Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

INTERNATIONAL REVIEW OF CYTOLOGY, SUPPLEMENT 17

The Structure of Bacteria and Molecular Biology of Viruses

P. J. KRELL AND T. J. BEVERIDGE

Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

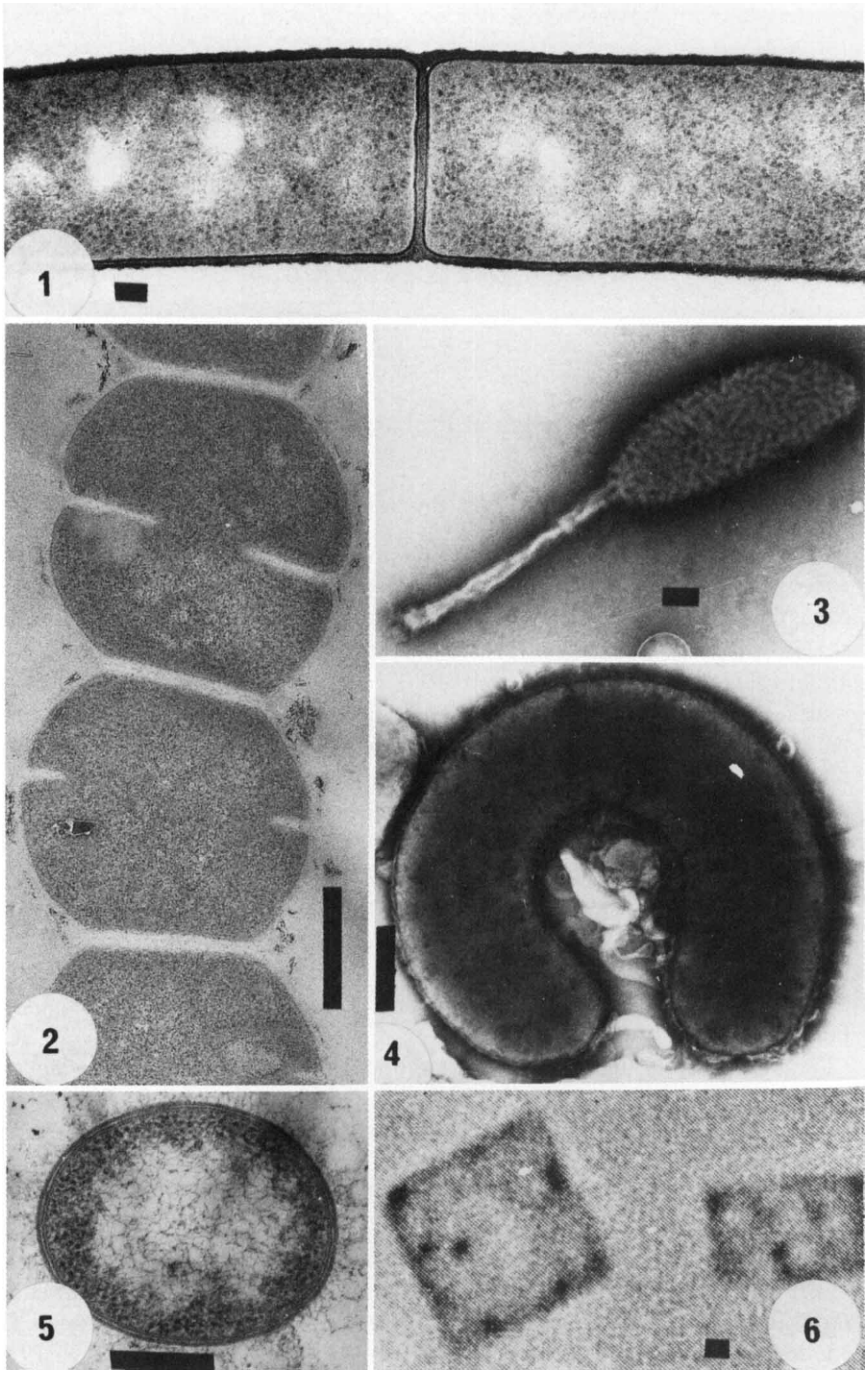
I. The Design of Prokaryotes and Viruses

A. BACTERIA

There is no doubt that the design of prokaryotes has been a tremendously successful feature of their existence. These designs have survived the test of time; the mineralized remnants of bacteria have been found in ancient cherts and shales which are approximately 3.6 billion years old (Banghoorn and Tyler, 1965; Pflug and Jaeschke-Bayer, 1979). Even today, bacteria remain one of the most successful life forms and are among the most abundant. Throughout time, the global cycling of biominerals has been primarily a microbial duty. Although microbial mass is very small when compared to total land and water mass, when the production rate of organic material is integrated over time, the total recycled mass approaches that of the Earth's mass (Abelson, 1957). Moreover, since average biomass contains metals at the 10,000 ppm level (Trudinger and Swaine, 1979) the inorganic mass recycled over time approaches the mass of the Earth's crust (Beveridge and Fyfe, 1985). Bacteria are important life forms and they have been in existence for billions of years. Like most successful man-made objects, their fortune must be due to an efficient blend between design and function.

Two completely autonomous prokaryotic groups are recognized today, the eubacteria and the archaebacteria (Fox *et al.*, 1980; Woese, 1981). Both groups have taken a common tact in their design rationale. They have remained small (usually about 1 to 2 μm in diameter) and have opted for simplicity. This is contrary to eukaryotic design which has utilized the principle of segregating function to distinct cellular organelles (e.g., mitochondria and nuclei), a trait which required greater space and has resulted in larger cells.

Unlike some eukaryotic cells, bacteria are not able to engulf particulate foodstuffs. Instead, they must rely entirely on diffusion. Since they are small, the application of low Reynolds number applies (about 10^{-4} to 10^{-5}; Purcell, 1977) and this means that their world is completely different from yours and mine.


1
2
3
4
5
6

Inertia is irrelevant, viscous forces predominate, and there is a total dependence on diffusion for survival. Accordingly, it becomes a matter of life and death that the proper balance between surface area and volume be established; it is a design problem, large cells with a low surface area to volume ratio will not survive.

As far as we know, bacteria do not possess manipulative ability to aid them grasp and internalize the nutrients that come their way. The excretion of exoenzymes may help them break up large molecular complexes into more manageable, smaller sized nutrients, but they are still under the constraints of diffusion. A simple design change can produce a significant increase in the surface area to volume ratio. For example, a typical, rod-shaped *Escherichia coli* cell has a surface of $\sim 18.5\ \mu m^2$ and a volume of $1.8\ \mu m^3$. A coccoid cell of similar volume would have a surface area of only $10.6\ \mu m^2$. Consequently, the rod has a surface area to volume ratio of 10:1, whereas the sphere has only 6:1. When it is a matter of diffusion, the rod wins every time!

Bacteria come in many shapes and forms; they can be spherical, rod-like, spiral, filamentous, comma-like, square, or ring-like (Figs. 1 to 6). One of the primary functions of these various designs is to increase the surface area to volume relationship; the greater the surface area, the greater the likelihood of encountering a diffusing substrate. Receptor proteins, transport proteins, and specialized pores must also contribute to ingestion, but it is the overall cellular design which provides a fundamental advantage. The same rationale explains the excretion of waste products. Bacteria cannot outrun their local environment; the viscous forces that predominate within this environment dictate that they must drag it around with them, so, once again, it ends up as a diffusion problem.

B. Viruses

Historically, both bacteria and viruses were discovered at the same time. In fact, all infectious agents were at one time collectively called viruses (virus = poisonous agent). After the introduction of porcelain filters, these "viruses" were subclassed as nonfilterable (i.e., bacteria) and filterable (i.e., viruses). The term filterable viruses was too wordy and as soon as the term bacteria was used to

---

FIG. 1. Thin section of a dividing *Bacillus subtilis* cell. This is representative of a "rod." (The bar in each figure denotes 100 nm and the large arrow in the freeze etchings shows the shadowing direction.)

FIG. 2. Thin section of a *Streptococcus pyogenes* "chain" of cells.

FIG. 3. Negative strain of a "stalked" bacterium *Caulobacter crescentus*.

FIG. 4. Negative stain of a "ring-shaped" bacterium, *Microcyclus flavus*.

FIG. 5. Thin section of an unidentified "coccus."

FIG. 6. Phase micrograph of a "square" bacterium. (From *J. Bacteriol.* **148,** 352–360, with permission of the author and the American Society for Microbiology.)

describe the nonfilterable, infectious agents, they were referred to simply as viruses (Hughes, 1977).

The first agent identified as a virus was tobacco mosaic virus (TMV). This initial discovery in 1882 by the Russian scientist Ivanowski was followed shortly by the 1898 discovery by Loeffler and Frosch that the foot and mouth disease of cattle was also a virus (in the same family as poliovirus). The first human disease identified as having a viral aetiology was yellow fever in 1900 by Sir Walter Reid. Several years later in 1916, Twort in England and d'Hérelle in France discovered viruses (the bacteriophage) which caused disease of bacteria (i.e., a pathogen of a pathogen!).

Except for their ability to cause disease, their macromolecular composition, and their use of the same genetic code, viruses have little in common with bacteria (or for that matter with eukaryotic cells). All viruses contain a central genome which consists of only one kind of nucleic acid (DNA or RNA) and is surrounded by a protective protein coat to form a nucleocapsid. Complex viruses have additional coats which are usually membranous. Poxviruses are the most complex and although they are almost the same size and almost as complex as a small bacterium, they differ, as do all viruses, from all cells in one fundamental characteristic: all viruses are obligate intracellular parasites. An individual virus (or virion) could survive without cells but could not replicate unless it can enter a living cell.

The structure of viruses reflects their association with the extracellular and intracellular environment. In its extracellular environment, viruses are in the form of mature virions, capable of protecting their sensitive genomes and transmitting their genetic information to new susceptible cells. In its intracellular environment, the protective coat is no longer needed and the virus structure changes. The viral genome is uncoated in the cell, allowing it to control the genetic machinery of its host. The genome then usurps cellular metabolism for the synthesis and eventual assembly of virus components (viral nucleic acid and proteins) into progeny virions.

The mature virus structure allows survival in the extracellular environment and specific association with cells; a change in this structure allows the replication of viruses. The morphology of the virion is dependent, in large part, on the structural information inherent in newly synthesized viral components (nucleic acid, proteins, membrane). These viral components can self-assemble, often without the need of a cellular scaffolding, into mature virions.

II. Bacterial Structure

A. The External Scaffolding

The external scaffolding of a bacterium is the cell wall. It is this structure which is responsible for cellular shape. Walls come in two basic varieties, gram-

positive and gram-negative, and the names refer to the manner in which eubacteria respond to a staining regimen first proposed by Christian Gram in 1884 (Gram, 1884). Since that time these bacteria have traditionally been divided into those that retain, within their cytoplasm, a crystal violet–iodide complex after ethanol washing (gram-positive) and those that do not (gram-negative). Actually, the staining response depends on the molecular fabric of the wall (Beveridge and Davies, 1983; Salton, 1963); gram-positive walls consist of a rather amorphous matrix (Fig. 7) of carbohydrate-containing material which shrinks in ethanol, thereby decreasing the wall's porosity and retaining the dye within the protoplast during the Gram stain. Gram-negative walls are mostly membranous (Fig. 8) and the ethanol extracts their lipid; this disrupts their integrity and liberates the stain from the protoplast (Beveridge and Davies, 1983). It is a simple and convenient staining regimen; Gram unwittingly, developed an assay which tells us the molecular format of the bacterial wall!

Not all bacteria fall into the easy categorization of the Gram reaction. Some varieties stain in a variable manner and these usually have atypical walls. For example, walls with a thinner amorphous layer than the gram-positive variety stain in this variable manner even though they often have a layer of protein or some other macromolecule on top (Fig. 10). It seems that the degree of gram positivity in these bacteria is somehow modulated by the age and nutritional status of each cell. Other bacteria do not possess the amorphous layer and are simply bound by a surface layer of protein (Fig. 9); this group stains gram-negative and usually belongs to the archaebacterial group.

Archaebacteria often prove to be an enigma to the Gram stain. This bacterial group rarely conforms to the eubacterial wall format; both the structure and chemistry can be different, and this is reflected in their staining response. For example, *Methanospirillum hungatei* is an archaebacterium which has a complexity of enveloping layers around the protoplast (Fig. 11); each is unique in terms of structure and chemistry (Stewart *et al.*, 1985; Beveridge *et al.*, 1985). It is a gram-negative bacterium, but the outermost layer (the sheath) is so impermeable to chemical agents that the stains may not even get into the cell. This may be the actual reason for the gram negativity and not that the crystal violet–iodide complex has been washed out.

1. *Gram-Positive Eubacterial Walls*

The amorphous matrix which comprises these walls consists primarily of one or two constituents. Peptidoglycan is a major component and is the rigid framework to which other wall polymers are attached. It consists of a repeating β(1-4)-linked dimer of *N*-acetylglucosaminyl-*N*-acetylmuramic acid which forms a linear polymer of 50 or more dimeric units. Each acetylmuramyl residue has a short peptide stem which is usually four or five amino acids long. The shorter peptides contribute to the cross-linking of neighboring peptidoglycan strands either through direct covalent bonding to similar but adjacent peptide stems or by

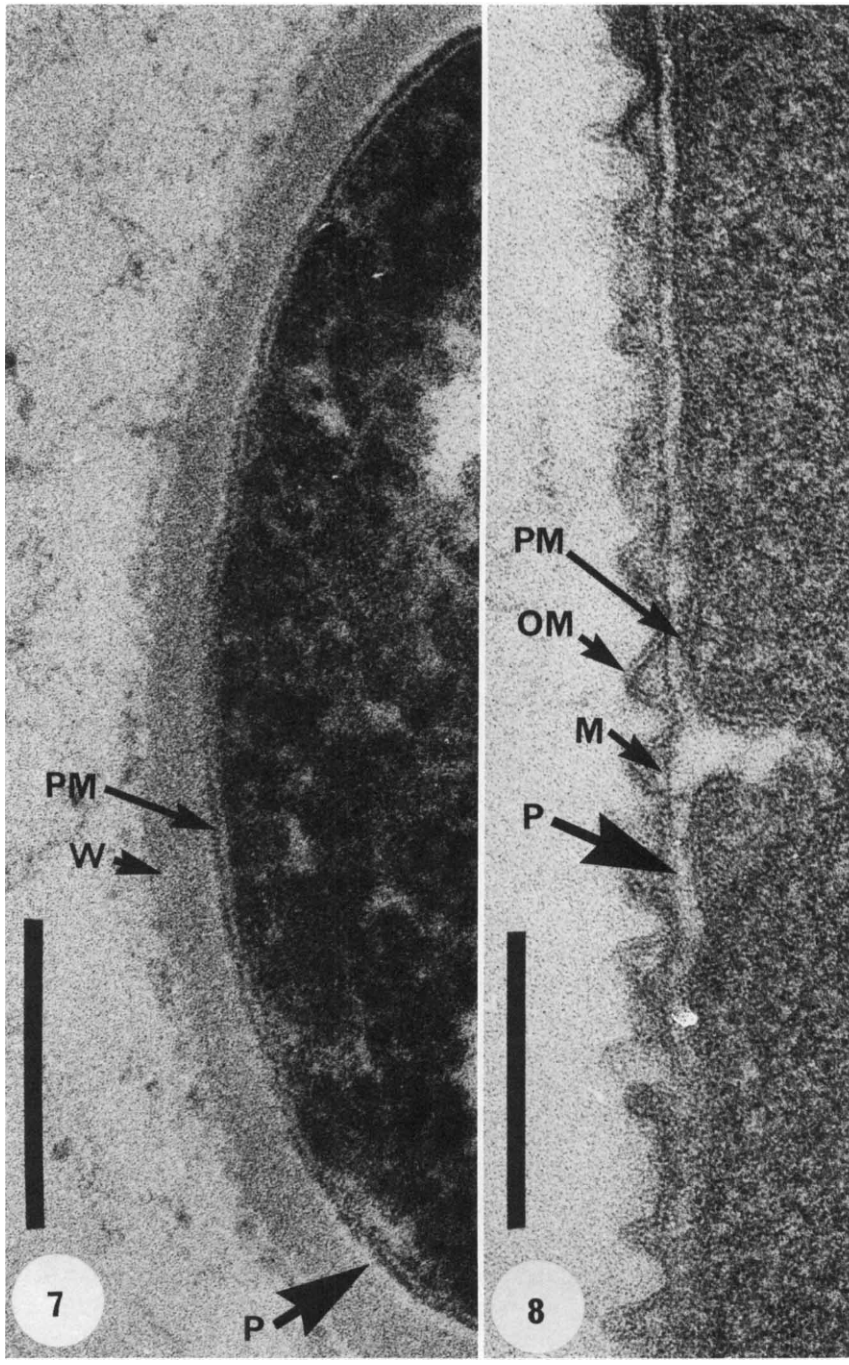


FIG. 7. Thin section of a *B. licheniformis* envelope which is representative of the gram-positive eubacterial variety.

FIG. 8. Thin section of an *Aquaspirillum serpens* envelope which is representative of the gram-negative eubacterial variety. PM, Plasma membrane; OM, outer membrane; M, murein or peptidoglycan layer; W, wall; and P, periplasmic space.

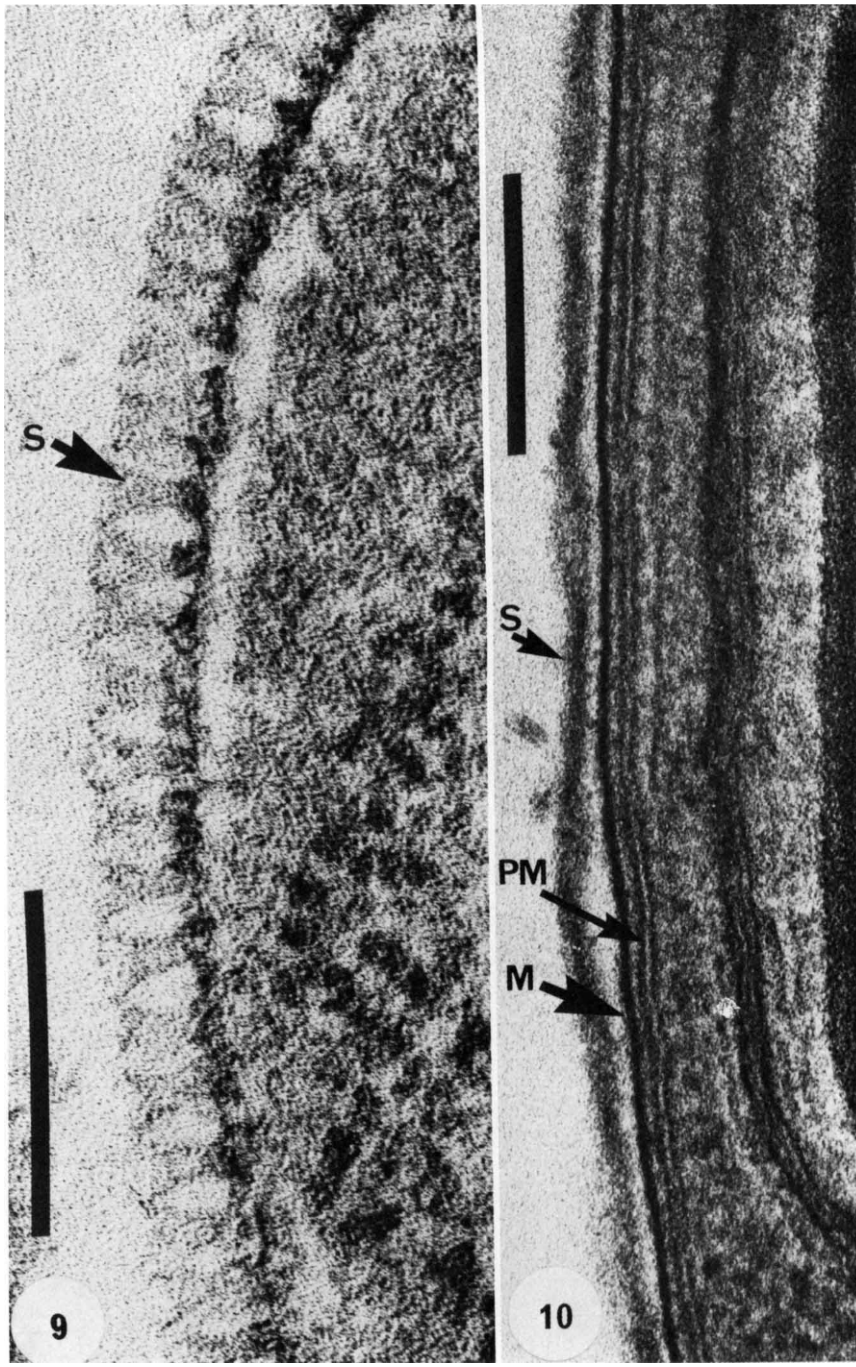


FIG. 9. Thin section of a cell wall which consist of only a surface array of globlet units. This bacterium has not yet been identified, but is suspected to be a *Methanococcus* spp.

FIG. 10. Thin section of a *B. sphaericus* envelope which is representative of a gram-variable variety. S, Surface array; M, murein layer; and PM, plasma membrane. The layers within the cytoplasm are structures of a developing spore.

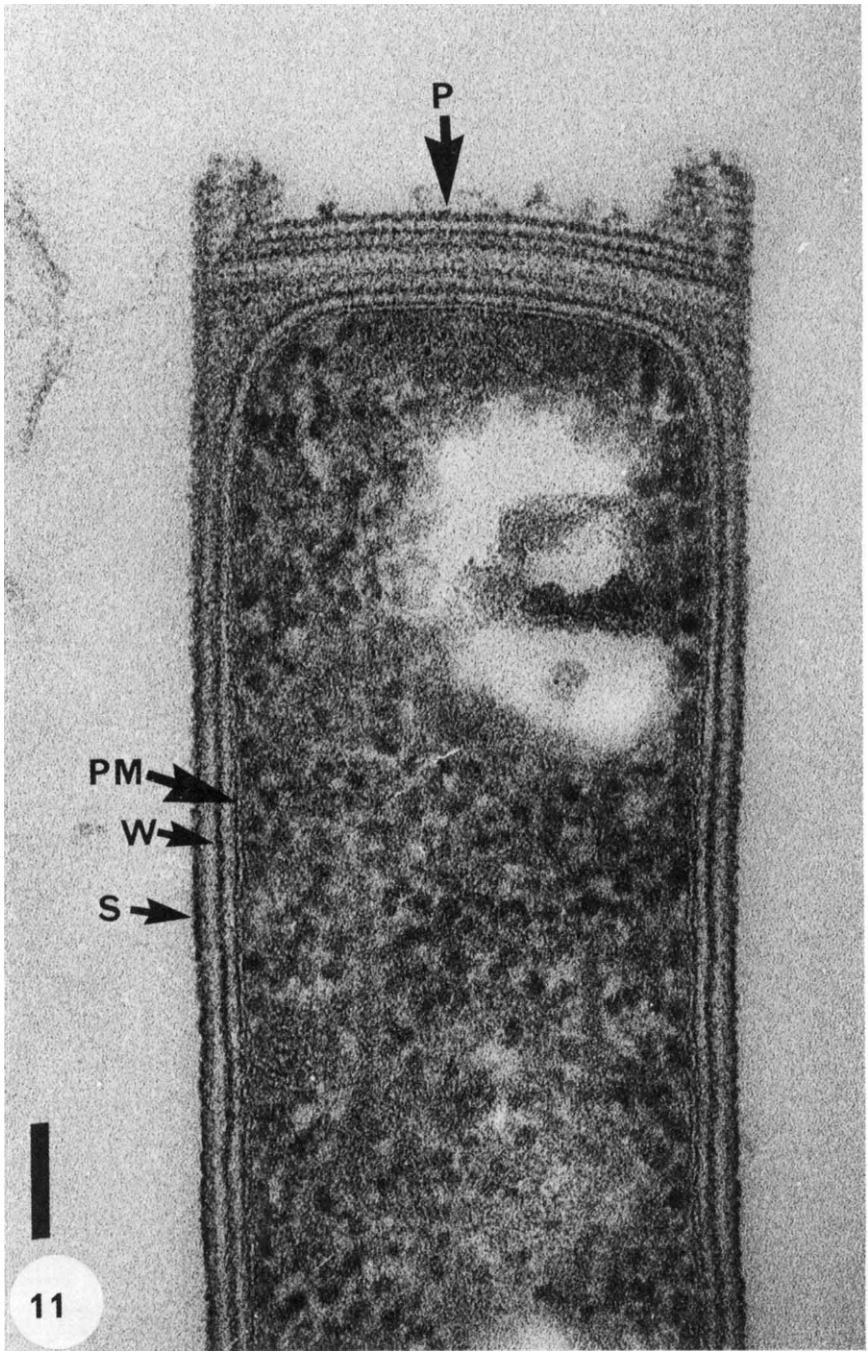


FIG. 11. Thin section of *Methanospirillum hungatei* showing the sheath (S), the wall (W), the plasma membrane (PM), and the end plug (P).

means of short, sometimes chemically dissimilar, peptide bridges between the adjacent peptide stems (Schleifer and Kandler, 1972). The number of interstrand linkages can vary between 10 and 50% of the available tetrapeptides and is a function of the bacterial species or strain. The end result of the interstrand linkage is to produce a large macromolecule, the murein sacculus, which completely surrounds the bacterial protoplast. Since each peptidoglycan strand is completely stabilized in three dimensions by interstrand linkage, the entire murein sacculus is an extremely resilient structure. Gram-positive walls can be 25 or more peptidoglycan strands thick. As expected, the thickness and the degree of cross-linkage all contribute to the degree of wall rigidity.

Other secondary polymers can be hooked into the peptidoglycan framework. These are usually teichoic acid (in *Bacillus* spp. this is a glycerol or ribitol-based polymer which is joined together by phosphodiester linkages into a flexible, linear strand) and teichuronic acid (a uronic acid-based polymer without phosphate, but possessing most of teichoic acid's physicochemical traits). Both of these polymers are covalently linked to the *N*-acetylmuramyl residue of the peptidoglycan and penetrate, we believe, throughout the entire murein framework and out above the solid wall surface into the external milieu. Other components can be found in gram-positive walls, but these are relatively minor constituents or are a specific trait of a particular bacterial group and will not be discussed (see Beveridge, 1981, for more details).

This interdigitation of the peptidoglycan and its secondary polymers produces an amorphous layer on the surface of the protoplast (Fig. 7). The very nature of the peptidoglycan cross-linkage suggests that, at least with this constituent, there should be a rather exact positioning for each macromolecular strand; the gram-positive wall should possess some degree of ordering (Hakenbeck *et al.*, 1983). This certainly is not evident in thin sections, but other physical techniques, such as X-ray diffraction, have detected lattice constants ranging from 0.44 × 0.83 nm (Formanek *et al.*, 1974) to 1.90 × 0.98 nm (Burge *et al.*, 1977). The secondary polymers, teichoic and teichuronic acids, are more flexible and not highly cross-linked; accordingly, the degree of their ordering should be determined only by the peptidoglycan scaffolding through which they penetrate.

In concert with one another, these polymers convey distinctive inherent properties to the gram-positive wall. The first is of utmost importance; the wall gives the bacterium its particular shape. It has been estimated that most gram-positive bacteria must cope with tremendous internal osmotic pressures which can approach 20 atmospheres. These pressures would be very difficult to withstand by the protoplast alone and would result in lysis. In the intact cell, the plasma membrane is pushed tightly against the wall so that the protoplast's shape mimics that formed by the wall. It therefore follows that shape and form in gram-positive bacteria are maintained by the wall and are a derivation of wall extension and growth. For gram-positive cocci and rods, it is our belief that extension, shape, and cellular growth are all intimately entwined and are dependent on one an-

other. This is called the "Surface Stress Theory" and can be explained in terms of the stresses which give soap bubbles their characteristic size and shape (Koch, 1983).

