Bacteriophages

Introductory article

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Bacteriophages are viruses that infect bacteria. They are ubiquitous in the biosphere, and have evolved a wide range of morphologies and replication mechanisms to enable them to infect many kinds of eubacteria and archaebacteria.

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Introduction

A bacteriophage is a virus that infects a bacterial cell. The name, usually shortened to 'phage', means 'eater of bacteria' and was coined by Felix d'Hérelle in recognition of the ability of the viruses he studied to lyse infected bacterial cells. The word 'bacteria' is used here as a general term for microorganisms in the domains Bacteria (eubacteria) and Archaea (archaebacteria).

Discovery

In 1915 and 1917, Frederick W. Twort, in London, and Felix d'Hérelle, in Paris, independently described viruses that destroyed bacteria. Twort published a single note on his observations and went on to other research, while d'Hérelle began years of studies on the nature of bacterial viruses. D'Hérelle's findings included development of the plaque assay for quantitating phages and description of the phage replication cycle, and was the beginning of modern virology.

Hosts

The range of hosts that can be infected by a particular phage is determined by which bacteria have specific sites for attachment of that phage. Some phages are extremely specific and infect only a single bacterial strain. Many phages infect phylogenetically closely related bacterial strains or species. Other phages infect bacteria in different genera, although these genera are usually phylogenetically related, e.g. enterobacteria.

Techniques

The presence of phages in a sample is most easily demonstrated by the ability of viable phages to infect and propagate in bacterial host cells. Experimentally, this is generally observed by spreading the putative phage-containing sample on a bacterial lawn growing on a solid medium (i.e. a layer of agar-containing culture medium in a Petri dish) and, after appropriate incubation conditions, looking for clear areas in the lawn. These clear areas, called 'plaques', result from the initial infection of a bacterial cell by a single viable phage, intracellular production of progeny phage and their release by cell lysis, and subsequent rounds of infection and lysis of nearby host cells in the lawn by nascent progeny phages. Hence, although a plaque is produced by the lysis of many infected cells, all phages in the plaque are the progeny of the single initial infectious phage, and phage titres are usually measured as 'plaque-forming units' (PFUs).

Isolation

Phage isolation requires a liquid sample that can be spread on lawns of possible bacterial host cells to produce plaques. Liquid specimens (e.g. sewage and marine samples) can be spread directly on bacterial lawns. Solid samples (e.g. soil, and plant and animal material) must be dispersed in liquid (either buffer or culture medium) before spreading. Solids and, in the case of nonsterile samples, contaminating microbial cells must be removed before spreading, usually by filtration through a 0.45-μm pore size filter.

Phage titres in most environmental samples may be so low that direct spreading yields no plaques. Such samples first must be either enriched using biological methods (i.e. infection of bacteria in liquid culture) or concentrated using physical (e.g. centrifugation) or chemical (e.g. separation by partitioning using an immiscible agent like polyethylene glycol) methods.

Propagation and maintenance

Most phages are propagated by infection of exponentially growing bacteria in liquid media, with incubation conditions (e.g. media, temperature and oxygen concentration) and times chosen to produce large progeny phage titres. However, some phages propagate best in bacteria growing on solid media. In these cases, phages are spread on bacterial lawns, incubated using appropriate conditions,

and progeny phages are harvested by washing. Phages in liquid or solid media are separated from cell debris and sterilized, usually by filtration through 0.45-μm pore size filters. Concentration may be achieved by physical and chemical methods, as noted above.

Long-term maintenance of viable phage samples requires different conditions for different phages. The most useful methods have been storage at 4°C or in liquid nitrogen, or in lyophiles.

Identification

Electron microscopy is the best initial method for phage identification because it enables the taxonomic family to be determined. Other important properties for phage classification are host range, nucleic acid structure (e.g.deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), single-stranded or double-stranded, and linear or circular). A variety of other properties have been used for studying closer taxonomic relationships, e.g. DNA–DNA hybridization, nucleic acid sequence similarities, serological crossreactivity, DNA replication and packaging mechanisms, and protein composition.

