertion in the chromosome is due to an identical IS in both plasmid and chromosome. Class 2 consists of transposons; these are blocks of genes capable of transposition in a way similar to

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(c) An actual case of linked operons defining, in this case, the two states of immunity and non-immunity to phage λ. The figure shows that part of the λ prophage genome which includes the immunity region. This particular prophage will not lyse the cell and produce λ phage particles because it carries mutations in the essential genes, N, O and P. In the upper figure, gene cI is transcribed from its promoter, p_{RM}; its product is λ repressor that binds to the left and right operator-promoter regions (o/p_L and o/p_R) to prevent leftward and rightward transcription. The presence of repressor in the cell also confers immunity against exogenous λ DNA. In the middle diagram, repressor is prevented from binding to o/p_L and o/p_R (technically this would be achieved by shifting a lysogen carrying a temperature sensitive mutation in gene cI to the non-permissive temperature). Leftward and rightward transcription take place. In the bottom diagram, the second state (non-immunity) is established because the product of the gene *cro* binds to the same regions as the cI product but with different consequences; in this case leftward and rightward transcription is allowed but transcription of cI is prevented.

the IS's. Both transposons and IS's contain a characteristic inverted repeat of DNA at the two ends. Class 3 contains only one known member; this is phage Mu1 — a temperate *E. coli* phage that establishes a lysogen by insertion at one of an enormous number of possible sites in the host chromosome.

Transcriptional and translational control

The characteristic transcriptional unit in prokaryotes is the operon. This consists of an RNA polymerase binding site (the promoter), a regulatory region (the operator) and a sequence of structural genes (or cistrons). Transcription of an operon can be regulated in one of four ways. (1) The operators are binding sites for allosteric regulatory proteins (the repressors). Repressor-operator interaction constitutes the fine control of gene expression through the processes of induction and repression. (2) RNA polymerase can only bind to certain promoters in the presence of cyclic AMP and a cyclic AMP binding protein (CAP). As the concentration of cyclic AMP in the cell is reduced in the presence of glucose and other catabolites, these substances reduce expression of certain operons ('the catabolite effect'). (3) The highly phosphorylated guanine nucleotides, ppGpp and pppGpp, affect the affinity of RNA polymerase for certain promoters. This is a coarse control of transcription and is significant in differentiation of just three classes of promoters, those for mRNA, tRNA and rRNA. (4) Finally, the DNA sequence that determines termination of transcription may play a regulatory role in those cases where the termination signal is only recognized as such by the polymerase in the presence of a termination factor, ρ. The removal of ρ can allow transcription of additional cistrons distal to the termination sequence.

The specificity of ribosomes and the protein factors required for protein biosynthesis can effect a degree of translational control. Ribosomes also interact with a mechanism of transcriptional control: the biosynthesis and turnover of ppGpp and pppGpp is under the control of ribosomal subunits not engaged in translation and also of uncharged tRNA molecules.

1.2.2 Radical changes in gene expression

Many of the changes introduced in section 1.1.2 and discussed in detail in the following chapters are associated with radical changes in the pattern of gene expression. Thus much of the molecular interpretation of developmental biology consists of an attempt to diagnose the basis of these changes in terms of the known nucleic acid molecular biology of prokaryotes. By way of introduction to the subject, this section surveys some theoretical possibilities with a view partly to paving the way to an understanding of those cases where the present state of knowledge can produce a plausible mechanism and partly to suggest a framework of ideas that can be tested when a new prokaryotic developmental system is studied for the first time.