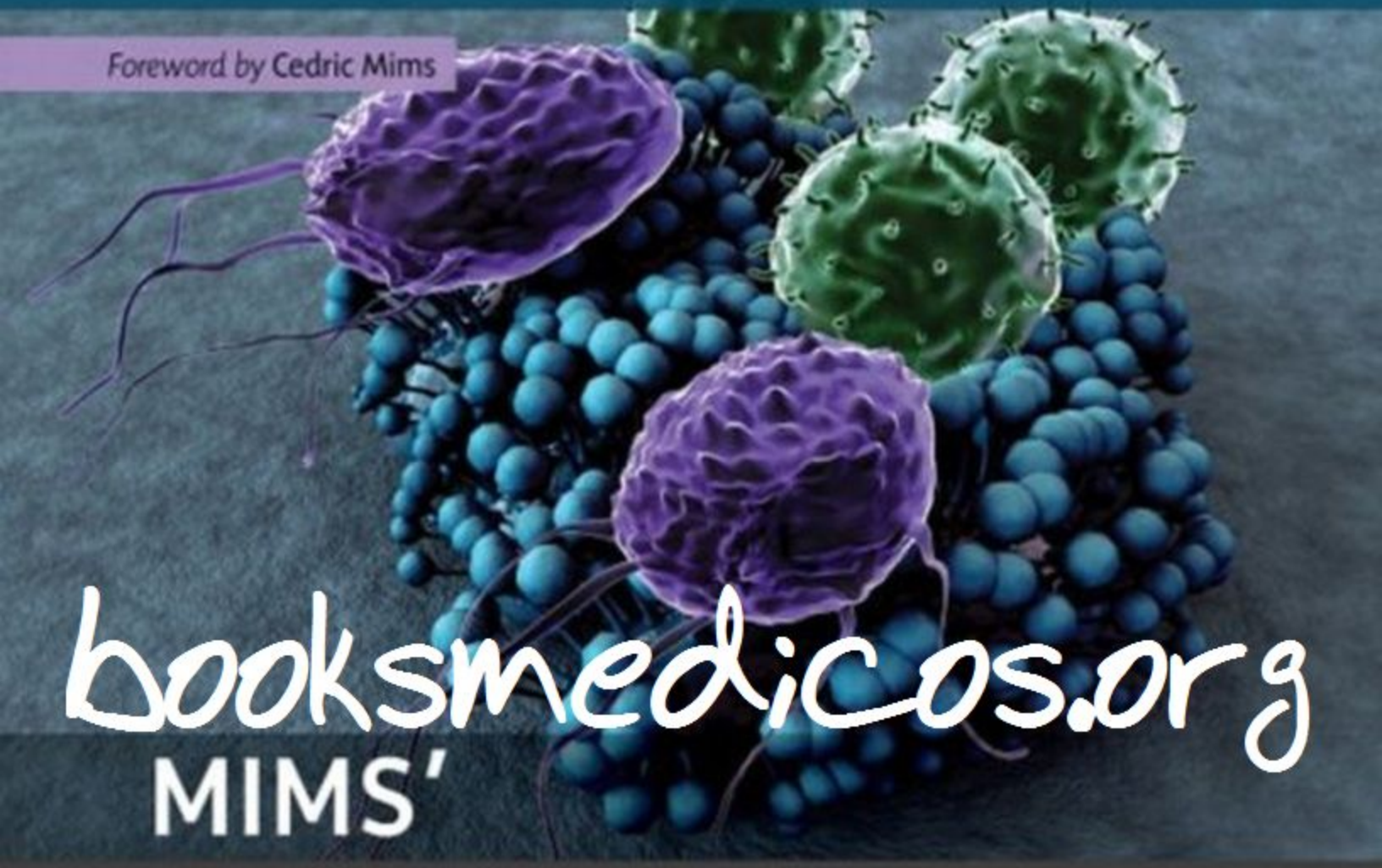
Richard V. Goering ■ Hazel M. Dockrell
Mark Zuckerman ■ Peter L. Chiodini

Foreword by Cedric Mims



MIMS' Medical Microbiology AND Immunology

SIXTH EDITION

ELSEVIER

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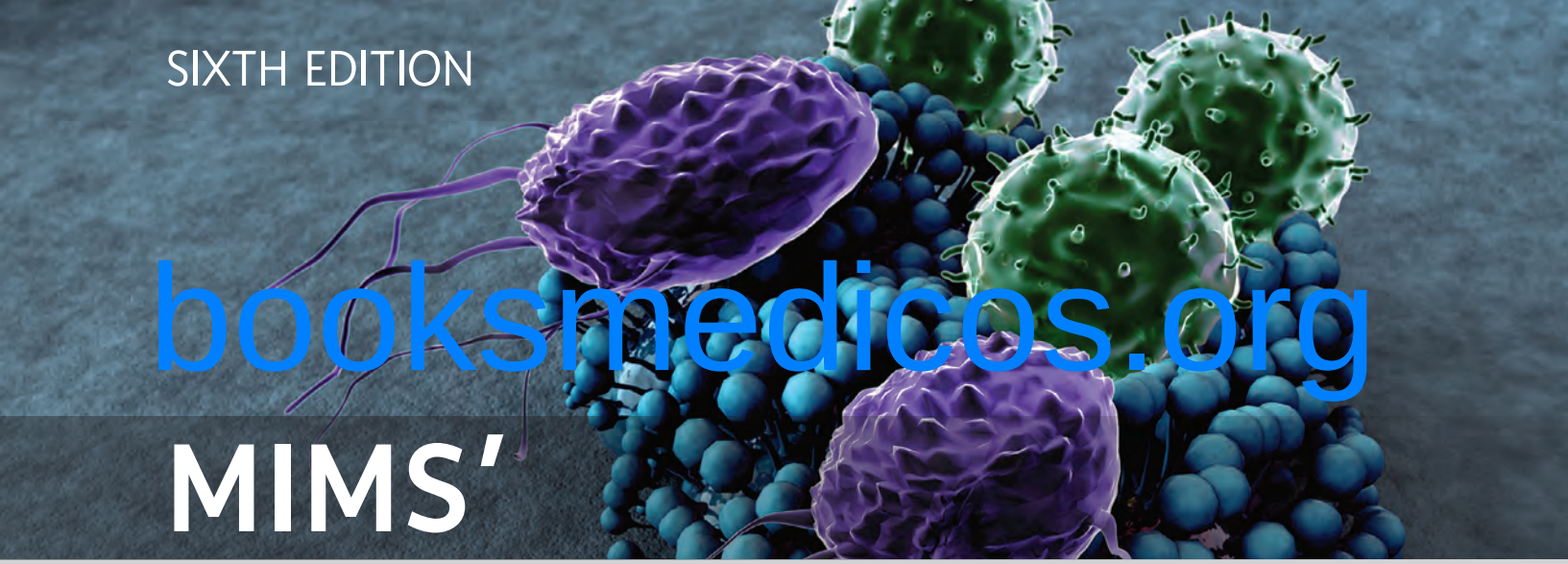

MIMS' Medical Microbiology AND Immunology

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Foreword by Cedric Mims

When I sat down with immunologist Ivan Roitt to think about writing this book, we agreed that it was to be more than a mere listing of microbial diseases with their diagnosis and treatment. All these infections result from the interplay between microbial cunning in relation to the immunological and inflammatory defences of the host, and Ivan's contribution meant that the immunology would be relevant and up-to-date.

During my 60 years as a physician and zoologist in England, America, Africa, and Australia, I have been able to study in some detail the mechanism by which microbial parasites enter the body, spread, and cause disease. It was always useful to think of those invaders as parasites, to look at it from their point of view, with the same forces governing the outcome in all cases, whether worms, bacteria, or viruses. It turns out that of all the different living species on earth, nearly half have opted for the parasitic way of life.

While the life of a parasite may sound attractive, with free board and lodging in or on the host, only a few invaders manage to survive those powerful defences. Over millions of years of evolution, their ability to avoid or evade the defences has been perfected and should never be underestimated.

Since the first edition of this book we have incorporated several improvements to make learning easier, including case studies, chapter key facts and chapter questions. My hope is that although what you learn from it will undoubtedly help you with final and board examinations, and although over the years many of the details may slip from your memory, you will have retained a useful way of looking at infectious diseases. To put it in military terms, every infection sets in train an armed conflict, with possible disease or death awaiting the loser.

This way of looking at infectious diseases will I hope stay with you and prepare you for the astonishing advances and the new treatments that await you during your career – in particular, new diseases from animals or birds, perhaps transmitted by biting insects or bats, as well as possible super-strains of influenza virus from birds that spread effectively in our species and make us ill, and also of course new antimicrobial drugs to which resistance is impossible. And we expect an unravelling of the influence on human health of that vast and mysterious collection of resident microbes living in our intestines.

I have always felt a personal as well as a scientific interest in these invaders. They killed both my parents when I was a child long before the development of antibiotics, and were responsible for my attacks of measles, mumps, diphtheria, whooping cough, tuberculosis, and much later Rift Valley Fever in Africa.

Cedric Mims
Canberra, Australia
October 2016

Preface to the sixth edition

Previous editions of *Mims' Medical Microbiology* have adopted the approach that the interaction between infectious disease and host response is best understood as a give-and-take conflict. The sixth edition continues this tradition, revising the title to *Mims' Medical Microbiology and Immunology* to better reflect the subject. Continued recognition of Cedric Mims' founding contribution to this work is seen not only in the title but also in the foreword to this sixth edition. Ivan Roitt, who played a major role in earlier editions, has relinquished his role as a main author and we gratefully acknowledge his contribution.

Overall, this edition benefits from significant revision in multiple areas. The introductory chapters continue to present fundamental principles of infectious agents and host defences but now include the newly recognized importance of the human microbiota. Subsequent chapters present an updated overview of the general principles behind the infectious agent – immune response conflict, followed by a chapter-specific consideration of system-oriented conflict scenarios. Final chapters provide a revised consideration of issues affecting diagnosis and control of the conflict especially centring on newer molecular (especially DNA-sequence-based) approaches.

Bibliographic references continue to include current Internet resources. Online access to interactive extras is provided via Elsevier's STUDENT CONSULT website (www.studentconsult.com) including questions and answers, mostly in USMLE format, the Pathogen Parade (infectious agent) index, and a new Vaccine Parade index.

Molecular approaches continue to inform and enlarge our understanding of pathogen–host interaction at a record pace. In this new edition of *Mims' Medical Microbiology and Immunology*, we believe the student will find a logical and unified approach to the subject that is readable, exciting, and informative.

Richard V Goering, Hazel M Dockrell,
Mark Zuckerman, Peter L Chiodini
2017

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A contemporary approach to microbiology

INTRODUCTION

Microbes and parasites

The conventional distinction between 'microbes' and 'parasites' is essentially arbitrary

Microbiology is sometimes defined as the biology of microscopic organisms, its subject being the 'microbes'. Traditionally, clinical microbiology has been concerned with those organisms responsible for the major infectious diseases of humans and whose size makes them invisible to the naked eye. Thus, it is not surprising that the organisms included have reflected those causing diseases that have been (or continue to be) of greatest importance in those countries where the scientific and clinical discipline of microbiology developed, notably Europe and the USA. The term 'microbes' has usually been applied in a restricted fashion, primarily to viruses and bacteria. Fungi and protozoan parasites have historically been included as more minor contributors, but in general they have been treated as the subjects of other disciplines (mycology and parasitology).

Although there can be no argument that viruses and bacteria are, globally, the most important pathogens, the conventional distinction between these as 'microbes' and the other infectious agents (fungi, protozoan, worm and arthropod parasites) is essentially arbitrary, not least because the criterion of microscopic visibility cannot be applied rigidly (Fig. Intro.1). Perhaps we should remember that the first 'microbe' to be associated with a specific clinical condition was a parasitic worm – the nematode *Trichinella spiralis* – whose larval stages are just visible to the naked eye (though microscopy is needed for certain identification). *T. spiralis* was first identified in 1835 and causally related to the disease trichinellosis in the 1860s. Viruses and bacteria comprise just over half of all human pathogen species (Table Intro.1).

THE CONTEXT FOR CONTEMPORARY MEDICAL MICROBIOLOGY

Many microbiology texts deal with infectious organisms as agents of disease in isolation, both from other infectious organisms and from the biological context in which they live and cause disease. It is certainly convenient to consider organisms group by group, to summarize the diseases they cause, and to review the forms of available control, but this approach produces a static picture of what is a dynamic relationship between the organism and its host.

Host response is the outcome of the complex interplay between host and parasite. Host response can be discussed in terms of pathological signs and symptoms and in terms of immune control, but it is better treated as the outcome of the complex interplay between two organisms – host and parasite; without this dimension a distorted view of infectious disease results. It simply is not true that 'microbe+host=disease', and clinicians are well aware of this. Understanding why it is that most host–microbe contacts do not result in disease, and what changes so that disease does arise, is as important as the identification of infectious organisms and a knowledge of the ways in which they can be controlled.

We therefore continue to believe that our approach to microbiology, both in terms of the organisms that might usefully be considered within a textbook and also in terms of the contexts in which they and the diseases they cause are discussed, provides a more informative and more interesting picture of these dynamic interrelationships. There are many reasons for having reached this conclusion, the most important being the following:

- A comprehensive understanding now exists at the molecular level of the biology of infectious agents and of the host–parasite interactions that lead to infection and disease. It is important for students to be aware of this understanding so that they can grasp the connections between infection and disease within both individuals and communities and to be able to use this knowledge in novel and changing clinical situations.
- It is now realized that the host's response to infection is a coordinated and subtle interplay involving the mechanisms of both innate and acquired resistance, and that these mechanisms are expressed regardless of the nature and identity of the pathogen involved. Our present understanding of the ways in which these mechanisms are stimulated and the ways in which they act is very sophisticated. We can now see that infection is a conflict between two organisms, with the outcome (resistance or disease) being critically dependent upon molecular interactions. Again, it is essential to understand the basis of this host–pathogen interplay if the processes of disease and disease control are to be interpreted correctly.

Emerging or re-emerging diseases continue to pose new microbiological problems

Three other factors have helped to mould our opinion that a broader view of microbiology is needed to provide a firm basis for clinical and scientific practice:

- There is an increasing prevalence of a wide variety of opportunistic infections in patients who are hospitalized or immunosuppressed. Immunosuppressive therapies are now common, as are diseases in which the immune system is compromised – notably, of course, acquired immunodeficiency disease (AIDS).
- Newly emerging disease agents continue to be identified, and old diseases previously thought to be under control, re-emerge as causes of concern. Of the 1407 species

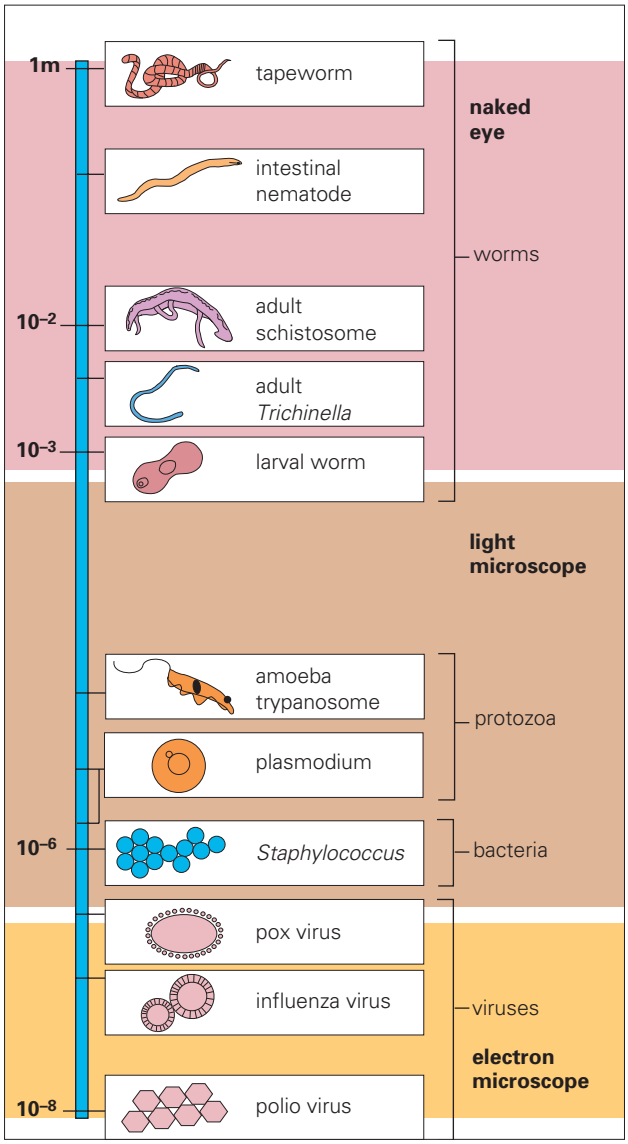

Figure Intro.1 Relative sizes of the organisms covered in this book.

Table Intro.1 Distribution of 1407 human pathogen species among the major groups of organisms (excluding arthropods)

Group	% of total
Viruses and prions	14–15
Bacteria	38–41
Fungi	22–23
Protozoa	4–5
Helminths	20

(Data from average of multiple studies summarized by Smith K.F., Guegan J.-F. Changing geographic distributions of human pathogens. *Annu Rev Ecol Evol* 2010; 41:231–250.)

identified as pathogenic for humans, 183 are regarded as emerging or re-emerging pathogens, almost half being viruses, some of animal origin (see Table Intro.1).

- Tropical infections are now of much greater interest. Clinicians see many tourists who have been exposed to the quite different spectrum of infectious agents found in tropical countries (at least 80 million people travel from resource-rich to resource-poor countries each year), and practising microbiologists may be called upon to identify and advise on these organisms. There is also greater awareness of the health problems of the resource-poor world.