Classification

A system for phage classification has been developed by the International Committee on Taxonomy of Viruses over the past few decades. Phages are currently (as of early 1999) classified in 13 families and 1 unassigned genus (**Table 1**). The three families of tailed phages have been grouped in the order *Caudovirales*. Other bacterial virus taxa contain phages with polyhedral, filamentous or more complex symmetry. In virus taxonomy, the name of an order ends in *-virales*, a family ends in *-viridae*, and a genus ends in *-virus*. Thus far, tailed phage genera have only been assigned vernacular names because of uncertainties regarding the necessary and sufficient parameters for defining these taxa. In each genus, a species for which considerable data are available is designated the type species.

Distribution in the Bacterial World

Phages that infect hosts in about 140 bacterial genera, including genera on most major eubacterial and archaebacterial phylogenetic branches, have been identified as of early 2000. As more diverse bacteria are studied, it is expected that most (if not all) eubacterial genera will be found to be hosts for one or more phages.

However, the situation for viruses infecting archaebacteria may be different. The archaebacterial phylogenetic tree consists of three branches: sulfur-reducing hyperthermophiles, methanogens, and uncultured hyperthermophiles. All archaebacterial viruses identified, thus far, infect genera on the sulfur-reducing hyperthermophile branch. It is not known whether the lack of viruses infecting hosts on the other two branches is due to some type of biological or physical chemical restriction or to the relatively few studies of archaebacterial viruses.

Virion Properties

The morphology of phage virions or particles has been determined largely by electron microscopy of negatively stained and frozen-hydrated samples. These results have been supplemented with data on virion composition and physical and chemical properties to elucidate the nature of phages.

Morphology

About 4800 phages have been reported, with 96% (about 4600 phages) having tailed phage morphology and 4% (about 170 phages) with polyhedral, filamentous or more complex morphology. Tailed phages have a protein head, containing a genome of linear, double-stranded DNA, and a protein tail that functions in phage–host cell attachment and DNA injection. The structure of phage heads is based on either an icosahedron (a polyhedron with 20 faces, each an equilateral triangle, and 12 vertices) or an elongated icosahedron. Three types of phage tails have been found: 24% of tailed phages have long, contractile tails; 62% have long, noncontractile tails; and 14% have short, noncontractile tails.

Of the nontailed phages, 60% have polyhedral morphology, 30% filamentous morphology and 10% more complex morphology. The polyhedral phages that have been studied are icosahedra, either naked or enveloped, with a protein shell or capsid containing the phage genome, which can be either single- or double-stranded DNA or RNA. Filamentous phages can be rigid or flexible protein filaments with helical symmetry, either naked or enveloped, containing a single-stranded DNA genome. A small number of phages with more complex morphologies (all containing genomes of double-stranded DNA) have been described, each having evolved to infect a novel type of host: enveloped, pleomorphic virions infect mycoplasmas, and lemon- and droplet-shaped virions infect archaebacteria.

The range of phage morphologies reflects selection for virions able to infect diverse bacteria and package different types of nucleic acids. Phages must attach and inject their genomes through a wide range of cell surfaces, including Gram-positive and Gram-negative eubacterial cell walls, chlamydial outer membranes, mycoplasmal cell membranes, and archaebacterial cell walls.