Another characteristic of gram-positive walls is that they are usually anionic. The peptidoglycan and each of the secondary polymers carry a net negative charge. The exception to this rule for peptidoglycan is when it is highly cross-linked and the constituent carboxylate groups are extensively substituted (e.g., the glutamic acid residues of the peptide stems can be amidated). This charge character means that the wall forms a cation "trap" and immobilizes counterions from the environment. The size and shape of the counterion are important for "correct" fit within the wall fabric, but metallic ions and protons are especially affected (Beveridge, 1984). This forms a type of ion exchange resin around the cell which buffers it from extremes of environmental pH or tonicity, thereby protecting the protoplast. At the same time, it is possible that essential metals are also immobilized by the wall which acts as a storehouse for their future use by the cell.

Since the constituent polymers within these walls are closely packed due to interstrand cross-linkage, the gram-positive wall provides a filtration barrier for the bacterium. Unless localized discontinuities exist within the fabric, only those molecules which will fit through the holes in the polymeric meshwork will be able to penetrate to the protoplast. Scherrer and Gerhardt (1971; Scherrer *et al.*, 1977) and Gerhardt and Scherrer (1974) have determined that the *Bacillus megaterium* wall can exclude macromolecules with a hydrodynamic radius of 9–12 nm. The outer surface meshwork of this wall is the most impermeable region and excludes polymers of greater than 1.1 nm radius. Our own work suggests that molecules about 0.3 to 0.9 nm can freely enter the *B. subtilis* wall (Beveridge and Davies, 1983; Beveridge and Jack, 1982).

2. *Gram-Negative Eubacterial Walls*

Clearly there are profound structural differences between gram-negative walls and gram-positive walls (cf. Figs. 8 and 7). Gram-negative walls consist of an external membrane (the outer membrane) which overlies a thin monolayer (the peptidoglycan which forms the murein sacculus). Each of these layers is distinctly different, in chemical terms, from each other and the underlying plasma membrane. During the last decade, there has been such a tremendous amount of research devoted toward the elucidation of these walls that this report can only give a broad overview.

The outer membrane consists of protein, phospholipid, and lipopolysaccharide (a distinctly prokaryotic macromolecule) which, at least in thin section, produces a bilayer format (Fig. 8). There is a tremendous asymmetry of the lipid constituents since almost all of the lipopolysaccharide is partitioned to the outermost leaflet, whereas the phospholipid (mostly phosphatidylethanolamine) is found in

the inner leaflet (Mühlradt and Goleck, 1985; Funahara and Nikado, 1980). Protein is distributed throughout the membrane and can be situated at the membrane surfaces (peripheral protein) or can span the hydrophobic domain (intrinsic protein). Most of the protein (at least in the outer membranes of the Enterobacteriaceae) exists as high levels of only 3 to 4 polypeptide species (DiRienzo *et al.*, 1978). One of these polypeptides, the lipoprotein, is a small α-helix consisting of 58 amino acids and possesses 3 fatty acid residues at its C-terminus. This effectively produces a molecule with an apolar end which is destined for the hydrophobic domain of the outer membrane and a polar end which, we believe, extends down through the lower leaflet. One out of every three lipoproteins in *E. coli* is covalently bonded to the peptidoglycan layer; the other two are not but are associated with each of the bound variety to make a trimer. Accordingly, these lipoprotein complexes form an actual chemical union between the outer membrane and the underlying peptidoglycan and effectively cement the two wall layers together.

Another important outer membrane polypeptide is the matrix or porin protein. In *E. coli* this protein consists of two chemical varieties whose levels are moderated by the osmolarity of the external milieu; each is a different gene product. The OmpF polypeptide is slightly larger and more basic than the OmpC polypeptide, but each has the same function within the outer membrane. They are intrinsic proteins which are closely associated with lipoprotein and lipopolysaccharide and form small, hydrophilic channels which span the bilayer. Hydrophilic molecules with molecular weights of about 600–1000 can percolate through these channels, but larger molecules are excluded; it is a sieving mechanism.

In thin section, the outer membrane presents the typical bilayered profile which we expect from a membrane (Fig. 8). The lipopolysaccharide–phospholipid asymmetry cannot be detected. Certainly it exists; it is just that both macromolecules have the capacity to bind the heavy metal stains used in electron microscopy. Our evidence suggests that it is the phosphoryl groups of both constituents which are responsible for the majority of metallic ion binding to the outer membrane (Ferris and Beveridge, 1984, 1986a,b). It is tempting to think that the bilayer format in Fig. 8 is actually a phosphoryl fingerprint of the lipopolysaccharide and phospholipid distribution.

The leaflets of the outer membrane are much more strongly bonded together than those of the plasma membrane. This is readily demonstrated by the freeze-fracturing technique; the outer membrane rarely fractures since the plasma membrane is preferred. Occasional "plateaus" of outer membrane can sometimes be seen on top of the plasma membrane in these images and have, traditionally, been attributed to "adhesion sites" between the two membranes of the envelope (Bayer and Leive, 1977). Their frequency can be increased by pretreatment of the cells with EDTA. More recent evidence suggests that the strength of this

interfacial bonding within the outer membrane is also attributable to the type and quantity of constituent inorganic cations since inappropriate cations increase the frequency of the plateaus (Ferris and Beveridge, 1986b). Metallic ions must therefore be intimately entwined with the chemical nature of intraleaflet bonding. Our experience with ion–outer membrane interaction leads us to conclude that calcium and magnesium, which are native to this membrane, so stabilize the outer membrane's hydrocarbon core that it cannot normally fracture (Ferris and Beveridge, 1985b).

The peptidoglycan layer which composes the murein sacculus of the cell is a thin electron-dense line, about 2.5 nm thick, in thin section (Fig. 8). The chemistry of this peptidoglycan exactly resembles that of the gram-positive variety, except it is almost always directly cross-linked (Schleifer and Kandler, 1972; Braun *et al.*, 1973) and secondary polymers (such as teichoic or teichuronic acids) are rarely, if ever, found (Beveridge, 1981).

The peptidoglycan layer is often closely apposed to the lower leaflet of the outer membrane. This, we suspect, is due to the chemical union which exists between certain outer membrane proteins (e.g., the lipoprotein) and the peptidoglycan; the bonding is strong and cannot easily be broken. Those gram-negatives which have, in thin section, a wavy outer membrane profile above the peptidoglycan, such as certain *Aquaspirillum* (Fig. 8). *Pseudomonas*, and *Zymomonas* spp., would (by analogy) have a more loosely bound format.

3. *Archaebacterial Walls*

There is tremendous diversity of wall structure and chemistry within the archaebacteria; there does not seem to be a simple common denominator (Kandler, 1982). *Methanosarcina, Methanobacteria,* and *Halococcus* have recognizably rigid walls, but most others do not. This is not to say that all other archaebacteria are pliable cells without distinctive shape, but only that the walls of these three, on isolation, maintained cellular shape, whereas the others do not. Each of the rigid types of walls is uniquely different in chemistry from those of gram-positive eubacteria. In fact, the methanobacterial wall contains *N*-acetyl-L-talosaminuronic acid, instead of muramic acid, in its distinctly different peptidoglycan which means it is certainly not murein; Kandler (1979) named it "pseudomurein" since it appears to perform the same function as murein. Some archaebacteria, such as *Methanospirillum* and *Methanothrix,* are bounded by a shape-forming sheath (Fig. 11) which is external to the wall and which compartmentalizes the bacteria into linear chains of cells separated from one another by complex "cell spacers." The sheath is extremely rigid and is composed of a crystalline array of proteinaceous subunits (Fig. 12) (Stewart *et al.*, 1985). This structure is so resilient to physical or chemical perturbation that we believe that intermolecular covalent bonding between the constituent subunits must exist (Beveridge *et al.*, 1985).

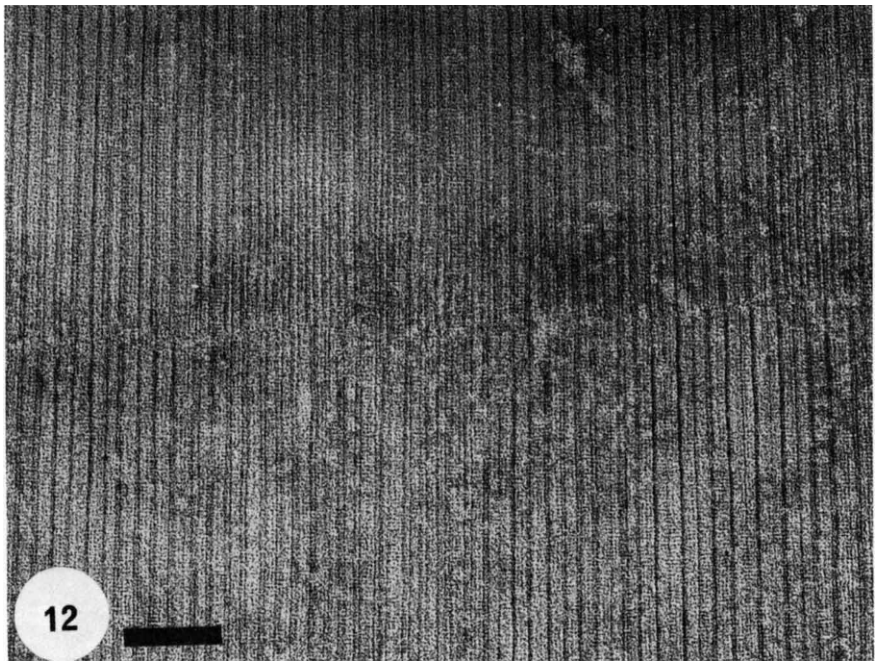


FIG. 12. Negative stain of the sheath of *Methanospirillum hungatei* showing its subunit structure (see Stewart *et al.*, 1985, for details).

Other archaebacteria, such as *Methanococcus* or *Sulfolobus*, are bounded by only a single unit layer of proteinaceous subunits (Fig. 13) (Jones *et al.*, 1977; Weiss, 1974). While these are paracrystalline arrays and may have a limited degree of shape-forming capacity, their bonding character makes the layer more flexible than that of pseudomurein. One archaebacterium, *Thermoplasma*, has no detectable wall material. Instead, it has a distinctive glycoprotein embedded in the plasma membrane (Young and Hough, 1979). The end result is a bacterium of indeterminate shape.

B. The Plasma Membrane

This membrane is also called the cytoplasmic or inner membrane and, in structural terms, is a bilayer (Figs. 7, 8, and 10). It is much more flexible than the outer membrane of gram-negative bacteria and under natural conditions is pressed tightly against the wall. This is because the plasma membrane ultimately discriminates as to what gets into and out of the cytoplasm of the cell with little regard to natural gradients or concentrations. This selectivity produces a distinctively different chemical environment within the cell than that of the external

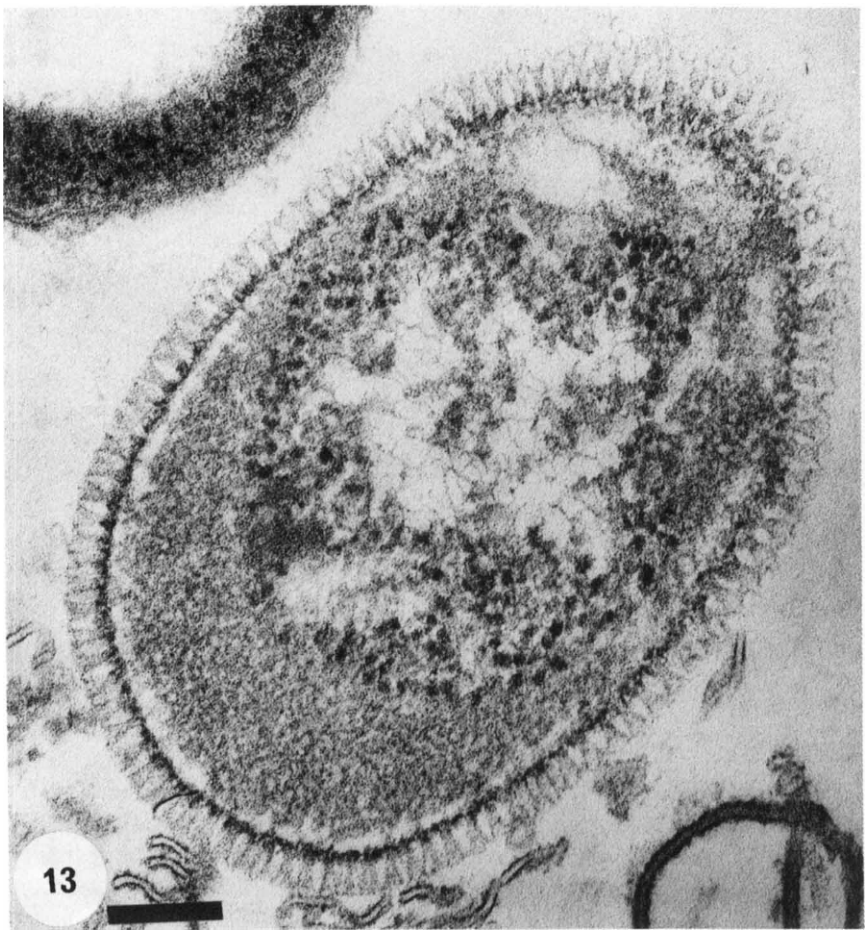


FIG. 13. Thin section of a *Methanococcus* sp. which has only a surface array for its wall.

milieu and results in a positive turgor pressure against the membrane; in gram-negatives this pressure is about 4 atmospheres, but in gram-positives it can reach as high as 20 atmospheres. This chemical discrimination by the plasma membrane also results in a proton and K^+ (versus Na^+) potential across the bilayer which is essential for an energized membrane and, in fact, the bacterium's vitality.

The plasma membrane is the major organelle of the bacterial cell since it not only confines the cytoplasm, but is also responsible for many of the essential cellular functions such as respiration, wall synthesis, ATPase activity, and nutrient transport. Most of the workload performed by the internal organelles of eukaryotic cells has been partitioned into this prokaryotic membrane.

The chemical character of the plasma membrane is much different than that of the outer membrane of gram-negative bacteria and is responsible for its innate pliability. Unlike eukaryotic membranes, sterols (e.g., cholesterol) are usually absent, but a wide range of protein and phospholipid is possible (Salton and Owen, 1976). The actual chemistry is made complicated because this membrane readily adapts to environmental stress and changes its constitution at the least provocation. In general, the predominant phospholipids are phosphatidylethanolamine, phosphatidlyglycerol, phosphatidylinositol, phosphatidylserine, and cardiolipin (White *et al.*, 1972; Ellar, 1978).

The proteinaceous component is even more diverse and depends not only on the environmental conditions, but also on cell age and taxonomy. For example, facultative anaerobic bacteria are able to grow both in the presence or absence of molecular oxygen. Under aerobic conditions, the entire respiratory chain is induced, synthesized, and located within the plasma membrane. Anaerobic conditions make most of this system functionless, and it is replaced by a system which uses a compound other than oxygen (e.g., hydrogen sulfide) as a terminal electron acceptor.

Also, bacteria do not contain an endoplasmic reticulum and a significant proportion of protein synthesis is carried out by polysomes attached to the plasma membrane. Recent reports suggest that these actively synthesizing regions are restricted to localized areas of plasma membrane and assist in membrane synthesis (Caulfield *et al.*, 1983; Marty-Mazar *et al.*, 1983). These regions also seem to be directly involved in the translocation of protein out of the protoplast and are also associated with the synthesis and reshaping of the peptidoglycan. Often when these ribosome-rich regions are negatively stained for electron microscopy, small "wrong-side-out" vesicles are seen with the ribosomes studding the outer surface (Fig. 14).

When bacteria are freeze-fractured. the predominant membrane fracture is through the hydrophobic domain of the plasma membrane. The fracture face of the plasma membrane exposes (we think) clumps of intrinsic protein that are organized into intramembraneous particles (Fig. 15). The reciprocal face of the membrane has fewer particles and contains numerous holes from which the particles have been knocked.

Although internal turgor pressure forces the plasma membrane against the wall, the adhesion is not quite as tight as one might expect. This is because there exists between the wall and the membrane a distinct region of plasm called the periplasm. A recent report suggests it may be a gel (Hobot *et al.*, 1984). In gram-negatives it extends up to the inner face of the outer membrane (Fig. 8) and in gram-positives, up to the internal face of the wall (Fig. 7). It is more difficult to see in gram-positives because their turgor pressure is usually greater.

The periplasm contains a variety of enzymes which are used to hydrolyze or process material which is coming into or going out of the bacterial protoplast. For example, oligosaccharides can be broken down into more nutritionally ac-

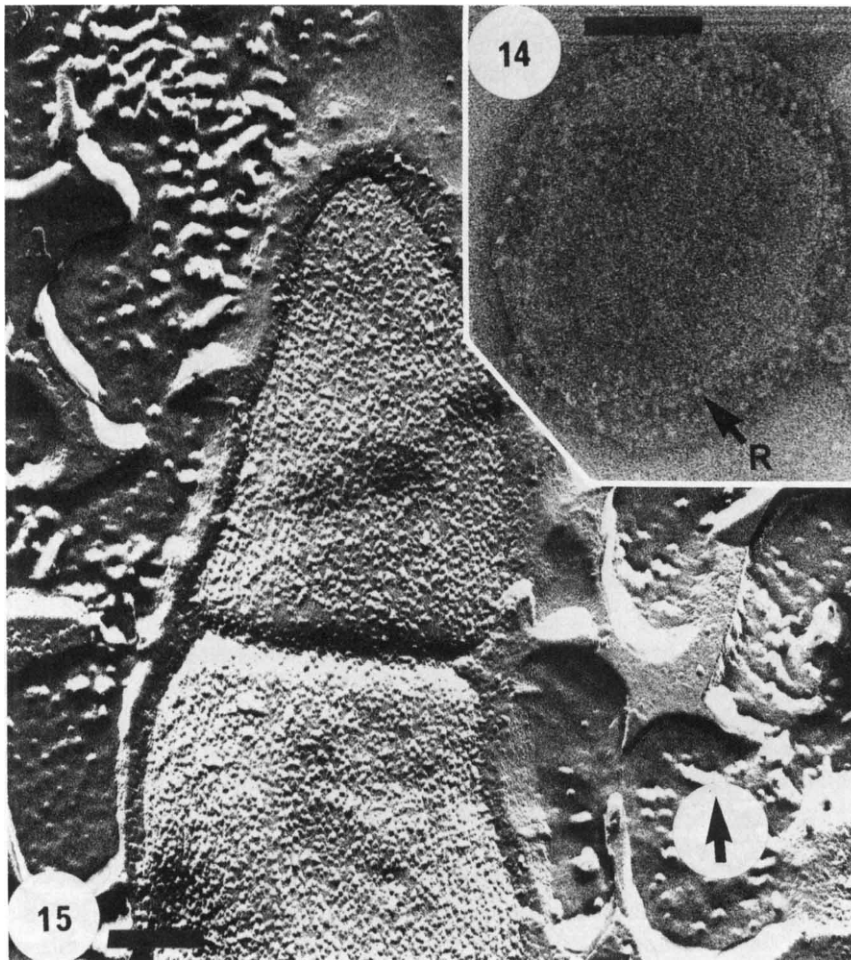


FIG. 14. Negative stain of an inverted (or "wrong-side-out") plasma membrane vesicle which is studded with ribosomes. One ribosome (R) is pointed out.

FIG. 15. A freeze-etching of *B. licheniformis* in which the plasma membrane has been cleaved down its middle to reveal proteinaceous particles.

ceptable di- and monosaccharides (e.g., glucose or maltose), or cell wall precursors can be subtly "matured" before insertion into the wall fabric. ["Adhesion sites" between plasma membrane and wall are also important for the transport of these precursors (Bayer, 1968, 1979; Beveridge, 1981).] In addition, unique proteins which are used to selectively bind nutrients (such as maltose or histidine) for eventual transport into the protoplast can be found in the periplasmic space (Lo, 1979).

Functionally, we have no clearcut way to define the borders of the periplasm. Many of the enzymes and binding proteins which we associate with it may actually be attached to the inner face of the wall or the outer face of the plasma membrane. By the periplasm's very nature, much of the material within its boundaries is in a transient state; although this material is being processed, it is just passing through.

C. Structures Associated with the Wall

1. *Capsules, Slime Layers, and Surface Arrays*

When we sample bacteria directly from their natural growth environment it is very rare that they do not have additional layers above their cell walls. Often, when these bacteria are taken back to the laboratory and subcultured several times on nutritionally complex media, these additional layers are lost. It is our belief that by taking them away from the "slings and arrows" of their native environment, we are inadvertently releasing them from the external stress which induced, or selected for, the production of this material. This is not unreasonable, since the bacterium's encapsulation would represent a major synthesis and energy drain which could be better redirected toward more beneficial routes by the bacterium within the sheltered confines of a laboratory environment.

One of the more frequently encountered additional layers on bacteria is a capsule. This lies immediately above the wall and can be found on either gram-positive or gram-negative varieties. It usually consists of a carbohydrate matrix, although, in rare instances, it can be entirely proteinaceous (e.g., the capsules of *B. anthracis* and *B. licheniformis* ATCC 9945A; Gardner and Troy, 1979).

This structure is extremely difficult to preserve for electron microscopy unless specialized procedures are used. It is extremely hydrated (up to 90% of its weight can be water) and, therefore, collapses on dehydration (a prerequisite for electron microscopy). In addition, capsules are thixotropic and, we believe, are continually alternating between a gel and liquid state. They consist of either hetero- or homopolymeric substances which are arranged in linear or branching formats; they are almost always highly acidic (Beveridge, 1981). This means that they are analogous to industrial anionic ion exchange resins and, therefore, interact strongly with cations in the environment. Accordingly, in nature it would be unusual for them to be free of metallic counterions. In fact, we make use of this trait for their preservation for electron microscopy by subjecting the bacteria to highly cationic, electron-dense stains (such as ruthenium red or alcian blue, Fig. 16) during the fixation process. Presumably, these stains replace native cations and effectively cement the fibers of the capsule together by electrostatic salt-bridging. An even better stabilization is mediated through the use of specific antibody to cross-link the capsular constituents together in the native extended format (Bayer and Thurow, 1977; Mackie *et al.*, 1979).

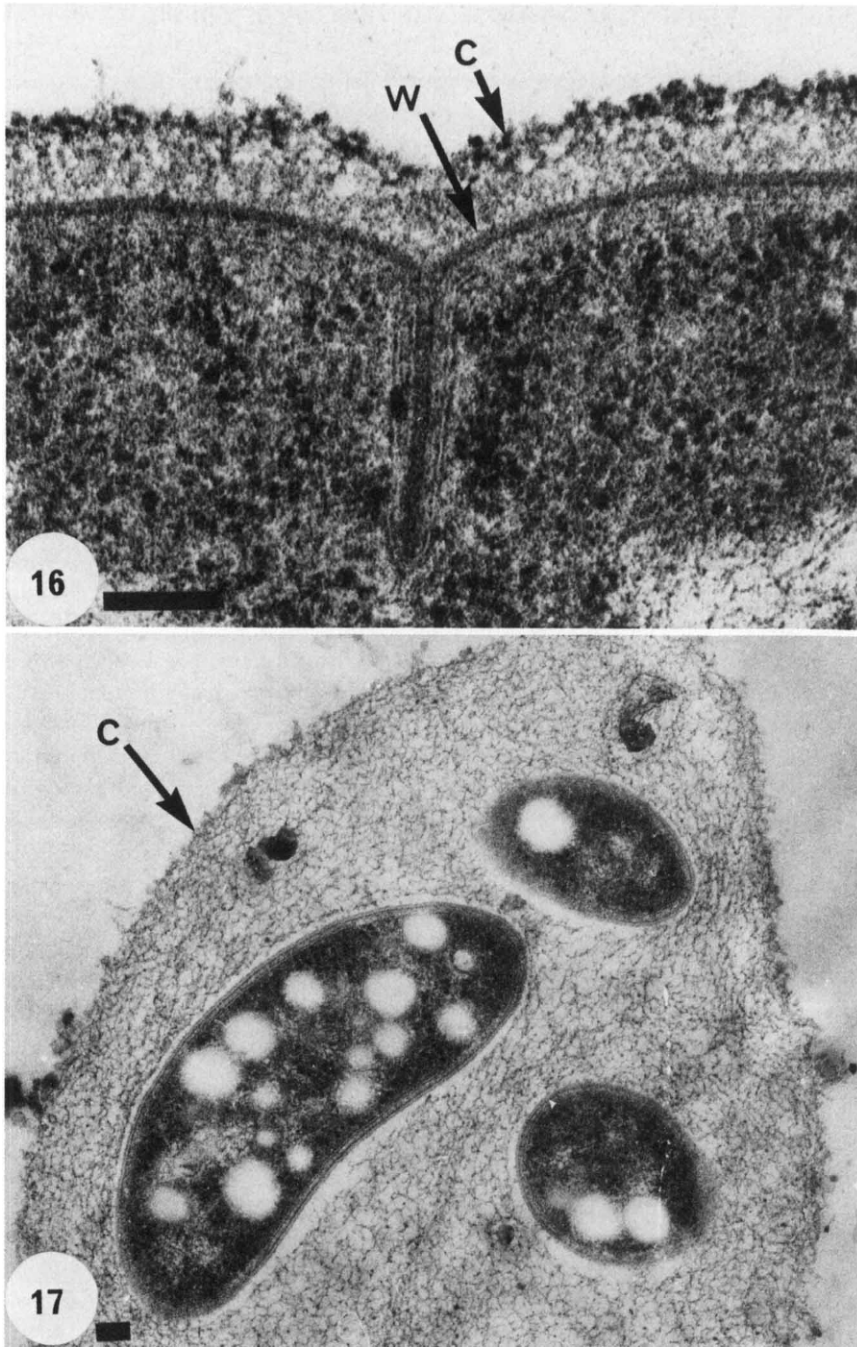


FIG. 16. Thin section of a ruthenium red stabilized capsule (C) which resides above the wall (W) of this unidentified gram-positive coccus from the bovine rumen.

FIG. 17. Thin section of part of a *Selenomonas* microcolony which is enshrouded in capsular material (C).

The difficulty in preserving capsules makes it awkward to estimate a typical size or shape for this structure. Some of those which have been stabilized by specific antibodies extend several micrometers from the bacterial surface (Bayer and Thurow, 1977). Other capsules are more compact and extend only nanometers above the cell, whereas others reach to neighboring cells and effectively join the cells to one another. This phenomenon can extend to large numbers of cells to produce an embedding matrix which holds microcolonies together (Fig. 17). There are several advantages for the bacteria to be enrobed in this amorphous matrix. Not only does it form an effective barrier against antagonistic molecules and abrasive forces in the environment, it also anneals the entire microcolony, as a consortium, to a solid interface. In a medical sense, capsules protect pathogenic bacteria from antibody and other serum components, as well as restricting phagocytosis by leukocytes (Costerton *et al.*, 1981b).

The differentiation between capsules and slime layers is vague. We have previously defined a slime layer to be a loose network of unordered gel or fibrils which extends from the bacterium's surface, and a capsule to be of a more compact nature (Beveridge, 1981). Slime layers are actually only very extensive capsules. Both are compositionally similar, but slime layers are so prodigiously manufactured and extend so far from the cell surface, that a major proportion sloughs-off and becomes free in the external milieu. This often increases the viscosity of the external fluid.

Both capsules and slime layers are considered to be distinct forms of the bacterial glycocalyx and are much more extensively covered by Costerton *et al.* (1981a,b) and Troy (1979).