Thus, a broader view of microbiology is necessary: one that builds on the approaches of the past, but addresses the problems of the present and of the future.

MICROBIOLOGY PAST, PRESENT AND FUTURE

The demonstration in the nineteenth century that diseases were caused by infectious agents founded the discipline of microbiology. Although these early discoveries involved tropical parasitic infections as well as the bacterial infections common in Europe and the USA, microbiologists increasingly focused on the latter, later extending their interests to the newly discovered viral infections. The development of antimicrobial agents and vaccines revolutionized treatment of these diseases and raised hopes for the eventual elimination of many of the diseases that had plagued the human race for centuries. Those in the resource-rich world learned not to fear infectious disease and believed such infections would disappear in their lifetime. To an extent, this was realized; through vaccination, many familiar childhood diseases became uncommon, and those of bacterial origin were more easily controlled by antibiotics. Encouraged by the eradication of smallpox during the 1970s, and the success of polio vaccines, the United Nations in 1978 announced programmes to obtain 'health for all' by 2000. However, this and other optimistic targets have required re-evaluation.

Infectious diseases are killers in both resource-rich and resource-poor countries

Globally, infectious diseases (especially lower respiratory infections) are second only to heart disease as most frequent cause of death. The World Health Organization (WHO) has now listed 12 antibiotic-resistant bacterial pathogens as priorities for the development of new antibiotics – 75% of which are categorized as critical or of high importance. However, infectious diseases are not evenly distributed worldwide (Fig. Intro.2)

The burden of infectious disease in the resource-poor world is especially concerning. Although sub-Saharan Africa has only about 10% of the world's population, it has the clear majority of AIDS infections and AIDS-related deaths, the highest HIV-TB co-infection rates and most of the global malaria burden. Tuberculosis (TB) and HIV-AIDS are of increasing importance in South-East Asia and the Pacific, where drug-resistant malaria is also common. Children younger than 5 years are most at risk from infectious diseases. It is obvious that the prevalence and importance of infectious diseases in the resource-poor world are directly linked to poverty.

Infections continue to emerge or re-emerge

On a worldwide basis, infectious diseases continue to emerge in the human population for the first time. Recent examples include the MERS coronavirus, the H7N9 avian influenza virus, and the Zika virus. Concern regarding spread of the Ebola virus and the lack of effective antibiotics for treating

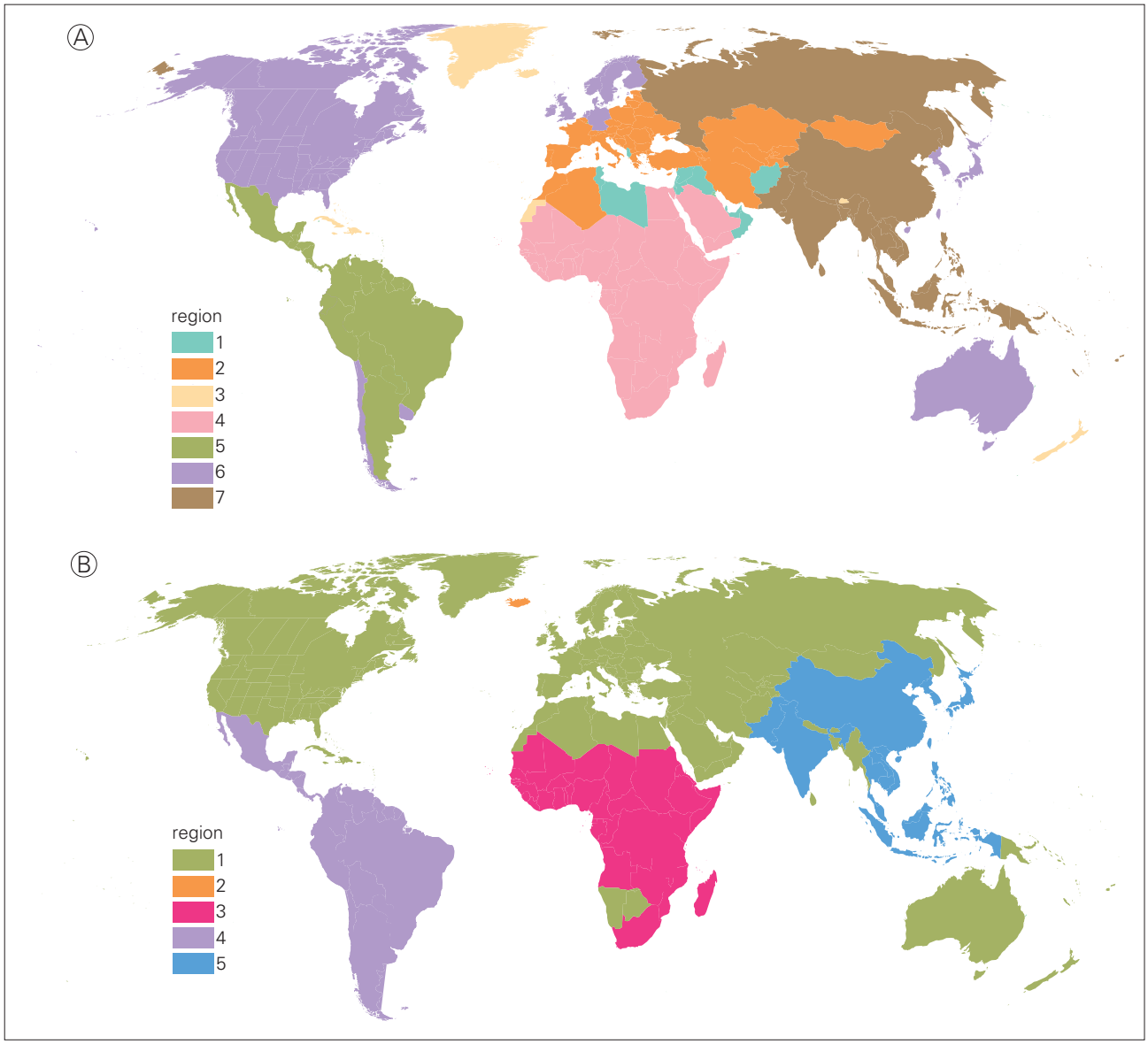

Figure Intro.2 Geographic distribution of 301 diseases in 229 countries. (A) 93 vector-associated diseases predominant in seven geographic regions and (B) 208 non-vector predominant in five geographic regions. Colours indicate groups of similar diseases (vector or non-vector) tending to predominate in specific geographic regions. (Redrawn from Just M.G., Norton J.F., Traud A.L. et al. [2014] Global biogeographic regions in a human-dominated world: the case of human diseases. *Ecosphere*. Chichester: John Wiley & Sons, Fig 1.)

bacterial infections (see above) further underscore the negative global impact of infectious diseases.

Modern lifestyles and technical developments facilitate transmission of disease

The reasons for the resurgence of infectious diseases are multiple. They include:

- New patterns of travel and trade (especially food commodities), new agricultural practices, altered sexual behaviour, medical interventions and overuse of antibiotics.
- The movement of multidrug-resistant bacteria, such as multiply resistant *Staphylococcus aureus* (MRSA), and virulent pathogens such as *Clostridium difficile* from the healthcare setting into the community. The issue of antimicrobial resistance is compounded in resource-poor countries by inability or unwillingness to complete programmes of treatment and by the use of counterfeit drugs with, at best, partial action. The World Health Organization (WHO) has now catalogued the existence of over 900 counterfeit medical products representing the full spectrum of medical therapies.
- Breakdown of economic, social and political systems especially in the resource-poor world has weakened medical services and increased the effects of poverty and malnutrition.
- The dramatic increase in air travel over the last few decades has facilitated the spread of infection and increased the threat of new pandemics. The Spanish influenza pandemic in 1918 spread along railway and sea links. Modern air

travel moves larger numbers of people more rapidly and more extensively and makes it possible for microbes to cross geographical barriers.

What of the future?

Predictions based on data from the United Nations and WHO give a choice of scenarios. Optimistically, the aging population, coupled with socioeconomic and medical advances, could be expected to see a fall in the problems posed by infectious disease, and a decrease in deaths from these causes. The pessimistic view is that population growth in resource-poor countries, especially in urban populations, the increasing gap between rich and poor countries and continuing changes in lifestyle will result in surges of infectious disease. Even in resource-rich countries, increasing drug resistance and a slowing of developments in new antimicrobials and vaccines will create additional problems in control. Added to these are three additional factors. These are:

- the emergence of new human infections such as a novel strain of influenza virus, or a new infection of wildlife origin
- climate change, with increased temperatures and altered rainfall adding to the incidence of vector-borne infection
- the threat of bioterrorism, with the possible deliberate spread of viral and bacterial infections to human populations with no acquired immunity or no history of vaccination.

One thing is certain: whether optimistic or pessimistic scenarios prove true, microbiology will remain a critical medical discipline for the foreseeable future.

THE APPROACH ADOPTED IN THIS BOOK

The factors outlined above indicate the need for a text with a dual function:

1. It should provide an inclusive treatment of the organisms responsible for infectious disease.
2. The purely clinical / laboratory approach to microbiology should be replaced with an approach that will stress the biological context in which clinical / laboratory studies are to be undertaken.

The approach we have adopted in this book is to look at microbiology from the viewpoint of the conflicts inherent in all host–pathogen relationships. We first describe the adversaries: the infectious organisms on the one hand, and the innate and adaptive defence mechanisms of the host on the other. The outcome of the conflicts between the two is then amplified and discussed system by system. Rather than taking each organism or each disease manifestation in turn, we look at the major environments available for infectious organisms in the human body, such as the respiratory system, the gut, the urinary tract, the blood and the central nervous system. The organisms that invade and establish in each of these are examined in terms of the pathological responses they provoke. Finally, we look at how the conflicts we have described can be controlled or eliminated, both at the level of the individual patient and at the level of the community. We hope that such an approach will provide readers with a dynamic view of host–pathogen interactions and allow them to develop a more creative understanding of infection and disease.

KEY FACTS

- Our approach is to provide a comprehensive account of the organisms that cause infectious disease in humans, from the viruses to the worms, and to cover the biological bases of infection, disease, host–pathogen interactions, disease control and epidemiology.
- The diseases caused by microbial pathogens will be placed in the context of the conflict that exists between them and the innate and adaptive defences of their hosts.
- Infections will be described and discussed in terms of the major body systems, treating these as environments in which microbes can establish themselves, flourish and give rise to pathological changes.

1 Pathogens as parasites

Introduction

The interaction between pathogen and host can be viewed as a parasitic relationship. The pathogenic process involves the establishment, persistence, and reproduction of the infecting agent at the expense of the host. How this is accomplished depends on multiple factors including microbial anatomy, size (macro vs micro parasites), and whether the organisms live inside or outside of host cells. Understanding these issues in the context of a classification system that provides a view of microbe interrelationships provides an important foundation for the study of pathogen-host interaction

THE VARIETIES OF PATHOGENS

Prokaryotes and eukaryotes

A number of important and distinctive biological characteristics must be taken into account when considering any microorganism in relation to infectious disease. In general, these can be considered in terms of comparative microbial anatomy – the way in which organisms are constructed, and particularly the way in which genetic material and other cellular components are organized.

All organisms other than viruses and prions are made up of cells

Although viruses have genetic material (DNA or RNA) they are not cellular, lacking cell membranes, cytoplasm and the machinery for synthesizing macromolecules, depending instead upon host cells for this process. Conventional viruses have their genetic material packed in capsids. The agents (prions) which cause diseases such as Creutzfeldt–Jakob disease (CJD), variant CJD and kuru in humans, and scrapie and bovine spongiform encephalopathy (BSE) in animals, lack nucleic acid and consist only of infectious proteinaceous particles.

All other organisms have a cellular organization, being made up of single cells (most 'microbes') or of many cells. Each cell has genetic material (DNA) and cytoplasm with synthetic machinery, and is bounded by a cell membrane.

Bacteria are prokaryotes; all other organisms are eukaryotes

There are many differences between the two major divisions – prokaryotes and eukaryotes – of cellular organisms (Fig. 1.1). These include the following.

In prokaryotes:

- a distinct nucleus is absent
- DNA is in the form of a single circular chromosome; additional 'extrachromosomal' DNA is carried in plasmids
- transcription and translation can be carried out simultaneously.

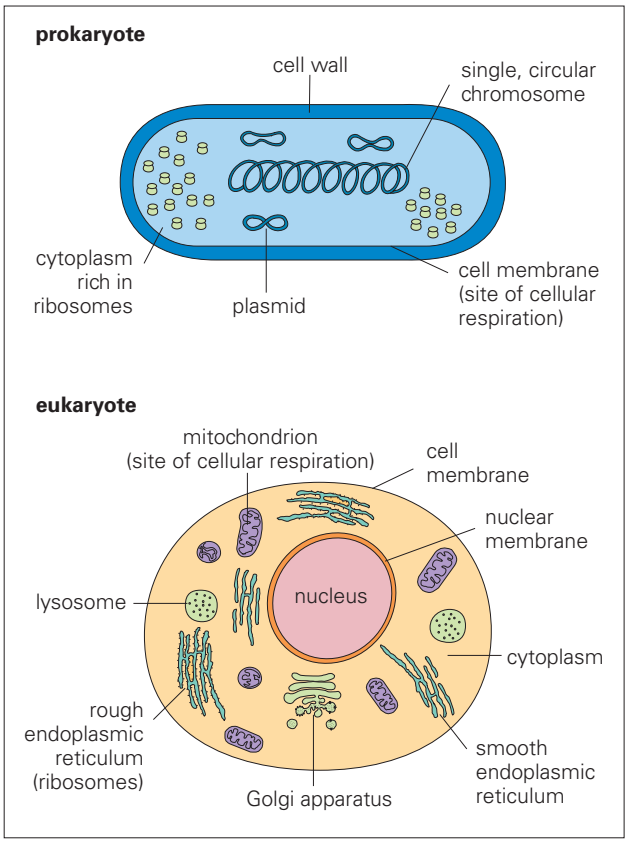

Figure 1.1 Prokaryote and eukaryote cells. The major features of cellular organization are shown diagrammatically.

In eukaryotes:

- DNA is carried on several chromosomes within a nucleus
- the nucleus is bounded by a nuclear membrane
- transcription and translation are carried out separately with transcribed messenger RNA (mRNA) moving out of the nucleus into the cytoplasm for ribosomal translation

- the cytoplasm is rich in membrane-bound organelles (mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes), which are absent in prokaryotes.

Gram-negative bacteria have an outer lipopolysaccharide-rich layer

Another important difference between prokaryotes and the majority of eukaryotes is that the cell membrane (plasma membrane) of prokaryotes is covered by a thick protective cell wall. In Gram-positive bacteria this wall, made of peptidoglycan, forms the external surface of the cell, whereas in Gram-negative bacteria there is an additional outer layer rich in lipopolysaccharides. These layers play an important role in protecting the cell against the host immune system and chemotherapeutic agents, and in stimulating certain pathological responses. They also confer antigenicity.

Microparasites and macroparasites

Microparasites replicate within the host

There is an important distinction between microparasites and macroparasites that overrides their differences in size. *Micro*parasites (viruses, bacteria, protozoa, fungi) replicate within the host and can, theoretically, multiply to produce a very large number of progeny, thereby causing an overwhelming infection. In contrast, *macro*parasites (worms, arthropods), even those that are microscopic, do not have this ability: one infectious stage matures into one reproducing stage and, in most cases, the resulting progeny leave the host to continue the cycle. The level of infection is therefore determined by the numbers of organisms that enter the body. This distinction between microparasites and macroparasites has important clinical and epidemiological implications.

The boundary between microparasites and macroparasites is not always clear. The progeny of some macroparasites do remain within the host, and infections can lead to the build-up of overwhelming numbers, particularly in immune-suppressed patients. The roundworms *Trichinella*, *Strongyloides stercoralis* and some filarial nematodes, and *Sarcoptes scabiei* (the itch mite), are examples of this type of parasite.

Organisms that are small enough can live inside cells

Absolute size has other biologically significant implications for the host–pathogen relationship, which cut across the divisions between micro- and macroparasites. Perhaps the most important of these is the relative size of a pathogen and its host's cells. Organisms that are small enough can live inside cells and, by doing so, establish a biological relationship with the host that is quite different from that of an extracellular organism – one that influences both disease and control.

LIVING INSIDE OR OUTSIDE CELLS

The basis of all host–pathogen relationships is the exploitation by one organism (the pathogen) of the environment provided by another (the host). The nature and degree of exploitation varies from relationship to relationship, but the pathogen's primary requirement is a supply of metabolic materials from the host, whether provided in the form of nutrients or (as in the case of viruses) in the form of nuclear synthetic machinery. The reliance of viruses upon host synthetic machinery requires an obligatory intracellular habit: viruses must live within host cells. Some other groups of pathogens (e.g. *Chlamydia*, *Rickettsia*) also live only within cells. In the remaining groups of pathogens, different species have adopted either the intracellular or the extracellular habit or, in a few cases, both. Intracellular microparasites other than viruses take their metabolic requirements directly from the pool of nutrients available in the cell itself, whereas extracellular organisms take theirs from the nutrients present in tissue fluids or, occasionally, by feeding directly on host cells (e.g. *Entamoeba histolytica*, the organism associated with amoebic dysentery). Macroparasites are almost always extracellular (though *Trichinella* is intracellular), and many feed by ingesting and digesting host cells; others can take up nutrients directly from tissue fluids or intestinal contents.