Table 1 Taxonomy of bacteriophages

Double-stranded (ds) DNA viruses

Order: *Caudovirales* (tailed phages, genome linear dsDNA)
- Family: *Myoviridae* (contractile tails)
  - Genus: 'T4-like viruses'
    - Type species: *Coliphage T4*
  - Genus: 'P1-like viruses'
    - Type species: *Coliphage P1*
  - Genus: 'P2-like viruses'
    - Type species: *Coliphage P2*
  - Genus: 'Mu-like viruses'
    - Type species: *Coliphage Mu*
  - Genus: 'SP01-like viruses'
    - Type species: *Bacillus phage SP01*
  - Genus: 'ΦH-like viruses'
    - Type species: *Halobacterium phage ΦH*
- Family: *Siphoviridae* (long, noncontractile tails)
  - Genus: 'λ-like viruses'
    - Type species: *Coliphage λ*
  - Genus: 'T1-like viruses'
    - Type species: *Coliphage T1*
  - Genus: 'T5-like viruses'
    - Type species: *Coliphage T5*
  - Genus: 'L5-like viruses'
    - Type species: *Mycobacterium phage L5*
  - Genus: 'c2-like viruses'
    - Type species: *Lactococcus phage c2*
  - Genus: 'ψM1-like viruses'
    - Type species: *Methanobacterium phage ψM1*
- Family: *Podoviridae* (short, noncontractile tails)
  - Genus: 'T7-like viruses'
    - Type species: *Coliphage T7*
  - Genus: 'P22-like viruses'
    - Type species: *Enterobacteria phage P22*
  - Genus: 'ϕ29-like viruses'
    - Type species: *Bacillus phage ϕ29*
- Family: *Tectiviridae* (lipid-containing, double icosahedral capsids, genome linear dsDNA)
  - Genus: *Tectivirus*
    - Type species: *Polyvalent phage PRD1*
- Family: *Corticoviridae* (lipid-containing, icosahedral capsid, genome circular dsDNA)
  - Genus: *Corticovirus*
    - Type species: *Alteromonas phage PM2*
- Family: *Plasmaviridae* (enveloped, pleomorphic, genome circular dsDNA)
  - Genus: *Plasmavirus*
    - Type species: *Acholeplasma phage L2*
- Family: *Lipothrixviridae* (enveloped, rod-shaped, genome linear dsDNA)
  - Genus: *Lipothrixvirus*
    - Type species: *Thermoproteus virus TTV1*
- Family: *Rudiviridae* (nonenveloped, rod-shaped, genome linear dsDNA)
  - Genus: *Rudivirus*
    - Type species: *Thermoproteus virus TTV4*

continued

Table 1 – *continued*

Family: *Fuselloviridae* (nonenveloped, lemon-shaped, genome circular dsDNA)
Genus: *Fusellovirus*
Type species: *Sulfolobus virus SSV1*
Unassigned genus SNDV (droplet-shaped, genome circular dsDNA)
Type species: *Sulfolobus virus SNDV*

Single-stranded (ss) DNA viruses
Family: *Inoviridae* (nonenveloped, filamentous or rod-shaped, genome circular ssDNA)
Genus: *Inovirus*
Type species: *Coliphage Ff*
Genus: *Plectrovirus*
Type species: *Acholeplasma phage L51*
Family: *Microviridae* (nonenveloped, icosahedral, genome circular ssDNA)
Genus: *Microvirus*
Type species: *Coliphage ϕX174*
Genus: *Spiromicrovirus*
Type species: *Spiroplasma phage SpV4*
Genus: *Bdellomicrovirus*
Type species: *Bdellovibrio phage MAC1*
Genus: *Chlamydiamicrovirus*
Type species: *Chlamydia phage Chp1*

Double-stranded RNA viruses
Family: *Cystoviridae* (enveloped, icosahedral, genome segmented linear dsRNA)
Genus: *Cystovirus*
Type species: *Pseudomonas phage ϕ6*

Positive-sense, single-stranded RNA viruses
Family: *Leviviridae* (nonenveloped, icosahedral, genome linear ssRNA)
Genus: *Levivirus*
Type species: Enterobacteriophage MS2
Genus: *Allolevirus*
Type species: Enterobacteriophage Qβ

Physical and chemical properties

Phage genomes can be single-stranded or double-stranded, linear or circular, DNA or RNA molecules. With one exception, phage genomes are single molecules. The exception is the cystoviruses, which have a segmented genome consisting of three molecules of linear double-stranded RNA. Phage genome sizes range from the leviviruses, with genomes about 3.5 kb (encoding 3–4 gene products), to the larger tailed phages, with genomes of several hundred kilobases (encoding hundreds of gene products). Many phage genome sequences have been determined and are available in databases (e.g. GenBank).

Paralleling the genome diversity of phages, their structural complexity ranges from the leviviruses, small icosahedral virions composed of 180 molecules of a coat protein and one molecule of an attachment protein, to the larger tailed phages, with virions of at least 40 different proteins, each present in from a few copies to hundreds of copies.

In addition to morphology and composition, a variety of physical and chemical properties have been measured for phage virions. Such studies have included X-ray diffraction and spectroscopic measurements to analyse virion molecular structure; sedimentation analysis to determine virion shape, size, and hydrodynamic properties; and sensitivity to inactivation by physical (e.g. heat and ultraviolet light) and chemical agents (e.g. lipid solvents and pH) to probe virion structure and repair capabilities.