Another type of layer which can occur above the native wall is the RS- or S-layer (Beveridge, 1981; Sleytr, 1978; Sleytr and Messner, 1983). These layers are composed of proteins or glycoproteins having molecular weights ranging from 40,000 to 200,000, and are remarkable in that they are thermodynamically derived, self-assemblies. All of the information which is required for assembly is carried within the proteinaceous subunits themselves and interaction between the subunits is so energetically favorable that they are driven to form an energetically closed layer which entirely surrounds the bacterial cell.

So far, four surface array formats have been found: square, tetragonal, hexagonal, and linear packings of subunits (Beveridge, 1981; Sleytr, 1978; Sleytr and Messner, 1983). Their paracrystalline regularity has been of great benefit to those of us in bacterial ultrastructure since this means they are amenable to optical or electron diffraction and computer-based Fourier enhancement of image (Figs. 18 and 19). Several of these arrays have now been resolved to 1–2 nm resolution (Sleytr and Messner, 1983).

These arrays can be found on both archaebacteria and eubacteria and on both gram-negative and gram-positive types. They can be found as multiple surface array layers (Beveridge and Murray, 1975, 1976; Stewart and Murray, 1982), or

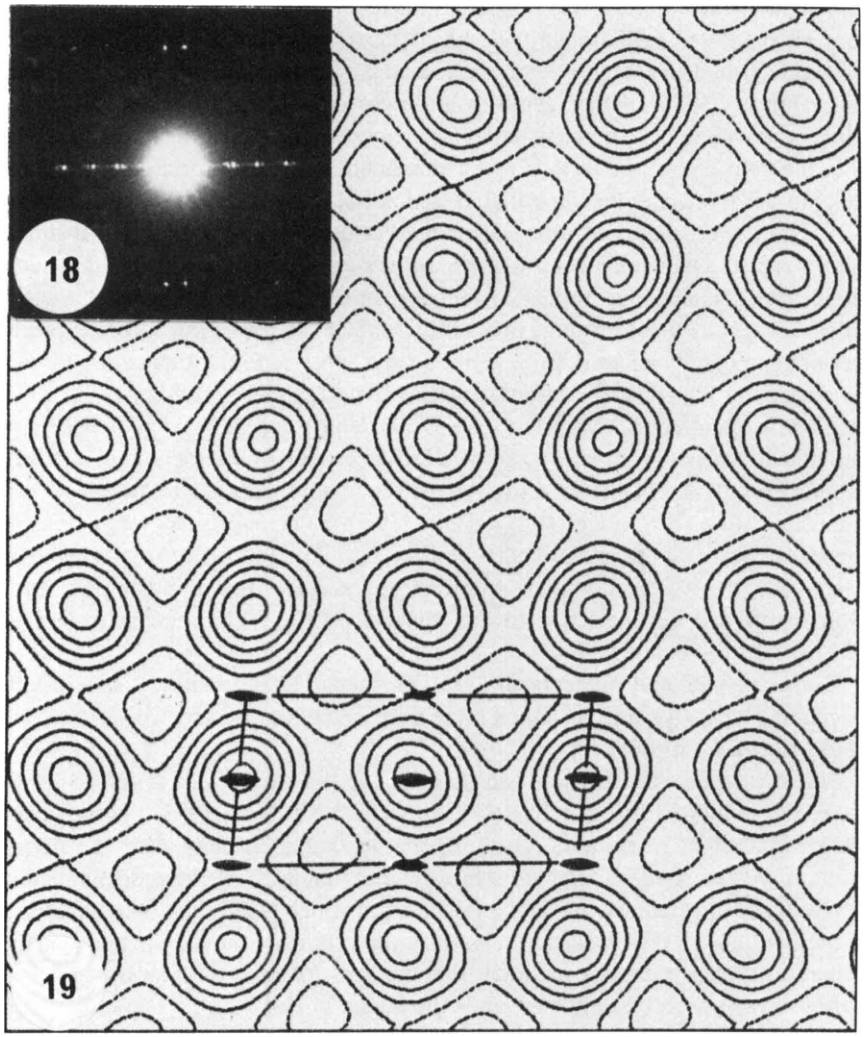


FIG. 18. Electron diffraction pattern of a *Methanospirillum hungatei* sheath similar to that seen in Fig. 12 which shows a 2.85 nm repeat.

FIG. 19. A contour plot of the lattice on the *M. hungatei* sheath. The p2 unit cell (which is outlined) is 5.70 × 2.85 nm and $\gamma = 87$. The longitudinal axis of the sheath is vertical to the page (Stewart *et al.*, 1984).

in combination with capsular material. Many of them have a reliance on Ca^{2+} or Mg^{2+} for their correct assembly and adhesion to the underlying wall (Koval and Murray, 1984).

Exact functions have been difficult to attribute to these layers since their existence in the native configuration can be determined only by electron micros-

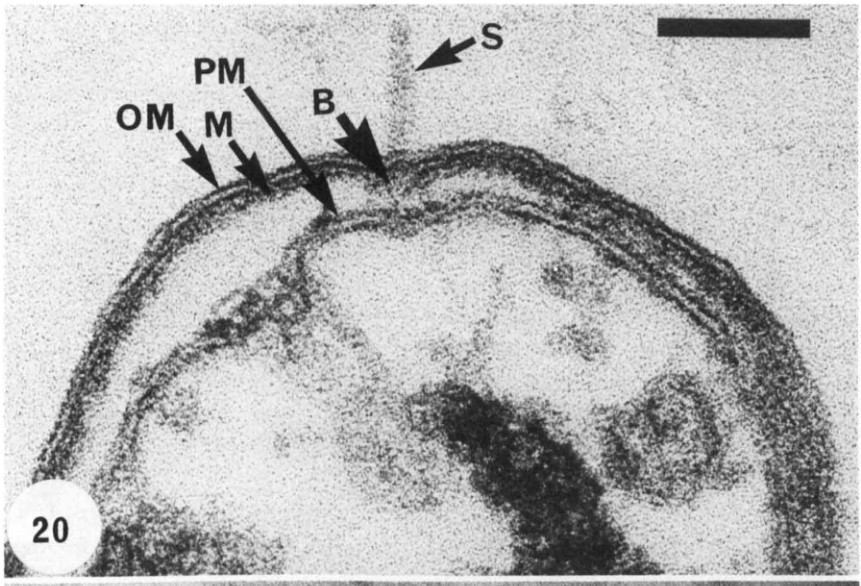
copy. The available evidence suggests that they have a multiplicity of function which ranges from providing a protective armor, to acting as a filtration barrier, to contributing to the maintenance of cell shape. Certainly, one species of *Aquaspirillum* is protected from predaceous *Bdellovibrio* attack by a surface array (Buckmire, 1971), and some archaebacteria have only these layers to define their shape (e.g., *Sulfolobus,* Weiss, 1974). The holes that permeate the arrays are large enough to allow nutrients to pass through, but would exclude foreign wall-degrading enzymes (Stewart and Beveridge, 1980). Possibly, the most exciting function has recently been attribted to the protein A layer of *Aeromonus salmonicida;* it has been implicated as being a virulence factor for this bacterium (Kay *et al.,* 1981). Loss of this protein array brings about a concomitant loss of virulence (Ishiguro *et al.,* 1981).

2. *Flagella*

Flagella are among the most complex organelles associated with the bacterial surface. They can be located at the polar end (or ends) or peritrichously arranged about the cell periphery. Their subunit arrangement was first described by electron microscopy (Kerridge *et al.,* 1962); they consist of a long spiral shaft about 10 to 20 μm long which is attached via a hook region to a complex organelle called a basal body (Fig. 20). The shaft consists of a helical arrangement of small protein "flagellin" subunits which assemble at the distal end of the shaft to produce a hollow cylinder, approximately 20 nm wide, with a distinct spiral wavelength. The shaft has a limited degree of flexibility and, when rotated about its central, long axis, acts as a propellor and pushes against the aqueous milieu.

The basal body is located at the proximal end of the shaft and consists of a series of rings attached to a central narrow rod. In gram-positive bacteria the basal body has two rings which are embedded in the plasma membrane; the hook lies above the bacterial surface and the narrow central rod traverses the wall. The gram-negative basal body has a total of four rings which are embedded into the bacterial envelope. To confuse the issue even more, several gram-negatives have a series of concentric membrane rings associated with the basal body which lie between the outer membrane and the peptidoglycan layer (Ferris *et al.,* 1984).

The flagellar basal complex is thought to act like a miniature electric motor and generates torque for rotation of the flagella shaft (Doetsch and Sjoblad, 1980). In *E. coli,* when the shaft is rotated counterclockwise, the bacterium "swims" in a straight line, when it is rotated clockwise, the bacterium "tumbles" (Berg, 1974). These observations have helped explain the tactic response of a number of different types of bacteria to stimuli in their environment. For example, *E. coli* is capable of swimming up a nutritional gradient; if a soluble fermentable carbohydrate is placed at one end of a light microscope slide and enough time is allowed to establish a chemical gradient by difusion, cells placed at the opposite end will move up the gradient by controlling their "tumbles" and "swims." For this type of response there must be a sensing/responding center to



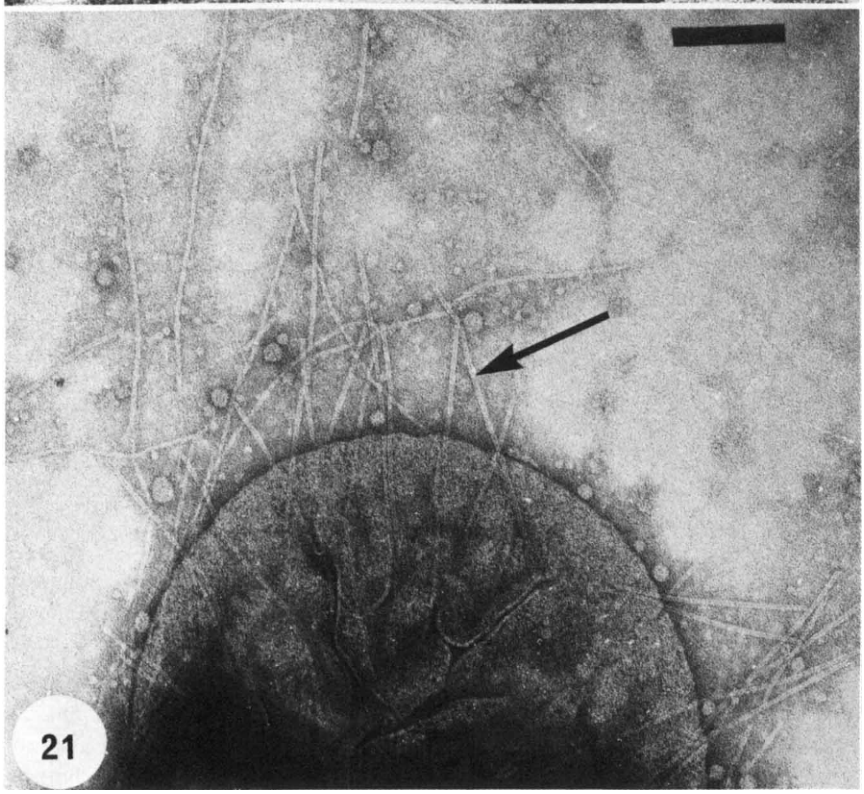


FIG. 20. Thin section of a polar end of *Pseudomonas aeruginosa* which shows the flagellar basal body (B) and the flagellar shaft (S). The basal body penetrates through the outer membrane (OM) and the murein or peptidoglycan layer (M), and inserts into the plasma membrane (PM).

FIG. 21. Negative stain of the pole of an *Escherichia coli* cell with type 1 pili studding its surface. An arrow points to a pilus.

control and coordinate the rotation of flagella. Indeed there is, and it is one of the most primitive sensory systems known; the entire phenomenon is called chemotaxis.

There are also other types of tactic responses detected in bacteria. Photosynthetic bacteria such as *Rhodospirillum* are able to perceive and react to light (phototaxis), aerobic bacteria are attracted to oxygen (positive aerotaxis), whereas anaerobic bacteria are repelled (negative aerotaxis), and magnetotactic bacteria are able to align themselves to and travel along magnetic fields. Toxic substances in the environment will repel some bacteria (negative chemotaxis).

A select group of bacteria is able to exhibit gliding motility over the surface of a solid interface. These bacteria do not possess flagella (or any other locomotory apparatus that we are aware of) and their actual mechanism of movement is not well understood, although they all appear to leave behind a trail of slime. The most recent explanation for this gliding movement is that the bacteria are able to excrete a surfactant from one of their poles which produces a driving force attributable to a localized change in the bacterium's surface tension (Dworkin *et al.*, 1983). The end result is that the bacterium is forced away from the surfactant and moves.

3. *Pili*

Pili are also referred to as fimbriae and are thin filamentous appendages which can stud the surface of many varieties of bacteria (Ottow, 1975). They were first discovered on *E. coli* (Brinton, 1965), but are now known (to name a few) to be a common feature of *Pseudomonas, Neisseria, Salmonella, Rhizobium, Caulobacter, Vibrio, Actinomyces,* and *Bacillus* (Fuerst and Hayward, 1969; Kumazawa and Yanagawa, 1972; Lotz and Pfister, 1975; Lagenaur and Agabian, 1977; Masuda *et al.*, 1981; DesRosier and Cano-Lara, 1981).

Pili are not as structurally complex as flagella; they are thinner (usually 3–10 nm in diameter), have no apparent wavelength, and do not possess a detectable basal complex (Fig. 21). Negatively stained pili are usually straight, but some types have a certain degree of flexibility (e.g., those of Providencia, Old and Scott, 1981). There are a wide variety of morphological types and their number can range from 1 to 300 (or more) per cell. In general, these different types can be placed in one of two categories: a rather large heterogeneous group with adhesive properties, and another smaller homogeneous group which is essential for conjugation [conjugation is a "sexual" process of gene transfer through an F-pilus from a (male) donor bacterium to a non-pilus containing (female) recipient]. This latter group of pili is commonly referred to as "F" or "sex" pili, whereas the adhesive variety are called fimbriae or "adhesins."

Sex pili are sometimes specified by conjugative plasmids (extrachromosomal DNA) of the F, I, or P types (Willetts, 1975). The pili of male cells recognize and adhere to specific receptors on the surface of female recipients. Only 1 or 2 sex pili are found per donor cell and they can be up to 20 μm in length. They

consist of a cylindrical assembly of "pilin" which, in *E. coli,* is an 11,400-Da polypeptide. The core of the pilus is empty and is large enough for the passage of donor DNA. At least 13 genes must function before F-pilin can be assembled at the cell surface; during this process an F-pilin pool can be found associated with the plasma membrane (Moore *et al.,* 1981).

The adhesive variety of fimbriae are more commonly found than F-pili and are usually not as long or as wide. Sometimes, these fimbriae also result in a particular type of motion referred to as "twitching" (e.g., the PSA fimbriae of *Pseudomonas aeruginosa,* Bradley, 1980). It is debatable whether or not this type of motility is actually a conscious form of lateral movement for the bacterium.

Their primary function is to act as adhesive agents and the binding can be either specific or nonspecific. For example, it is not unusual in nature to find a variety of bacteria stuck to the substratum by these fimbriae. Presumably, the attraction to the substratum is electrostatic or through hydrophilic/hydrophobic interaction. On the other hand, the *E. coli* K88 adhesin specifically attaches to the intestinal epithelium of pigs and this is an important factor in porcine intestinal infections by enterotoxigenic strains (Jones and Rutter, 1972). Also, the urogenital pathogen, *Neisseria gonorrhoeae,* owes much of its virulence to its ability for adherence to the epithelium of the urogenital tract.

Not only do fimbriae interact with inanimate objects and host tissues, groups of fimbriated bacteria sometimes entwine their fimbriae to form thick aggregations of cells (Ottow, 1975). This usually happens during the late stages of growth and is a method of suspending the bacterial mass at the air–liquid interface of the growth vessel. This ensures that the culture receives the highest possible concentration of dissolved oxygen for continuation of growth.

D. Internal Structures

1. *Structures Associated with the Plasma Membrane*

a. *Mesosomes.* The term "mesosomes" was coined by FitzJames in 1960, and refers to intracytoplasmic extensions which are continuous with the plasma membrane and which are commonly found in thin sections of bacteria. They are frequently encountered at division sites (Fig. 22) and seem to be associated with the bacterial chromosome. Since their first detection, there has been controversy as to their reality. More recent observations suggest that mesosomes are actually an artifact of the chemical fixation process which is preliminary to plastic embedding and thin sectioning (Higgins *et al.,* 1981). They are rarely seen in freeze-etchings of rapidly frozen cells, but can be induced by prefixation before the freezing process. It is possible that chemical fixatives condense the bacterial chromosome and, because the chromosome is attached to the plasma membrane at several sites, this pulls the membrane into the cytoplasm at the attachment sites.

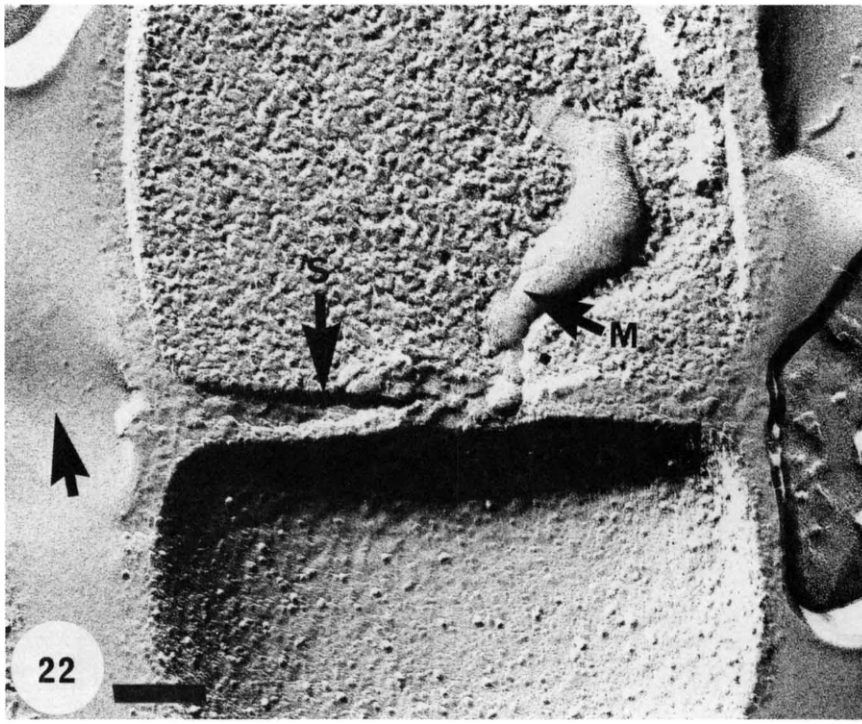


FIG. 22. Freeze etching of a dividing *Bacillus anthracis* cell showing a septum (S) and a mesosome (M) which is derived and attached to the plasma membrane. Although this cell has not been fixed, chemical fixation increases the frequency of mesosomes.

b. *Other Intracytoplasmic Membranes.* There are a variety of other internal membranes in bacteria. They all are derivatives of the plasma membrane. These internal membrane systems are a function of particular metabolic traits of characteristic bacteria. For example, photosynthetic bacteria are unique due to their photosynthetic apparatus which is contained in intracytoplasmic membrane systems. The Rhodospirillaceae (purple, nonsulfur bacteria), Chromatiaceae (purple, sulfur bacteria), Chlorobiaceae (green and brown bacteria), Chloroflexaceae (filamentous gliding green bacteria), and Cyanobacteriaceae (blue-green bacteria) all have these membranes. Also, internal membrane systems can be found in nitrifying bacteria, methane-oxidizing bacteria, and, possibly, methanogens. As with the photosynthetic bacteria, these membrane systems also possess the enzymatic apparatus which makes each group metabolically unique. It is as if the normal complement of plasma membrane per cell does not possess the surface area required for the additional burden of extra enzymatic equipment; the added area is obtained by the infolding of the plasma membrane and the eventual partitioning into that membrane of the distinct metabolic system.

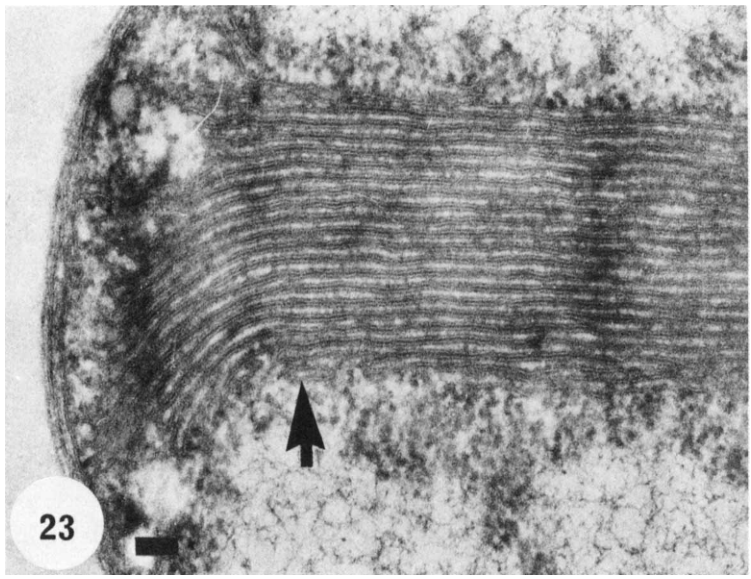


FIG. 23. Thin section of a *Nitrosococcus* (*Nitrosocystis*) *oceanus* cell, which is a nitrifying bacterium, showing the internal stacked membrane system (arrow). (From Murray and Watson, 1965, *J. Bacteriol.* **89,** 1594–1609, with permission of the authors and the American Society for Microbiology.)

An in-depth treatment of the intracytoplasmic membranes of bacteria is beyond the scope of this review and the reader is referred to Beveridge (1985).

These membranes appear to arise by an infolding of the plasma membrane and, once formed, remain as its continuation. It is not unusual for a major proportion of the cytoplasm to be occupied by these membranes (Fig. 23) and a variety of structural forms are available. For example, photosynthetic membranes can range from simple intrusions (e.g., those in *Rhodopseudomonas spheroides*), to tubular stacks (e.g., those in *Thiocapsa pfennigii*), to extremely ordered lamellar stacks (e.g., those in *R. palustris*) (Pfennig, 1977; Varga and Staehelin, 1983). Those photosynthetic membranes which have been isolated and purified from select species clearly demonstrate that the photosynthetic apparatus is concentrated within these internal membranes; they are analogous to the thylakoid stacks of eukaryotic cells.

Those photosynthetic membranes that are most closely related to higher cells are contained by the cyanobacteria (blue-green algae) which constitute the largest, most diverse, widely distributed assemblage of photosynthetic bacteria. Unlike the green or purple bacteria, their photosynthesis is oxygenic and, appropriately, their chlorophyll is identical to higher plants (Stanier and Cohen-Bazire, 1977). Their light-harvesting pigments are located in separate particles (phy-

cobilisomes) which are arranged in regular rows along the outer faces of the photosynthetic membranes. These membranes are arranged as flattened sacs aligned to the cell periphery under the plasma membrane.

2. *Cell Inclusions*

Two types of intracytoplasmic particles can be found within bacteria, those which are enclosed by a membrane and those which are not. Gas vacuoles, polyhydroxybutyrate (PHB) bodies, sulfur granules, carboxysomes, and magnetosomes are examples of the former, whereas polyphosphate and glycogen granules have no membrane and are free within the cytoplasm.

Polyhydroxybutyrate bodies are among the most common internal granule and are unique to bacteria. They consist of a mixed β-hydroxy fatty acid polymer with chain lengths generally 4–8 carbons long. Physical studies have indicated that the bodies are in a crystalline state, that calcium is the preferred salt form, and that the polymer is stabilized by carbonyl/methyl interaction to form a right-hand helix. Polyhydroxybutyrate is actually a carbon storage product for the bacterium and, sometimes, the bodies can fill a major proportion of the internal cell volume. Typically, a body is spherical and has a diameter of 0.1 to 0.5 μm (Fig. 24).

Because of their lipoid nature, polyhydroxybutyrate bodies are extremely difficult to retain in thin sections. They are hydrophobic and there are very few available bonds for fixatives to cross-link. Consequently, the organic solvents used for dehydration before plastic embedding extract most of their substance. They are better seen in freeze-etchings since they are physically fixed *in situ* by the freezing process. If the fracture plane traverses a body, enough energy is imparted to the polymeric network to produce a localized melt. The ground substance of the particle is therefore stretched out, like toffee, into an atypical extension (Fig. 24). More infrequently, the entire ground substance will be popped out during the fracture and the smooth surface of a nonunit membrane (approximately 3–4 nm thick) will be exposed.

A less frequently encountered internal particle is the gas vacuole. Although these occur in a diverse spectrum of planktonic bacteria, which includes both eubacteria and archaebacteria, they are restricted to those that require floatation devices; gas vacuoles contribute to bacterial buoyancy. They are remarkably ordered hollow structures, are oblate spheroids, and are 65–115 nm in diameter and 200–1000 nm long (Armstrong and Walsby, 1981). Their shape is tailored by their 2.8–3.5 nm proteinaceous subunits which self-assemble into a 2 nm monolayer. The end result is a very resilient structure which is capable of withstanding internal pressures of up to 50–100 atmospheres (Hemmingsen and Hemmingsen, 1980). Since the vacuoles trap gas, buoyancy regulation is the most obvious function, but they may also regulate the cell's surface to volume ratio or, even, shield the cell from too much light (Walsby, 1972).

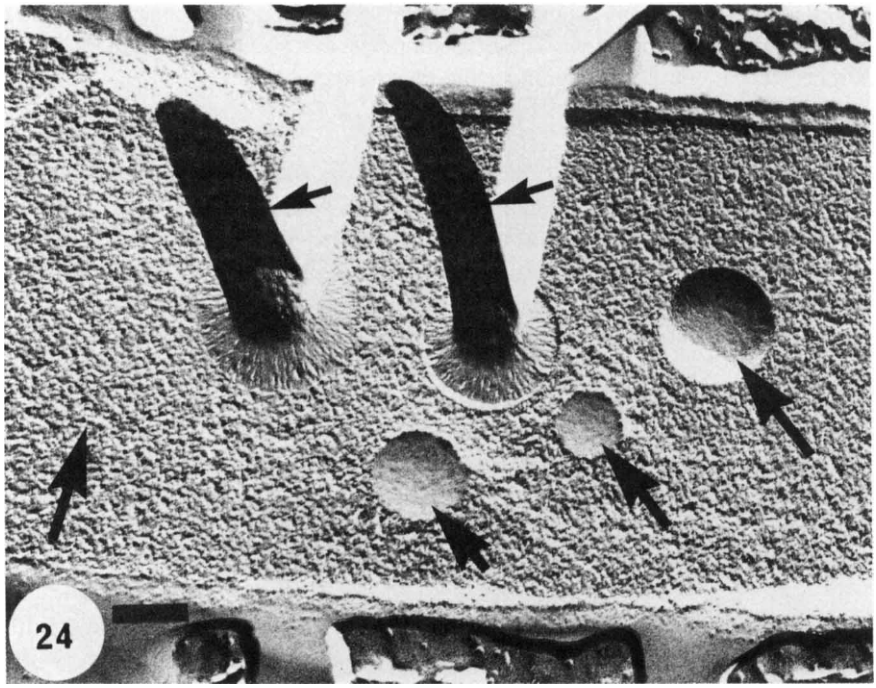


FIG. 24. Freeze etching of a *B. anthracis* cell showing polyhydroxybutyrate granules. Two granules have fractured through their middles and this has produced a localized melt in the polyhydroxybutyrate resulting in a "pulled toffee" appearance (small arrows). In three other granules, the polyhydroxybutyrate has been entirely knocked out leaving a smooth membrane behind (large arrows). The arrow at lower left denotes shadow direction.