Pathogens within cells are protected from many of the host's defence mechanisms

As will be discussed in greater detail in Chapter 15, the intracellular pathogens pose problems for the host that are quite different from those posed by extracellular organisms. Pathogens that live within cells are largely protected against many of the host's defence mechanisms while they remain there, particularly against the action of specific antibodies. Control of these infections depends therefore on the activities of intracellular killing mechanisms, short-range mediators or cytotoxic agents, although the latter may destroy both the pathogen and the host cell, leading to tissue damage. This problem, of targeting activity against the pathogen when it lives within a vulnerable cell, also arises when using drugs or antibiotics, as it is difficult to achieve selective action against the pathogen while leaving the host cell intact. Even more problematic is the fact that many intracellular pathogens live inside the very cells responsible for the host's immune and inflammatory mechanisms and therefore depress the host's defensive abilities. For example, a variety of viral, bacterial and protozoal pathogens live inside macrophages, and several viruses (including human immunodeficiency virus, HIV) are specific for lymphocytes.

Intracellular life has many advantages for the pathogen. It provides access to the host's nutrient supply and its genetic machinery and allows escape from host surveillance and antimicrobial defences. However, no organism can be wholly intracellular at all times: if it is to replicate successfully, transmission must occur between the host's cells, and this inevitably involves some exposure to the extracellular environment. As far as the host is concerned, this extracellular phase in the development of the pathogen provides an opportunity to control infection through defence mechanisms such as phagocytosis, antibody and complement. However, transmission between cells can involve destruction of the initially infected cell and so contribute to tissue damage and general host pathology.

Living outside cells provides opportunities for growth, reproduction and dissemination

Extracellular pathogens can grow and reproduce freely, and may move extensively within the tissues of the body. However, they also face constraints on their survival and development. The most important is continuous exposure to components of the host's defence mechanisms, particularly antibody, complement and phagocytic cells.

The characteristics of extracellular organisms lead to pathological consequences that are quite different from those associated with intracellular species. These are seen most dramatically with the macroparasites, whose sheer physical size, reproductive capacity and mobility can result in extensive destruction of host tissues. Many extracellular pathogens have the ability to spread rapidly through extracellular fluids or to move rapidly over surfaces, resulting in a widespread infection within a relatively short time. The rapid colonization of the entire mucosal surface of the small bowel by *Vibrio cholerae* is a good example. Successful host defence against extracellular parasites requires mechanisms that differ from those used in defence against intracellular parasites. The variety of locations and tissues occupied by extracellular parasites also poses problems for the host in ensuring effective deployment of defence mechanisms. Defence against intestinal parasites requires components of the innate and adaptive immune systems that are quite distinct from those effective against parasites in other sites, and those living in the lumen may be unaffected by responses operating in the mucosa. These problems in mounting effective defence are most acute where large macroparasites are concerned, because their size often renders them insusceptible to defence mechanisms that can be used against smaller organisms. For example, worms cannot be phagocytosed; they often have protective external layers, and can actively move away from areas where the host response is activated.

SYSTEMS OF CLASSIFICATION

Infectious diseases are caused by organisms belonging to a very wide range of different groups – prions, viruses, bacteria, fungi, protozoa, helminths (worms) and arthropods. Each has its own system of classification, making it possible to identify and categorize the organisms concerned. Correct identification is an essential requirement for accurate diagnosis and effective treatment. Identification is achieved by a variety of means, from simple observation to molecular analysis. Classification is being revolutionized by the application of genome sequencing. Many of the major pathogens in all categories have now been sequenced and this is allowing not only more precise identification but also a greater understanding of the interrelationships of members within each taxonomic group.

The approaches used vary between the major groups. For the protozoa, fungi, worms and arthropods, the basic unit of classification is the species, essentially defined as a group of organisms capable of reproducing sexually with one another. Species provide the basis for the binomial system of classification, used for eukaryote and some prokaryote organisms. Species are in turn grouped into a 'genus' (closely related but non-interbreeding species). Each organism is identified by two names, indicating the 'genus' and the 'species' respectively, for example, *Homo sapiens* and *Escherichia coli*. Related genera are grouped into progressively broader and more inclusive categories.

Classification of bacteria and viruses

The concept of 'species' is a basic difficulty in classifying prokaryotes and viruses, although the categories of genus and species are routinely used for bacteria. Classification of bacteria uses a mixture of easily determined microscopic, macroscopic and biochemical characteristics, based on size, shape, colour, staining properties, respiration and reproduction, and a more sophisticated analysis of immunological and molecular criteria. The former characteristics can be used to divide the organisms into conventional taxonomic groupings, as shown for the Gram-positive bacteria in Fig. 1.2 (see also Ch. 2).

Correct identification of bacteria below the species level is often vital to differentiate pathogenic and non-pathogenic forms

Correct treatment requires correct identification. For some bacteria, the important subspecies groups are identified on the basis of their immunological properties. Cell wall, flagellar and capsule antigens are used in tests with specific antisera to define serogroups and serotypes (e.g. in salmonellae, streptococci, shigellae and *E. coli*). Biochemical characteristics can be used to define other subspecies groupings (biotypes, strains, groups). For example, *Staphylococcus aureus* strains typically release a beta-haemolysin (causing red blood cells to lyse). Production of other toxins is also important in differentiating between

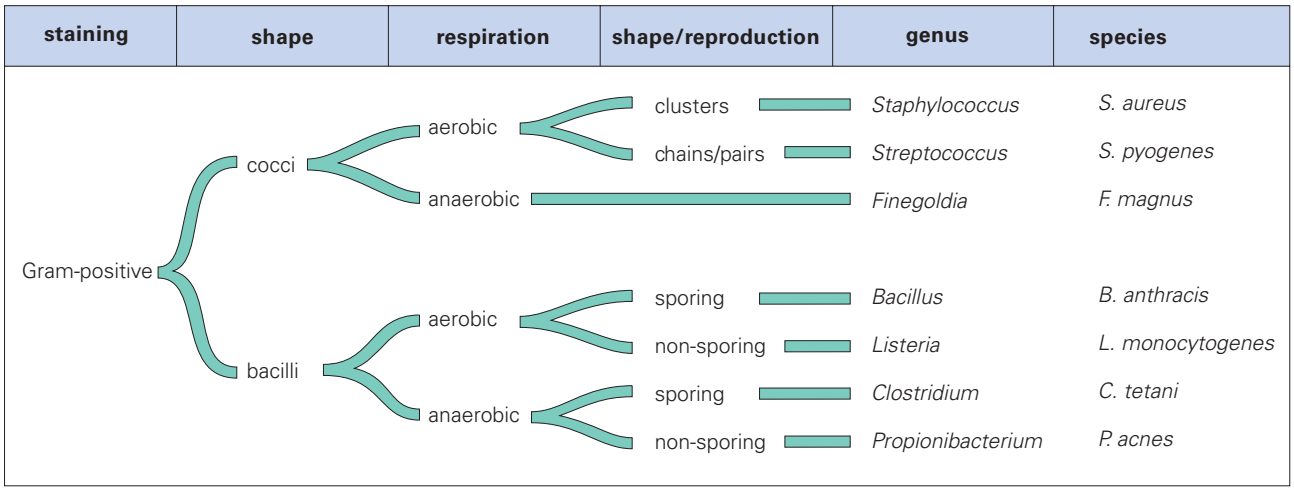

Figure 1.2 How the structural and biological characteristics of bacteria can be used in classification, taking Gram-positive bacteria as an example.

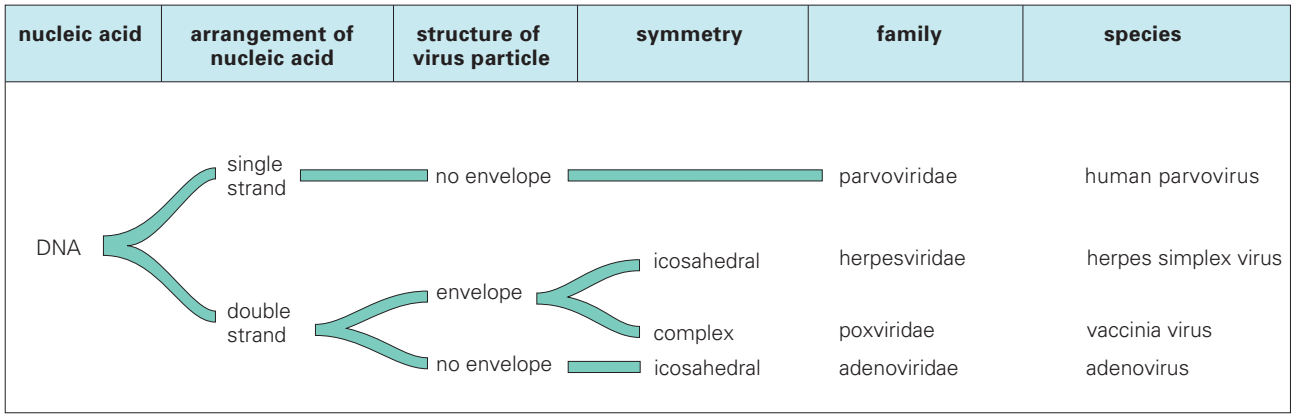

Figure 1.3 How the characteristics of viruses can be used in classification, taking DNA viruses as an example.

groups, as in *E. coli*. Antibiotic susceptibility can also be helpful in identification. Matrix-assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry is being increasingly used as a rapid and cost-effective means of identification. Direct genetic approaches are also used in identification and classification such as the use of the polymerase chain reaction (PCR) and probes to detect organism-specific sentinel DNA sequences. These tests are particularly useful for those organisms which grow poorly or not at all in vitro.

Classification of viruses departs even further from the binomial system

Virus names draw on a wide variety of characteristics (e.g. size, structure, pathology, tissue location or distribution). Groupings are based on characteristics such as the type of nucleic acid present (DNA or RNA), the symmetry of the virus particle (e.g. icosahedral, helical, complex) and the presence or absence of an external envelope, as shown for the DNA viruses in Fig. 1.3 (see also Ch. 3). The equivalents of subspecies categories are also used including serotypes, strains, variants and isolates and are determined primarily by serological reactivity of virus material. The influenza virus, for example, can be considered as the equivalent of a genus containing three types (A, B, C). Identification can be carried out using the stable nucleoprotein antigen, which differs between the three types. The neuraminidase and haemagglutinin antigens are not stable and show variation within types. Characterization of these antigens in an isolate enables the particular variant to be identified, haemagglutinin (H) and neuraminidase (N) variants being designated by numbers (e.g. H5N1, the variant associated with fatal avian influenza; see Ch. 20). A further example is seen in adenoviruses, for which the various antigens associated with a component of the capsid can be used to define groups, types and finer subdivisions. The rapid rate of mutation shown by some viruses (e.g. HIV) creates particular problems for classification. The population present in a virus-infected individual may be genetically quite diverse and may best be described as a quasispecies – representing the average of the broad spectrum of variants present.

Classification assists diagnosis and the understanding of pathogenicity

Prompt identification of organisms is necessary clinically so that diagnoses can be made and appropriate treatments advised. To understand host–parasite interactions, however, not only should the identity of an organism be known, but also as much as possible of its general biology; useful predictions can then be made about the consequences of infection. For these reasons, in subsequent chapters, we have included outline classifications of the important pathogens, accompanied by brief accounts of their structure (gross and microscopic), modes of life, molecular biology, biochemistry, replication and reproduction.

KEY FACTS

- Organisms that cause infectious diseases can be grouped into seven major categories: prions, viruses, bacteria, fungi, protozoa, helminths and arthropods.
- Identification and classification of these organisms are important parts of microbiology and are essential for correct diagnosis, treatment and control.
- Each group has distinctive characteristics (structural and molecular make-up, biochemical and metabolic strategies, reproductive processes) which determine how the organisms interact with their hosts and how they cause disease.
- Many pathogens live within cells, where they are protected from many components of the host's protective responses.

2 The bacteria

Introduction

Although free-living bacteria exist in huge numbers, relatively few species cause disease. The majority of these are well known and well studied; however, new pathogens continue to emerge and the significance of previously unrecognized infections becomes apparent. Good examples of the latter include Ebola virus disease and Zika fever, while infection with *Legionella*, the cause of Legionnaires' disease and gastric ulcers associated with *Helicobacter pylori* infection, are good historical bacterial examples.

Bacteria are single-celled prokaryotes, their DNA forming a long circular molecule, but not contained within a defined nucleus. Many are motile, using a unique pattern of flagella. The bacterial cell is surrounded by a complex cell wall and often a thick capsule. They reproduce by binary fission, often at very high rates, and show a wide range of metabolic patterns, both aerobic and anaerobic. Classification of bacteria uses both phenotypic and genotypic data. For clinical purposes, the phenotypic data are of most practical value, and rest on an understanding of bacterial structure and biology (see Fig. 32.2). Detailed summaries of members of the major bacterial groups are given in the Pathogen Parade (see online appendix).

STRUCTURE

Bacteria are 'prokaryotes' and have a characteristic cellular organization

The genetic information of bacteria is carried in a long, double-stranded (ds), circular molecule of deoxyribonucleic acid (DNA) (Fig. 2.1). By analogy with eukaryotes (see Ch. 1), this can be termed a 'chromosome', but there are no introns; instead, the DNA comprises a continuous coding sequence of genes. The chromosome is not localized within a distinct nucleus; no nuclear membrane is present and the DNA is tightly coiled into a region known as the 'nucleoid'. Genetic information in the cell may also be extrachromosomal, present as small circular self-replicating DNA molecules termed plasmids. The cytoplasm contains no organelles other than ribosomes for protein synthesis. Although ribosomal function is the same in both pro- and eukaryotic cells, organelle structure is different. Ribosomes are characterized as 70 S in prokaryotes and 80 S in eukaryotes (the 'S' unit relates to how a particle behaves when studied under extreme centrifugal force in an ultracentrifuge). The bacterial 70 S ribosome is specifically targeted by antimicrobials such as the aminoglycosides (see Ch. 34). Many of the metabolic functions performed in eukaryote cells by membrane-bound organelles such as mitochondria are carried out by the prokaryotic cell membrane. In all bacteria except mycoplasmas, the cell is surrounded by a complex cell wall. External to this wall may be capsules, flagella and pili. Knowledge of the cell wall and these external structures is important in diagnosis and pathogenicity and for understanding bacterial biology.

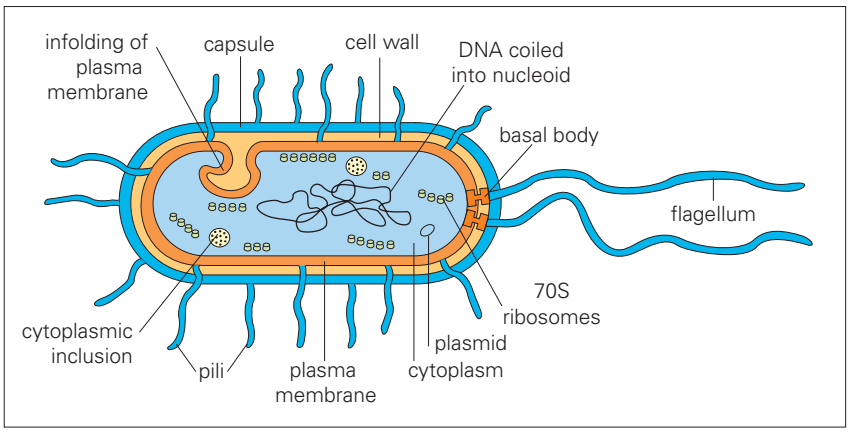

Figure 2.1 Diagrammatic structure of a generalized bacterium.

Bacteria are classified according to their cell wall as Gram-positive or Gram-negative

Gram staining is a basic microbiological procedure for identification of bacteria (see Ch. 32). The main structural component of the cell wall is 'peptidoglycan' (mucopeptide or murein), a mixed polymer of hexose sugars (*N*-acetylglucosamine and *N*-acetylmuramic acid) and amino acids (Fig. 2.2):

- In Gram-positive bacteria, the peptidoglycan forms a thick (20–80 nm) layer external to the cell membrane, and may contain other macromolecules.
- In Gram-negative species, the peptidoglycan layer is thin (5–10 nm) and is overlaid by an outer membrane, anchored to lipoprotein molecules in the peptidoglycan layer. The principal molecules of the outer membrane are lipopolysaccharides and lipoprotein.

The polysaccharides and charged amino acids in the peptidoglycan layer make it highly polar, providing the bacterium with a thick hydrophilic surface. This property allows Gram-positive organisms to resist the activity of bile in the intestine. Conversely, the layer is digested by lysozyme, an enzyme present in body secretions, which therefore has bactericidal properties. Synthesis of peptidoglycan is disrupted by beta-lactam and glycopeptide antibiotics (see Ch. 34).

In Gram-negative bacteria, the outer membrane is also hydrophilic, but the lipid components of the constituent molecules give hydrophobic properties as well. Entry of hydrophilic molecules such as sugars and amino acids is necessary for nutrition and is achieved through special channels or pores formed by proteins called 'porins'. The lipopolysaccharide (LPS) in the membrane confers both antigenic properties (the 'O antigens' from the carbohydrate chains) and toxic properties (the 'endotoxin' from the lipid A component; see Ch. 18).

While staining weakly Gram-positive, mycobacteria also possess an outer membrane, which contains a variety of complex lipids (mycolic acids). These create a waxy layer, which both alters the staining properties of these organisms (the so-called acid-fast bacteria) and gives considerable resistance to drying and other environmental factors. Mycobacterial cell wall components also have a pronounced adjuvant activity (i.e. they promote immunological responsiveness).