Replication

The process in which a phage infects a bacterial cell and produces progeny virions is called the lytic, vegetative or productive cycle – lytic because, for most phages, infected

cells must lyse to release progeny phages. In certain intracellular physiological situations, some phages do not replicate and instead produce latent infections. This is called a lysogenic cycle and latently infected cells are called lysogens. Phages that only produce a lytic infection are called lytic or virulent phages. Phages that produce either a lytic or a lysogenic infection are called lysogenic or temperate phages.

Lytic cycle

The steps in a lytic phage infection are attachment (or adsorption), penetration (or injection), multiplication (or replication), assembly and packaging (or maturation), and release.

Attachment (adsorption)

Attachment begins with random collisions between a phage and a bacterial cell, leading to specific interactions between virion and cell surface components. These cell components are called receptors and are normal constituents of cell wall polysaccharides, pili or flagella. Different phages adsorb to different specific receptors.

Penetration (injection)

Penetration is the entry of an adsorbed virion or its nucleic acid into a host cell. For most phages, only virus nucleic acid enters. Although virions of a few of the smaller phages penetrate the cell wall, only virus nucleic acid enters the cytoplasm. Penetration of Gram-negative bacteria by tailed phages appears to involve fusion of bacterial outer and inner membranes, induced by the adsorbed phage tail, followed by DNA injection. However, for other phages, the mechanism by which the virus genome passes from the virion through the cell surface and into the cytoplasm is not understood.

Multiplication (replication)

Multiplication requires expression of the injected virus genome to take over the host cell's biosynthetic machinery for synthesis of the viral nucleic acids and proteins needed to assemble progeny virions. Phages with small DNA genomes (and, hence, limited coding capacity) use host cell DNA and RNA polymerases for DNA replication and transcription, while phages with large DNA genomes (and, hence, greater coding capacity) generally encode phage DNA and RNA polymerases. RNA phages encode an RNA-dependent RNA-polymerase (also called a replicase or transcriptase) for phage RNA replication and transcription.

There is no temporal regulation of gene expression for small phage genomes: essentially all phage genes begin to be transcribed and translated soon after injection, and continue to be expressed throughout infection. In contrast, multiplication of phages with large DNA genomes requires temporal regulation of phage macromolecular syntheses, usually occurring in three phases: synthesis of early gene products, then of progeny virus DNA, and finally of late gene products. Phage early gene products shut down the host cell's DNA, RNA and protein syntheses, adapt cellular biosynthetic machinery for phage syntheses, and start phage DNA replication. Late genes encode the products required for assembly of progeny phage virions.

Assembly and packaging (maturation)

Assembly of phage structural components is by self-assembly, frequently involving scaffolding or other morphogenetic proteins and specific proteolytic cleavages of structural proteins by phage-encoded proteases to induce conformational changes and form new binding sites for further assembly. In most phage assembly processes, progeny virus nucleic acid is packaged into almost completed capsids, thereby assuring that progeny genomes are packaged into correctly assembled structures. However, in some phage assembly processes, the capsid is assembled around, or coassembled with, progeny virus genomes. For tailed phages with large DNA genomes, heads and tails are assembled in independent pathways, DNA is packaged and completed heads and tails are joined, assuring both that progeny DNA is packaged in correctly assembled heads and that tails only go on to heads containing correctly packaged DNA.

Release

Release of most progeny phages requires bacterial cell lysis. In these cases, a virus gene product is produced that gradually hydrolyses the host cell peptidoglycan structure, thereby weakening it enough so that internal cell osmotic pressure can eventually cause cell lysis. The exceptions to release by cell lysis are filamentous phages, which are released by extrusion through host cell membranes, and mycoplasmal phages, which are released by budding from their wall-less host cells. Productive infections lead to release of 20–1000 progeny phages per infected cell, depending on the specific phage and cell growth conditions.