Carboxysomes, or polyhedral bodies, are particles which act as a reservoir for an enzyme, ribulose-1,5-biphosphate carboxylase-oxygenase, which is essential for some bacteria's net assimilation of inorganic carbon. They have been seen in the cytoplasm of cyanobacteria, nitrifying bacteria, and thiobacilli (Shiveley, 1974; Allen, 1984). The internal ground substance of carboxysomes has a granular substructure which is attributed to the enzyme and is enclosed by a single, 3–5 nm membrane to produce a 100 nm particle. One report suggests that these structures may also contain a small amount of circular double-stranded (ds)DNA (Westphal *et al.*, 1979).

Sulfur granules are unique to bacteria which are able to oxidize hydrogen sulfide and grow in its presence. These bacteria typically form internal deposits of elemental sulfur which are 0.1–1.0 μm in diameter and which are enclosed in a nonunit membrane composed of protein globules 2.5 nm wide (Nicholson and Schmidt, 1971). For some reason, sulfur granules are rarely retained in thin section and they are better detected by negative stains.

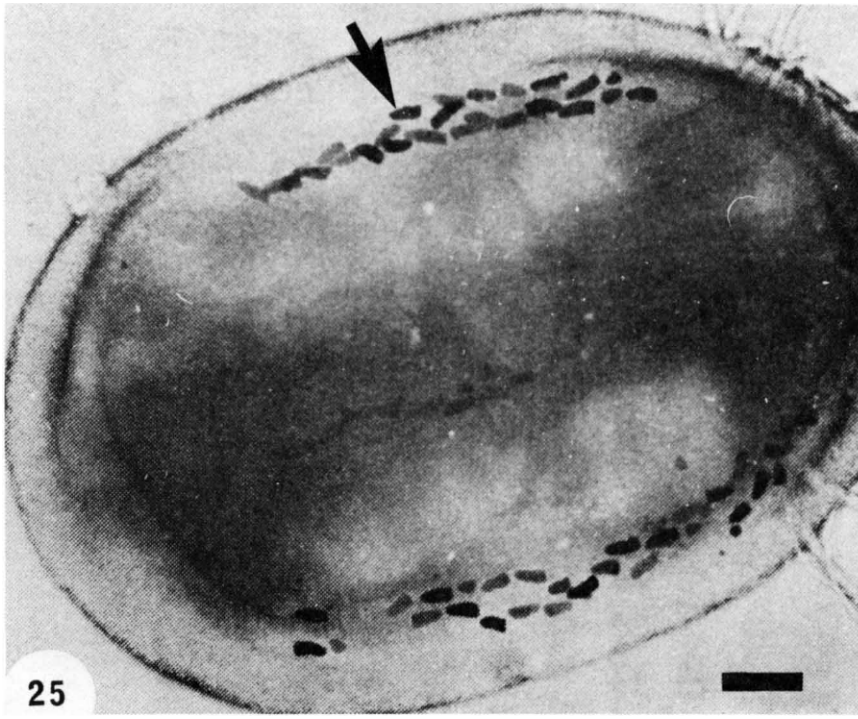


FIG. 25. Negative stain of an unidentified magnetotactic bacterium showing chains of magnetosomes (one chain is pointed out by the arrow). (From Blakemore, 1982. *Annu. Rev. Microbiol.* **36,** 217–238, with permission of the author and the publisher.)

Possibly the most unusual inclusion body is the magnetosome. These particles of magnetite (Fe_3O_4) which are enveloped by a thin (3.0 nm) nonunit membrane. They are generally cubic to octagonal, 25–55 nm on each side, and are distributed in chains within the cytoplasm, usually close to the plasma membrane, along the longitudinal axis of the cell (Fig. 25). Magnetosomes are in the single magnetic domain size range for Fe_3O_4 and the chain arrangement impacts a permanent magnetic dipole moment to the cells. Their function is to orient magnetotactic bacteria to geomagnetic fields so that they can swim parallel to them (Blakemore, 1982). In practice this means that the bacteria are able to distinguish their vertical alignment in aqueous sediments and, consequently, migrate toward regions of optimal oxygen tension. Since most magnetotactic bacteria are microaerophilic, they migrate away (or downward) from the oxygenic surface sediments.

The production of magnetosomes is a complex situation which is only partially understood. Magnetite is not a natural bacterial mineral and it must, somehow, be manufactured within the cell. Both low-density and high-density hydrous

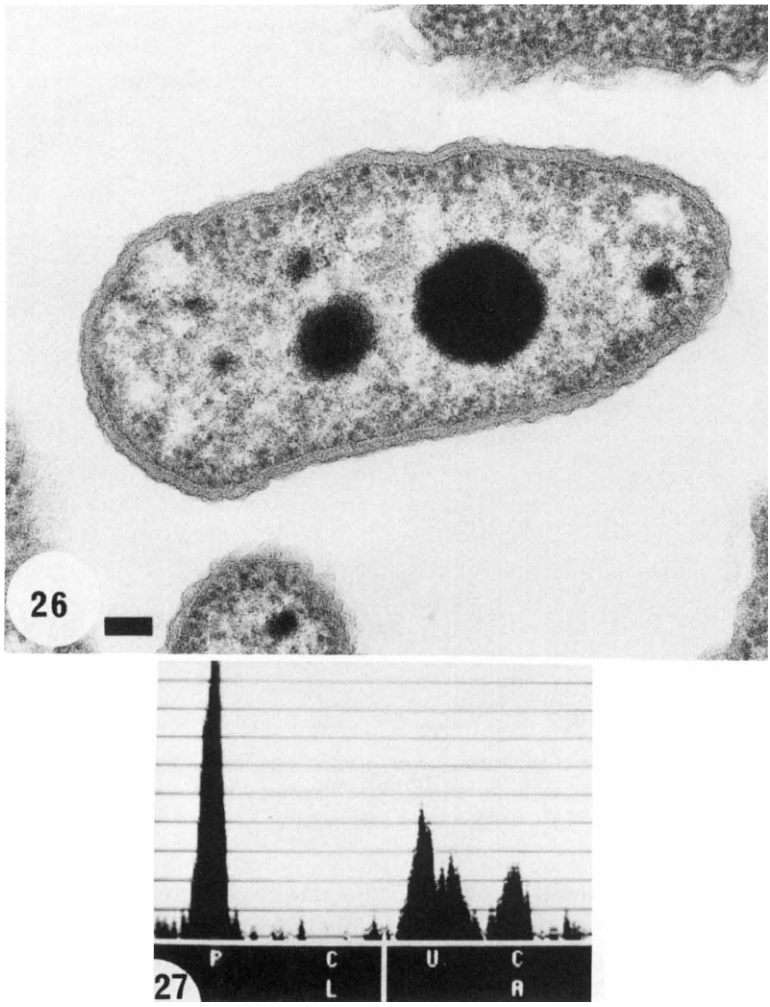


FIG. 26. Thin section of a *Pseudomonas aeruginosa* cell containing electron-dense polyphosphate granules.

FIG. 27. Energy dispersive X-ray analysis spectrum of one of the polyphospate granules in Fig. 26. Calcium and phosphorus are seen. The uranium is due to uranyl acetate which has been used as a contrasting stain for electron microscopy.

ferric oxides exist, in conjunction with magnetite, within the cell and a ferrous complex is associated with the bacterial envelope (Frankel *et al.*, 1983; R. B. Frankel, personal communication). It is possible that these ancillary iron compounds are transient states of the iron during its transport into the cell and conversion to Fe_3O_4.

Glycogen granules are an example of a cytoplasmic inclusion which is not enclosed by a membrane (an exception is certain *Clostridia* spp.; Laishley *et al.*, 1973). They, like polyhydroxybutyrate bodies, are storage depots for carbon and consist of highly branched, large-molecular-weight polymers of glucoside which form amorphous bodies 20–100 nm in diameter scattered throughout the cytoplasm. Glycogen granules do not have an apparent substructure and appear as an amorphous substance which is readily stained by heavy metal salts in thin sections.

Polyphosphate granules (also called volutin or metachromatic granules) are also free within the cytoplasm and are not encased by a membrane (Fig. 26). As their name implies, they are mostly phosphate, but it is in the form of high-molecular-weight, linear polymers or lower molecular weight, cyclic tri- to hexametaphosphates (Harold, 1966). Calcium is often in great abundance within the granules (Fig. 27) and sometimes iron, magnesium, sulfur, and chlorine can also be detected (Scherer and Bochem, 1983). They have an enormous size range (50 to 1000 nm) but are commonly 250 nm in diameter. Due to their high phosphorus content, even unstained sections of the granules have a high degree of electron opacity; staining with heavy metal salts increases their density, presumably by displacing a proportion of their natural counterions.

3. *Nucleoplasm*

Unlike eukaryotic cells, bacteria do not possess a clearly defined nucleus. There is no nuclear envelope to partition the chromatin from the cytoplasm. Each bacterial cell contains one chromosome and, possibly, one or more extrachromosomal closed, circular elements called plasmids. All of these chromosomal elements lie within the nucleoplasm of the cell, and this region is called the bacterial nucleoid.

It is difficult to accurately describe the outermost limits of or the actual shape of the nucleoid. It is an extremely delicate structure and, since it is not contained within a protective membranous envelope, it is particularly sensitive to fixation and dehydration. This fact is aptly demonstrated by the nucleoid's appearance after different fixative regimens; osmium tetroxide condenses the DNA fiber into a tightly wound skein in the center of the cell (Fig. 28), whereas glutaraldehyde leaves it more loosely arranged throughout a major proportion of the cytoplasm (Fig. 29). Snap-frozen cells which have been frozen-fractured and -etched have been difficult to interpret due to the granularity of the cytoplasmic ground substance; the DNA is impossible to discern unless it has previously been stabilized and condensed by chemical fixation (Woldringh and Nanninga, 1976; Daneo-Moore *et al.*, 1980).

Another complicating factor is that the bacterial chromosome is highly anionic. Accordingly, it is exquisitely sensitive to counterions and, in fact, can swell or contract depending on the ion balance of the internal milieu. If cations,

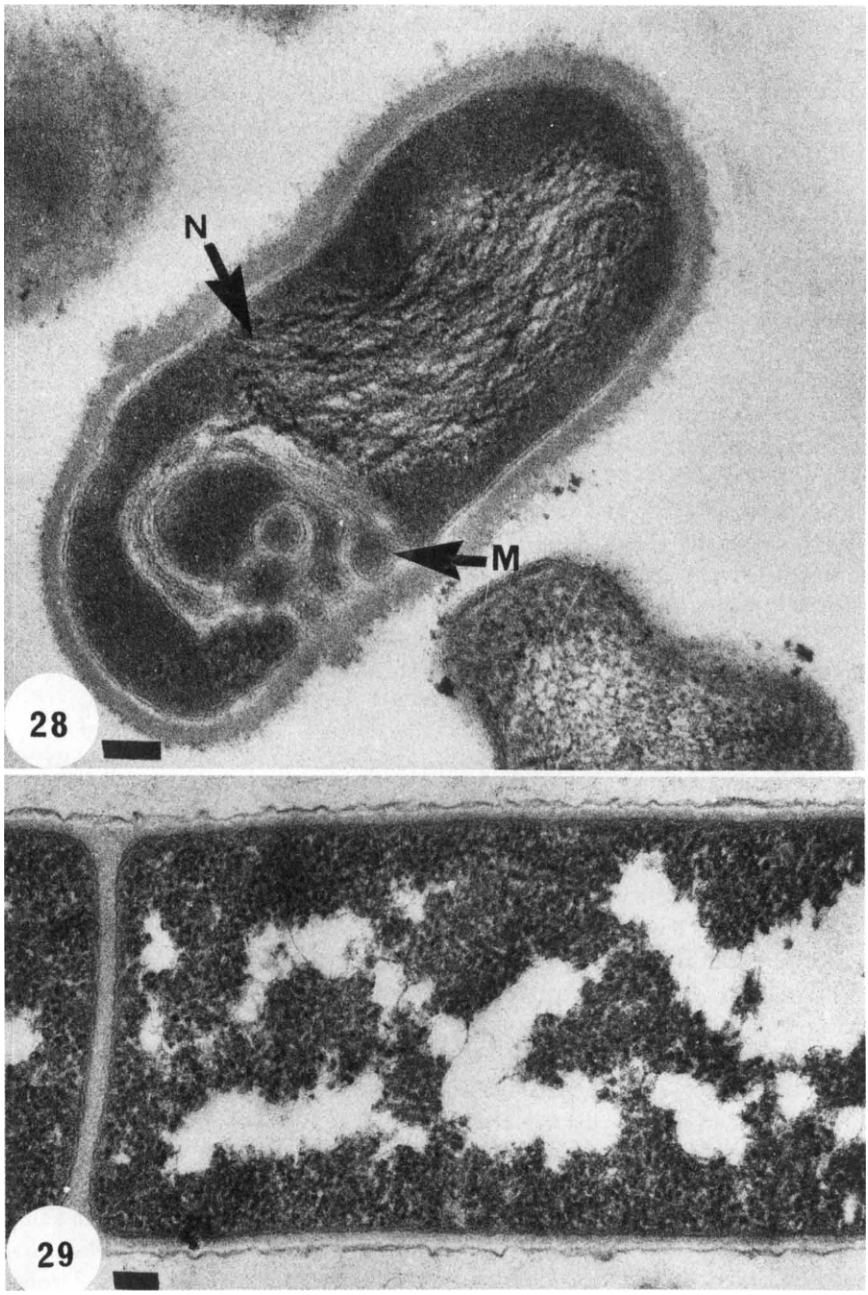


FIG. 28. Thin section of a *Corynebacterium parvum* cell which has been chemically fixed in osmium tetroxide. The nucleoid (N) is condensed and is attached to a mesosome (M).

FIG. 29. Thin section of a glutaraldehyde fixed *Bacillus licheniformis* cell. The nucleoid is distributed throughout the electron translucent regions within the cytoplasm.

such as magnesium or calcium, are present, the overall charge density is reduced and salt-bridging fuses adjacent regions of the fiber together. If they are absent, the folding of the fiber becomes more diffuse.

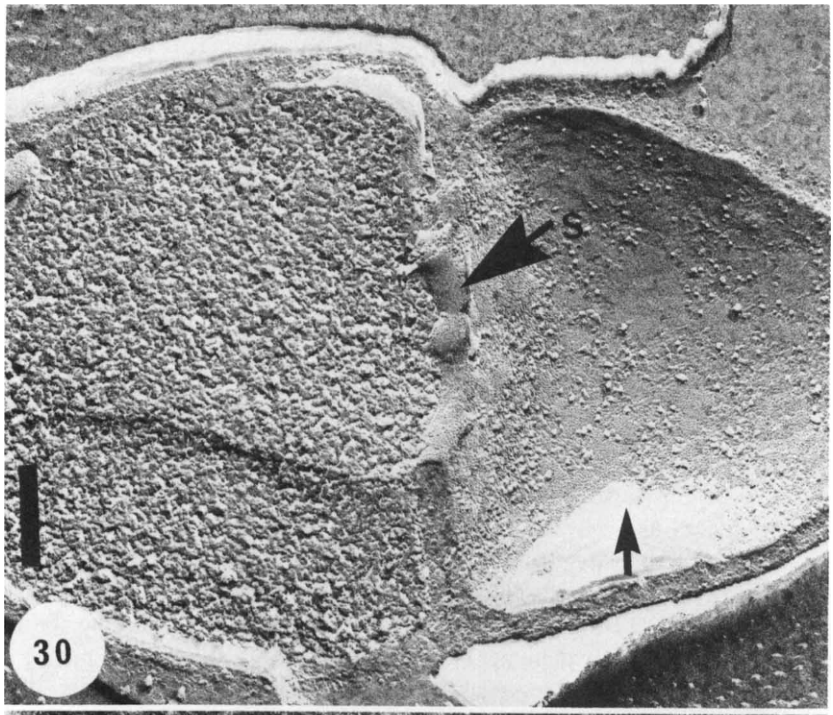
The bacterial chromosome can be readily isolated from the cell and exists as a DNA–ribonucleoprotein complex. In *E. coli,* its molecular weight is about 2.5×10^9 and, if completely unwound, is a circle approximately 1 mm in circumference; this is large enough to accommodate approximately four million nucleotide base pairs! The interaction between all the component parts of the chromosome is complicated and not completely understood. The end result is a highly folded and supercoiled dsDNA fiber.

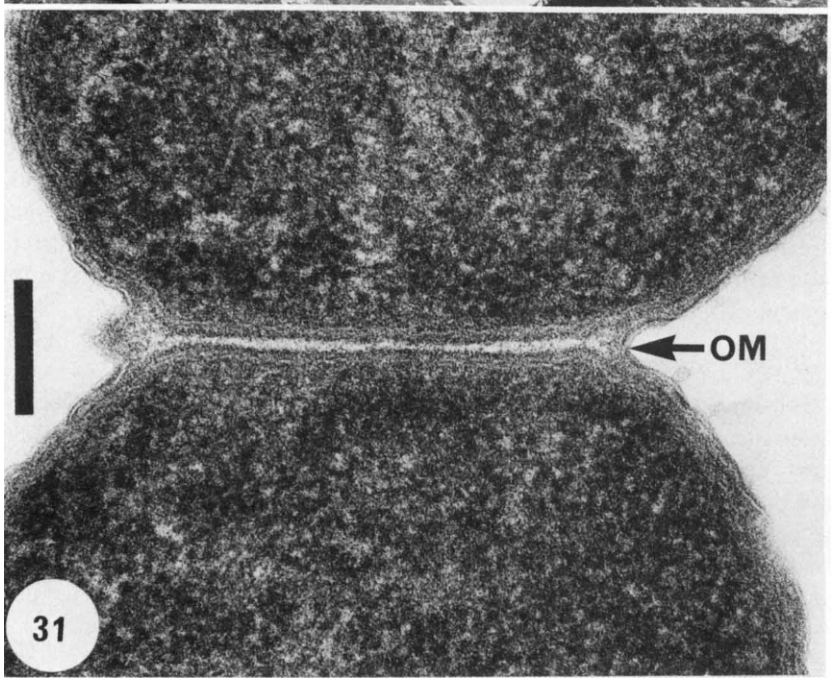
We believe the isolated chromosome is more tightly folded and condensed than in its *in vivo* state. This high tension is attributed to associated ribonucleoprotein since, when these constituents are digested away, the DNA fiber unfolds and its circularity becomes quite evident (Pettijohn, 1976). *In vivo* studies have revealed the chromosome to be in a more relaxed state within the cell (only 60–70% of its mass is supercoiled) and there are 43 + 10 domains of tension (folds) along its length (Sinden and Pettijohn, 1981). Unlike isolated chromosomes, these domains are independent of nascent RNA in the intact cell. To complicate the issue further, it is not unusual for rapidly growing bacteria (*E. coli* can have a doubling time of 20 minutes) to have multiple division forks along the chromosome's length; each new daughter cell must obtain a full DNA complement. To aid genome replication and segregation to each daughter cell, discrete sites of the chromosome are linked to the plasma membrane and even, possibly, to the wall (Sargent *et al.*, 1983; Doyle *et al.*, 1980; Koch *et al.*, 1981).

It is therefore apparent that the bacterial nucleoid must be a dynamic structure. Certainly, it is responsible for carrying the genomic message and must be continually active in transcription. At the same time the chromosome, itself, is in an active state of replication. It would appear, then, that the nucleoid is always in an active state of transition within a growing cell and this, we believe, is the biggest hurdle to surmount in defining its ultrastructure. If we do manage to accurately stabilize it, we would succeed in viewing its finite structure for only one brief moment of the transition process.

4. *Division Sites*

In nature, doubling times of 2 to several hours are not unusual. Deep sea bacteria can take weeks or even months to divide; their metabolism is unusually slow. Yet, under optimal growth conditions, many bacteria in the laboratory have doubling times that can be measured in fractions of an hour. Bacteria have the potential to grow and divide at rates that are extraordinary by eukaryotic standards. The difference between natural and laboratory cultures is that in nature optimal growth conditions are rarely encountered; most bacteria are under growth-limiting conditions.


S
30


OM
31

We have recognized two major formats for bacterial division, septation and constriction. Gram-positive and several types of gram-negative bacteria divide by septation. For example, bacilli are rod-shaped bacteria which elongate as they grow; their width remains constant (~0.5 to 1.0 μm). At a distinct cell length which is unique to each species (usually about 3 to 5 μm), the plasma membrane begins to pinch-in around the entire growth of the rod. As it grows in, it is followed by new wall fabric so that an aperture of membrane and wall begins to bisect the bacterium (Fig. 30). This aperture eventually closes and two identical, small, joined, daughter cells are formed where once there was a longer parent cell. The wall septum which still joins the two cells together now splits down its center and this separates the cells. This entire division process is called "septation."

Zymomonas mobilis is an example of a gram-negative bacterium which divides by septation (Beveridge *et al.*, 1984). The gram-negative process mimics the gram-positive one, except that the outer membrane remains at the bacterial surface outside the division aperture (Fig. 31); the plasma membrane and the peptidoglycan layer form the aperture. As the cells separate, the peptidoglycan splits down the middle and this splitting is followed by an ingrowth of outer membrane. At the time of actual cell separation, both daughter cells are completely encompassed by the outer membrane.

More often, gram-negative bacteria seem to divide by constriction. This is a pinching-in of the entire cell envelope (Fig. 32) until contact is made at the cell center. All envelope layers reanneal and two intact daughter cells are formed. There is no rigid structure analogous to a septum in this process. It is possible that this constrictive process is an artifact that has been induced by the processing of the bacteria preliminary to electron microscopy (Burdett and Murray, 1974; Gilleland and Murray, 1975). Clearly, gram-negative cell envelopes are more delicate than their gram-positive counterparts and their septa, if they exist, could be destroyed.

III. Structure of Viruses

Although different viruses vary in the details of their structure and composition, there are some common architectural principles and components to all viruses. By definition, all viruses contain a nucleic acid genome (either DNA or

FIG. 30. Freeze etching of a dividing *Bacillus licheniformis* cell showing the closing septum aperture (S). The left side of the cell has cleaved through the cytoplasm, whereas the right side has cleaved through the middle of the plasma membrane.

FIG. 31. Thin section of a dividing *Zymomonas mobilis* cell showing a completed septum. The outer membrane (OM) of this gram-negative cell is outside the septum and will grow inwards during cell separation. (From Beveridge *et al.*, 1984, *Can. J. Microbiol.* **30**, 1283–1289, with permission of the authors and the National Research Council of Canada.)

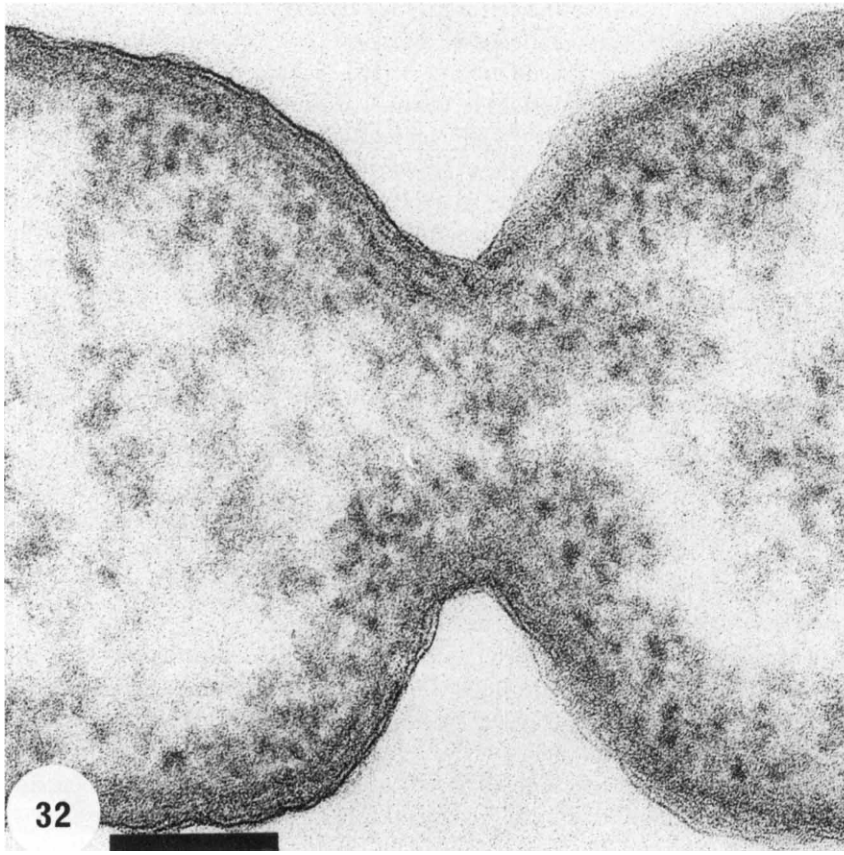


FIG. 32. Thin section of a dividing *Pseudomonas aeruginosa* cell which is representative of a gram-negative constrictive type of division. The entire cell envelope is pinching the cell in two.

RNA) surrounded by a protective coat. Viruses exist in many shapes and sizes (Fig. 33), but most follow simple architectural constructions in their design.

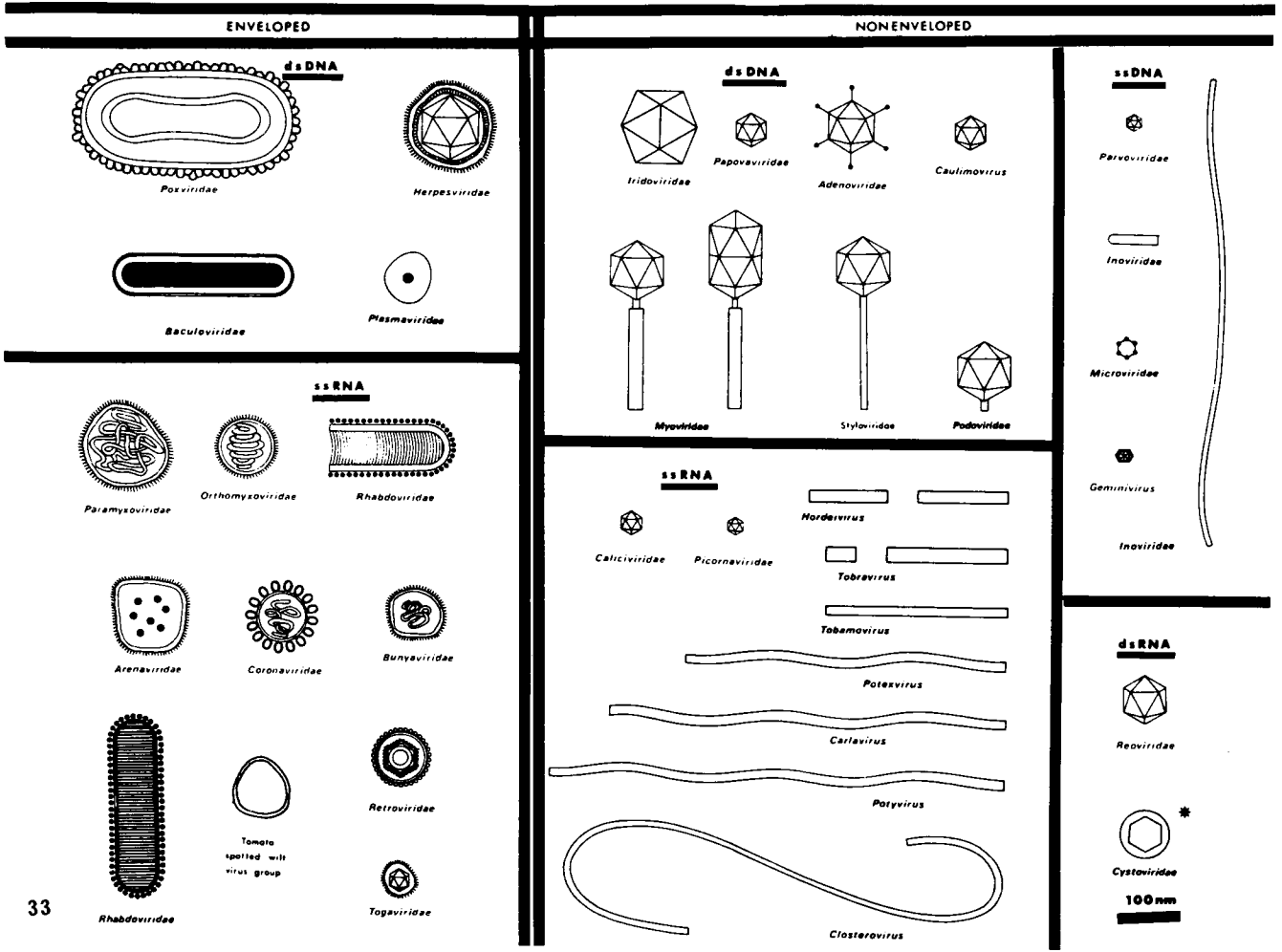
A. THE GENOME

The nucleic acid genome of DNA viruses can vary greatly in conformation among different viruses. It can be linear, circular, double stranded (ds), or single stranded (ss). The viral genes may be contiguous on a single molecule or may be segregated to different molecules (segmented genomes). Viral linear DNAs can have ends which differ in conformation. The ssDNA of parvovirus has a terminal

hairpin at both ends (Lusby *et al.*, 1980; Berns, 1984). The ends of poxvirus dsDNA are covalently joined, resulting in no free 3′-OH or 5′-PO_4 at either end (Holowczak, 1982). The dsDNA of phage λ has single-stranded (12 nucleotides long) ends which are complementary to each other allowing the DNA to circularize after infecting a bacterium (Friedman *et al.*, 1984). The dsDNA of bacteriophage T4 has the same sequence at both ends allowing for homologous recombination between ends of the same DNA molecule to generate a circular DNA or between other copies of viral DNA to form linear concatenated DNA containing several contiguous genomes in an infected cell (Mathews *et al.*, 1983). The 5′ ends of the dsDNA of adenoviruses are covalently attached to a polypeptide complexed with a cytidine nucleotide (VPg, for virion genome linked protein) which acts as a primer (providing a free 3′-OH end) for DNA synthesis during replication of the adenovirus genome (Desiderio and Kelly, 1981).