External to the cell wall may be an additional capsule of high molecular weight polysaccharides (or amino acids in anthrax bacilli) that gives a slimy surface. This provides protection against phagocytosis by host cells and is important in determining virulence. With *Streptococcus pneumoniae* infection, only a few capsulated organisms can cause a fatal infection, but unencapsulated mutants cause no disease.

The cell wall is a major contributor to the ultimate shape of the organism, an important characteristic for bacterial identification. In general, bacterial shapes are categorized as spherical (cocci), rods (bacilli) or helical (spirilla) (Fig. 2.3), although there are variations on these themes.

Many bacteria possess flagella

Flagella are long helical filaments extending from the cell surface, which enable bacteria to move in their environment. These may be restricted to the poles of the cell, singly (polar) or in tufts (lophotrichous), or distributed over the general surface of the cell (peritrichous). Bacterial flagella are structurally quite different from eukaryote flagella. In addition, the forces that result in movement are generated quite differently, being proton dependent (i.e. driven by movement of hydrogens across the cell membrane) in prokaryotes but adenosine triphosphate (ATP) dependent in eukaryotes. Motility allows positive and negative responses to environmental stimuli such as chemicals (chemotaxis). Flagella are built of protein components (flagellins), which are strongly antigenic. These antigens, the H antigens, are important targets of protective antibody responses.

Pili are another form of bacterial surface projection

Pili (fimbriae) are more rigid than flagella and function in attachment, either to other bacteria (the 'sex' pili) or to host cells (the 'common' pili). Adherence to host cells involves

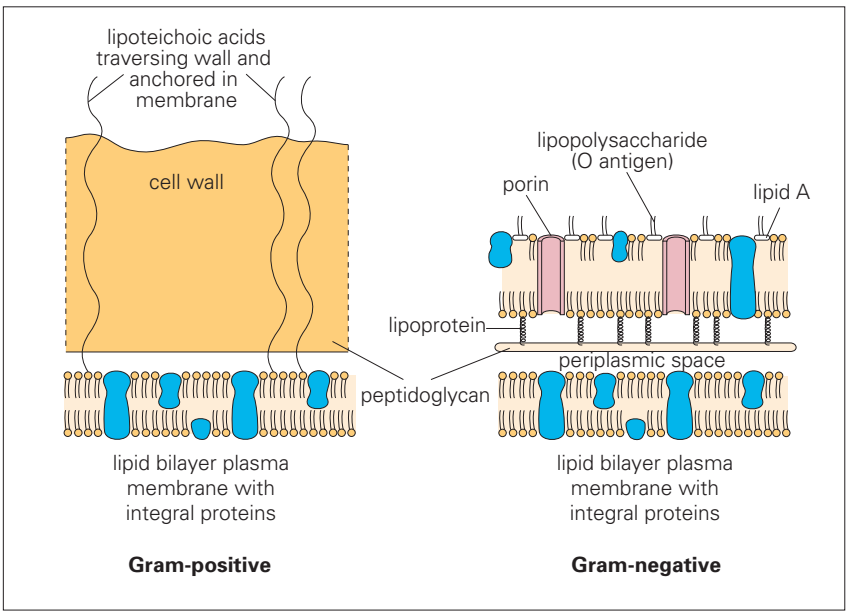

Figure 2.2 Construction of the cell walls of Gram-positive and Gram-negative bacteria.

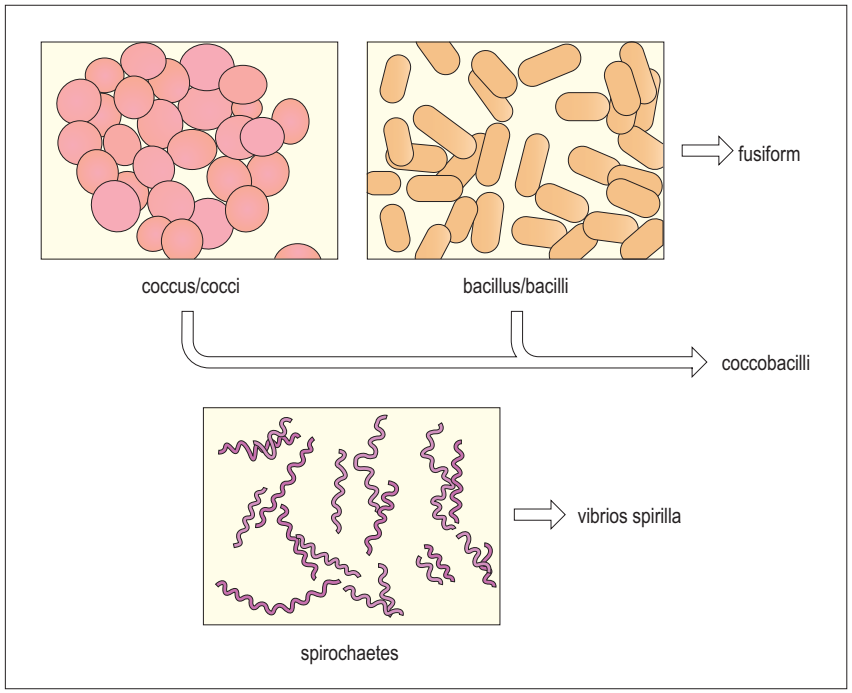

Figure 2.3 The three basic shapes of bacterial cells.

specific interactions between component molecules of the pili (adhesins) and molecules in host cell membranes. For example, the adhesins of *Escherichia coli* interact with fucose / mannose molecules on the surface of intestinal epithelial cells (see Ch. 23). The presence of many pili may help to prevent phagocytosis, reducing host resistance to bacterial infection. Although immunogenic, their antigens can be changed, allowing the bacteria to avoid immune recognition. The mechanism of 'antigenic variation' has been elucidated in organisms such as the gonococci and is known to involve recombination of genes coding for 'constant' and 'variable' regions of pili molecules.

NUTRITION

Bacteria obtain nutrients mainly by taking up small molecules across the cell wall

Bacteria take up small molecules such as amino acids, oligosaccharides and small peptides across the cell wall. Gram-negative species can also take up and use larger molecules after preliminary digestion in the periplasmic space. Uptake and transport of nutrients into the cytoplasm is achieved by the cell membrane using a variety of transport mechanisms including facilitated diffusion, which utilizes a carrier to move compounds to equalize their intra- and extracellular concentrations, and active transport, where energy is expended to deliberately increase intracellular concentrations of a substrate. Oxidative metabolism (see below) also takes place at the membrane–cytoplasm interface.

Some species require only minimal nutrients in their environment, having considerable synthetic powers, whereas others have complex nutritional requirements. *E. coli*, for example, can be grown in media providing only glucose and inorganic salts; streptococci, on the other hand, will grow only in complex media providing them with many organic compounds. Nevertheless, all bacteria have similar general nutritional requirements for growth, which are summarized in Table 2.1.

All pathogenic bacteria are heterotrophic

All bacteria obtain energy by oxidizing preformed organic molecules (carbohydrates, lipids and proteins) from their environment. Metabolism of these molecules yields ATP as an energy source. Metabolism may be aerobic, where the final electron acceptor is oxygen, or anaerobic, where the final acceptor may be an organic or inorganic molecule other than oxygen.

- In aerobic metabolism (i.e. aerobic respiration), complete utilization of an energy source such as glucose produces 38 molecules of ATP.
- Anaerobic metabolism utilizing an inorganic molecule other than oxygen as the final hydrogen acceptor (anaerobic respiration) is incomplete and produces fewer ATP molecules than aerobic respiration.
- Anaerobic metabolism utilizing an organic final hydrogen acceptor (fermentation) is much less efficient and produces only two molecules of ATP.

Anaerobic metabolism, while less efficient, can thus be used in the absence of oxygen when appropriate substrates are available, as they usually are in the host's body. The requirement for oxygen in respiration may be 'obligate' or it may be 'facultative', some organisms being able to switch between aerobic and anaerobic metabolism. Those that use fermentation pathways often use the major product pyruvate in secondary fermentations by which additional energy can be generated. The interrelationship between these different metabolic pathways is illustrated in Fig. 2.4.

The ability of bacteria to grow in the presence of atmospheric oxygen relates to their ability to deal enzymatically with

Table 2.1 Major nutritional requirements for bacterial growth

Element	Cell dry weight (%)	Major cellular role
Carbon	50	Molecular 'building block' obtained from organic compounds or CO_2
Oxygen	20	Molecular 'building block' obtained from organic compounds, O_2 or H_2O; O_2 is an electron acceptor in aerobic respiration
Nitrogen	14	Component of amino acids, nucleotides, nucleic acids and coenzymes obtained from organic compounds and inorganic sources such as $NH4^+$
Hydrogen	8	Molecular 'building block' obtained from organic compounds, H_2O, or H_2; involved in respiration to produce energy
Phosphorus	3	Found in a variety of cellular components including nucleotides, nucleic acids, lipopolysaccharide (lps) and phospholipids; obtained from inorganic phosphates (PO_4^{3-})
Sulphur	1–2	Component of several amino acids and coenzymes; obtained from organic compounds and inorganic sources such as sulphates (SO_4^{2-})
Potassium	1–2	Important inorganic cation, enzyme cofactor, etc., obtained from inorganic sources

Table 2.2 Bacterial classification in response to environmental oxygen

Environmental oxygen			
Category	**Present**	**Absent**	**Oxygen-detoxifying enzymes (e.g. superoxide dismutase, catalase, peroxidase)**
Obligate aerobe	Growth	No growth	Present
Microaerophile	Growth in low oxygen levels	No growth	Some enzymes absent; reduced enzyme concentration
Obligate anaerobe	No growth	Growth	Absent
Facultative (anaerobe/aerobe)	Growth	Growth	Present

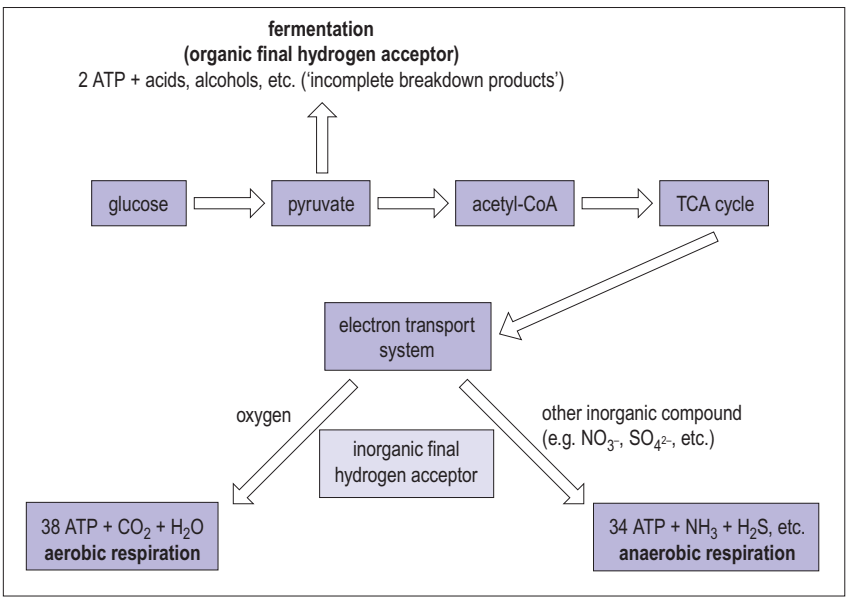

Figure 2.4 Catabolic breakdown of glucose in relationship to final hydrogen acceptor.

potentially destructive intracellular reactive oxygen species (e.g. free radicals, anions containing oxygen, etc.) (Table 2.2). The interaction between these harmful compounds and detoxifying enzymes such as superoxide dismutase, peroxidase and catalase is illustrated in Fig. 2.5 (also see Ch. 10 and Box 10.2).

GROWTH AND DIVISION

The rate at which bacteria grow and divide depends in large part on the nutritional status of the environment. The growth and division of a single *E. coli* cell into identical 'daughter cells' may occur in as little as 20–30 min in rich laboratory

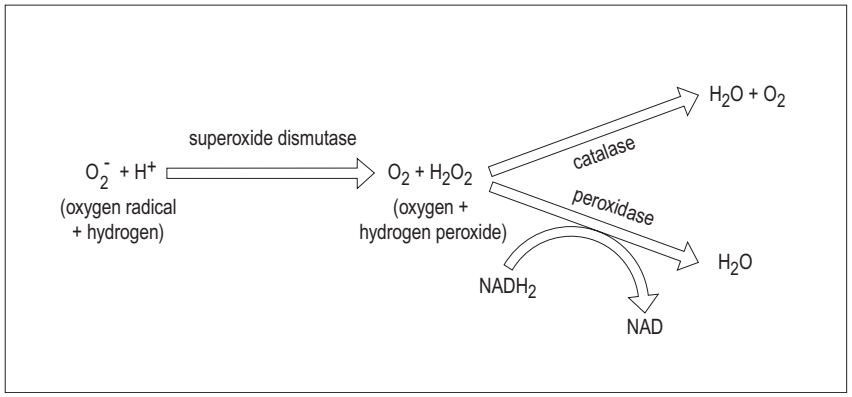

Figure 2.5 Interaction between oxygen detoxifying enzymes.

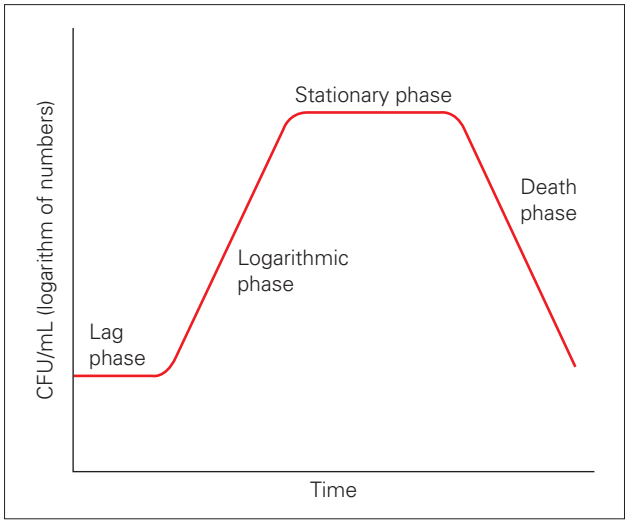

Figure 2.6 The bacterial growth curve. CFU, colony-forming units.

media, whereas the same process is much slower (1–2 h) in a nutritionally depleted environment. Conversely, even in the best environment, other bacteria such as *Mycobacterium tuberculosis* may grow much more slowly, dividing every 24 h. When introduced into a new environment, bacterial growth follows a characteristic pattern depicted in Fig. 2.6. After an initial period of adjustment (lag phase), cell division rapidly occurs, with the population doubling at a constant rate (generation time), for a period termed log or exponential phase. As nutrients are depleted and toxic products accumulate, cell growth slows to a stop (stationary phase) and eventually enters a phase of decline (death).

A bacterial cell must duplicate its genomic DNA before it can divide

All bacterial genomes are circular, and their replication begins at a single site known as the origin of replication (termed OriC). A multienzyme replication complex binds to the origin and initiates unwinding and separation of the two DNA strands, using enzymes called helicases and topoisomerases (e.g. DNA gyrase). Each of the separated DNA strands serves as a template for DNA polymerase. The polymerization reaction involves incorporation of deoxyribonucleotides, which correctly base pair with the template DNA. Two characteristic replication forks are formed, which proceed in opposite directions around the chromosome. Each of the two copies of the total genetic information (genome) produced during replication comprises one parental strand and one newly synthesized strand of DNA.

Replication of the genome takes approximately 40 min in *E. coli*, so when these bacteria grow and divide every 20–30 min they need to initiate new rounds of DNA replication before an existing round of replication has finished to provide complete chromosomal copies at an accelerated rate. In such instances, daughter cells inherit DNA that has already initiated its own replication.

Replication must be accurate

Accurate replication is essential because DNA carries the information that defines the properties and processes of a cell. It is achieved because DNA polymerase is capable of proofreading newly incorporated deoxyribonucleotides and excising those that are incorrect. This reduces the frequency of errors to approximately one mistake (an incorrect base pair) per 10^{10} nucleotides copied.

Cell division is preceded by genome segregation and septum formation

The process of cell division (or septation) involves:

- segregation of the replicated genomes
- formation of a septum in the middle of the cell
- division of the cell to give separate daughter cells.

The septum is formed by an invagination of the cytoplasmic membrane and ingrowth of the peptidoglycan cell wall (and outer membrane in Gram-negative bacteria). Septation and DNA replication and genome segregation are not tightly coupled, but are sufficiently well coordinated to ensure that the overwhelming majority of daughter cells have the correct complement of genomic DNA.

The mechanics of cell division result in reproducible cellular arrangements, when viewed by microscopic examination. For example, cocci dividing in one plane may appear chained (streptococci) or paired (diplococci), while division in multiple planes results in clusters (staphylococci). As with cell shape, these arrangements have served as an important characteristic for bacterial identification.

Bacterial growth and division are important targets for antimicrobial agents

Antimicrobials that target the processes involved in bacterial growth and division include:

- quinolones (ciprofloxacin and levofloxacin), which inhibit the unwinding of DNA by DNA gyrase during DNA replication
- the many inhibitors of peptidoglycan cell wall synthesis (e.g. beta-lactams such as the penicillins, cephalosporins and carbapenems, and glycopeptides such as vancomycin).

These are considered in more detail in Chapter 34.

GENE EXPRESSION

Gene expression describes the processes involved in decoding the 'genetic information' contained within a gene to produce a functional protein or ribonucleic acid (RNA) molecule.

Most genes are transcribed into messenger RNA (mRNA)

The overwhelming majority of genes (e.g. up to 98% in *E. coli*) are transcribed into mRNA, which is then translated into proteins. Certain genes, however, are transcribed to produce ribosomal RNA species (5 S, 16 S, 23 S), which provide a scaffold for assembling ribosomal subunits; others are transcribed into transfer RNA (tRNA) molecules, which together with the ribosome participate in decoding mRNA into functional proteins.