Lysogenic cycle

Most phages are temperate and can produce either lytic or lysogenic infections. For these phages, after adsorption and penetration, a temperate phage early gene product is affected by the cell's intracellular physiological condition. If intracellular conditions (e.g. adenosine triphosphate (ATP) and amino acid concentrations) are not good for a productive phage infection, the phage early gene product induces synthesis of another phage protein which is a repressor. This repressor blocks further synthesis of phage genes and a latent infection, called lysogeny, is established. The latent phage genome is called a 'prophage'. Most

prophage genomes are integrated into the host cell's chromosome, but a few persist as plasmids. In either case, prophages replicate along with the cell genome and a lysogen produces a clone of cells, each containing a prophage.

The lysogenic state can continue indefinitely. However, if the host cell encounters a potentially lethal situation (e.g. damage to the chromosomal DNA), most prophages undergo induction and produce progeny phages. This occurs because, as the cell activates its DNA repair genes, the new intracellular conditions lead to degradation of the repressor, which allows synthesis of phage genes to begin, eventually leading to production of progeny phages and cell lysis. For prophages integrated in the cell chromosome, induction begins with excision of the phage genome from the cell genome. Induction can also occur spontaneously at low frequency, so most cultures of lysogenic bacteria contain free phages produced by the small fraction of spontaneously induced lysogens in the culture.

Some temperate phages carry genes that can change the phenotype of a lysogenized bacterial cell. These genes are expressed from the integrated prophage genome and can affect host cell surface properties (e.g. *Salmonella phage* ε can carry a gene that changes the structure of the host cell's outer membrane lipopolysaccharide) or pathogenicity (e.g. *Corynebacterium phage* β can carry the diphtheria toxin gene).

Other types of host–virus relationship

There are two types of persistent phage infection distinct from lysogeny: pseudolysogeny and steady state infection. Pseudolysogeny occurs when only some cells in a culture are infected, so the culture contains phage-infected cells and uninfected cells. This situation can arise if the host cell culture is a mixture of cells with different sensitivities to phage infection. Steady state infection occurs when all cells are infected by a noncytocidal phage (e.g. filamentous phages that are released by extrusion), so all cells continue to grow, although usually more slowly compared with uninfected cells, while releasing progeny phages.

Ecology

Since viruses are obligate intracellular parasites, the habitats of phages are bacteria and phage ecological diversity reflects bacterial ecological diversity. Therefore, phages are found wherever bacteria are found. Bacteria are ubiquitous in the biosphere, growing on the surfaces of and within plants and animals, in biofilms on many organic and inorganic materials, in diverse terrestrial and aquatic habitats, and in a variety of extreme environments (e.g. thermophiles and psychrophiles, acidophiles and alkaliphiles, halophiles, xerophiles, and barophiles). Lysogenic bacteria are generally distributed in all these habitats and, because of spontaneous induction, free phages are usually present as well.

Phages titres in most natural habitats are low but there are exceptions in a few ecosystems where bacterial titres are high: phage titres can reach 10^9 PFU g^{-1} in human faeces and 10^7 PFU mL^{-1} in sewage. On a global scale, the number of phages is enormous: the biosphere contains about 10^{30} prokaryotes and, since environmental samples have about 10 times more tailed phages than bacteria, the biosphere must contain at least 10^{31} phages.

Recent studies have identified bacteria that have been unable to be cultivated in many habitats. These data indicate that only a few per cent of bacterial species in natural habitats can be cultivated using current methods. Therefore, since phages are presently recognized by their ability to form plaques on lawns of cultivated bacteria, it will be interesting to see whether future studies of uncultivated bacteria identify new phage taxa.

Plasmids, Episomes and Bacteriocins

Several types of genetic elements and gene products, other than phages, are found in some bacteria. Plasmids are extrachromosomal double-stranded DNAs that replicate independently of the bacterial chromosome and regulate their replication so the number of plasmid copies per cell is relatively constant. A plasmid can be a circular or linear DNA molecule, vary in size from a few kilobase pairs to hundreds of kilobase pairs, and have a copy number ranging from one per cell to several hundred per cell. Depending on its size, plasmids encode from a few to hundreds of gene products and, although plasmid-encoded gene products are rarely essential for bacterial growth, some provide the cell with a selective advantage for growth in particular situations.

Some plasmids can exist either as extrachromosomal DNAs or integrated into the cell chromosome. Such plasmids are called 'episomes'. The terms 'plasmid' and 'episome' are sometimes used interchangeably.