Although there is a great variety in the conformation of the ends of viral DNA, the ends all serve similar functions in genome replication during the infection cycle; all ensure replication of the entire genome. For all but one family of DNA viruses, each virion (mature virus particle) contains a single molecule of DNA. One exception to this rule is in the insect polydnaviruses in which several different-sized, circular, viral DNAs may be encapsidated within a single virion (Stoltz *et al.*, 1984).

Viral RNA genomes are different in conformation and organization among the different RNA viruses. All viral RNAs are linear. Although most RNA viruses have ssRNA genomes, three groups, the reoviruses, birnaviruses and the bacterial cystoviruses, have dsRNA (Mathews, 1982; Dobos *et al.*. 1979). Single-stranded genomes of the plus (+) sense, as in polioviruses, can act directly as a messenger RNA once it is uncoated in the cytoplasm of a suitable cell. Other viruses have ssRNA of the negative (−) sense, and must be transcribed to positive (+) sense RNA which can direct virus-specific protein synthesis. Although some RNA genomes are without alterations, the ends of others may have different structures or specialized sequences. The RNAs of togaviruses, coronaviruses, and plant viruses have a 7 methyl guanosine 5′ "cap" common to that on many eukaryotic messenger RNAs (Joshi and Haenni, 1984). In the RNA of the picornaviruses and several plant viruses a polypeptide (VPg) is covalently attached to the 5′ end (Dasgupta, 1983). The (+)RNAs of the picornaviruses and some plant viruses, have a 3′ poly(A) tail, similar to the 3′ poly(A) tail of many eukaryotic mRNAs (Dasgupta, 1983). The (+)RNA of other plant viruses have 3′ sequences which are similar to those of some tRNAs (notably tRNAs for Tyr, Val, and His). It is easy to see how a cell could be tricked into translating a viral (+)RNA if it has the 5′ cap (or related structure) and the 3′ poly(A) tail typical of eukaryotic mRNAs!


ENVELOPED
NONENVELOPED
dsDNA
Poxviridae
Herpesviridae
Baculoviridae
Plasmaviridae
ssRNA
Paramyxoviridae
Orthomyxoviridae
Rhabdoviridae
Arenaviridae
Coronaviridae
Bunyaviridae
Rhabdoviridae
Tomato spotted wilt virus group
Retroviridae
Togaviridae
dsDNA
Iridoviridae
Papovaviridae
Adenoviridae
Caulimovirus
Myoviridae
Styloviridae
Podoviridae
ssRNA
Caliciviridae
Picornaviridae
Hordeivirus
Tobravirus
Tobamovirus
Potexvirus
Carlavirus
Potyvirus
Closterovirus
ssDNA
Parvoviridae
Inoviridae
Microviridae
Geminivirus
Inoviridae
dsRNA
Reoviridae
Cystoviridae
100nm

Other virus RNAs have "special sequences" at their ends. The 3′ and 5′ ends of the (−)ssRNA of arenaviruses are self-complementary (cohesive); this allows them to circularize into a partially dsRNA circle. The 5′ end of the ssRNA of retroviruses (the only virus with a diploid genome) has sequences which allow hybridization between the 5′ ends of two identical ssRNAs to form a partially duplex dimer which is encapsidated in the mature virion (Varmus, 1984).

In most cases the special ends of viral nucleic acid are important for the virus infection cycle and participate in genome replication or translation. Since many of these viral events mimic those found at the cellular level, several of thse viruses (both DNA and RNA types) have been used as models for studying DNA replication and transcription and mRNA translation in cellular systems. The different types of genome (and morphology) of viruses in different families (or groups) are summarized in Fig. 33.

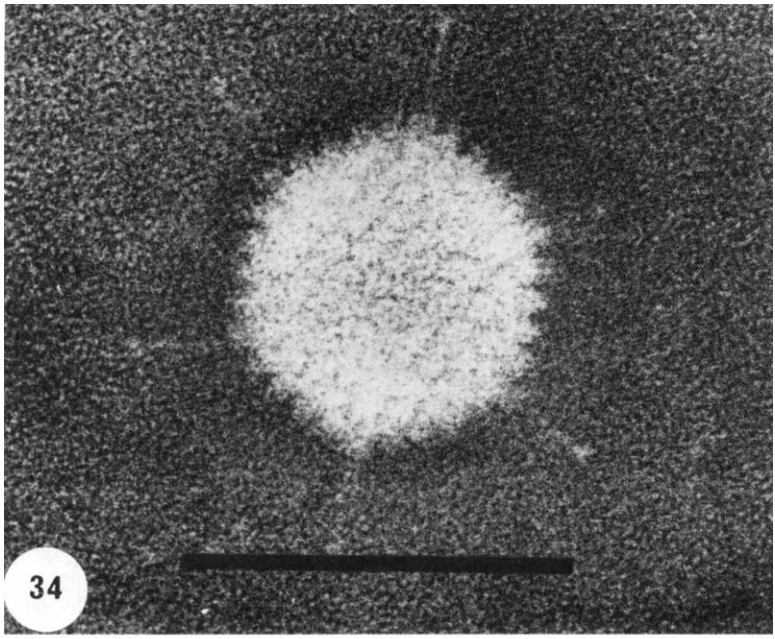
B. Simple Capsid Structures

Because of their small genome, viruses cannot code for a wide range of polypeptides. Consequently, the design of most viruses incorporates many copies of one or a limited number of polypeptide species some of which possess the innate ability to self-crystallize into a capsid surrounding the viral genome. These polypeptides contain information for self-assembly into a nucleocapsid, genome encapsidation, and the localization of surface determinants responsible for virus–cell interactions. Viruses have consequently evolved highly efficient architectural forms of nucleocapsids which utilize the least number of polypeptide species possible (thereby limiting the amount of viral genetic information required for capsid morphogenesis).

One form of nucleocapsid is the icosahedron (Figs. 34–37). While the design of the geodesic dome (based on icosahedral symmetry) has been largely credited to the architect Buckminster Fuller—viruses have been using this same architectural principle for eons. However, instead of using steel and plastic, they fabricate their icosahedrons out of proteins which, together with viral nucleic acids and accessory proteins, self-assemble into virions (often within the viroplasm of infected cells). An icosahedron is an isometric (spherical) structure requiring many copies of at least two different polypeptides. In one of the simplest ico-

---

Fig. 33. Composite of some virus families and groups divided into enveloped and nonenveloped groups and subdivided according to nucleic acid type and strandedness. (Modified from Mathews, 1982.)



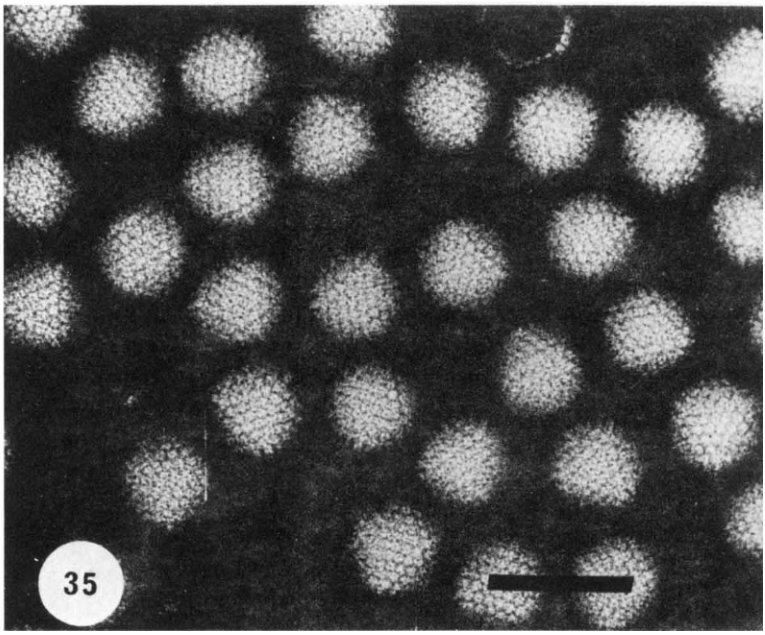


FIG. 34. Negative stain of adenovirus showing spikes from 6 of 12 pentons. (From Williams and Fisher, 1974, with permission of the authors. Courtesy of Charles C. Thomas, publisher.)

FIG. 35. Lower magnification of Fig. 34, showing icosahedral substructure of adenovirus. (From Williams and Fisher, 1974, with permission of the authors and the publisher.)

sahedral viruses (e.g., parvoviruses), one polypeptide species forms hexons (six hexon protomers per hexon) which have a central hole and six, equal-sized edges, whereas another polypeptide species forms pentons (five penton protomers per penton) possessing a central hole and five equal sized edges. A protomeric subunit of a hexon or penton does not necessarily consist of a single polypeptide. Often a protomer is an aggregate of two or more different polypeptides. Each of these units (hexons and pentons) is termed a capsomer and is formed by self-assembly. Once formed, they interact with one another and the genome to produce an icosahedral nucleocapsid which always consists of 12 corner pentons usually aligned with and bonded to 20 (or more) hexons. The smallest number of capsomers (with the exception of ϕX174 and polyoma virus whose virions consist only of 12 pentons, Baker *et al.*, 1983) is 32 (12 pentons plus 20 hexons), with a minimum of 180 protomers ($12 \times 5 = 60$ penton protomers plus $20 \times 6 = 120$ hexon protomers). This is the structure of the parvovirus nucleocapsid which has a diameter of 20 nm. Larger icosahedral nucleocapsids require more (or larger) hexons, but always have 12 pentons. For example, papoviruses (Fig. 36) have 72 capsomers (diameter of 50 nm), adenoviruses (Figs. 34 and 35) have 252 capsomers (12 pentons and 240 hexons, and a capsid diameter of 80 nm), and herpesviruses have 162 capsomers (150 hexons, larger than those of adenoviruses) and a nucleocapsid diameter of 100 nm).

The other basic architectural construct for nucleocapsids is a helical one (Figs. 38 and 39). A helical nucleocapsid requires only one polypeptide species: in this case, self-assembly of the capsomers usually occurs in concert with the genomic nucleic acid. The diameter of the resulting rod-shaped nucleocapsid depends on the size of the polypeptide and its chemical association with adjacent identical polypeptides and nucleic acid. The length of the helical nucleocapsid is often dictated by the size of the genome. These rod-shaped nucleocapsids can be either rigid (e.g., tobacco mosaic virus; Fig. 38) or flexible (e.g., most plant RNA viruses; Fig. 39). The rigidity of the nucleocapsid is a reflection of the strength of chemical bonding between adjacent capsomers. As a general rule (bacuolviruses and Inoviridae excepted), practically all viruses with a helical nucleocapsid have an RNA genome.

C. Complex Capsid Structures

Although nucleocapsids with icosahedral and helical symmetry are the two major architectural forms, some more complex structures also exist. In some bacteriophages (Figs. 40–42), both types of symmetry apply; the head (containing the nucleic acid) can be totally (e.g., T1; Fig. 41) or partially (e.g., T2; Fig. 40) icosahedral, whereas the tail exhibits helical symmetry (Reanney and Acker-

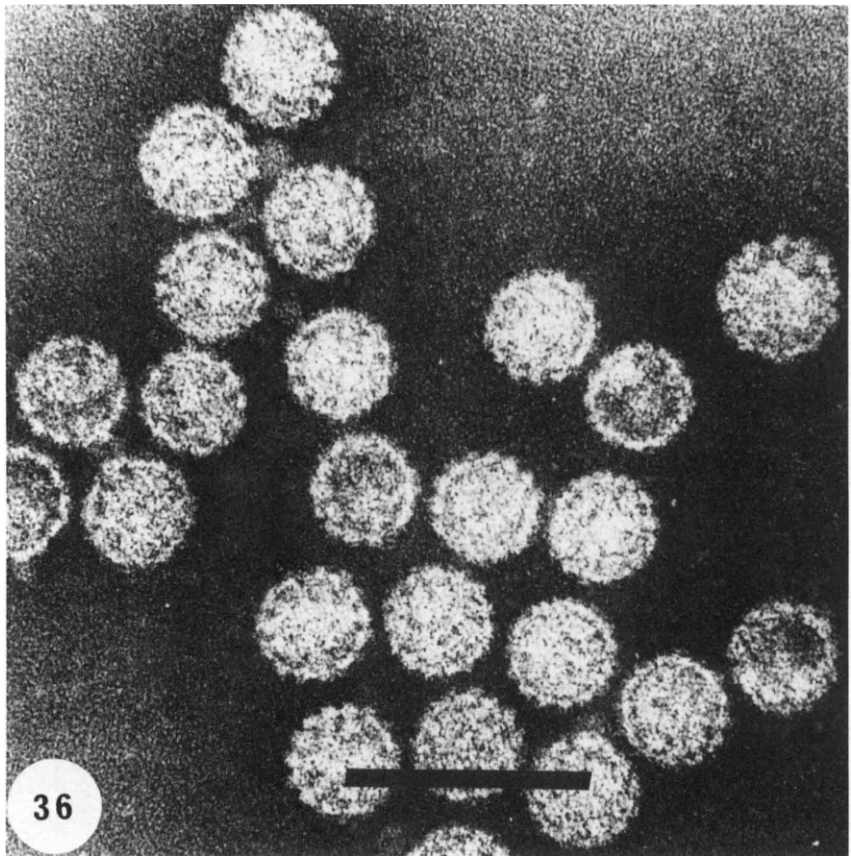


FIG. 36. Negative stain of papovirus SV40. (From Wiliams and Fisher, 1974, with permission of the authors. Courtesy of Charles C. Thomas, publisher.)

mann, 1982). Additional structures (e.g., tail fibers, spikes, and sheaths; Fig. 40) can also be attached. In a few viruses (bacterial Plasmaviridae and Arenaviridae), a noncrystalline pleomorphic (or polyhedral) nucleocapsid is formed. The rhabdovirus nucleocapsid, although helically organized, is in the shape of a bullet with conical and flat ends. The nucleocapsid of the gemini viruses of plants appear like twin, fused, partially completed icosahedrons (hence their name).

Most virus families have nonenveloped nucleocapsids (considered as naked viruses). However, the viruses of about a dozen families are enveloped such that the nucleocapsid is surrounded by a lipoprotein envelope often with easily recognizable glycoprotein spikes. For example, the icosahedral nucleocapsid of her-

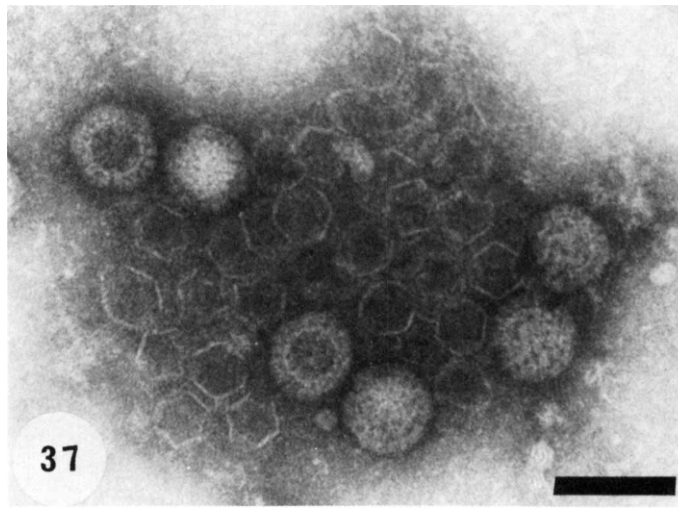


FIG. 37. Negative stain of reovirus (infectioius terosinovitis virus). Two virions show the double capsid nature of reovirus. Many empty capsids also visible (photo courtesy of Dr. Jan Thorsen, University of Guelph, Canada).

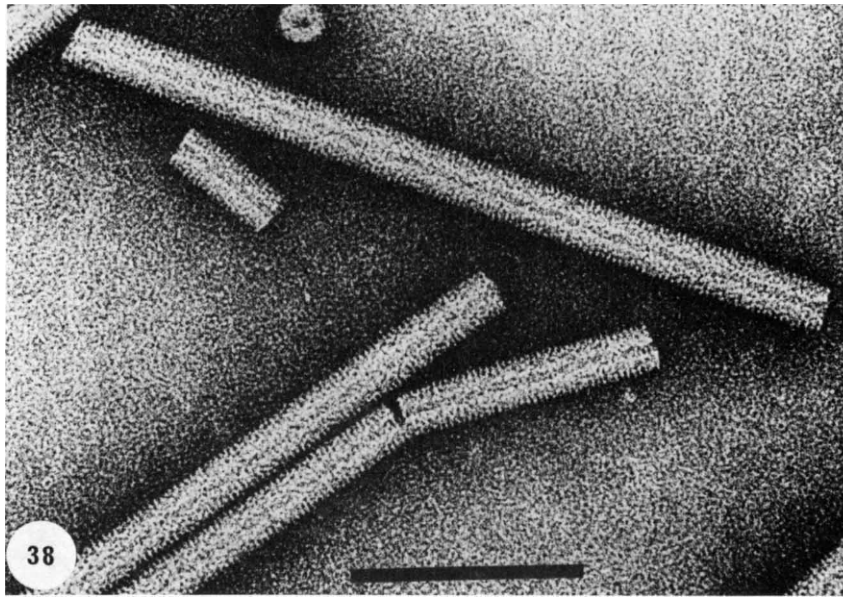


FIG. 38. Negative stain of the tobamovirus tobacco mosaic virus. (From Williams and Fisher, 1974, with permission of the authors. Courtesy of Charles C. Thomas, publisher.)

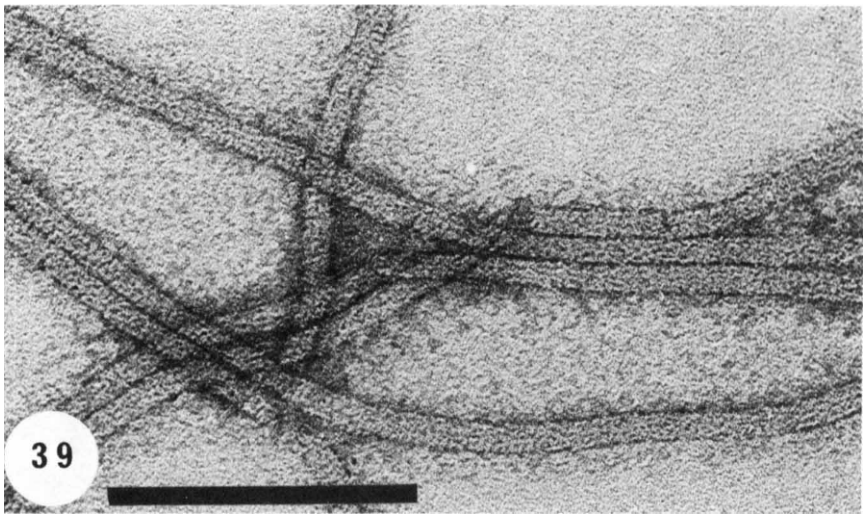


FIG. 39. Negative stain of a flexuous rod plant virus. Clover yellow mosaic virus. The virus was kindly supplied by J. Bancroft, University of Western Ontario, Canada.

pesvirus and togavirus, and the helical nucleocapsid of influenza virus and rabies virus, are surrounded by a complex spike containing membrane (Fig. 43). These membranes are not only protective but are also important to virus adsorption during the initial stages of infection.

The insect baculoviruses (and reoviruses) can exist in two morphological forms (Tinsley and Harrap, 1978; Tweeten *et al.*, 1981). The dsDNA containing helical nucleocapsid of baculovirus is enveloped; this is the form in which the virus spreads from cell to cell. The enveloped nucleocapsid can in turn be surrounded by a dense proteinaceous "occlusion body." It is in this form that the virus is transmitted from insect to insect. The insect polydnaviruses (Fig. 44) have a complex structure consisting of an ovoid nucleocapsid surrounded by two membranes, the inner derived *de novo,* the outer derived from the plasma membrane of the infected cell.

The brick-shaped poxvirus (Fig. 45) is the largest (400 × 200 nm) and by far the most complex virus known. The dsDNA genome is contained in a membrane-bound, elongated nucleoid surrounded by two or more proteinaceous lateral bodies. The external envelope contains lipid and proteins organized into a regular globular surface substructure.

IV. The Virus Replication Cycle

The structure of the mature virion protects the genome from the physical environment. It is also well designed for its own replication. The outer layer of

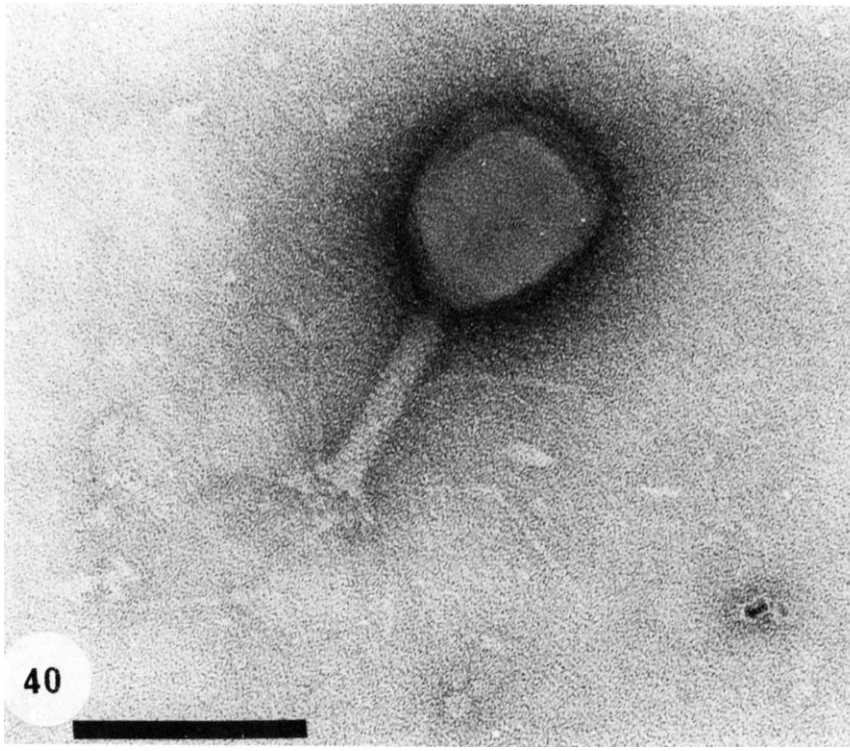


FIG. 40. Negative stain of the myovirus T4.

the virus permits specific interaction with host cells, leading to infection, whereas the viral genome and associated components allow the virus to take control of the cellular metabolism and redirect it for the synthesis of viral components. This section will deal with the virus replication cycle in which all of the viral components are synthesized, the viral genome is replicated, and progeny virions are formed and released.

All viruses must ultimately interact with host cells so that at least the viral genome becomes intracellular and can direct cellular metabolism to produce progeny virions. When progeny virions are released from the infected cell they can then attack other cells and initiate additional replication cycles. While the molecular details of virus replication are as varied as the number of different types of viruses, there is a common overall strategy. The basic stages of a virus replication cycle are transmission, adsorption, penetration, genome uncoating. virus genome expression (genome replication, transcription, and virus protein synthesis), virus morphogenesis, and virion release.

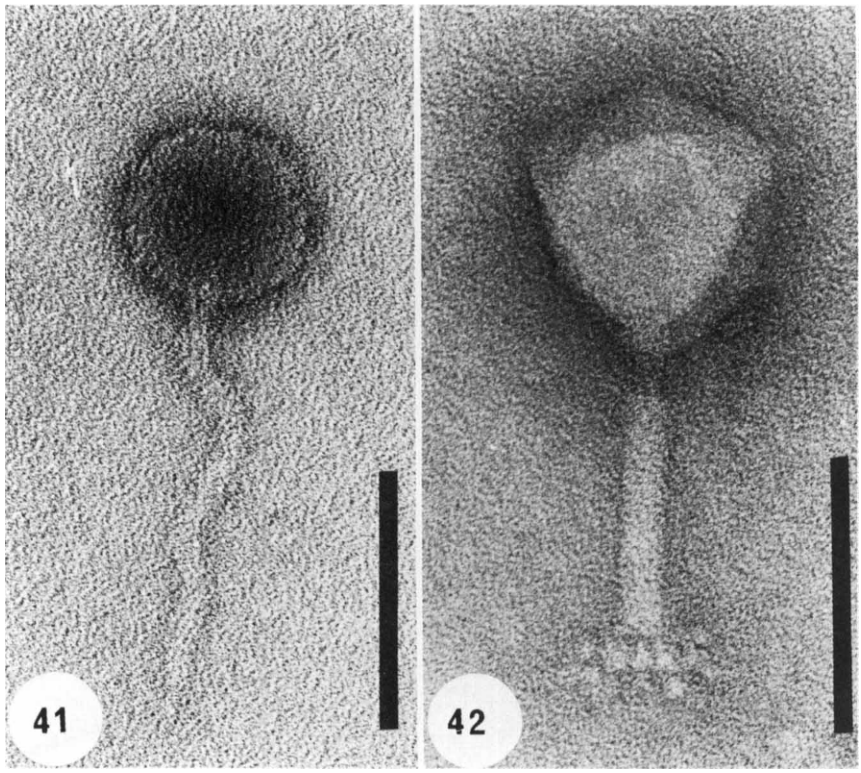


FIG. 41. Negative stain of the stylovirus T.
FIG. 42. Negative stain of the myovirus from *Yersinia ruckeri*.

A. TRANSMISSION

Virus transmission can be horizontal, in which virus is transmitted directly from infected to noninfected hosts or, rarely, vertical if it is transmitted from parents to offspring, often over several generations (Mims, 1981).