Transcription

The DNA is copied by a DNA-dependent RNA polymerase to yield an RNA transcript. The polymerization reaction involves incorporation of ribonucleotides, which correctly base pair with the template DNA.

Transcription is initiated at promoters

Promoters are nucleotide sequences in DNA that can bind the RNA polymerase. The frequency of transcription initiation can be influenced by many factors, for example:

- the exact DNA sequence of the promoter site
- the overall topology (supercoiling) of the DNA
- the presence or absence of regulatory proteins that bind adjacent to and may overlap the promoter site.

Consequently, different promoters have widely different rates of transcriptional initiation (of up to 3000-fold). Their activities can be altered by regulatory proteins. Sigma factor (a protein specifically needed to begin RNA synthesis) plays an important role in promoter recognition. The presence of several different sigma factors in bacteria enables sets of genes to be switched on simply by altering the level of expression of a particular sigma factor (e.g. spore formation in Gram-positive bacteria).

Transcription usually terminates at specific termination sites

These termination sites are characterized by a series of uracil residues in the mRNA following an inverted repeat sequence, which can adopt a stem-loop structure (which forms as a result of the base-pairing of ribonucleotides) and interfere with RNA polymerase activity. In addition, certain transcripts terminate following interaction of RNA polymerase with the transcription termination protein, rho.

mRNA transcripts often encode more than one protein in bacteria

The bacterial arrangement seen for single genes (promoter–structural-gene–transcriptional-terminator) is described as monocistronic. However, a single promoter and terminator may flank multiple structural genes, a polycistronic arrangement known as an operon. Operon transcription thus results in polycistronic mRNA encoding more than one protein (Fig. 2.7). Operons provide a way of ensuring that protein subunits that make up particular enzyme complexes or are required for a specific biological process are synthesized simultaneously and in the correct stoichiometry. For example, the proteins required for the uptake and metabolism of lactose are encoded by the lac operon. Many of the proteins responsible for the pathogenic properties of medically important microorganisms are likewise encoded by operons, for example:

- cholera toxin from *Vibrio cholerae*
- fimbriae (pili) of uropathogenic *E. coli*, which mediate colonization.

Translation

The exact sequence of amino acids in a protein (polypeptide) is specified by the sequence of nucleotides found in the mRNA transcripts. Decoding this information to produce a protein is achieved by ribosomes and tRNA molecules in a process known as translation. Each set of three bases (triplet) in the mRNA sequence corresponds to a codon for a specific amino acid. However, there is redundancy in the triplet code resulting in instances of more than one triplet encoding the same amino acid (i.e. also referred to as code degeneracy). Thus, a total of 64 codons encode all 20 amino acids as well as start and stop signal codons.

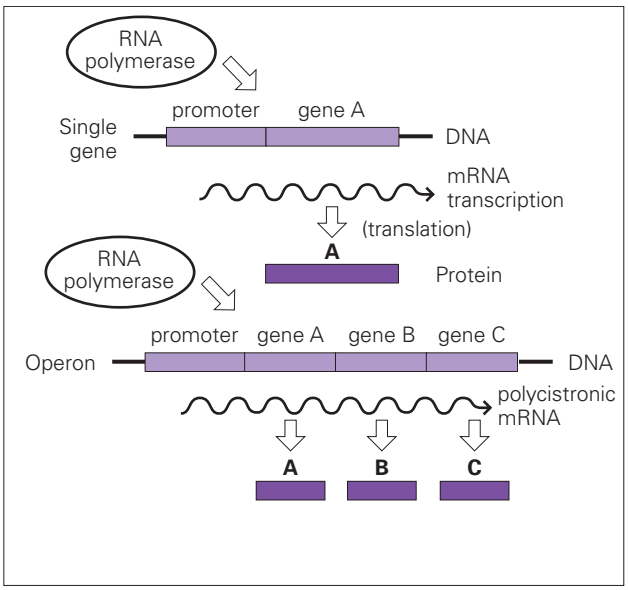

Figure 2.7 Bacterial genes are present on DNA as separate discrete units (single genes) or as operons (multigenes), which are transcribed from promoters to give, respectively, monocistronic or polycistronic messenger RNA (mRNA) molecules; mRNA is then translated into protein.

Translation begins with formation of an initiation complex and terminates at a STOP codon

The initiation complex comprises mRNA, ribosome and an initiator tRNA molecule carrying formylmethionine. Ribosomes bind to specific sequences in mRNA (Shine–Dalgarno sequences) and begin translation at an initiation (START) codon, AUG (i.e. the bases adenosine, uracil, guanine), which hybridizes with a specific complementary sequence (the anti-codon loop) of the initiator tRNA molecule. The polypeptide chain elongates as a result of movement of the ribosome along the mRNA molecule and the recruitment of further tRNA molecules (carrying different amino acids), which recognize the subsequent codon triplets. Ribosomes carry out a condensation reaction, which couples the incoming amino acid (carried on the tRNA) to the growing polypeptide chain.

Translation is terminated when the ribosome encounters one of three termination (STOP) codons: UGA, UAA or UAG.

Transcription and translation are important targets for antimicrobial agents

Such antimicrobial agents include:

- inhibitors of RNA polymerase, such as rifampicin
- a wide array of bacterial protein synthesis inhibitors including macrolides (e.g. erythromycin, aminoglycosides, tetracyclines, chloramphenicol, lincosamides, streptogramins, and oxazolidinones) (see Ch. 34).

Regulation of gene expression

Bacteria adapt to their environment by controlling gene expression

Bacteria show a remarkable ability to adapt to changes in their environment. This is predominantly achieved by controlling gene expression, thereby ensuring that proteins are produced only when and if they are required. For example:

- Bacteria may encounter a new source of carbon or nitrogen and as a consequence switch on new metabolic pathways that enable them to transport and use such compounds.
- When compounds such as amino acids are depleted from a bacterium's environment, the bacterium may be able to switch on the production of enzymes that enable it to synthesize de novo the particular molecule it requires.

Expression of many virulence determinants by pathogenic bacteria is highly regulated

This makes sense as it conserves metabolic energy and ensures that virulence determinants are produced only when their particular property is needed. For example, enterobacterial pathogens are often transmitted in contaminated water supplies. The temperature of such water will probably be lower than 25°C and low in nutrients. However, upon entering the human gut there will be a striking change in the bacterium's environment – the temperature will rise to 37°C, there will be an abundant supply of carbon and nitrogen and a low availability of both oxygen and free iron (an essential nutrient). Bacteria adapt to such changes by switching on or off a range of metabolic and virulence-associated genes.

The analysis of virulence gene expression is one of the fastest-growing aspects of the study of microbial pathogenesis. It provides an important insight into how bacteria adapt to the many changes they encounter as they initiate infection and spread into different host tissues.

The most common way of altering gene expression is to change the amount of mRNA transcription

The level of mRNA transcription can be altered by altering the efficiency of binding of RNA polymerase to promoter sites. Environmental changes such as shifts in growth temperature (from 25°C to 37°C) or the availability of oxygen can change the extent of supercoiling in DNA, thereby altering the overall topology of promoters and the efficiency of transcription initiation. However, most instances of transcriptional regulation are mediated by regulatory proteins, which bind specifically to the DNA adjacent to or overlapping the promoter site and alter RNA polymerase binding and transcription. The regions of DNA to which regulatory proteins bind are known as operators or operator sites. Regulatory proteins fall into two distinct classes:

- those that increase the rate of transcription initiation (activators)
- those that inhibit transcription (repressors) (Fig. 2.8).

Genes subject to positive regulation need to bind an activated regulatory protein (apoinducer) to promote transcription initiation. Gene transcription subject to negative regulation is inhibited by the binding of repressor proteins.

The principles of gene regulation in bacteria can be illustrated by the regulation of genes involved in sugar metabolism

Bacteria use sugars as a carbon source for growth and prefer to use glucose rather than other less well-metabolized sugars. When growing in an environment containing both glucose and lactose, bacteria such as *E. coli* preferentially metabolize glucose and at the same time prevent the expression of the lac operon, the products of which transport and metabolize lactose (Fig. 2.9). This is known as catabolite repression. It occurs because the transcriptional initiation of the lac operon is dependent upon a positive regulator: the cyclic adenosine monophosphate (cAMP)-dependent catabolite activator protein (CAP), which is activated only when cAMP is bound. When bacteria grow on glucose the cytoplasmic levels of cAMP are low and so CAP is not activated. CAP is therefore unable to bind to its DNA binding site adjacent to the lac promoter and facilitate transcription initiation by RNA polymerase. When the glucose is depleted, the cAMP concentration rises resulting in the formation of activated cAMP–CAP complexes, which bind the appropriate site on the DNA, increasing RNA polymerase binding and lac operon transcription.

CAP is an example of a global regulatory protein that controls the expression of multiple genes; it controls the expression of over 100 genes in *E. coli.* All genes controlled by the same regulator are considered to constitute a regulon (see Fig. 2.8). In addition to the influence of CAP on the lac operon, the operon is also subject to negative regulation by the lactose repressor protein (LacI, see Fig. 2.9). LacI is encoded by the *lacI* gene, which is located immediately upstream of the lactose operon and transcribed by a separate promoter. In the absence of lactose, LacI binds specifically to the operator region of the lac promoter and blocks transcription. An inducer molecule, allolactose (or its non-metabolizable homologue,

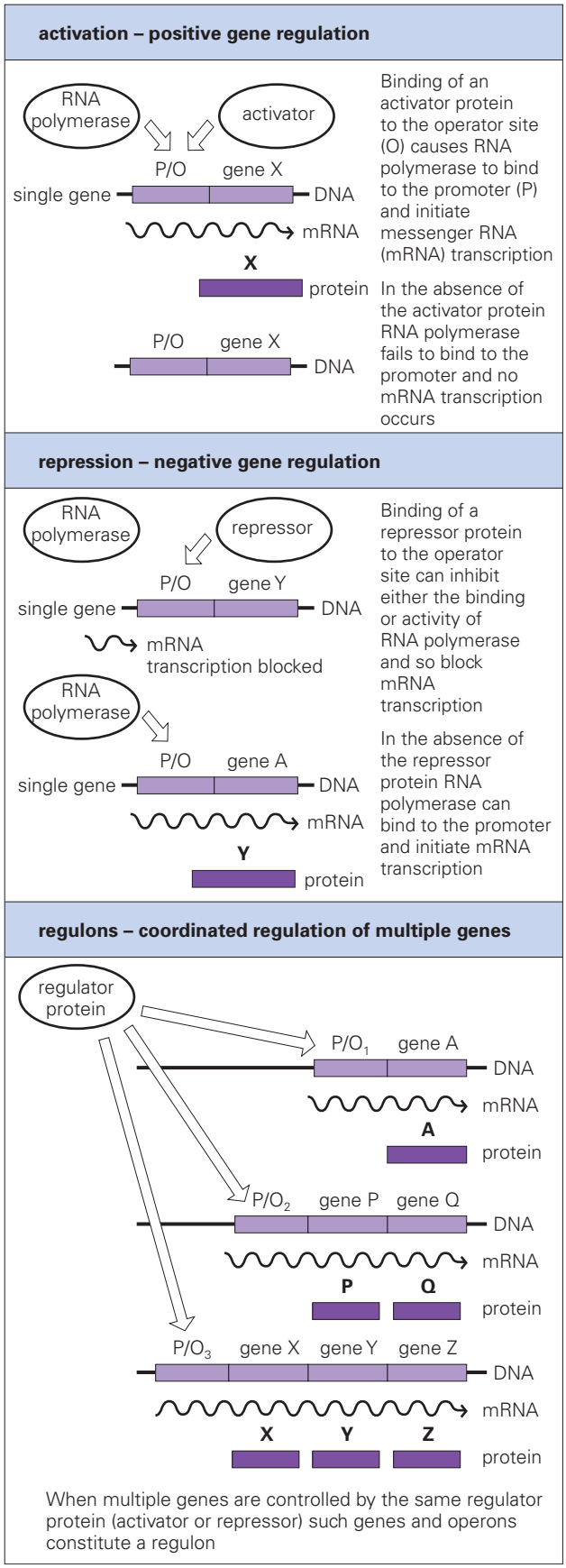

Figure 2.8 Expression of genes in bacteria is highly regulated, enabling them to switch genes on or off in response to changes in available nutrients or other changes in their environment. Genes and operons controlled by the same regulator constitute a regulon.

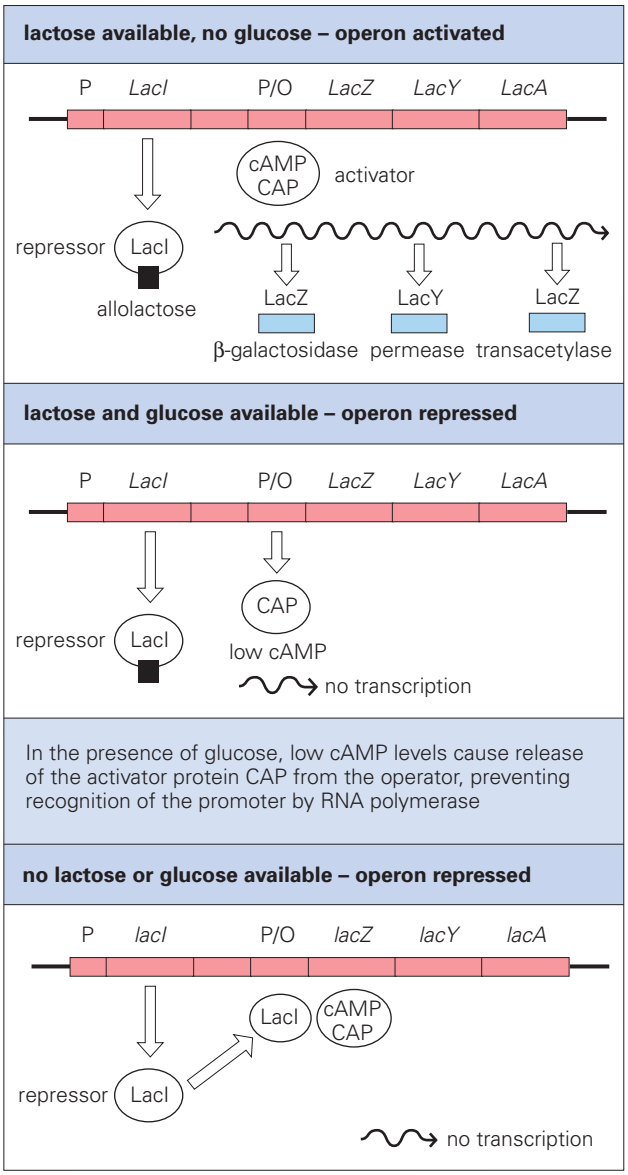

Figure 2.9 Control of the lac operon. Transcription is controlled by the lactose repressor protein (LacI, negative regulation) and by the catabolite activator protein (CAP, positive regulation). In the presence of lactose as the sole carbon source for growth, the lac operon is switched on. Bacteria prefer to use glucose rather than lactose, so if glucose is also present the lac operon is switched off until the glucose has been used.

isopropyl-thiogalactoside – IPTG) is able to bind to LacI, causing an allosteric change in its structure. This releases it from the DNA, thereby alleviating the repression. The lac operon therefore illustrates the fine tuning of gene regulation in bacteria – the operon is switched on only if lactose is available as a carbon source for cell growth, but remains unexpressed if glucose, the cell's preferred carbon source, is also present.

Expression of bacterial virulence genes is often controlled by regulatory proteins

An example of such regulation is the production of diphtheria toxin by *Corynebacterium diphtheriae* (see Ch. 19), which is subject to negative regulation if there is free iron in the growth environment. A repressor protein, DtxR, binds iron and undergoes a conformational change that allows it to bind with high affinity to the operator site of the toxin gene and inhibit transcription. When *C. diphtheriae* grow in an environment with a very low concentration of iron (i.e. similar to that of human secretions), DtxR is unable to bind iron, and toxin production occurs.

Many bacterial virulence genes are subject to positive regulation by 'two-component regulators'

These two-component regulators typically comprise two separate proteins (Fig. 2.10):

- one acting as a sensor to detect environmental changes (such as alterations in temperature)
- the other acting as a DNA-binding protein capable of activating (or repressing in some cases) transcription.

Bacteria may possess multiple two-component regulators recognizing different environmental stimuli. Thus, bacteria residing in more complex environments tend to carry increased numbers of two-component regulators.

In *Bordetella pertussis*, the causative agent of whooping cough (see Ch. 20), a two-component regulator (encoded by the bvg locus) controls expression of a large number of virulence genes. The sensor protein, BvgS, is a cytoplasmic membrane-located histidine kinase, which senses environmental signals (temperature, Mg^{2+}, nicotinic acid), leading to an alteration in its autophosphorylating activity. In response to positive regulatory signals such as an elevation in temperature, BvgS undergoes autophosphorylation and then phosphorylates, so activating the DNA-binding protein BvgA. BvgA then binds to the operators of the pertussis toxin operon and other virulence-associated genes and activates their transcription.

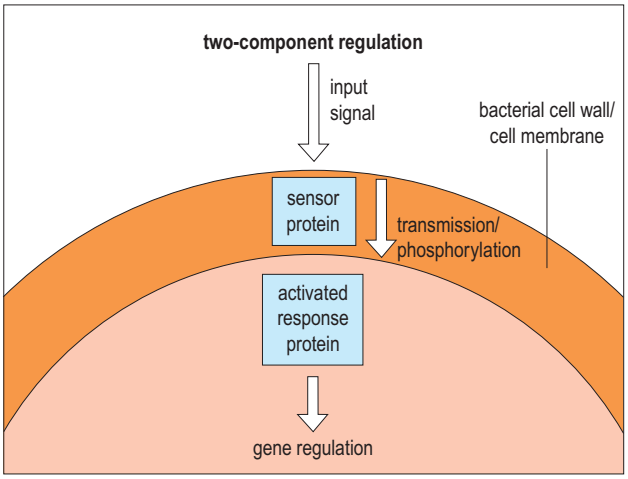

Figure 2.10 Two-component regulation is a signal transduction process that allows cellular functions to react in response to a changing environment. An appropriate environmental stimulus results in autophosphorylation of the sensor protein, which, by a phosphotransfer reaction, activates the response protein that affects gene regulation.

In *Staphylococcus aureus*, a variety of virulence genes are influenced by global regulatory systems, the best studied and most important of which is a two-component regulator termed accessory gene regulator (*agr*). Agr control is complex in that it serves as a positive regulator for exotoxins secreted late in the bacterial life cycle (post-exponential phase) but behaves as a negative regulator for virulence factors associated with the cell surface.

The control of virulence gene expression in *V. cholerae* is under the control of ToxR, a cytoplasmic membrane-located protein, which senses environmental changes. ToxR activates both the transcription of the cholera toxin operon and another regulatory protein, ToxT, which in turn activates the transcription of other virulence genes such as toxin-co-regulated pili, an essential virulence factor required for colonization of the human small intestine.

In some instances the pathogenic activity of bacteria specifically begins when cell numbers reach a certain threshold

Quorum sensing is the mechanism by which specific gene transcription is activated in response to bacterial concentration. While quorum sensing is known to occur in a wide variety of microorganisms, a classic example is the production of biofilms by *Pseudomonas aeruginosa* in the lungs of cystic fibrosis (CF) patients. The production of these tenacious substances allows *P. aeruginosa* to establish serious long-term infection in CF patients, which is difficult to treat (see Ch. 20; Fig. 20.23). As illustrated in Fig. 2.11, when quorum-sensing bacteria reach appropriate numbers, the signalling compounds they produce are at sufficient concentration to activate transcription of specific response genes such as those related to biofilm production. Current research is aimed at better understanding the quorum-sensing process in different bacterial pathogens and exploring potential therapeutic approaches (e.g. inhibitory compounds) to interfere with this coordinated mechanism of bacterial virulence.

SURVIVAL UNDER ADVERSE CONDITIONS

Some bacteria form endospores

Certain bacteria can form highly resistant spores – endospores – within their cells, and these enable them to survive adverse conditions. They are formed when the cells are unable to grow (e.g. when environmental conditions change or when nutrients are exhausted), but never by actively growing cells. The spore has a complex multilayered coat surrounding a new bacterial cell. There are many differences in composition between endospores and normal cells, notably the presence of dipicolinic acid and a high calcium content, both of which are thought to confer the endospore's extreme resistance to heat and chemicals.

Because of their resistance, spores can remain viable in a dormant state for many years, re-converting rapidly to normal existence when conditions improve. When this occurs,

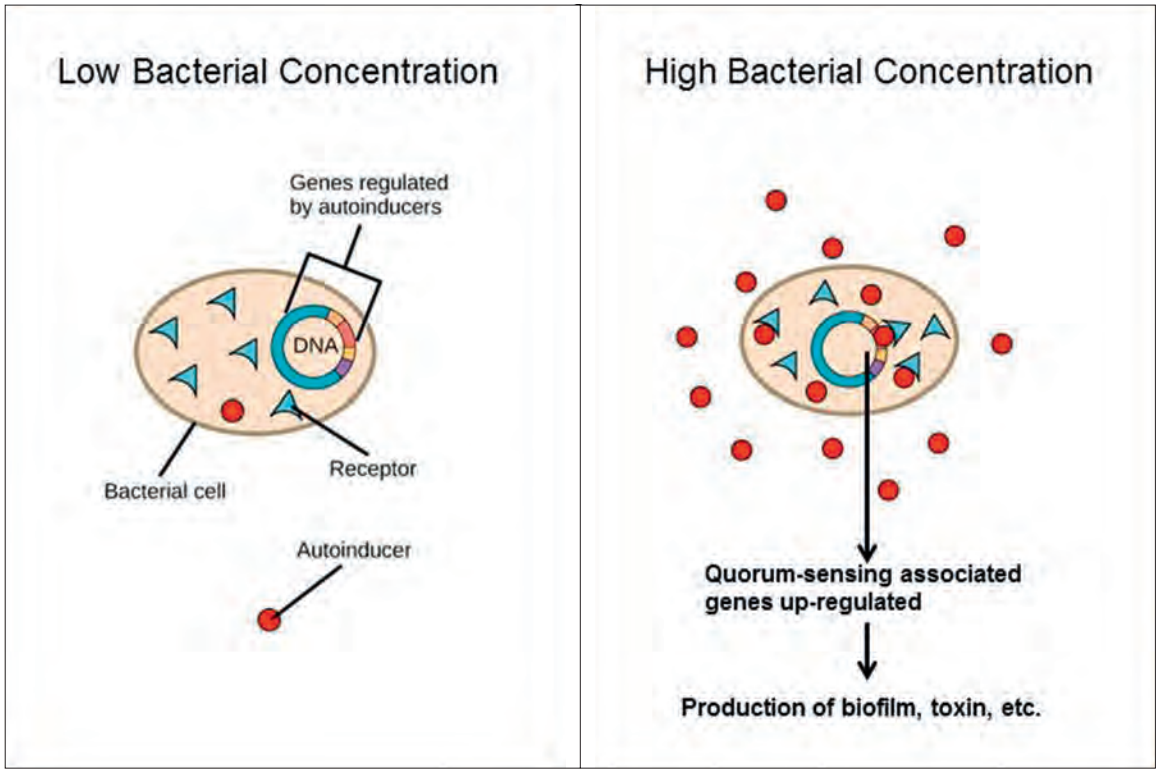

Figure 2.11 Quorum sensing bacteria produce autoinducer signaling compounds which, in sufficient concentration, bind to receptors that activate transcription of specific response genes (e.g. for biofilm production, etc.). (Adapted from https://www.boundless.com/biology/textbooks/boundless-biology-textbook/cell-communication-9/signaling-in-single-celled-organisms-86/signaling-in-bacteria-391-11617/images/fig-ch09_04_02/.)

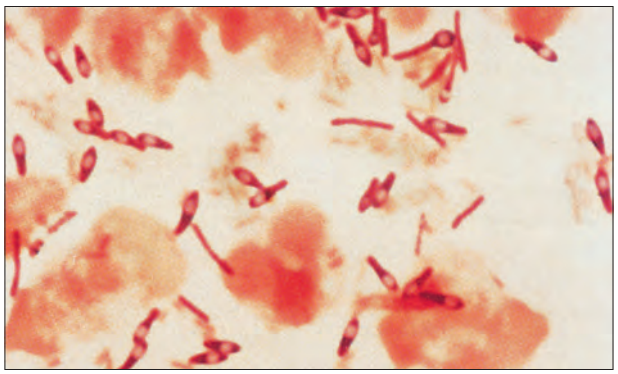

Figure 2.12 *Clostridium tetani* with terminal spores.

a new bacterial cell grows out from the spore and resumes vegetative life. Endospores are abundant in soils, and those of the *Clostridium* and *Bacillus* are a particular hazard (Fig. 2.12). Tetanus and anthrax caused by these bacteria are both associated with endospore infection of wounds, the bacteria developing from the spores once they are in appropriate conditions.

MOBILE GENETIC ELEMENTS

The bacterial chromosome represents the primary reservoir of genetic information within the cell. However, a variety of additional genetic elements may also be present which are capable of independently moving to different locations within a cell or between cells (also termed horizontal gene transfer).

Many bacteria possess small, independently replicating (extrachromosomal) nucleic acid molecules termed plasmids and bacteriophages

Plasmids are independent, self-replicating, circular units of dsDNA, some of which are relatively large (e.g. 60–120 kb) whereas others are quite small (1.5–15 kb). Plasmid replication is similar to the replication of genomic DNA, though there are differences. Not all plasmids are replicated bidirectionally – some have a single replication fork, others are replicated like a 'rolling circle'. The number of plasmids per bacterial cell (copy number) varies for different plasmids, ranging from 1 to 1000s of copies per cell. The rate of initiation of plasmid replication determines the plasmid copy number; however, larger plasmids generally tend to have lower copy numbers than smaller plasmids. Some plasmids (broad-host-range plasmids) are able to replicate in many different bacterial species; others have a more restricted host range.

Plasmids contain genes for replication, and in some cases for mediating their own transfer between bacteria (*tra* genes). Plasmids may additionally carry a wide variety of additional genes (related to the overall size of the plasmid) which can confer a variety of advantages to the host bacterial cell (e.g. antibiotic resistance, toxin production).

Widespread use of antimicrobials has applied a strong selection pressure in favour of bacteria able to resist them

In the majority of cases, resistance to antimicrobials is due to the presence of resistance genes on self-transferrable

(conjugative) plasmids (R plasmids; see Ch. 34). These are known to have existed before the era of mass antibiotic treatments, but they have become widespread in many species as a result of selection. R plasmids may carry genes for resistance to multiple antimicrobials. For example, one of the earliest-studied R plasmids, R100, confers resistance to sulphonamides, aminoglycosides, chloramphenicol, and tetracycline, and there are many others carrying genes for resistance to an even greater spectrum of antimicrobials. R plasmids can recombine, resulting in individual replicons encoding new combinations of multiple drug resistance.

Plasmids can carry virulence genes

Plasmids may encode toxins and other proteins that increase the virulence of microorganisms. For example:

- The virulent enterotoxinogenic strains of *E. coli* that cause diarrhoea produce different types of plasmid-encoded enterotoxins that alter the secretion of fluid and electrolytes by the intestinal epithelium (see Ch. 23).
- In *Staph. aureus*, both an enterotoxin and a number of enzymes involved in bacterial virulence (haemolysin, fibrinolysin) are encoded by plasmid genes.

The production of toxins by bacteria, and their pathological effects, is discussed in detail in Chapter 18.

Plasmids are valuable tools for cloning and manipulating genes

Molecular biologists have generated a wealth of recombinant plasmids to use as vectors for genetic engineering (Fig. 2.13). Plasmids can be used to transfer genes across species barriers so that defined gene products can be studied or synthesized in large quantities in different recipient organisms.

Bacteriophages are bacterial viruses that can survive outside as well as inside the bacterial cell

Bacteriophages differ from plasmids in that their reproduction usually leads to destruction of the bacterial cell. In general, bacteriophages consist of a protein coat or head (capsid), which surrounds nucleic acid which may be either DNA or RNA but not both. Some bacteriophages may also possess a tail-like structure which aids them in attaching to and infecting their bacterial host. As illustrated in Fig. 2.14 for DNA-containing bacteriophages, the virus attaches and injects its DNA into the bacterium, leaving the protective protein coat behind. Virulent bacteriophages instigate a form of molecular mutiny to commandeer cellular nucleic acid and protein to produce new virus DNA and protein. Many new virus particles (virions) are then assembled and released into the environment as the bacterial cell ruptures (lyses), thus allowing the cycle to begin again.

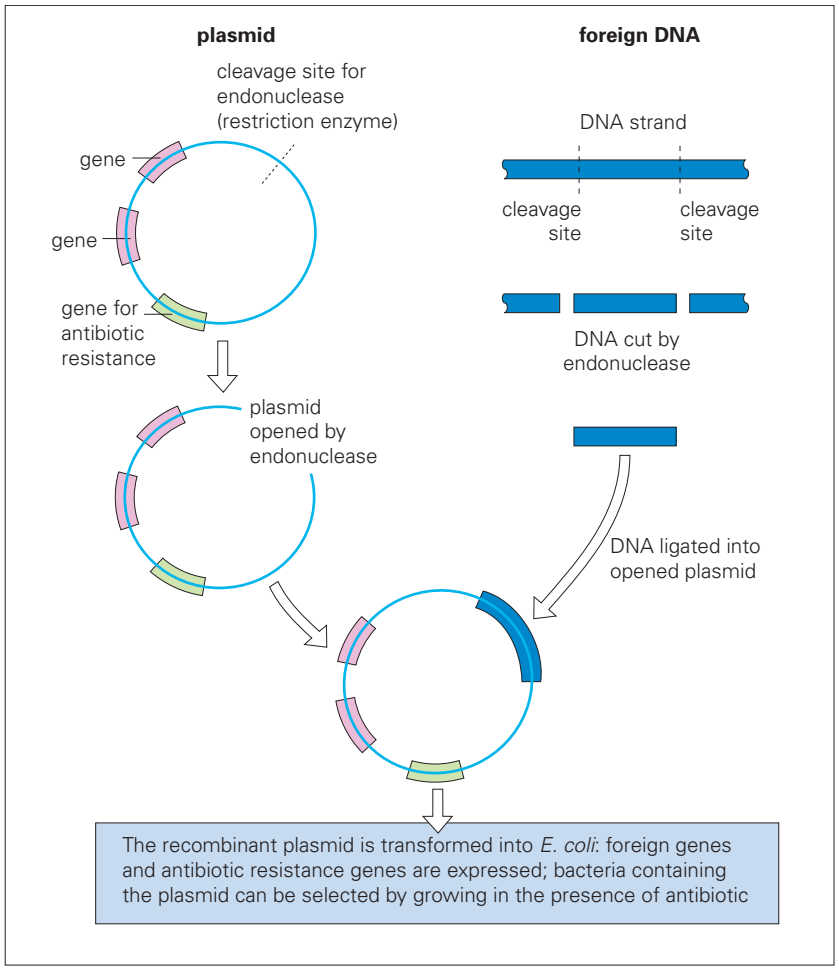

Figure 2.13 The use of plasmid vectors to introduce foreign DNA in *E. coli* – a basic step in gene cloning.

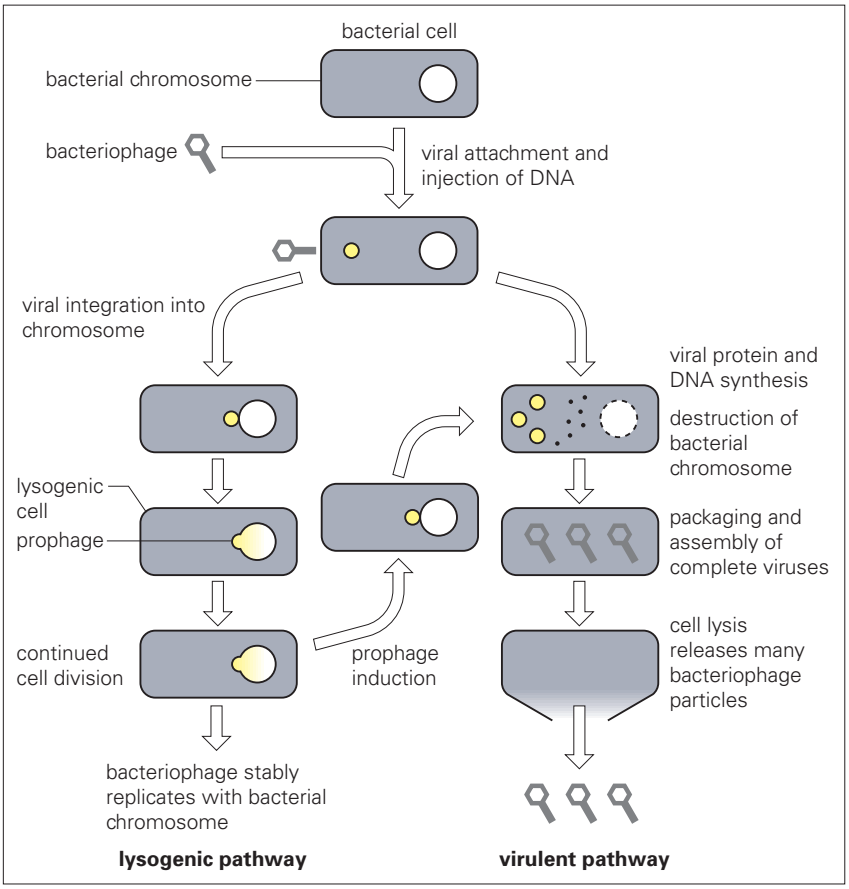

Figure 2.14 The life cycle of bacteriophages.

While destruction of the host is always the direct consequence of virulent bacteriophage infection, temperate bacteriophages may exercise a 'choice'. Following infection, they may immediately reproduce in a manner similar to their virulent counterparts. However, in some instances they may insert into the bacterial chromosome. This process, termed lysogeny, does not kill the cell as the integrated viral DNA (now called a prophage) is quiescently carried and replicated within the bacterial chromosome. New characteristics may be expressed by the cell as a result of prophage presence (prophage conversion), which, in some instances, may increase bacterial virulence (e.g. the gene for diphtheria toxin resides on a prophage). Nevertheless, this latent state is eventually destined to end, often in response to some environmental stimulus inactivating the bacteriophage repressor which normally maintains the lysogenic condition. During this induction process, the viral DNA is excised from the chromosome and proceeds to active replication and assembly, resulting in cell lysis and viral release.

Whether virulent or temperate, bacteriophage infection ultimately results in death of the host cell which, given current problems with multiple resistance, has sparked a renewed interest in their use as 'natural' antimicrobial agents. However, a variety of issues related to dosing, delivery, quality control, etc. have impeded the use of 'bacteriophage therapy' in routine clinical practice.