Horizontal transmission occurs at two levels: organism to organism (important to viral epidemiology) for metazoan hosts and cell to cell for both unicellular (e.g., bacteria, protozoa) or multicellular hosts.

Virus can be spread by air (e.g., aerosols from a sneeze), soil (e.g., plant viruses), or water (e.g., polio virus), or by biological means from an "infected" organism to a susceptible organism often but not necessarily of the same species. Biological transmission is more efficient because the number of virions transmitted (i.e., inoculum size) is generally higher than the dilute numbers encountered in air, water, or soil. Often, if a potential host is under stress, the infection is easier to establish. Only those viruses which can survive the environmental insults of the external environment remain infectious. Temperature, dryness, pH,

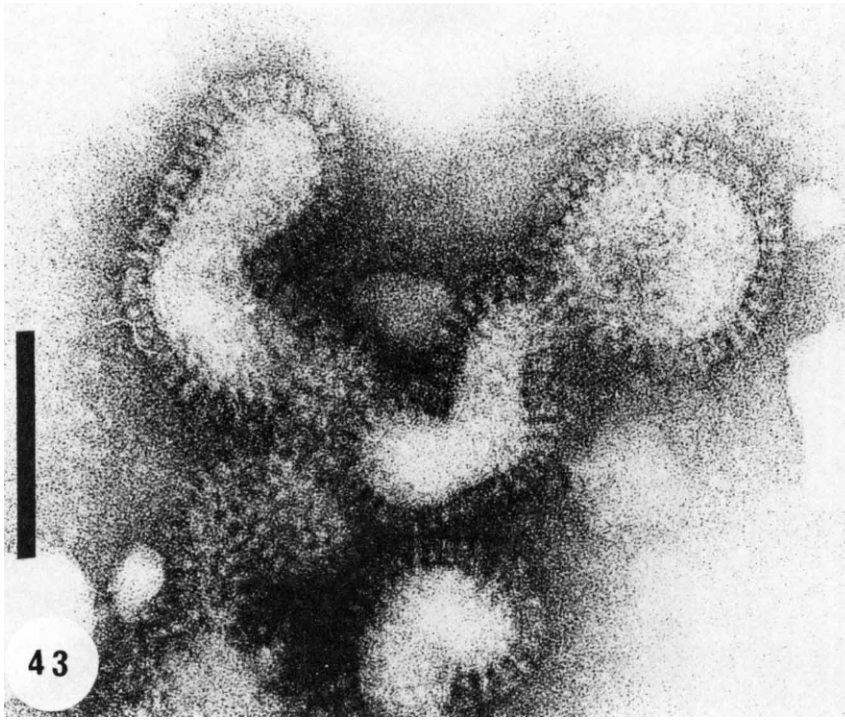


FIG. 43. Negative stain of the paramyxovirus influenza virus. Note the pleomorphic shape and the spike containing envelope.

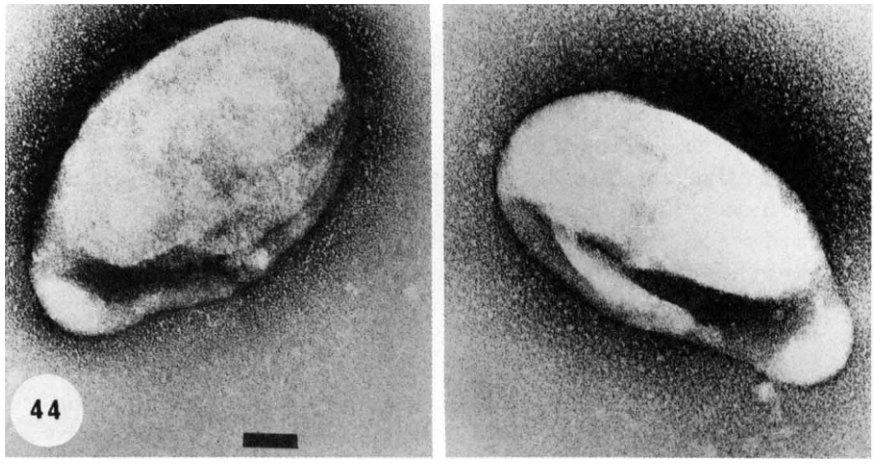


FIG. 44. Negative stain of a large complex enveloped virus from a wasp *Campoletis sonorensis*.

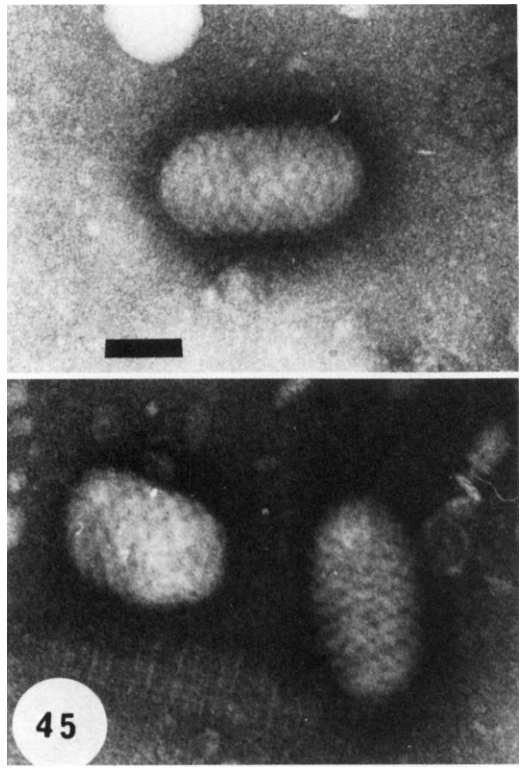


FIG. 45. Negative stain of the poxvirus Orf virus (from sheep). Note the surface substructure of this large complex virus.

ultraviolet irradiation, etc. all take their toll. Certain viruses are transmitted by vectors such as insects or fungi.

Cell to cell transmission occurs when virus is released from an infected cell which is near an uninfected cell, such as in a suspension of bacteria or in an organized tissue (e.g., adjacent intestinal epithelial cells). Since, theoretically, a single virion can initiate virus replication and the number of progeny virions produced per cell (burst size) is high, the virions released from an infected cell and transmitted through the liquid media surrounding the cells could infect many neighboring susceptible cells. After several cycles of cell to cell transmission, the whole population of susceptible cells (bacterial or tissue) could become infected. Note that some cells may be refractory to infection and could remain uninfected. In a metazoan organism, tissue to tissue and organ to organ transmission is often through the circulatory or lymphatic system. As an example, although the primary site of poliovirus infection is the intestinal mucosa, progeny

virions can spread to lymph nodes which drain into the circulatory system and result in a viremia (virus in the blood). The circulatory system can then disseminate the virus throughout the body. If the virus breaks the blood/brain barrier, the infection can spread to nerve cells and the classic symptoms of paralysis are produced.

Many plant viruses rely on both physical transmission (e.g., contaminated soil or water) or transmission by insect vectors which have fed on infected plants. Progeny virions are then transmitted from cell to cell usually through the intracellular cytoplasmic chanels, the plasmodesmata (Hirth and Richards, 1981).

In vertical transmission (parents to offspring), virus can be transmitted to the fertilized egg from either virion-contaminated sperm or egg or, in some cases (e.g., RNA tumor virus), the viral genetic material is directly (by integration into chromosomal DNA) or indirectly (extrachromosomal) associated with the nuclear DNA (Bishop, 1983; Varmus, 1984) of one or both germ cells (sperm or egg). Virus replication may remain dormant (or latent) and will be initiated only at a later stage of embryo development or after maturity. In some cases the virus may remain quiescent during the life of an "infected" individual but can still be transmitted vertically to their progeny; it can be activated in succeeding generations. Although virus infection is persistent (Norkin, 1982), it may not be recognized for several generations and only under certain circumstances (errors in DNA metabolism, environmental factors, etc.) does the virus initiate an overt infection.

B. Adsorption

During transmission, virions can be considered as inanimate objects buffeted by the forces present in their immediate environment (winds, water flow, mucous flow mediated by ciliary action, blood flow, paristalsis, etc.). Since viruses have no locomotory apparatus and cannot direct their motion to their cellular host, the initial interaction between viruses and cells is of a random transient nature.

Since the charge on the outer surfaces of both viruses and cells is usually negative at pH 7.0, the initial virus–cell interaction may be by cross-linking through divalent cations (probably magnesium). If a virus and susceptible cell are in proper juxtaposition, specific interaction between a component on the virion surface and a receptor on the cell surface results. For example, after a series of random collisions between a T4 virion and an *E. coli* cell, a tail fiber of T4 interacts specifically but reversibly with an outer membrane cell receptor (Mathews *et al.*, 1983). Similarly, a protein of the poliovirus capsid associates specifically but reversibly with a glycoprotein cell receptor in the membrane of an intestinal epithelial cell (Medrano and Green, 1973). This initial specific binding is followed by an irreversible adsorption in which more adsorption

specific proteins of the virus attach to more cell receptors, thus stabilizing the virus attachment to the outer surface of the cell. It is at this point that the "host specificity" of many viruses is determined. If a receptor required for adsorption is missing from the cell surface (or hidden under a mucous layer or covered with antibodies), then virus adsorption cannot occur and the cell cannot be infected. The reason poliovirus infection is limited to primates is that only primate cells have complementary receptors (Cooper, 1977). The tissue specificity (i.e., only intestinal mucosa, some viscera and nerve are infected) of poliovirus lies in the lack of receptors in cells of the nonsusceptible tissues. The initial adsorption may be consolidated by interaction of additional capsid proteins and more complementary cell receptors. If a cell is surrounded by a layer such as the capsule of bacteria, the virus must first penetrate this to approach membrane-bound cell receptors. The capsule of *E. coli* K30 is first hydrolyzed by an enzyme associated with the tip of the tail of a podovirus like T7. Once the capsule layer has been penetrated, virus adsorption can occur. In some viruses, the adsorption phase is circumvented altogether and virions enter cells directly through a mechanical break in the cell membrane or are deposited by insects (e.g., aphids feeding on plants) which transmit the virus while feeding on intracellular components. The latter two methods are the prevalent means of infection by plant viruses.

C. Penetration

The virus genome must eventually enter a cell for virus replication to proceed. This can be achieved by several mechanisms which range from having the whole virion enter (e.g., plant viruses) to having only viral nucleic acid enter the cell (e.g., bacteriophage). Many more viruses penetrate the cell membrane by some intermediate method.

In general, naked viruses (adenovirus, picornavirus) penetrate by one of two mechanisms. In one, the virion is thought to pass directly through the membrane intact. Presumably the membrane would have to rapidly repair the rent. The thermodynamic requirement for an intact plasma membrane, however, would not favor such a mechanism, although it may occur on rare occasion. More often virions appear to be engulfed by physical extensions ot the cell membrane, resulting in the virion being enclosed within an endocytic vesicle. The vesicles break down (by virion hydrolases?) and the nucleocapsids are released into the cytoplasm. In some cases intact nucleocapsids may directly penetrate the endocytic membrane.

Membrane-bound viruses also penetrate cells by endocytosis and are often seen in phagocytic vesicles (lysosomes). It is not known if these virions are ultimately destroyed within the vesicle or whether this is an obligatory step in releasing the genome (uncoating) from the nucleocapsid. The infectious core

could subsequently be released into the cytoplasm by direct passage through the membrane. In other cases, the lysosomal membranes fuse with the viral membrane and the entire nucleocapsid is released into the cytoplasm (Lenard and Miller, 1982; Simons *et al.*, 1983).

A second mode of entry for enveloped viruses (e.g., rhabdoviruses, herpesviruses, and influenza viruses) is by fusion between the viral envelope and the cell membrane (Matlin and Simons, 1983; Fuller *et al.*, 1984; Lamb and Choppin, 1983). In these cases the viral membrane remains as an integral part of the plasma membrane.

In all of the above examples, either the whole virion or at least the viral nucleocapsid enters the cytoplasm. For some viruses, however, notably most bacterial viruses, the virion nucleocapsid remains adsorbed to the outer cell surface (wall or pili; Mathews *et al.*, 1983; Friedman *et al.*, 1984). Only the viral genome and some associated proteins enter the cells' cytoplasm, leaving behind an empty nucleocapsid. Injection of viral DNA by T even phages is dependent on a conformational change of the virus tail triggered by the adsorption to the cell wall (Mathews *et al.*, 1983). The energy released from this conformational change forces the tail tube through the cell envelope into the cytoplasm. The viral DNA is released directly into the cytoplasm through the tube. Some animal viruses (e.g., poliovirus) may also leave their nucleocapsid coat at the membrane releasing only the genome into the cell.

D. Uncoating

Irrespective of its mode of entry the virus or virus core must localize in the correct intracellular compartment (nucleus or cytoplasm) and has to be "uncoated" (freed from at least some structural virion components) for gene expression. In general, the genome of a DNA virus replicates in the nucleus whereas that of a RNA virus usually replicates in the cytoplasm. There are, of course, exceptions. The DNA poxviruses and iridoviruses are uncoated and remain in the cytoplasm, whereas the RNA orthomyxoviruses (influenza) replicate in the nucleus (Lamb and Choppin, 1983). The genomes of bacterial viruses are uncoated as a consequence of penetration and they remain either attached to the plasma membrane or reside within the protoplasm.

For viruses which undergo a part of their replication in the nucleus, the genome has to be targeted for the nucleus. The viral core (containing the viral DNA genome) is released from the virion in the cytoplasm and either enters the nucleus, presumably through nuclear pores, or, as has been postulated for adenoviruses, the core empties the viral DNA into a nuclear pocket while the rest of the core remains outside the nucleus (Morgan *et al.*, 1969).

A rather interesting mechanism of uncoating has been described for two families of insect DNA viruses: the baculoviruses and the polydnaviruses. For some

of these viruses the helical nucleocapsid orients and moves toward the nucleus (presumably directed by cytoskeletal components) where it attaches at right angles to the nuclear pore by means of a tail-like structure penetrating the pore. Uncoating ensues and viral DNA appears to be injected into the nucleus leaving the nucleocapsid behind (Stoltz and Vinson, 1979). It therefore appears that some eukaryotic viruses have evolved (by convergent evolution?) a method of uncoating directly into the nucleus which is analogous to that used by some bacterial viruses to uncoat into a prokaryotic host.

Uncoating of reoviruses is also somewhat unusual. The virion appears to penetrate by phagocytosis and is partially uncoated within lysosomes. The resultant subviral particle (SVP) containing the viral core of 10 segments of dsRNA and the inner capsid is then released intact into the cytoplasm. The 10 segments remain associated with this core throughout the replication cycle.

An even more complex process occurs with poxviruses which require a newly synthesized viral protein for uncoating (Wittek, 1982; Joklik and Becker, 1964). The first stage of uncoating is initiated by host enzymes which degrade the viral membrane and some of the protein surrounding the nucleoprotein core. The DNA of this partially uncoated core is then partially transcribed (25%). Among the "early" transcripts is one for a special viral "uncoating protein" which continues the uncoating process until the final release and expression of poxvirus DNA.

E. Genome Expression and Replication

The viral genome, once uncoated and in the appropriate intracellular compartment, can be expressed and replicated; the appropriate genes are transcribed to viral mRNAs (which are then used to translate viral polypeptides) and copies of the genome are replicated. Two alternative life cycles could be adopted at this stage. In one, the genetic information of certain viruses is integrated into host DNA, producing the temperate (or lysogenic) state where no phage (as for bacteriophage λ) are produced or (in retrovirus replication) where the provirus is integrated with host DNA (transformation) but has concomitant virus production (Nash, 1981; Norkin, 1982; Friedman *et al.*, 1984). In the second, a lytic (or virulent) state ensues in which progeny virions are produced and released from infected cells. This is often referred to as a productive infection and this is the life cycle that will be discussed below.

There is a great diversity in the mechanisms used for virus genome expression and replication. This diversity relates in part to the different types of genomes; type of nucleic acid (RNA or DNA), strandedness (ss or ds) and conformation (circular, or linear), and physical organization (single or segmented genomes). The mechanisms used also depend on the cellular compartment in which the viral genome resides and on the genetic organization of the viral genome.

It is difficult to generalize this aspect of the virus replication cycle. Virus genome expression and replication of DNA viruses will be discussed under headings which reflect the type, conformation, and genetic organization of the different genomes.

1. *DNA Viruses*

The DNA viruses can be organized into four major groups: those with a small circular dsDNA (e.g., papovaviruses), those with linear dsDNA (e.g., adenoviruses and herpesvirus), those with ssDNA (e.g., parvoviruses and some filamentous bacterial viruses), and those which replicate in the cytoplasm (e.g., poxvirus). Genome expression and replication can generally be divided into 3 phases: early transcription and translation (prior to DNA replication), onset of DNA replication, and late transcription and translation.

a. *Supercoiled dsDNA Viruses.* Papovaviruses such as SV40, which was isolated from monkey kidney cell cultures and which can cause tumors in hamsters, are probably the easiest DNA viruses to understand with respect to gene expression and replication. The genetically conservative circular genome with only four (some overlapping) genes coding for at least five polypeptides is one of the smallest dsDNA-containing viruses known. While the viral genome can become integrated, often resulting in cell transformation and tumor induction (Norkin, 1982), they also have a productive life cycle in permissive cells. Although five mRNAs are ultimately transcribed, some of the mRNA transcripts are from overlapping regions of the viral DNA. Transcription is regulated, as with most DNA viruses, into "early" and "late" phases, separated by the onset of DNA replication (Hauswirth and Berns, 1979; Challberg and Kelly, 1982; Keller and Alwine, 1984). There are two early transcripts from SV40 gene A which are required for DNA synthesis. They are of different sizes but have common leader sequences and other overlapping sequences (the result of the splicing of larger primary transcripts). The first early transcript (2230 kb) codes for "large T" protein which binds to the virus origin of replication on the supercoiled SV40 DNA and appears to be needed for initiation of viral DNA replication (Hutchinson *et al.*, 1978). The second early transcript (2500 kb) codes for "small t" protein which may lead to cell transformation. The intranuclear viral DNA, although supercoiled (26 negative supercoils per molecule), is similar to host chromosomal DNA in that it becomes associated with host cell histones (Shelton *et al.*, 1978) and is organized into typical nucleosomes (24 nucleosomes per molecule). The early viral polypeptides stimulate the synthesis of cellular DNA, histones, and enzymes involved with DNA synthesis. The cells thus will have all the precursors needed for the replication of viral DNA. Replication of SV40 DNA is similar to that of plasmid DNA in bacteria (Hauswirth and Bern, 1979; Challberg and Kelly, 1982). DNA replication is initiated by large T and a host DNA polymerase, and replication proceeds bidirectionally

until the supercoiled daughter molecules are separated. The two late genes are expressed after the initiation of DNA replication. Three virion polypeptides (VP1, VP2, and VP3) are derived from two primary transcripts. One of two transcripts from one late gene has the same nucleotide sequence but is two-thirds the size of the other transcript. The shorter one codes for VP3, while the longer one codes for the related VP2. The VP1 transcript is from the second late gene, and although the primary transcript is the same sequence as that for VP2 and VP3, it is spliced so that the VP1 transcript is initiated out of phase with VP2 and VP3 transcripts. These transcripts and their translation products continue to be synthesized as viral DNA replication proceeds.

b. *Linear dsDNA Nuclear Viruses.* Viruses, like adenovirus and herpesvirus with larger linear duplex genomes (35 kb and 150 kb, respectively), have more complex mechanisms for gene expression and genome replication. The genome is uncoated in the nucleus and their transcription is divided into early (subdivided into immediate early and delayed early) and late phases separated by the onset of viral DNA replication. Early transcription (and translation) is required to provide the enzymes and substrates needed for viral DNA replication, while late transcripts are required for synthesis of virion proteins, progeny virus assembly, and virus release.

In adenovirus-infected cells, host RNA polymerase II (responsible for transcription of host mRNA genes) transcribes the five early genes on the adenovirus DNA (Logan and Shenk, 1982). Both strands of viral dsDNA (13% of one and 14% of the other) at both ends of the genome are used as templates for the synthesis of the five early transcripts. The primary transcripts are processed in the nucleus and are then translated (in the cytoplasm) into five virus proteins including an ssDNA binding protein, a DNA polymerase, and a VPg (all needed for viral DNA replication), and E1A protein (which regulates the temporal order of transcription of the early genes).

The adenovirus genome is about 35 kb long, has terminally repeated inverted sequences (of more than 100 base pairs), and has a virion protein (VPg) covalently attached to the DNA at the 5′ end of both strands (Lechner and Kelly, 1977; Desiderio and Kelly, 1981; Challberg *et al.*, 1980; Ostrove *et al.*, 1983). DNA replication can initiate at either end. In order for replication to proceed from one end, the strand containing the 5′ end (with the VPg) must be displaced to expose the 3′ end of the template strand. A primer for synthesis of the daughter strand from this template is also required. This is provided by a soluble VPg precursor (a product of the early genes) containing a cytidine nucleotide (Mitra, 1980; Challberg and Kelly, 1982). This complex attaches to the 3′ terminal guanine nucleotide partly by complementary GC base pairing and provides a terminal 3′ hydroxyl site for priming. Replication then proceeds by displacing the opposite strand and a viral DNA polymerase utilizes the nondisplaced parental strand as template (Ostrove *et al.*, 1983). The ssDNA binding protein functions in strand

displacement and probably protects the exposed ss regions from nucleases. The DNA polymerase probably displaces the ssDNA binding protein from the template strand as DNA synthesis continues. After synthesis the progeny DNA strand is the exact length of the parental template strand and can be used for another round of replication.

The late phase of genome expression occurs after the onset of virus DNA replication and is dependent on synthesis of adenovirus DNA (Schneider *et al.*, 1984). Twelve of the 13 late transcripts arise from one parental strand from an internal region of about 80% of the genome length whereas the other transcript is derived from the opposite strand. All primary transcripts are processed (splicing, capping, polyadenylation) in the nucleus. The primary late transcripts can be placed into five groups. All the transcripts in one group have coterminal (same nucleotide sequence) 3′ ends and identical nontranslated leader sequences at the 5′ end. They are of similar length and represent two or more contiguous genes. The transcripts of each group are processed (i.e., spliced; Nevins and Chen-Kiang, 1981) to contain a common 5′ leader sequence (the same in all transcripts of one group). The transcripts are further processed to contain sequences representing only one of the 3 or 4 contiguous genes. Although some primary transcripts contain RNA for more than one gene, the processing removes all but the RNA for one of the genes (in some primary transcripts a given sequence may be an exon which is spliced to the leader seqeunce, whereas in another primary transcript, that same sequence could be an intron and will be removed). In some cases a mature transcript is produced with contiguous sequences for two genes but during translation only the 5′ proximal sequence is translated.

The late transcripts are further processed by "capping" of the 5′ end with 7 methyl Gppps. This cap is identical to that used by host mRNAs, and is probably added by host enzymes. This 5′ cap allows the viral mRNA to attach and be translated by cellular ribosomes. Further processing of the transcript includes polyadenylation of the 3′ end. Adenosine nucleotides are added sequentially to the 3′ end of the transcripts (up to 200 per end) by a host nuclear poly(A) polymerase. The primary viral transcripts are thus processed (splicing out of introns, 5′ capping and 3′ polyadenylation) by the same mechanisms and enzymes the host uses for processing many ot its own mRNA transcripts. The viral DNA has evolved to have the correct sequences properly located to fool the cell's transcription and processing machinery which allows expression of the viral genome. When these viral transcripts reach the cytoplasm they "look" just like host mRNAs and are translated as such. The polypeptides, translated from these late transcripts, are largely the structural virion polypeptides which are needed for virus assembly and release (Cepko and Sharp, 1982). In addition to synthesis of viral mRNA, adenovirus synthesizes small (160 bases) RNAs late in infection which appear to have a role in controlling translation of viral mRNAs (Schneider *et al.*, 1984).

c. *ssDNA Viruses.* The parvovirus ssDNA genome has sequences at both the 3′ and 5′ end which form terminal hairpins (duplex regions). The genome is of limited complexity (2.2 kb) and codes for the 3 virion structural proteins. The replication of parvovirus DNA is cell cycle dependent and occurs during the S phase of cell division (Challberg and Kelly, 1982; Mitra, 1980). Because of its limited genome size, the parvovirus is completely dependent on host cell DNA replication enzymes and nucleotides (which are at high levels only during S phase). Several models, too complex (and hypothetical) to discuss here, have been proposed to account for parvovirus genome replication. They suggest that the hairpin duplex at the 3′ end provides the primer (rather than using RNA or VPg) for template-directed DNA synthesis. All models suggest a series of foldings (to provide a primer) and unfolding (to provide more template) of both the original parental strand and the newly synthesized daughter strand. Eventually, ds, replicative form (RF), twice the length of the native ss form, is produced (Berns, 1984). Unit length ss parvovirus DNAs appear to be removed from these RFs by a specific endonucleolytic cleavage during encapsidation. The nonencapsidated RF is used as template to make more RF. After several cycles of parvovirus DNA replication, host RNA polymerase II transcribes the (−)strand DNA to form primary transcripts. The three cytoplasmic transcripts are generated by differential splicing of a single class of primary transcripts. The mature transcripts may share some identical sequences and are translated into virion polypeptides (VP1, VP2, and VP3) which share some amino acid sequence homology. Although little research has been done on parvoviruses in the past, now that a human parvovirus has been identified (Cotmore and Tattersall, 1984), more detailed analysis of the infection cycle of parvoviruses will surely be forthcoming.

d. *dsDNA Cytoplasmic Viruses.* Poxviruses (and iridoviruses) are DNA viruses which replicate entirely within the cytoplasm and therefore code for many of their own DNA metabolizing enzymes. For this reason they have, by necessity, one of the largest viral genomes known (180 kb). Essentially, poxviruses have to construct their own "micronuclei" (Moss, 1974) within the cytoplasm to provide the enzymatic and structural components required for DNA replication.

The poxvirus genome is partially expressed during uncoating by a virion RNA polymerase which transcribes 25% of the viral DNA. The transcripts are processed by virion enzymes within the core to produce the "immediate early" mRNAs (Dales and Pogo, 1982). One of the early translation products is an "uncoating" protein which liberates the rest of the DNA from the core. Synthesis of the "delayed early" transcripts then proceeds culminating in the synthesis of some viral enzymes (e.g., thymidine kindase and DNA polymerase) which are required for nucleotide metabolism, DNA synthesis, and transcript processing.

Once the virus DNA synthesizing enzymes are available, poxvirus DNA rep-

lication can begin. Although the mechanism of replication is unknown, electron microscopy has revealed some putative replication intermediates (RI). Of particular interest are the ends of the parental DNA which are cross-linked by oligonucleotides (Baroudy *et al.*, 1982) suggesting that poxvirus DNA replicates in a circular form. However, other models of poxvirus DNA replication exist to account for electron microscopic and isotope labeling studies of concatameric forms of presumably replicating viral DNA (Pogo *et al.*, 1984).

After initiation of DNA replication, the "late" mRNA transcripts appear. Although the products of most of these late transcripts are virion proteins, one of the late gene products further prevents translation of early viral mRNAs even though they continue to be transcribed and processed. Poxviruses are thus unique since they control gene expression at the level of translation (other viruses usually control gene expression at the level of transcription).

2. *RNA Viruses*

All RNA viruses have a unique replication problem; they must replicate and be transcribed in a cellular environment which uses RNA derived exclusively from transcription of DNA, for translation. The host contains only DNA-dependent RNA polymerases. RNA-dependent RNA polymerases have been detected only in some plants and in these only at very low levels. All RNA viruses must, therefore, code for their own RNA-dependent RNA polymerase which acts as a transcriptase for virus mRNA synthesis or as a replicase which uses an RNA template to synthesize progeny RNAs. The one exception is the RNA tumor virus (retrovirus) which codes for an RNA-dependent DNA polymerase (reverse transcriptase) to make a DNA copy of the RNA template. This DNA copy can then act as a template for RNA transcription using the DNA-dependent RNA polymerases of the host (Palese and Roizman, 1980).