Transposition

Transposable elements are DNA sequences that can jump (transpose) from a site in one DNA molecule to another in a cell. This movement does not rely on host-cell (homologous) recombination pathways which require extensive similarity between the resident and incoming DNA. Instead, movement involves short target sequences in the recipient DNA molecule where recombination / insertion is directed by the mobile element (site-specific recombination).

While plasmid transfer involves the movement of genetic information between bacterial cells, transposition is the movement of such information between DNA molecules. The most extensively studied transposable elements are those found in *E. coli* and other Gram-negative bacteria, although examples are also found in Gram-positive bacteria, yeast, plants and other organisms.

Insertion sequences are the smallest and simplest 'jumping genes'

Insertion sequence elements (ISs) are generally <2 kb in length and only encode functions such as the transposase enzyme, which is required for transposition from one DNA site to another. At the ends of ISs, there are usually short inverted repeat sequences (36 nucleotides long in IS*911*), which are also important in the process of locating and inserting into a DNA target (Fig. 2.15A). During the transposition process, a portion of the target sequence is duplicated, resulting in short direct repeat sequences (the same sequence in the same orientation) on each side of the newly inserted IS element. Many aspects of the target selection process remain unclear. While adenine / thymine (A / T)-rich regions of DNA appear to be preferred, some ISs are highly selective, whereas others

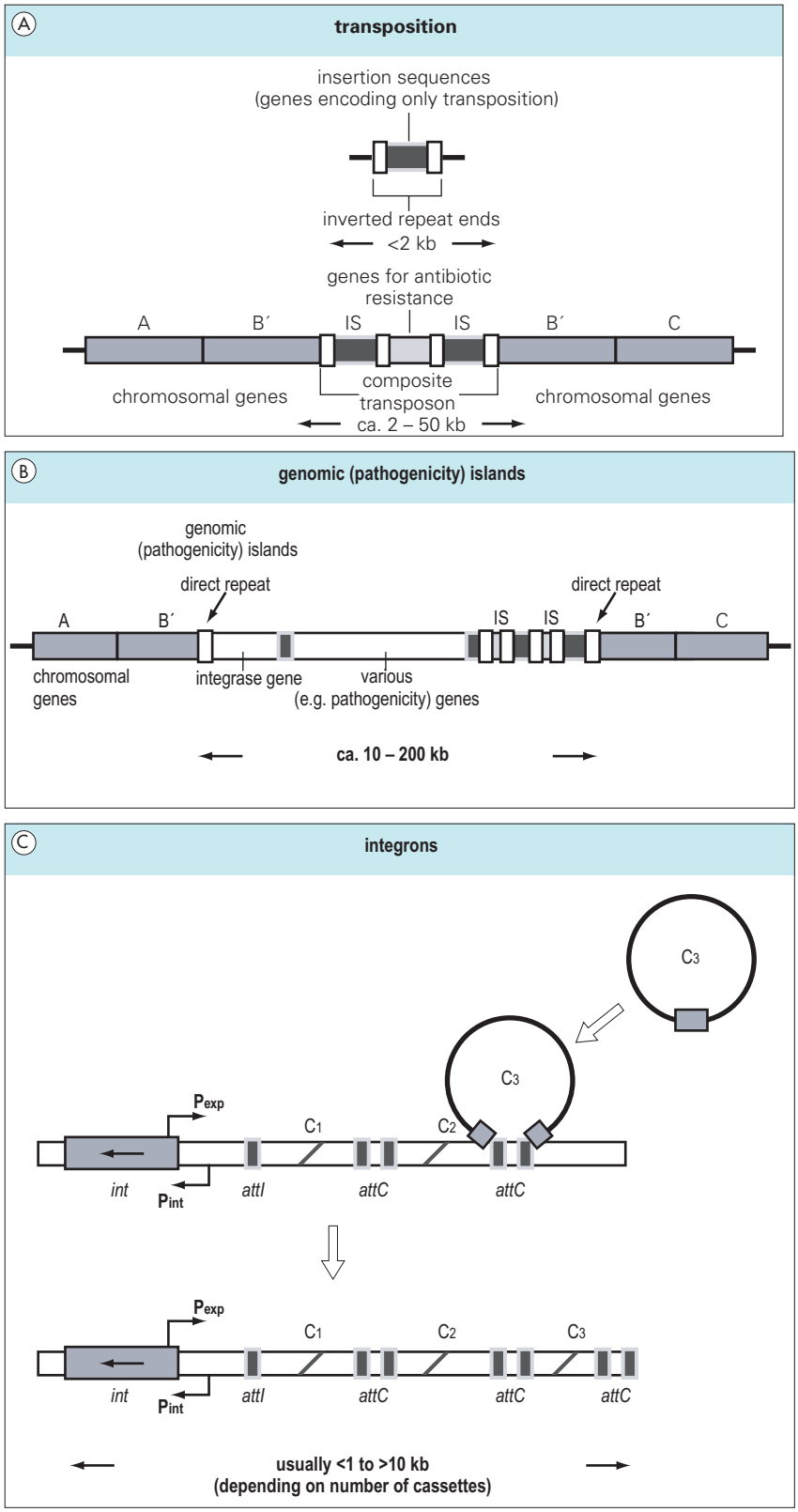

Figure 2.15 (A) Transposons (jumping genes) can move from one DNA site to another; they inactivate the recipient gene into which they insert. Transposons often contain genes which confer resistance to antibiotics. (B) Genomic islands are regions of DNA with 'signature sequences' (e.g. direct repeats) indicative of mobility. Their encoded functions increase bacterial fitness (e.g. pathogenicity). (C) Integrons are genetic regions into which independent open reading frames, also termed gene cassettes, can integrate and become functional (e.g. under control of the promoter P_{exp}). The integration process occurs by site-specific recombination between circular cassettes and their recipient integron, which is directed by an integrase gene (*intI*) with promoter P_{int} and an associated attachment site (*attI*).

are generally indiscriminate. Transposition does not rely on enzymatic processes typically used by the cell for homologous recombination (recombination between highly related DNA molecules) and is thus termed 'illegitimate recombination'. The result is a number of ISs in bacterial genomes. For example, some *E. coli* strains carry 19 copies of IS*629*, and three copies of IS*677*. Multiple IS copies serve an important function as 'portable regions of homology' throughout a bacterial genome where homologous recombination may occur between different DNA regions or molecules (e.g. chromosome and plasmid) carrying the same IS sequence. Two IS elements inserting relatively near to each other would allow the entire region to become transposable, further promoting the potential for genetic movement and exchange in bacterial populations.

Transposons are larger, more complex elements, which encode multiple genes

Transposons are >2 kb in size and contain genes in addition to those required for transposition (often encoding resistance to one or more antibiotics) (Fig. 2.15A). Furthermore, virulence genes, such as those encoding heat-stable enterotoxin from *E. coli*, have been found on transposons.

Transposons can be divided into two classes:

1. composite transposons, where two copies of an identical IS element flank antibiotic-resistance genes (kanamycin resistance in Tn5)
2. simple transposons, such as Tn3 (encoding resistance to beta-lactams).

ISs at the ends of composite transposons may be either in the same or in an inverted orientation (i.e. direct or indirect repeats). Although part of the composite transposon structure, the terminal IS elements are fully intact and capable of independent transposition.

Simple transposons move only as a single unit, containing genes for transposition and other functions (e.g. antibiotic resistance) with short, inversely oriented sequences (indirect repeats) at each end.

Mobile genetic elements promote a variety of DNA rearrangements which may have important clinical consequences

The ease with which transposons move into or out of DNA sequences means that transposition can occur:

- from host genomic DNA harbouring a transposon to a plasmid
- from one plasmid to another plasmid
- from a plasmid to genomic DNA.

Transposition onto a broad-host-range self-transferrable (conjugative) plasmid can lead to the rapid dissemination of resistance among different bacteria. The transposition process (whether by ISs or transposons) can be deleterious if insertion occurs within, and disrupts, a functional gene. However, transpositional mutagenesis has been effectively utilized in the molecular biology laboratory to produce extremely specific mutations without the harmful secondary effects often seen with more generally acting chemical mutagens.

Other mobile elements also behave as portable cassettes of genetic information

Pathogenicity islands (Fig. 2.15B) are a special class of mobile genetic elements containing groups of coordinately controlled virulence genes, often with ISs, direct repeat sequences, etc. at their ends. Though originally observed in uropathogenic *E. coli* (encoding haemolysins and pili), pathogenicity islands have now been found in a number of additional bacterial species including *H. pylori*, *V. cholerae*, *Salmonella* spp., *Staph. aureus* and *Yersinia* spp. Such regions are not found in non-pathogenic bacteria, may be quite large (up to hundreds of kilobases), and may be unstable (spontaneously lost). Differences in DNA sequence (guanine+cytosine [G+C] content) between such elements and their host genomes and the presence of transposon-like genes support speculation regarding their origin and movement from unrelated bacterial species. The term 'genomic island' has been given to DNA sequences similar to pathogenicity islands but not contributing directly to virulence or pathogenicity.

Integrons are mobile genetic elements that are able to use site-specific recombination to acquire new genes in 'cassette-like' fashion and express them in a coordinated manner (Fig. 2.15C). Integrons lack terminal repeat sequences and certain genes characteristic of transposons but, similar to transposable elements, often carry genes associated with antibiotic resistance (see Fig. 34.5).

Another important type of mobile element includes staphylococcal cassette chromosomes (SCCs) such as SCC*mec*, which not only encodes methicillin resistance but also serves as a recombinational hot spot for the acquisition of other mobile sequences. SCCs influencing virulence and antimicrobial resistance include SCC*capI* encoding capsular polysaccharide I and SCC_{476} and SCC*mercury* conferring resistance to fusidic acid and mercury, respectively. The arginine catabolic mobile element (ACME) is a cassette-like element potentially contributing to the virulence of the important USA300 community-associated methicillin-resistant *Staph. aureus* (MRSA) strain originally reported in the United States but now globally disseminated. An example of the interrelationship between the bacterial core genome and additional mobile genetic elements is depicted in Fig. 2.16.

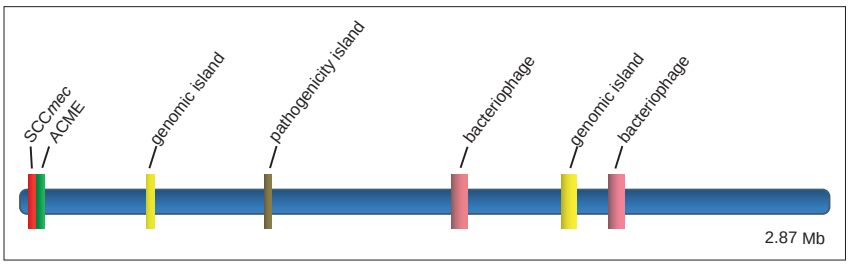

Figure 2.16 Linear depiction of the interrelationship between the USA300 MRSA core genome and key mobile genetic elements SCC*mec*, ACME, two different bacteriophages, two different genomic islands, and a pathogenicity island encoding antibiotic resistance and a variety of virulence factors.

MUTATION AND GENE TRANSFER

Bacteria are haploid organisms, their chromosomes containing one copy of each gene. Replication of the DNA is a precise process resulting in each daughter cell acquiring an exact copy of the parental genome. Changes in the genome can occur by two processes:

- mutation
- recombination.

These processes result in progeny with phenotypic characteristics that may differ from those of the parent. This is of considerable significance in terms of virulence and drug resistance.

Mutation

Changes in the nucleotide sequence of DNA can occur spontaneously or under the influence of external agents

While mutations may occur spontaneously as a result of errors in the DNA replication process, a variety of chemicals (mutagens) brings about direct changes in the DNA molecule. A classic example of such an interaction involves compounds known as nucleotide-base analogues. These agents mimic normal nucleotides during DNA synthesis but are capable of multiple pairing with a counterpart on the opposite strand. While 5-bromouracil is considered a thymine analogue, for example, it may also behave as a cytosine, thus allowing the potential for a change from T–A to C–G in a replicating DNA duplex. Other agents may cause changes by inserting (intercalating) and distorting the DNA helix or by interacting directly with nucleotide bases to alter them chemically.

Regardless of their cause, changes in DNA may generally be characterized as follows:

- Point mutations – changes in single nucleotides, which alter the triplet code. Such mutations may result in:
  - no change in the amino acid sequence of the protein encoded by the gene, because the different codons specify the same amino acid and are therefore silent mutations
  - an amino acid substitution in the translated protein (missense mutation), which may or may not alter its stability or functional properties
  - the formation of a STOP codon, causing premature termination and production of a truncated protein (nonsense mutation).
- More comprehensive changes in the DNA, which involve deletion, replacement, insertion or inversion of several or many bases. The majority of these changes are likely to harm the organism, but some may be beneficial and confer a selective advantage through the production of different proteins.

Bacterial cells are not defenceless against genetic damage

As the bacterial genome is the most fundamental molecule of identity in the cell, enzymatic machinery is in place to protect it against both spontaneously occurring and induced mutational damage. As illustrated in Fig. 2.17, these DNA repair processes include the following:

- Direct repair, which either reverses or simply removes the damage. This may be regarded as 'first-line' defence. For example, abnormally linked pyrimidine bases in DNA (pyrimidine dimers) resulting from ultraviolet radiation are directly reversed by a light-dependent enzyme through a repair process known as photoreactivation.
- Excision repair, where damage in a DNA strand is recognized by an enzymatic 'housekeeping' process and excised, followed by repair polymerization to fill the gap using the intact complementary DNA strand as a template. This is also a primary form of defence, as the goal is to correct damage before it encounters and potentially interferes with the moving DNA replication fork. Some of these housekeeping genes are also part of an inducible system (SOS repair), which is activated by the presence of DNA damage to quickly respond and effect repair.
- 'Second line' repair, which operates when DNA damage has reached a point where it is more difficult to correct. When normal DNA replication processes are blocked, permissive systems may allow the interfering damage to be inaccurately corrected, allowing errors to occur but improving the probability of cell survival. In other instances, where damage has passed the DNA replication fork, post-replication or recombinational repair processes may 'cut and paste' to construct error-free DNA from multiple copies of the sequence found in parental and daughter strands.

Bacterial DNA repair has provided a model for understanding similar, more complex processes in humans

DNA repair mechanisms appear to be present in all living organisms as a defence against environmental damage. The study of these processes in bacteria has led to an important understanding of general principles that apply to higher organisms, including issues of cancer and aging in humans. For example, several human disorders are known to be DNA-repair related, including:

- xeroderma pigmentosum, characterized by extreme sensitivity to the sun, with great risk for development of a variety of skin cancers such as basal cell carcinoma, squamous cell carcinoma and melanoma
- Cockayne syndrome, characterized by progressive neurological degeneration, growth retardation, and sun sensitivity not associated with cancer
- trichothiodystrophy, characterized by mental and growth retardation, fragile hair deficient in sulphur, and sun sensitivity not associated with cancer.

Gene transfer and recombination

New genotypes can arise when genetic material is transferred from one bacterium to another. In such instances, the newly transferred DNA is expressed when it:

- inserts into or recombines with the genome of the recipient cell
- or is on a plasmid capable of replication in the recipient without recombination.

Recombination can bring about large changes in the genetic material and, as these events usually involve functional genes, they are likely to be expressed phenotypically. DNA can be transferred from a donor cell to a recipient cell by:

- transformation
- transduction
- conjugation.

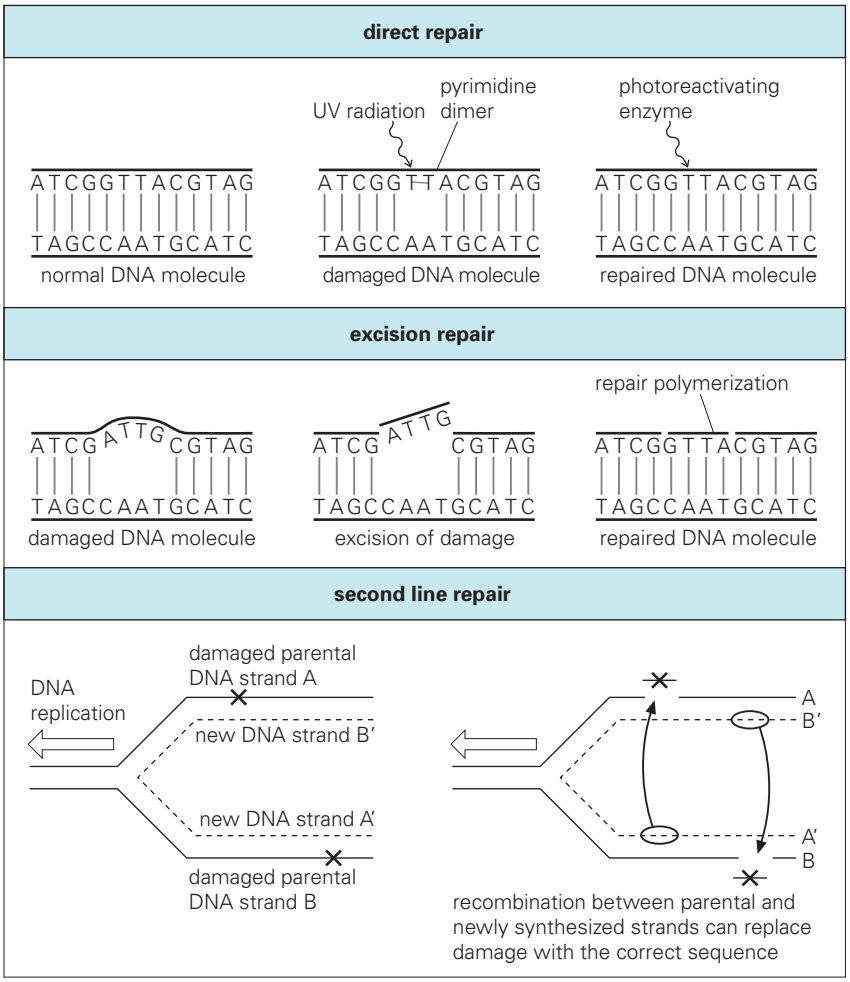

Figure 2.17 Mechanisms of DNA repair.

Transformation

Some bacteria can be transformed by DNA present in their environment

Certain bacteria such as *S. pneumoniae*, *Bacillus subtilis*, *Haemophilus influenzae* and *Neisseria gonorrhoeae* are naturally 'competent' to take up DNA fragments from related species across their cell walls. Such DNA fragments may result from lysis of organisms, the release of their DNA and its cleavage into smaller fragments, which are then available for uptake by available (competent) recipient cells. Once taken into the cell, chromosomal DNA must recombine with a homologous segment of the recipient's chromosome to be stably maintained and inherited. If the DNA is completely unrelated, the absence of homology prevents recombination and the DNA is degraded. However, plasmid DNA may be transformed into a cell and expressed without recombination. Thus, transformation has served as a powerful tool for molecular genetic analysis of bacteria (Fig. 2.18).

Most bacteria are not naturally competent to be transformed by DNA, but competence can be induced artificially by treating cells with certain bivalent cations and then subjecting them to a heat shock at 42°C or by electric shock treatment (electroporation).

Prior to uptake by competent cells, DNA is extracellular, unprotected and thus vulnerable to destruction by environmental extremes (e.g. DNA-degrading enzymes – DNases). Thus, it is the least important mechanism of gene transfer from the standpoint of clinical relevance (e.g. probability of transfer within a patient).

Transduction

Transduction involves the transfer of genetic material by infection with a bacteriophage

During the process of virulent bacteriophage replication (or temperate bacteriophages direct replication upon infection, rather than lysogeny), other DNA in the cell (genomic or plasmid) is occasionally erroneously packaged into the virus head, resulting in a 'transducing particle', which can attach to and transfer the DNA into a recipient cell. If chromosomal, the DNA must be incorporated into the recipient genome by homologous recombination to be stably inherited and expressed. As with transformation, plasmid DNA may be transduced and expressed in a recipient without recombination. In either case, this type of gene transfer is known as generalized transduction (see Fig. 2.18).

Another form of transduction occurs with 'temperate' bacteriophages, since they may integrate at specialized attachment sites in the bacterial genome. As the resulting prophages prepare to enter the lytic cycle, they occasionally incorrectly excise from the site of attachment. This can result in

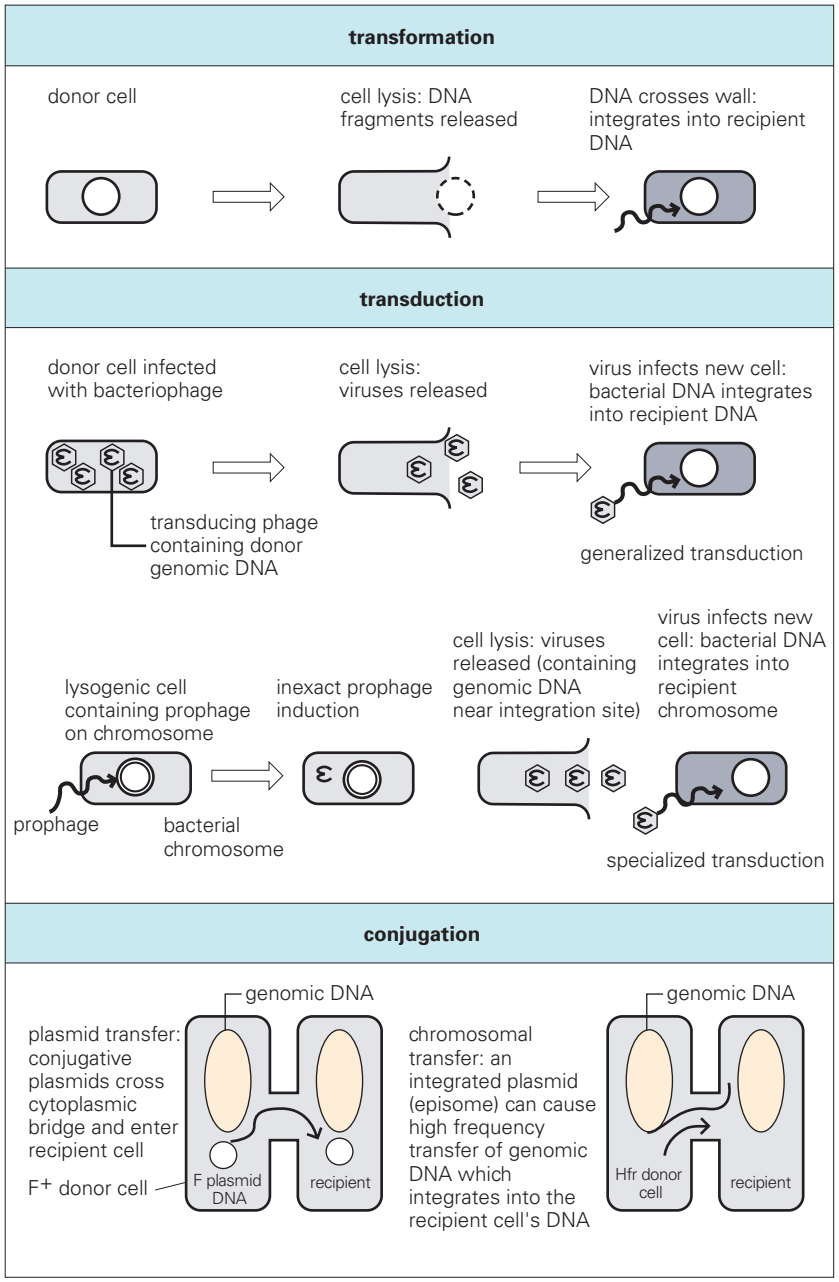

Figure 2.18 Different ways in which genes can be transferred between bacteria. With the exception of plasmid transfer, donor DNA integrates into the recipient's genome by a process of either homologous or illegitimate (in the case of transposons) recombination.

phages containing a piece of bacterial genomic DNA adjacent to the attachment site. Infection of a recipient cell then results in a high frequency of recombinants where donor DNA has recombined with the recipient genome in the vicinity of the attachment site. As this 'specialized transduction' is based on specific chromosome–prophage interaction, only genomic DNA, and not plasmids, is transferred by this process.

In contrast to transformation, transduced DNA is always protected, thus increasing its probability of successful transfer and potential clinical relevance. However, bacteriophages are extremely host-specific 'parasites' and therefore unable to move any DNA between bacteria of different species.

Conjugation

Conjugation is a type of bacterial 'mating' in which DNA is transferred from one bacterium to another

Conjugation is dependent upon the *tra* genes found in 'conjugative' plasmids, which, among other things, encode instructions for the bacterial cell to produce a sex pilus – a tube-like appendage which allows cell-to-cell contact to ensure the protected transfer of a plasmid DNA copy from a donor cell to a recipient (see Fig. 2.18). Since the *tra* genes take up genetic space, 'conjugative' plasmids are generally larger than non-conjugative ones.

Occasionally, conjugative plasmids such as the fertility plasmid (F plasmid or F factor) of *E. coli* integrate into the

bacterial genome (e.g. facilitated by identical IS elements on both molecules as noted earlier), and such integrated plasmids are called episomes. When an integrated F episome attempts conjugative transfer, the duplication-transfer process eventually moves into regions of adjacent genomic DNA, which are carried along from the donor cell into the recipient. Such strains, in contrast to cells containing the unintegrated F plasmid, mediate high-frequency transfer and recombination of genomic DNA (Hfr strains). However, conjugation with Hfr donor cells does not result in complete transfer of the integrated plasmid. Thus, the recipient cell does not become Hfr and is incapable of serving as a conjugation donor. The circular nature of the bacterial genome and the relative 'map' positions of different genes were established using interrupted mating of Hfr strains.

When a non-conjugative plasmid is present in the same cell as a conjugative plasmid, they are sometimes transferred together into the recipient cell by a process known as mobilization. Conjugative transfer of plasmids with resistance genes has been an important cause of the spread of resistance to commonly used antibiotics within and between many bacterial species, since no recombination is required for expression in the recipient. Of all the mechanisms for gene transfer, this rapid and highly efficient movement of genetic information through bacterial populations is clearly of the highest clinical relevance.

THE GENOMICS OF MEDICALLY IMPORTANT BACTERIA

Bacteria have been historically identified and characterized by phenotypic methods. However, advances in molecular biology have increasingly focused attention on analysis of the bacterial genome as it represents the ultimate source of information regarding bacterial identity, potential for pathogenicity, etc.

Various targeted approaches to the detection and utilization of genomic sequence information exist

Methods such as the polymerase chain reaction (PCR) and nucleic acid probes have clearly had a pivotal role in providing sequence-based answers to clinical microbiology questions (see Ch. 37).

- *Identification and classification.* The genes encoding ribosomal RNA (16 S, 23 S and 5 S) are typically found together in an operon where their transcription is coordinated (Fig. 2.19). This rDNA operon is found at least once and often in multiple copies distributed around the chromosome, depending on the bacterial species (*Borrelia burgdorferi* has one copy; *Clostridium difficile* may have up to 12 copies). While the rDNA operon contains many conserved sequences (identical in different bacterial species), a portion of the 16 S- and 23 S-encoding regions have been found to be species specific. In between them, an 'internally transcribed spacer' (ITS) region exhibits sequence variability that may be analysed in PCR products providing utility in differentiating closely related bacterial isolates. Such information may also allow the rapid identification, classification and epidemiology of clinically important microorganisms (see Chs. 32 and 37).
- *Resistance to antimicrobial agents.* Genes specifically mediating antimicrobial resistance are well known (see Ch. 34) and may be detected by a variety of targeted genomic approaches including PCR and probes.
- *Molecular epidemiology.* While a variety of phenotypic and genotypic methods have been employed to assess interrelationships in clinical isolates (see Ch. 37), epidemiological analysis has now moved toward sequence-based approaches. In contrast to earlier methods, sequence data are highly portable (internet transfer, etc.), less ambiguous (encoded entirely in the characters A, T, G and C, corresponding to the four bases adenine, thymine, guanine and cytosine, respectively), and easily stored in databases.

Microarrays provide a more global targeted genomic analysis

DNA microarrays are a means for the 'parallel processing' of genomic information. Traditionally, molecular biology has operated by analysing one gene in one experiment. Although yielding important information, this approach is time consuming and does not afford ready access to the information (chromosomal organization and multiple-gene interaction) contained within genomic-sequence databases. Microarrays acquire information from multiple queries simultaneously posed to a genomic-sequence database (parallel processing). DNA microarrays are based on the principles of nucleic hybridization (A pairs with T; G pairs with C). While there are a number of variations on the theme, the general format is the arrangement of samples (e.g. gene sequences) in a known matrix on a solid support (nylon, glass, etc.). Using specialized robotics, individual 'spots' may be less than 200 mm in diameter, allowing a single array (often called a DNA chip) to contain thousands of spots. Different fluorescently labelled probes of known sequence may then be simultaneously applied followed by monitoring to detect whether complementary binding has occurred.

DNA microarrays have been especially useful in the identification of mutations and studies on bacterial gene expression

In a number of instances, specific point mutations are clinically important in pathogenic bacteria. Since these changes involve only one nucleotide base they are often referred to as single nucleotide polymorphisms (SNPs). Resistance to the quinolone class of antibiotics, for example, may result from a single base change within the bacterial *gyrA* gene (see Ch. 34). In the past, such mutations have been detected by PCR amplification of

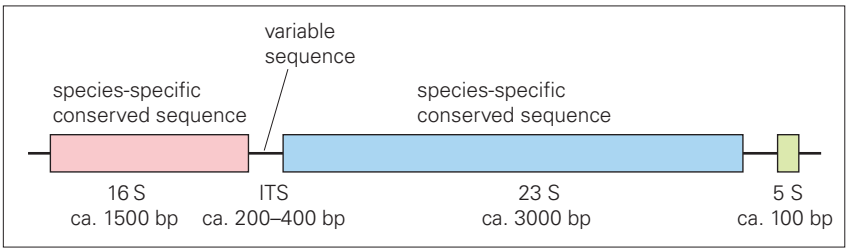

Figure 2.19 Typical arrangement of the bacterial operon encoding ribosomal RNA. Sizes of the genes for 16 S, 23 S, 5 S rRNA and the internally transcribed spacer (ITS) region are indicated in nucleotide base pairs (bp). Regions encoding sequences helpful for species identification or epidemiology are indicated.

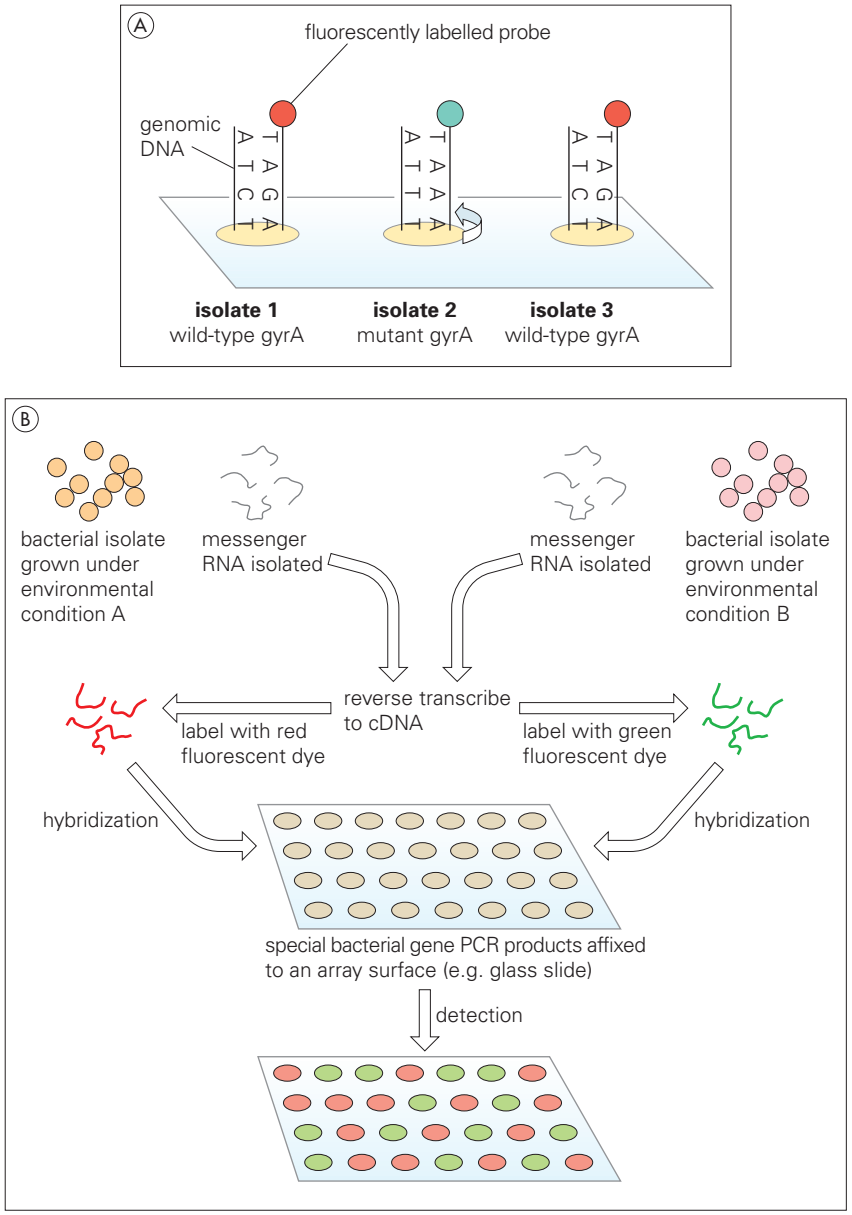

Figure 2.20 (A) Microarray detection of mutations and (B) analysis of gene expression.

the desired *gyrA* region followed by DNA sequencing and analysis. As illustrated in Fig. 2.20A, DNA microarrays allow *gyrA* amplicons from different bacterial isolates to be applied to the same chip. Two *gyrA* probes (wild type, fluorescently labelled red; mutant, fluorescently labelled green) are applied to the array under conditions so stringent that only 100% homology will result in hybridization. In this way, the presence or absence of the specific mutation may be quickly and accurately assessed in a large number of isolates simultaneously.

Studies of gene expression are extremely important to the understanding of numerous bacterial processes, including virulence. For example, analysis might involve a comparison of gene expression (transcription) in an organism under different environmental conditions (Fig. 2.20B). In such an experiment, genomics can provide data allowing sequences from every known chromosomal gene of the organism to be applied to a unique position on the chip. Messenger RNA (the result of gene expression) may be isolated from the same bacteria grown under either environmental condition A or B. Using the enzyme reverse transcriptase in a process similar to that naturally employed by retroviruses (see Ch. 3), the mRNA is copied into complementary DNA (termed cDNA). Different fluorescent dyes (red or green) are bound to the A or B cDNA, respectively, which is then allowed to hybridize to complementary sequences on the chip. Array spots with red fluorescence will indicate genes expressed in environment A. Those appearing green will correspond to genes active in environment B, while yellow spots (red+green) will indicate genes active under both conditions.

Sequence of the entire bacterial chromosome (whole genome sequencing; WGS) represents the most global approach to genomic analysis

Targeted sequence-based genomic analysis continues to be of great value in providing results rapidly and comparatively inexpensively. However, the specific nature of these assays