As with DNA viruses, there is a tremendous diversity in the strategies for gene expression and replication of RNA viruses (Reanney, 1982). At its simplest level, the overall strategy depends on the strandedness (ss or ds), sense [(+) or (−) of ss viral RNA] and the structural organization of the viral RNA (as single molecules, as a segmented ss genome or as a segmented ds genome). A problem with eukaryotic RNA viruses is that eukaryotic systems use only monocistronic mRNA (with a single ribosome binding site) for translation. For RNA viruses with several genes (usually fewer than 10), different mechanisms have evolved to accommodate this problem. For picornaviruses, translation from a monocistronic mRNA results in a "multigene" giant peptide which is subsequently cleaved. In (−)RNA viruses transcription of subgenomic monocistronic mRNAs initiates in regions equivalent to promoters on a larger (−)RNA template. In reoviruses and orthomyxoviruses the genome is organized into single-gene-sized segments.

Genome expression and replication of only four groups of RNA viruses differ-

ing in genome organization will be discussed here. This should help exemplify the inherent diversity of strategies which RNA viruses use to circumvent similar problems of gene expression and replication. The four groups are (1) those with a large monocistronic (+)RNA genome, (2) those with a multigenic (+)RNA genome, (3) those with a (−)RNA genome, and (4) those with a segmented, dsRNA genome. As in the earlier discussion, genome expression will be discussed at the level of transcription (and possible processing) of viral mRNA from a genomic RNA template.

a. *(+)RNA Viruses.* Two families of eukaryotic RNA viruses with an ss "infectious" (+)RNA genome are the picornaviruses and togaviruses. [The retrovirus genome is also (+)RNA, but is not infectious and will be discussed separately below.] Purified virion (+)RNA when introduced into cells by transfection is translated directly into a large polypeptide. The virion RNA is a multigenic but monocistronic (only one site for initiation of translation) mRNA. The 5′ and 3′ ends of viral (+)RNAs are often modified to mimic host mRNAs. Although the monocistronic RNAs of most eukaryotic (+)RNA viruses have a 5′ methylated cap and a 3′ poly(A) tail, other variations exist. Several plant picornaviruses (phytopicornaviruses; Joshi and Haenni, 1984) possess a 3′ CCA terminal sequence which can be charged with an amino acid (just like tRNA). The (+)RNA of other plant viruses have neither a 5′ cap nor a 3′ poly(A) tail. The (+)RNA of one group of phytopicornaviruses and of all animal picornaviruses has a VPg covalently linked to the 5′ end. Since these genomes are generally small (3 kb for phytopicornaviruses, 7 kb for animal picornaviruses and calicyviruses, 12 kb for togaviruses, and 20 kb for cornaviruses), the overall genetic complexity is limited to only a few genes. One gene codes for an RNA-dependent RNA polymerase and the others code for virion structural polypeptides and some enzymes (cleavage enzymes). The following comments apply only to picornaviruses, but the general strategy for gene expression and replication is similar to that of all (+)RNA viruses.

The (+)RNA of poliovirus (a picornavirus) is 7433 nucleotides long, has a VPg at the 5′ end, and a poly(A) at the 3′ end (Kitamura *et al.*, 1981; Dasgupta, 1983). The 5′ VPg is cleaved off and the RNA is translated immediately into a polygenic precursor polypeptide (Racaniello and Baltimore, 1981). One product of processing of this polygenic precursor polypeptide is the virus RNA-dependent RNA polymerase (or replicase; Flanegan and Baltimore, 1979; Hanecak *et al.*, 1982; Jacobson and Baltimore, 1968). Since the replicase is essential for genome replication, virion RNA translation has to precede virus RNA replication. Genome replication occurs within intracytoplasmic complexes which contain partially ds replicative intermediates (RIs). An RI contains a full length (+)RNA and several partial length (−)RNAs. The newly synthesized poliovirus VPg precursor is embedded in cytoplasmic membranes and is coupled to poly(U) which base pairs with the poly(A) of the (+) template 3′ end; replication begins

after VPg is cleaved from its precursor [the 5′ end of (−)RNA is a poly(U) copy of the 3′ poly(A) tail of the template]. Replication of this progeny RNA strand continues along the parental template. A second round of replication initiates on the (+)RNA template before replication of the first (−)RNA is complete. This second (−)RNA also uses VPg as a primer and the same (+)RNA as template. Several rounds of replication can be initiated with different VPg primers at the 3′ end of the parental strand before the first transcript has been completed and freed of the parental (+) strand. This RI has a core genome-length parental (+)RNA to which is attached (via RNA replicase), at various sites along its length, varying lengths of partially replicated (−)RNA. After several hundred (−)RNAs have been synthesized, they in turn act as templates for new RIs during the synthesis of virion (+)RNA (Racaniello and Baltimore, 1981). Only virus (+)RNAs which have the VPg at the 5′ end removed are used as mRNAs for the synthesis of more polygenic virus protein (and consequent processing to more RNA replicase, VPg, and other viral polypeptides; Jacobson and Baltimore, 1968). The (+)RNAs which retain the VPg are destined for encapsidation into viral procapsids or continue to serve as templates for RNA synthesis.

There is one notable variation in the production of virus mRNA from RIs. Togavirus virion RNA has a 5′ cap (no VPg) and a 3′ poly(A) tail. Two sizes of RNA are transcribed, one a full-length virion RNA which could be used as mRNA or virion RNA, and another, shorter (+)RNA equivalent to one-third of the 3′ end of the (+)RNA. Synthesis of the shorter RNA is initiated internally at a site two-thirds of the way from the 3′ end of the full-length (−) strand. These shorter (+)RNAs are not encapsidated but are synthesized in high amounts and are used as mRNA. Since these RNAs code for some virion polypeptides, this process allows for high levels of the virus structural polypeptides which are needed in greater amounts than others (e.g., RNA replicase and other enzymes).

Although retroviruses also have (+)RNA genomes (two molecules per virion), they have a completely different life cycle (Varmus, 1984; Bishop, 1983). After uncoating, a virion-associated, RNA-dependent DNA polymerase (i.e., reverse transcriptase) begins to synthesize DNA from the RNA template (using a cell tRNA as primer). This polymerase also has RNase H activity which hydrolyzes the original RNA template from the RNA/DNA duplex. The copy ssDNA is now a template for "second strand" DNA synthesis, also performed by the polymerase. As a consequence of complex DNA rearrangements, mediated by terminal complementary redundant sequences (copied from virion RNAs), the ds linear viral DNA circularizes, moves to the nucleus, and integrates into host DNA. There it resides as a provirus. The proviral DNA is replicated during normal cell division such that at least one copy is present in all progeny cells. Once activated, the proviral DNA is used as a template by host RNA polymerase to synthesize full-length viral (+)RNA transcripts which are subsequently capped and polyadenylated. After transport to the cytoplasm, they are translated

into viral proteins and encapsidated. A shorter transcript results by further processing of these primary transcripts in the nucleus. In some retroviruses these shorter viral mRNAs code for the virion envelope glycoprotein. The overall mechanism of retrovirus genome expression and replication utilizes a reverse transcribed, integrated, proviral DNA as a template for both virion RNA and viral mRNAs. Of interest is the observation that some retroviruses (as well as some adenoviruses, herpesviruses, and papovaviruses) cause neoplasias (tumors) in many animal species (Kriegler *et al.*, 1984; Varmus, 1984). However, this is a complex topic, beyond the scope of this chapter, and will not be dealt with further.

b. *(−)RNA Viruses.* The RNAs of (−)RNA viruses are "noninfectious" and cannot be translated. They also require a viral RNA-dependent RNA polymerase for RNA replication. Since they cannot synthesize this enzyme from their (−)RNA template [in contrast to the (+)RNA viruses], the RNA replicase is virion associated and interacts with virion RNA during uncoating.

There are two major groups of (−)RNA viruses: those with a single multigenic genome and those with a segmented genome. Although some similarities in genome expression exist, they are sufficiently different to warrant separate discussions.

Influenza virus, an orthomyxovirus, has a segmented, ss (−)RNA genome (Lamb and Choppin, 1983). There are eight segments of RNA; each is associated with its own helical nucleocapsid but they are collectively enveloped in one virion. All virion RNAs have similar 5′ ends (a sequence of 13 nucleotides ending in Appp) and a highly conserved 3′ end of 13 nucleotides. Each RNA codes for a different polypeptide (sometimes in precursor form) including the RNA transcriptase. Uncoating of the virion, unlike that of other RNA viruses, occurs in the nucleus. The core remains intact as a transcription unit with the virion RNA transcriptase.

Essentially two types of transcripts are generated, mRNAs for translation of virion polypeptides, and cRNA (c for copy) to be used as template for synthesis of virion (−)RNA. Virus mRNA synthesis occurs first and results from incomplete transcription of the 8 parental (−)RNAs (the 3′ terminal 17 nucleotides are not copied), using a host nuclear RNA oligonucleotide as a primer. Several transcripts may be simultaneously transcribed from a single parental template as described for poliovirus transcription. The 8 mRNA species are capped by a mechanism in which 5′ caps (including 10 to 15 of the 5′ terminal nucleotides) are cannibalized from host mRNAs and spliced onto the 5′ end of the nascent viral mRNAs (Breidis *et al.*, 1982). These are then polyadenylated and move to the cytoplasm to be translated. Influenza viruses are therefore dependent on continued host mRNA synthesis for two reasons: (1) so that host RNAs can be used as primers for viral mRNA transcription, and (2) so that 5′ caps are available for viral mRNAs. The second class of transcripts (cRNA) is also synthesized

from the (−)RNAs in the transcription complex, and is dependent on some of the proteins translated from the viral mRNAs. These proteins modify the RNA transcriptase so that it can copy the complete parental (−)RNA. These RNAs are not capped nor polyadenylated, but remain in the nucleus to serve as templates for the synthesis of (−) virion RNA. The eight different virion RNAs are synthesized on RIs as described earlier for picornavirus RNAs. The progeny virion (−)RNAs remain in the nucleus where they become encapsidated in helical nucleocapsids.

Most (−)RNA viruses, unlike the orthomyoviruses described before, have a nonsegmented polygenic genome. The virus families included in this group are the Paramyxoviridae and Rhabdoviridae. These viruses uncoat in the cytoplasm, releasing a core transcription complex of RNA transcriptase, virion (−)RNA, and other associated proteins. Transcription of rhabdovirus RNA from a promoter at the 3′ end of the template (−)RNA using a virion transcriptase results in the synthesis of a full-length template RNA and five smaller subgenomic mRNAs (six in paramyoxvirus-infected cells). Since the parental RNA has only one "promoter," it is difficult to rationalize the generation of five subgenomic mRNAs from five different but contiguous regions of the parental virion (−)RNA (Banerjee *et al.*, 1977). One model suggests that the RNA transcriptase initiates at a 3′ end, transcribes a 47-base leader RNA, and continues to a poly(U) termination signal (adjacent to a second initiation signal), terminates (releasing the leader and the first subgenomic mRNA), and reinitiates transcription at the second initiation site. A sequential series of initiation, transcription, and termination steps results in the generation of all five primary transcripts. These short transcripts are capped and polyadenylated by viral enzymes and serve as mRNAs for virus polypeptide synthesis (Banerjee, 1980). Any full-length, (+)RNA transcripts that arise are used as templates for the synthesis of full-length virion (−)RNAs. A newly synthesized viral RNA replicase (different from the virion RNA transcriptase) may be needed for the generation of progeny virion (−)RNAs from this template. Virion RNA replication occurs on characteristic RIs. The progeny full-length (−)RNAs subsequently became encapsidated by nucleocapsid proteins.

c. *Segmented dsRNA Viruses.* There are only three groups of dsRNA viruses, the reoviruses and birnaviruses (Dobos *et al.*, 1979) of eukaryotes and cystoviruses of prokaryotes. Only the reoviruses will be discussed here. The reovirus gene is organized in a complex of 10 (or 11) separate dsRNAs; each RNA represents a different gene (Fields and Greene, 1982; Joklik, 1981). After the reovirus virion is partially uncoated in the lysosome (the outer of the two concentric capsids is removed) it is released into the cytoplasm as a "subviral particle" containing all 10 dsRNAs, the RNA transcriptase and other associated viral proteins. Each dsRNA is simultaneously used for synthesis of full-length virus mRNAs by the virion RNA transcriptase (Yamakawa *et al.*, 1982). These

mRNAs are capped by complexes containing four viral enzymes associated with the subviral particle, and appear to be extruded through pores in the subviral particles. All parental RNA strands remain in a duplex form in the subviral particle for continuous synthesis of viral mRNAs and only the (−)RNA strand is transcribed.

Once sufficient viral mRNA molecules are synthesized, and sufficient viral polypeptides are translated and accumulated, these components aggregate in the cytoplasm to form the equivalent of subviral particles initially containing one copy of (+)RNA for each of the 10 segments. Each (+)RNA of the complex is "back transcribed" to the dsRNA form by virus replicase proteins. The newly synthesized (−)RNA strand remains associated with the (+)RNA as virion dsRNA. In reoviruses, RNA replication is conservative [i.e., only the (−) strand of the duplex RNA is copied, and serves as template for synthesis of the complementary virion (+)RNA and both parental strands remain associated during replication]. This is in contrast to semiconservative replication of duplex DNA in which both strands of the duplex are simultaneously used as template and the two parental strands end up in different progeny dsDNAs. The newly formed replication complexes act as subviral particles and synthesize more viral mRNAs which are translated into more virion polypeptides and form yet more replication complexes.

3. *Virus Protein Synthesis*

Virus-specific translation is directed by viral mRNA. The major translation products are virion polypeptides (structural and enzymatic) directly associated with mature extracellular virions, and nonvirion polypeptides (usually enzymatic) which are intracellular viral gene products transiently involved in some aspect of virus replication (sometimes referred to as ICP, infected cell proteins or NSVP, nonstructural viral polypeptides). Viral mRNAs can be transcribed from either DNA or RNA templates as described earlier. Often the viral mRNAs of eukaryotic origin are processed either completely or partially by 5′ capping (e.g., with 7-methyl Gppp), 3′ polyadenylation, internal methylation, and splicing out of introns. The processing enzymes can be of host or viral origin. These processed viral mRNAs are often indistinguishable from host mRNAs and are readily translated by host ribosomes (Banerjee, 1980).

Although viral protein synthesis is often regulated by primary transcription, regulation at the posttranscriptional, translational, or posttranslational level also occurs. The temporal order of virus protein synthesis in poxvirus- and adenovirus-infected cells is regulated by transcription from early and late viral genes. Regulation of expression at the posttranscriptional level may apply in part to the herpesviruses which continuously synthesize primary transcripts from most of its genome (Honess and Roizman, 1974). Here two immediate early proteins are required for processing of primary transcripts of the "delayed early" genes. The delayed early proteins, in turn, inhibit translation of the immediate

early proteins (a form of feedback inhibition at the genetic level) and are also required for processing of late transcripts. Herpesviruses are good examples of coordinate regulation of expression since they control both processing and translation of viral mRNAs in a temporal manner.

There appears to be little temporal regulation of virus protein synthesis in cells infected with RNA viruses. However, the levels of different virus proteins may vary radically. Since virion structural proteins are generally required in greater amounts than nonstructural (enzymatic) viral proteins, RNA viruses have evolved several mechanisms to regulate the respective levels. Regulation can be at the level of promoter affinity to RNA transcriptase. This dictates the rate of initiation of transcription of different genes (RNA or DNA) from different promoters. In the case of polygenic (−)RNA viruses, the order of the genes on the virion template is important. Genes at the 3′ end are transcribed more often than those at the 5′ end. Transcription always initiates at the 3′ proximal initiation site. It is only after the complete transcription of the 3′ proximal genes that the RNA transcriptase can transcribe the next downstream (5′ proximal) gene, etc. The RNA transcriptase can dissociate from the template before it reaches some of the downstream 5′ proximal initiation sites. Consequently, transcripts in the 5′ proximal direction are synthesized at progressively lower levels. The number of copies of a given polypeptide translated (which depends on the number of mRNA molecules available) therefore depends on the relative positions of the genes. The genes for structural virion polypeptides are therefore often located at the 3′ end of the (−)RNA template while genes for viral enzymes are located at the 5′ end.

Posttranslational modifications accompany the maturation of many viral proteins. As an example, the large polyprotein translated from the mRNA of poliovirus contains the contiguous amino acid sequences for the 7 mature (and independent) viral polypeptides. After a cascade of autolytic cleavages, seven poliovirus polypeptides are freed from each other (Hanecak *et al.* 1982). Other modifications can also occur, most notably, the glycosylation of membrane-specific viral polypeptides. This normally occurs in endoplasmic reticulum and Golgi membranes (Braell *et al.*, 1984; Burke and Warren, 1984; Matlin and Simons, 1983). In addition, certain viral enzymes are often modified by phosphorylation or methylation. These posttranslational modifications are often, but not exclusively, mediated by viral enzymes (Laver and Valentine, 1969; Caton *et al.*, 1982; Colman *et al.*, 1983; Varghese *et al.*, 1983).

The requirement for these types of modifications affords viruses with other levels with which to regulate virus replication.

V. Virus Morphogenesis

Toward the latter stages of infection, cellular metabolism is almost exclusively directed toward synthesis of virion polypeptides which are involved in virus

morphogenesis (the assembly of a mature viral form from pools of viral nucleic acids, protein and virion-specific membranes). The "instructions" for this morphogenesis are encoded in the inherent structural (size and shape) and chemical (charged groups, hydrophobic regions) characteristics of the component parts.

Although virus maturation can be considered a self-assembly process, it usually proceeds in a cascade of steps in which certain structures (e.g., procapsids and scaffolding) form first, thereby allowing the interaction of other viral components. The number and complexity of morphogenetic steps depend to a large degree on the morphology of the virus. Clearly viruses with naked (i.e., nonenveloped) nucleocapsids mature differently from viruses which need to acquire a membrane envelope. Virus maturation will be discussed in terms of the different morphological types of viruses.

A. Nucleocapsids and Nonenveloped Viruses

1. *Helical Nucleocapsids*

The simplest structure of any virus is that of the helical nucleocapsid and the best examples of this are rod-shaped plant RNA viruses. The assembly of nucleocapsid protein and virion RNA is coordinated and sequential; RNA and polypeptides (from the pool of virion precursors) attach to one another by single step kinetics. Several interactions which dictate the ultimate shape of the nucleocapsids come into play. The RNA fits into a groove on each capsid protein and is stabilized by ionic interactions. The capsid proteins are wedge-shaped with the narrow end of the wedge associated with the RNA so that the growing structure aggregates into a cylindrical configuration. Side to side interactions between the proteins stabilize this configuration. There are also fairly strong interactions between capsid proteins in adjacent layers. The final structure is a string of capsomers arranged in a shallow helix and held together by RNA; this is the thermodynamically most favorable configuration and accommodates the structural and chemical interactions of all component parts. The length of the helical nucleocapsid is directly related to the length of the viral nucleic acid. The diameter of the rod, however, is dictated largely by the shape and chemical interactions between adjacent capsid proteins.

As one example, the assembly of the helical nucleocapsid of tobacco mosaic virus (TMV) occurs in a complex sequence of steps (Hirth and Richards, 1981). The initiation complex for assembly is an aggregate (generated by self assembly) of 33 capsid monomers in a double disk. An assembly initiation site on the ssRNA, several hundred nucleotides from the 3′ end, attaches to this disc. The rod grows by aggregation of capsid monomers or aggregates of three or four monomers with the RNA in a helical fashion. As assembly proceeds, the RNA is pulled through the hollow core in a 3′ to 5′ direction. As the RNA interacts with

the protein monomers (three nucleotides binding to three basic amino acids in the groove of each monomer), the 5′ end is threaded through the axial channel, leaving the 5′ free end emerging at the initial site of aggregation and an RNA loop at the active site of aggregation and elongation. The final structure is the rigid TMV helical nucleocapsid with a diameter of 15 nm, a length of 300 nm, and a pitch of 2.3 nm.

2. *Icosahedral Nucleocapsids*

The size (and organization) of icosahedral nucleocapsids is dependent solely on the characteristics of the polypeptides making up the capsomers (hexons and pentons; Putnak and Phillips, 1981). Some of the smallest icosahedral nucleocapsids have 12 pentons and 20 hexons (e.g., parvoviruses, diameter 20 nm). The nucleocapsid of the bacteriophage, ϕX174, is the smallest icosahedral virus and is unusual in that it consists solely of 12 pentons. Larger nucleocapsids also have 12 pentons but have more than 20 hexons. Papovaviruses (diameter 45–55 nm) have 60 hexons, adenoviruses (diameter 70–90 nm) have 240 hexons, and the large herpesvirus icosahedral nucleocapsid (diameter 100–110 nm) has 150 hexons. The size of the capsomers can also vary. Although herpesvirus nucleocapsids are larger then adenoviruses, they have fewer but larger hexons. The assembly of the icosahedral nucleocapsids of some icosahedral RNA viruses occurs by coaggregation of the viral RNA and capsomers. The hexons and pentons are probably assembled into a hollow capsid from their respective precursors prior to nucleocapsid assembly. The viral nucleic acid interacts with this procapsid and becomes internalized. Internalization is specific between the virion nucleic acid and its corresponding procapsid and is often accompanied by processing (precursor cleavage) of procapsid polypeptides into virion polypeptides. Coincident with the internalization of picornavirus (+)RNA into picornavirus procapsids is the cleavage of the procapsid polypeptide VP to the mature virion VP2 and VP4 polypeptides (Putnak and Phillips, 1981; Hanecak *et al.*, 1982). Procapsid polypeptides pVI and pVIII (p for precursor) of adenovirus are cleaved into the smaller virion polypeptides VI and VIII during their assembly (Cepko and Sharp, 1982). Also, during the morphogenesis of adenoviruses, the VPg 85K precursor associated with the viral DNA is cleaved to a 55K VPg and becomes specifically associated with procapsid polypeptides (Desiderio and Kelly, 1981). The proteolytic cleavages accompanying morphogenesis may be a mechanism for providing the energy needed for internalization of the viral genome.

B. Enveloped Viruses

The nucleocapsids (helical or icosahedral) of enveloped viruses are assembled essentially as described above. Nucleocapsids of cytoplasmic viruses (e.g., para-

myxoviruses, togaviruses) are assembled in cytoplasmic viroplasmic centers (where virion polypeptides and nucleic acids are synthesized and accumulated). Morphogenesis of nuclear viruses requires the transport of viral polypeptides from the cytoplasm (where they are synthesized on host polysomes) to the site of assembly (viroplasm) in the nucleus. Once all virion components reach a critical concentration, morphogenesis of the nucleocapsid begins.

The membranes of enveloped viruses are derived either from existing cellular membranes (plasma or nuclear) that have been modified by viral activity or are synthesized *de novo* without utilizing preexisting membranes.

The membranes of viruses that bud through the plasma membrane (e.g., orthomyxoviruses, paramyxoviruses, and rhabdoviruses) are modified by the insertion and processing of viral polypeptides in cellular membranes (Lamb and Choppin, 1983; Caton *et al.*, 1982; Matlin and Simons, 1983; Varghese *et al.*, 1983; Colman *et al.*, 1983; Laver and Valentine, 1969; Rose and Gallione, 1981; Braell *et al.*, 1984; Fuller *et al.*, 1984; Saraste and Kuismanen, 1984). Viral membrane polypeptides are synthesized on the cellular endoplasmic reticulum (ER) by host membrane-bound polysomes (containing viral mRNA). Many of these viral polypeptides are processed (e.g., glycosylated) in the ER and Golgi apparatus, usually by host enzymes, in a manner similar to that for host membrane glycoproteins. Although viruses cannot metabolize their own lipids (poxvirus may be an exception), they can select the lipids they need from among those present in the membranes. This could occur in conjunction with the placement of viral membrane proteins. The envelopes of two different influenza viruses (A and B) replicating in the same cell type can have different proportions of various lipids. As membrane morphogenesis proceeds the modified membrane becomes continuous with the plasma membrane.

Some enveloped viruses such as the rhabdoviruses and paramyxoviruses direct the establishment of a layer of viral matrix protein on the cytoplasmic side of the plasma membrane (Rose and Gallione, 1981). This matrix layer acts as a nucleating layer for nucleocapsid morphogenesis and budding, and may add increased stability to the virion envelope.

Some enveloped viruses, like herpesviruses, acquire their membrane from the nuclear membrane. The inner nuclear membrane of herpesvirus-infected cells is modified by the insertion and processing of herpesvirus proteins (e.g., hemagglutinin) which probably occurs in specialized ER on host membrane-bound polysomes containing the appropriate herpesvirus mRNAs (Roizman and Furlong, 1974; Ben Porat and Kaplan, 1972).

A third type of membrane is utilized by only a few virus families, notably poxviruses, baculoviruses, and polydnaviruses, and is made *de novo* without the intervention of a preexisting membrane. Although it is difficult to rationalize the thermodynamic forces involved, electron micrographs depict partially complete membrane components at various stages of morphogenesis in the viroplasm

(Moss, 1974; Stoltz and Vinson, 1979). This could represent the only situation in biology where membrane is not made from preexisting membrane and the morphogenesis of these viral membranes provides an excellent model for studying the general question of membrane biogenesis.

C. Complex Viruses

Because of its overall structural complexity, the morphogenesis of poxvirus is somewhat unusual. Granular and fibrillar material aggregate with partially complete membrane fragments (synthesized *de novo*) within the cytoplasmic viroplasm. Condensation of material within the completed membrane results in a dense but still immature viral nucleoid. A second envelope then encloses the developing nucleoid, proteinaceous lateral bodies and other virion components (e.g., RNA transcriptase). The poxvirus membranes often contain lipids which are different from those of the cellular membrane, suggesting that some of the poxvirus polypeptides must be involved in lipid metabolism. Once the outer envelope is complete, morphogenesis continues both within the membrane (condensation to a denser structure) and on the outer surface of the membrane (development of a distinct surface substructure). The sequence of events is necessarily very complex and probably proceeds by a cascade of morphogenetic events. Viral DNA and associated proteins condense with the inner envelope to form an immature nucleoid and morphogenesis continues until a large, highly condensed, and organized brick-shaped virion is produced. The complex structures surrounding the nucleoid no doubt have a protective advantage and this was no doubt partly responsible for the difficulty in eradicating smallpox (Behbehani, 1983).

Morphogenesis of some insect viruses (baculoviruses, entomopoxviruses, and reoviruses) often produces an occluded and a nonoccluded form (Harrap and Payne, 1979). The nucleocapsids of nonoccluded forms are enveloped (by *de novo* membrane) and can spread infection to adjacent cells when released. The enveloped nucleocapsids of occluded forms become surrounded (singly or multiply) in a dense crystalline polyhedral layer (hence the name nuclear polyhedrosis viruses for baculoviruses and cytoplasmic polyhedrosis viruses for the insect reoviruses). The membrane of the nucleocapsids destined for occlusion is probably modified to act as nucleating sites for the crystallization of the occlusion body protein. The large occlusion body (up to 15 μm diameter) protects the virus from the physical factors present in their immediate environment and is transmitted to other insects which feed on contaminated cadavers. The occlusion body protein has an alkaline protease which dissolves the occlusion body crystal in the alkaline environment of the insect gut, thereby freeing the virions to infect gut epithelial cells.

VI. Virus Release

Once virion morphogenesis is complete, the virus can be released from the infected cell and initiate additional virus infection cycles in adjacent noninfected cells.

The release of enveloped viruses is often coincident with completion of virion morphogenesis. The morphogenesis of orthomyxoviruses, paramyxoviruses, and rhabdoviruses, for example, involves the association of virus nucleocapsids with the cytoplasmic side of membrane regions which have been modified to contain viral glycoproteins (Klenk and Choppin, 1970; Fuller *et al.*, 1984). This association is probably directed and stabilized by the viral matrix protein layer in the appropriate regions of the plasma membrane (Rose and Gallione, 1981). After the initial association, the nucleocapsid starts to "bud" out of the cell by pushing the modified membrane out. Budding is completed when the membrane pinches together behind the nucleocapsid and separates it from the cell; the virions are now extracellular.

Herpesviruses acquire their envelope in a similar manner except that they bud through the inner nuclear (virus-modified) membrane into the perinuclear space. They can subsequently become transported through the cisternal space of the ER (which is continuous with the perinuclear space) into the extracellular environment (Roizman and Furlong, 1974).

Viruses with nonenveloped nucleocapsids (adenoviruses, reoviruses, and picornaviruses) and with *de novo* acquired envelopes are released by either slow extrusion through the membrane (without damaging it) or liberation after cell lysis (a consequence of virus infection). In either case, many virions stay associated with the cell (or cell debris) and are not involved in reinfection.

Virus release from plants is usually by transport of the naked nucleocapsids from an infected cell to a noninfected adjacent cell via the interconnecting plasmadesmata. Theoretically cell infection by (+)RNA plant viruses could be transmitted by passage of just (+)RNA through the plasmadesmata. This is undoubtedly the way plant viroids (naked circular infectious RNAs) spread throughout an affected plant (Sanger, 1980). Plant viruses can also be released after physical damage (insects, abrasions, agricultural machinery) of the cell wall and membrane.

Bacterial viruses are released from infected cells by two major routes. Many of the larger viruses have a late gene which codes for a cell-specific lysozyme which degrades the bacterial wall. For example, in cells infected with λ, the level of lysozyme increases throughout infection until it is sufficient to cause cell lysis (Friedman *et al.*, 1984). Some of the ssDNA viruses (e.g., fd and M13) are released through the cell wall in a manner similar to budding. Associated with the membrane at the site of exit are the viral capsid proteins. As the viral DNA is

pushed through the membrane, it becomes encapsidated with viral capsid proteins removed from the membrane. During this budding, the GpV protein which is associated with and protects the viral DNA inside the cell is displaced by the capsid protein as the nucleoprotein core moves through the membrane (Ray, 1977).

The intracellular assembly of a complex bacteriophage like T4 and λ is a well orchestrated series of morphogenetic events involving several separate, independent, assemblages (e.g., head, tail, tail fibers). These involve sophisticated scaffolding structures and culminate in the assembly of an intact infectious virion. Some of these morphogenetic events are associated with processing of precursor proteins of the maturing structure. These types of morphogenesis are very detailed and cannot be covered here. The reader is referred to Mathews *et al.* (1983) and Friedman *et al.* (1984) for more information.

Once viruses are released from their intracellular viroplasms they are then capable of spreading the infection to neighboring cells or transmitting it to other susceptible, free-living individuals (unicellular or multicellular) it comes in contact with. The virus infection cycle has thus come full cycle to increase the total number of virions in the immediate area. Several new rounds of virus replication in new cells (or new organisms) are then initiated.

The preceding is a brief overview of the molecular biology of viruses and their interaction with their cellular hosts. For a more comprehensive treatment of the discipline of virology, the reader is directed to the following texts on the subject: "Virology," by Heinz Fraenkel-Conrat and Paul C. Kimball, "Microbiology," Third Edition, by Bernard D. Davis, Renato Dulbecco, Herman E. Eisen, and Harold S. Ginsberg (especially Section V, Virology, by R. Dulbecco and H. S. Ginsberg), and "Virology," chief editor Bernard N. Fields. The latter text is the most recent (1984) and comprehensive (over 1600 pages) overview. For quick information on viral taxonomy and morphology, the reader is referred to "Classification and Nomenclature of Viruses," the fifth report of the International Committee on Taxonomy of Viruses (1985) by R. E. F. Mathews and "An Electron Micrographic Atlas of Viruses" by Robley C. Williams and Harold W. Fisher. Many more texts on virology are, of course, available from most academic libraries.

ACKNOWLEDGMENTS

The authors are indebted to W. Johnson for typing the manuscript. The research from the authors' laboratories has been supported by operating grants from the Natural Science and Engineering Council of Canada (T.J.B. and P.J.K.), the Medical Council of Canada (T.J.B.), and the Weston Research Centre of Toronto, Canada (T.J.B.).

REFERENCES

Abelson, P. (1957). *Ann. N.Y. Acad. Sci.* **69,** 276–285.

Allen, M. M. (1984). *Annu. Rev. Microbiol.* **38,** 1–25.

Archer, D. B., and King, N. R. (1984). *J. Gen. Microbiol.* **130,** 167–172.

Armstrong, R. E., and Walsby, A. E. (1981). *In* "Organization of Prokaryotic Cell Membranes" (B. K. Ghosh, ed.), Vol. II. CRC Press, Boca Raton, Florida.

Baker, T. S., Caspar, D. L. D., and Murakami, W. T. (1983). *Nature (London)* **303,** 446–448.

Banerjee, A. K. (1980). *Microbiol. Rev.* **44,** 175–205.

Banerjee, A. K., Abraham, G., and Colonno, R. J. (1977). *J. Gen. Virol.* **34,** 1–8.

Banghoorn, E. S., and Tyler, S. A. (1965). *Science* **147,** 563–577.

Baroudy, B. M., Venkatesan, S.. and Moss, B. (1982). *Cell* **28,** 315–324.

Bayer, M. E. (1968). *J. Gen. Microbiol.* **53,** 395–404.

Bayer, M. E. (1979). *In* "Bacterial Outer Membranes: Biogenesis and Functions" (M. Inouye, ed.), pp. 167–202. Wiley, New York.

Bayer, M. E., and Leive, L. (1977). *J. Bacteriol.* **130,** 1364–1381.

Bayer, M. E., and Thurow, H. (1977). *J. Bacteriol.* **130,** 991–936.

Behbehani, A. M. (1983). *Microbiol. Rev.* **47,** 455–509.

Ben Porat, T., and Kaplan, A. S. (1972). *Nature (London)* **235,** 165.

Berg, H. C. (1974). *Nature (London)* **249,** 77–79.

Berns, K. I., ed. (1984). "The Parvoviruses." Plenum, New York.

Beveridge, T. J. (1981). *Int. Rev. Cytol.* **72,** 229–317.

Beveridge, T. J. (1984). *In* "Current Perspectives in Microbial Ecology" (M. J. Klug and C. A. Reddy, eds.), pp. 601–607. American Society for Microbiology, Washington, D.C.

Beveridge, T. J. (1985). *In* "Bacteria in Nature: A treatise on the Interactions of Bacteria and their Habitats" (E. R. Leadbetter and J. S. Poindexter, eds.). Vol. 2. Plenum, New York.

Beveridge, T. J., and Davies, J. A. (1983). *J. Bacteriol.* **156,** 846–858.

Beveridge, T. J., and Fyfe, W. S. (1985). *Can. J. Earth Sci.* **22,** 1892–1898.

Beveridge, T. J., and Jack, T. (1982). *J. Bacteriol.* **149,** 1120–1123.

Beveridge, T. J., and Murray, R. G. E. (1975). *J. Bacteriol.* **124,** 1529–1544.

Beveridge, T. J., and Murray, R. G. E. (1976). *Can. J. Microbiol.* **12,** 567–582.

Beveridge, T. J., Harris, R., Fein, J., Lawford, H., and Lawford, R. (1984). *Can. J. Microbiol.* **30,** 1283–1289.

Beveridge, T. J., Stewart, M., Doyle, R. J., and Sprott, G. D. (1985). *J. Bacteriol.* **162,** 728–737.

Bishop, J. M. (1983). *Annu. Rev. Biol.* **52,** 301–354.

Blakemore, R. P. (1982). *Annu. Rev. Microbiol.* **36,** 217–238.

Bradley, D. E. (1972). *Genet. Res.* **19,** 39–51.

Bradley, D. E. (1980). *Can. J. Microbiol.* **26,** 146–154.

Braell, W. A., Balch, W. E., Dobbertin, D. C., and Rothman, J. E. (1984). *Cell* **39,**511–524.

Braun, V., Gnirke, H., Henning, U., and Rehn, K. (1973). *J. Bacteriol.* **114,** 1264–1270.

Breidis, D. J., Lamb, R. A., and Choppin, P. W. (1982). *Virology* **116,** 581–588.

Brinton, C. C., Jr. (1965). *Trans. N.Y. Acad. Sci.* **27,** 1003–1054.

Buckmire, F. L. A. (1971). *Bacteriol. Proc. Am. Soc. Microbiol.* p. 43.

Burdett, I. D. J., and Murray, R. G. E. (1974). *J. Bacteriol.* **119,** 303–324.

Burge, R. E., Fowler, A. G., and Reaveley, D. A. (1977). *J. Mol. Biol.* **117,** 927–953.

Burke, B., and Warren, G. (1984). *Cell* **36,** 847–856.

Caton, A. J., Brownles, G. G., Yewdell, J. W., and Gerhardt, W. (1982). *Cell* **31,** 417–427.

Caulfield, M. P., Tai, P. C., and Davis, B. D. (1983). *J. Bacteriol.* **156,** 1–5.

Cepko, C. L., and Sharp, P. A. (1982). *Cell* **31,** 402–415.

Challberg, M. D., and Kelly, T. J. (1982). *Annu. Rev. Biochem.* **51,** 901–934.
Challberg, M. D., Desiderio, S. V., and Kelly, T. J., Jr. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77,** 5105–5109.
Colman, P. M., Varghese, J. N., and Laver, W. G. (1983). *Nature (London)* **303,** 41–44.
Cooper. P. D. (1977). *Compr. Virol.* **9,** 133–207.
Costerton, J. W., Irvin, R. T., and Cheng, K.-J. (1981a). *Annu. Rev. Microbiol.* **35,** 299–324.
Costerton, J. W., Irvin, R. T., and Cheng, K.-J. (1981b). *Crit. Rev. Microbiol.* **8,** 303–338.
Cotmore, S. F., and Tattersall, P. (1984). *Science* **226,** 1161–1165.
Dales, S., and Pogo, B. G. T. (1982). *In* "The Biology of Poxvirus." Springer-Verlag, New York.
Daneo-Moore, L., Dicker, D., and Higgins, M. L. (1980). *J. Bacteriol.* **141,** 928–937.
Dasgupta, A. (1983). *Virology* **128,** 252–259.
Davis, B. D., Dulbecco, R., Eisen, H. N., and Ginsberg, H. S., eds. (1980). "Microbiology," 3rd Ed. Harper & Row, New York.
Desiderio, S. V., and Kelly, T. J. (1981). *J. Mol. Biol.* **145,** 19–37.
DesRossier, J. P., and Cano-Lara, J. (1981). *J. Bacteriol.* **145,** 613–619.
DiRienzo, J. M., Nakamura, K., and Inouye, M. (1978). *Annu. Rev. Biochem.* **47,** 481–532.
Dobos, P., Hill, B. J., Hallett, R., Kells, D. T. C., Becht, H., and Tenninges, D. (1979). *J. Virol.* **32,** 593–605.
Doetsch, R. N., and Sjoblad, R. D. (1980). *Annu. Rev. Microbiol.* **34,** 69–108.
Doyle, R. J., Streips, U. N., Imada, S., Fan, V. S. C., and Brown, W. C. (1980). *J. Bacteriol.* **144,** 957–966.
Dulbecco, R., and Ginsberg, H. S. (1980). *In* "Microbiology" (B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg, eds.), 3rd Ed., pp. 853–1261. Harper & Row, New York.
Dworkin, M., Keller, K. H., and Weisberg, D. (1983). *J. Bacteriol.* **155,** 1367–1371.
Ellar, D. J. (1978). *In* "Comparison to Microbiology. Selected Topics for Further Study" (A. T. Bull and P. M. Meadows, eds.). Longman, London.
Ferris, F. G., and Beveridge, T. J. (1984). *FEMS Microbiol. Lett.* **24,** 43–46.
Ferris, F. G., and Beveridge, T. J. (1986a). *Can. J. Microbiol.* **32,** 52–55.
Ferris, F. G., and Beveridge, T. J. (1986b). *Can. J. Microbiol.* **32,** 594–601.
Ferris, F. G., Beveridge, T. J., Marceau-Day, M. L., and Larson, A. D. (1984). *Can. J. Microbiol.* **30,** 322–333.
Fields, B. N. (1984). *In* "Virology" (D. M. Knipe, R. M. Chanock, J. C. Helnick, B. Roizman, and R.E. Shope, eds.). Raven, New York.
Fields, B. N., and Greene, M. I. (1982). *Nature (London)* **300,** 19–23.
FitzJames, P. C. (1960). *J. Biophys. Biochem. Cytol.* **8,** 507–528.
Flanegan, J. B., and Baltimore, D. (1979). *J. Virol.* **29,** 352–360.
Formanek, H., Formanek, S., and Wawra, H. (1974). *Eur. J. Biochem.* **46,** 279–294.
Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibsons, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrsen, K. R., Chen, K. N., and Woese, C. R. (1980). *Science* **209,** 457–463.
Fraenkel-Conrat, H., and Kimball, P. C., eds. (1982). *In* "Virology," pp. i–406. Prentice Hall, Toronto.
Frankel, R. B., Papaefthymiou, G. C., Blakemore, R. P., and O'Brien, W. (1983). *Biochim. Biophys. Acta* **763,** 147–159.
Friedman, D.I., Olson, E. R., Georgopoulos, C., Tilly, K., Herskowitz, I., and Banuett, F. (1984). *Microbiol. Rev.* **48,** 299–325.
Fuerst, J. A., and Hayward, A. C. (1969). *J. Gen. Microbiol.* **58,** 227–237.
Fuller, S., von Bonsdorff, C. H., and Simons, K. (1984). *Cell* **38,** 65–77.
Funahara, Y., and Nikado, H. (1980). *J. Bacteriol.* **141,** 1463–1465.

Gardner, J. M., and Troy, F. A., II (1979). *J. Biol. Chem.* **254,** 6232–6269.
Gerhardt, P., and Scherrer, R. (1974). *Proc. Intersect. Congr. Int. Am. Microbiol. Soc.* **1,** 506–513.
Gilleland, H. E., Jr., and Murray, R. G. E. (1975). *J. Bacteriol.* **121,** 721–725.
Gram, C. (1984). *Fortschr. Med.* **2.** 185–189.
Hakenbeck, R., Holtje, J.-V., and Labischinski, H., eds. (1983). *In* "The Target of Penicillin." De Gruyter, Berlin.
Hanecak, R., Semler, B. L., Anderson, C. W., and Wimmer, E. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 3972–3977.
Harold, F. M. (1966). *Bacteriol. Rev.* **30,** 772–794.
Harrap, K. A., and Payne, C. C. (1979). *Adv. Virus Res.* **25,** 273.
Hauswirth, W. W., and Berns, K. I. (1979). *Virology* **93,** 57–68.
Hemmingsen, B. B., and Hemmingsen, E. A. (1980). *J. Bacteriol.* **143,** 841–846.
Higgins, M. L., Parks, L. C., and Daneo-Moore, L. (1981). *In* "Organization of Prokaryotic Cell Membranes" (B. K. Ghosh, ed.), Vol. II. CRC Press, Boca Raton, Florida.
Hirth, L., and Richards, K. E. (1981). *Adv. Virus Res.* **26,** 145–199.
Hobot, J. A., Carlemalm, E., Villiger, W., and Kellenberger, E. (1984). *J. Bacteriol.* **160,** 143–152.
Holowczak, J. A. (1982). *Curr. Top. Microbiol. Immunol.* **97,** 27–79.
Honess, R. W., and Roizman, B. (1974). *J. Virol.* **14,** 8–19.
Hughes, S. S. (1977). *In* "The Virus: A History of the Concept," pp. 1–140. Science History Publications, New York.
Hutchinson, M. A., Hunter, T., and Eckhart, W. (1978). *Cell* **15,** 65–77.
Ishiguro, E. E., Kay, W. W., Ainsworth, T., Chamberlain, J. B., Austen, R. A., Buckley, J. T., and Trust, T. J. (1981). *J. Bacteriol.* **148,** 333–340.
Jacobson, M. F. and Baltimore, D. (1968). *Proc. Natl. Acad. Sci. U.S.A.* **61,** 77–84.
Joklik, W. K. (1981). *Microbiol. Rev.* **45,** 483–501.
Joklik, W. K., and Becker, Y. (1964). *J. Mol. Biol.* **10,** 452–474.
Jones, J. B., Bowers, B., and Stadtman, T. C. (1977). *J. Bacteriol.* **130,** 1357–1363.
Jones, G. W., and Rutter, J. M. (1972). *Infect. Immun.* **5,** 595–605.
Joshi, S., and Haenni, A. L. (1984). *FEBS Lett.* **177,** 163–174.
Kandler, O. (1979). *Naturwissenschaften* **66,** 95–105.
Kandler, O. (1982). *Zentralbl. Baktariol. Hyg., I. Abt. Orig.* **C3,** 149–160.
Kay, W. W., Buckley, J. T., Ishiguro, E. E., Phipps, B. M., Monette, J. P. L., and Trust, T. J. (1981). *J. Bacteriol.* **147,** 1077–1084.
Keller, J. M., and Alwine, J. C. (1984). *Cell* **36,** 381–389.
Kerridge, D., Horne, R. W., and Glauert, A. M. (1962). *J. Mol. Biol.* **4,** 227–238.
Kitamura, N., Semler, B. L., Rothberg, P. G.. Larsen, G. R., Adler, C. J., Dorner A. J., Emini, E. A., Hanecak, R., Lee, J. J., van der Werf, S., Anderson, C. W., and Wimmer, E. (1981). *Nature (London)* **291,** 547–553.
Klenk, H. D., and Choppin, P. W. (1970). *Virology* **40,** 939–947.
Koch, A. L. (1983). *Adv. Microbiol. Physiol.* **24,** 301–367.
Koch, A. L., Mobley, H. L. T., Doyle, R. J., and Streips, U. N. (1981). *FEMS Microbiol. Lett.* **12,** 201–208.
Koval, S. F., and Murray, R. G. E. (1984). *Can. J. Biochem. Cell Biol.* **62,** 1181–1189.
Kriegler, M., Perez, C. F.. Hardy, C., and Botchan, M. (1984). *Cell* **38,** 483–491.
Kumazawa, N., and Yanagawa, R. (1972). *Infect. Immun.* **5,** 27–30.
Lagenaur, C., and Agabian, N. (1977). *J. Bacteriol.* **131,** 340–346.
Laishley, E. J., MacAlister, T. J., Clements, I., and Young, C. (1973). *Can. J. Microbiol.* **19,** 991–994.
Lamb, R. J., and Choppin, P. W. (1983). *Annu. Rev. Biochem.* **52,** 467–506.

Laver, W. G., and Valentine, R. C. (1969). *Virology* **38,** 105–119.
Lechner, R. L., and Kelly, T. J., Jr. (1977). *Cell* **12,** 1007–1200.
Lenard, J., and Miller, D. K. (1982). *Cell* **28,** 5–6.
Lo, T. C. Y. (1979). *Can. J. Biochem.* **57,** 289–301.
Logan, J. S., and Shenk, T. (1982). *Microbiol. Rev.* **46,** 377–383.
Lotz, W., and Pfister, H. (1975). *J. Virol.* **16,** 725–728.
Lusby, E., Fife, K. H., and Berns, K. I. (1980). *J. Virol.* **34,** 402–409.
Mackie, E. B., Brown, K. N., Lam, J., and Costerton, J. W. (1979). *J. Bacteriol.* **138,** 609–617.
Marty-Mazars, D., Horiuchi, D., Tai, P. C., and Davis, B. D. (1983). *J. Bacteriol.* **154,** 1381–1388.
Masuda, N., Ellen, R. P., and Grove, D. A. (1981). *J. Bacteriol.* **147,** 1095–1104.
Mathews, C. K., Kutter, E. M., Mosiq, G., and Berget, P. B., eds. (1983). *In* "Bacteriophage T4'." American Society for Microbiology, Washington, D.C.
Mathews, R. E. F. (1982). *In* "Classification and Nomenclature of Viruses." Karger, New York.
Matlin, K., and Simons, K. (1983). *Cell* **34,** 233–243.
Matlin, K. S., Reggio, H., Helenius, A., and Simons, K. (1982). *J. Mol. Biol.* **156,** 609–631.
Medrano, L., and Green, H. (1973). *Virology* **54,** 515–524.
Mims, C. A. (1981). *Microbiol. Rev.* **45,** 267–286.
Mitra, S. (1980). *Annu. Rev. Genet.* **14,** 347–397.
Moore, D., Sowa, B. A., and Ippen-Ihler, K. (1981). *J. Bacteriol.* **146,** 251–259.
Morgan, C.. Rosenkranz, H. S., and Mednis, B. (1969). *J. Virol.* **4,** 777–796.
Moss, B. (1974). *Compr. Virol.* **3,** 405–474.
Mühlradt, P. F., and Golecki, J. R. (1975). *Eur. J. Biochem.* **51,** 343–352.
Murray, R. G. E., and Watson, S. W. (1965). *J. Bacteriol.* **89,** 1594–1609.
Nash, H. A. (1981). *Annu. Rev. Genet.* **15,** 143–167.
Nevins, J. R., and Chen-Kiang, S. (1981). *Adv. Virus Res.* **26,** 1–35.
Nicolson, G. L., and Schmidt, G. L. (1971). *J. Bacteriol.* **105,** 1142–1148.
Norkin, L. C. (1982). *Microbiol. Rev.* **46,** 384–425.
Old, D. C., and Scott, S. S. (1981). *J. Bacteriol.* **146,** 404–408.
Ostrove, J. M., Rosenfeld, P., Williams, J., and Kelly, T. J., Jr. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 935–939.
Ottow, J. C. G. (1975). *Annu. Rev. Microbiol.* **29,** 79–108.
Palese, P., and Roizman, B. (1980). *Ann. N.Y. Acad. Sci.* **354,** i–507.
Pettijohn, D. E. (1976). *Crit. Rev. Biochem.* **4,** 175–202.
Pfennig, N. (1977). *Annu. Rev. Microbiol.* **31,** 275–290.
Pflug, H. D., and Jaescke-Boyer, H. (1979). *Nature (London)* **280,** 483–486.
Pogo, B. G. T., Berkowitz, E. M., and Dales, S. (1984). *Virology* **132,** 436–444.
Purcell, E. M. (1977). *Am. J. Phys.* **45,** 3–11.
Putnak, J. R., and Phillips, B. A. (1981). *Microbiol. Rev.* **45,** 287–315.
Racaniello, V. R., and Baltimore, D. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 4887–4891.
Ray, D. C. (1977). *Comp. Virol.* **7,** 105–178.
Reanney, D. C. (1982). *Annu. Rev. Microbiol.* **36,** 47–73.
Reanney, D. C., and Ackermann, H. W. (1982). *Adv. Virus Res.* **27,** 205–280.
Roizman, B., and Furlong, D. (1974). *Compr. Virol.* **3,** 229–403.
Rose, J. K., and Gallione, C. J. (1981). *J. Virol.* **39,** 519–528.
Salton, M. R. J. (1963). *J. Gen. Microbiol.* **30,** 223–235.
Salton, M. R. J., and Owen, P. (1976). *Annu. Rev. Microbiol.* **30,** 451–482.
Sanger, H. C. (1980). *Ann. N.Y. Acad. Sci.* **354,** 251–278.
Saraste, J., and Kuismanen, E. (1984). *Cell* **38,** 535–549.
Sargent, M. G., Bennett, M. F., and Burdett, I. D. J. (1983). *J. Bacteriol.* **154,** 1389–1396.

Scherer, P. A., and Bochem, H.-P. (1983). *Can. J. Microbiol.* **29,** 1190–1199.
Scherrer, R., and Gerhardt, P. (1971). *J. Bacteriol.* **114,** 888–890.
Scherrer, R., Berlin, E., and Gerhardt, P. (1977). *J. Bacteriol.* **129,** 1162–1164.
Schleifer, K. H., and Kandler, O. (1972). *Bacteriol. Rev.* **36,** 407–477.
Schneider, R. J., Weinberger, C., and Shenk, T. (1984). *Cell* **37,** 291–298.
Shelton, E. R., Wassarman, P. M., and DePamphilis, M. C. (1978). *J. Mol. Biol.* **125.** 491–514.
Shiveley, J. M. (1974). *Annu. Rev. Microbiol.* **28,** 167–187.
Simons, K., Garoff, H., and Helenius, A. (1983). *Sci. Am.* **246,** 58–66.
Sinden, R. R., and Pettijohn, D. E. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 224–228.
Sleytr, U. B. (1978). *Int. Rev. Cytol.* **53,** 1–64.
Sleytr, U. B., and Messner, P. (1983). *Annu. Rev. Microbiol.* **37,** 311–339.
Stanier, R. Y., and Cohen-Bazire, G. (1977). *Annu. Rev. Microbiol.* **31,** 225–274.
Stewart, M., and Beveridge, T. J. (1980). *J. Bacteriol.* **142,** 302–309.
Stewart, M., and Murray, R. G. E. (1982). *J. Bacteriol.* **150,** 348–357.
Stewart, M., Beveridge, T. J., and Sprott, G. D. (1984). *Proc. EMSA* **42,** 214–215.
Stewart, M., Beveridge, T. J., and Sprott, G. D. (1985). *J. Mol. Biol.* **183,** 509–515.
Stoltz, D. B., and Vinson, S. B. (1979). *Adv. Virus Res.* **24,** 125–171.
Stoltz, D. B., Krell, P., Summers, M. D., and Vinson, S. B. (1984). *Intervirology* **21,** 1–4.
Tinsley, T. W., and Harrap, K. A. (1978). *Compr. Virol.* **12,** 1–101.
Troy, F. A., II. (1979). *Annu. Rev. Microbiol.* **33,** 519–560.
Trudinger, P. A., and Swaine, D. J., eds. (1979). *In* "Biogeochemical Cycling of Mineral-forming Elements," p. 612. Elsevier, Amsterdam.
Tweeten, R. A., Bulla, L. A., Jr., and Consigli, R. A. (1981). *Microbiol. Rev.* **45,** 379–408.
Varga, A. R., and Staehelin, L. A. (1983). *J. Bacteriol.* **154,** 1414–1430.
Varghese, J. N., Laver, W. G., and Colman, P. M. (1983). *Nature (London)* **303,** 35–40.
Varmus, H. E. (1983). *Annu. Rev. Genet.* **18,** 553–612.
Walsby, A. E. (1972). *Bacteriol. Rev.* **36,** 1–32.
Weiss, R. L. (1974). *J. Bacteriol.* **118,** 275–284.
Westphal, K., Bock, E., Cannon, E., and Shively, J. M. (1979). *J. Bacteriol.* **140,** 285–288.
White, D. A., Lennarz, W. J., and Schnaitman, C. A. (1972). *J. Bacteriol.* **109,** 686–690.
Willetts, N. S. (1975). *In* "Microbial Drug Resistance" (S. Mitsuhashi and H. Hashimoto, eds.). Univ. Park Press, Baltimore.
Williams, R. C., and Fisher, H. W. (1974). "An Electron Micrographic Atlas of Viruses." Charles C. Thomas, Springfield, Illinois.
Wittek, R. (1982). *Experientia* **38,** 285–310.
Woese, C. R. (1981). *Sci. Am.* **244,** 98–122.
Woldringh, C. L., and Nanninga, N. (1976). *J. Bacteriol.* **127,** 1455–1464.
Yamakawa, M., Shatkin, A. J., and Furuichi, Y. (1982). *Virology* **118,** 157–168.
Young, L., and Hough, A. (1979). *Biochim. Biophys. Acta* **556,** 265–277.