One type of plasmid-encoded gene product in some bacteria is an agent, called a 'bacteriocin', which is toxic to related bacteria. Therefore, bacteriocins provide the bacteria that produce them with a selective advantage against other bacteria trying to grow in the same ecological niche.

Origin and Evolution

Genome sequences of many viruses and cells have recently become available. Analysis of these data indicate phylogenetic relatedness between viruses within many families (and, in some cases, between virus families within orders)

and between virus and cell genes. Hence, the generally accepted model for the origin of viruses is that mobile genetic elements capable of autonomous replication (such as plasmids, episomes, transposons and retrotransposons) acquired the cell genes that gave them an extracellular infectious capability, enabling them to be horizontally transmitted between cells. Virus evolution would have then proceeded by point mutations, insertions, deletions and changes in reading frames. Recombination and reassortment would have led to modular evolution, in which gene domains or contiguous genes move between virus genomes and between virus and cell genomes. Questions remain about whether there was one or more origin of viruses; in particular, whether there was a single origin of RNA and DNA viruses, and whether RNA viruses may be descendants of the 'RNA World' hypothesized to have been the precursor to cells with DNA-based genomes.

Phages probably evolved with their bacterial hosts aeons before the evolution of eukaryotes. Since tailed phages infect cells in the domains Bacteria and Archaea, tailed phages probably evolved early in cellular evolution, perhaps before these two domains diverged from their common ancestor, but definitely before the eubacterial and archaebacterial biosynthetic machinery diverged significantly. The double-stranded DNA genomes of tailed phages are mosaics of virus and cell genes, presumably the result of modular evolution with horizontal transfer of genes between phage genomes and between phage and bacterial genomes. There has been less analysis of phages with icosahedral and filamentous morphology but, because their hosts are usually obligate or facultative aerobes, these types of phages may have evolved more recently, after the evolution of an oxygen-containing atmosphere.

Applications

Phages have been used as tools in molecular biology and biotechnology, antibacterial agents, markers and test objects, and reagents for typing bacteria. In addition, phages are significant contaminants in a number of industrial processes.

Phages were important experimental organisms in the origin and development of molecular biology, and continue as essential systems in many aspects of molecular biology and biotechnology. For example, several tailed phages with double-stranded DNA and their derivatives (e.g. phages λ, P1 and Mu, and constructs containing parts of phage λ and plasmid DNAs) are major cloning vectors; filamentous phages with single-stranded DNA (e.g. phage M13 and its variants) have been genetically engineered to serve as cloning vectors, DNA sequencing templates, and vectors for phage display (i.e. expression of cloned peptides for selection and cloning); and phage Mu, in which replication involves integration of double-stranded Mu DNA into the host cell's genome, is used for random mutagenesis and gene transfer.

D'Hérelle originally recognized the possibility of using phages as therapeutic antibacterial agents for certain diseases. Experiments at that time and into the 1920s were popularized in Sinclair Lewis's 1924 novel *Arrowsmith*. The controversial results of phage therapy kept this approach from being adopted in most countries, and the advent of antibiotics in the 1940s generally eliminated interest in this type of therapy. However, phage therapy continued to be studied and used in France, Poland and parts of the former Soviet Union. In the past few years there has been renewed interest in evaluating phage therapy, as the work from Eastern Europe has become known and translated into English and as antibiotic-resistant bacteria have become a growing threat to public health.

The small size of phage particles and the sensitivity of phage assays (a single phage can be detected as a plaque-forming unit) have made phages useful markers and test objects for a variety of industrial applications. These include measuring disinfectant efficacy, evaluating air filter and aerosol sampling capacities, and monitoring water movements in natural environments.

Phage typing was an early epidemiological tool for classifying bacteria. Sets of typing phages were established for many human, animal and plant pathogenic bacteria. In practice, samples of phages from a typing set were spotted on lawns of bacteria of interest and, after incubation, bacteria were classified based on their resistance to infection by members of the phage set. Phage typing has been replaced almost completely by a variety of molecular methods for bacterial epidemiology (e.g. determination of molecular markers like antibiotic resistance and plasmid profiles).

Finally, although not a desired application, phages are important contaminants in a number of industrial microbial fermentation processes; for example production of antibiotics, organic solvents and dairy products. Phage contamination leads to abnormal fermentations and can cause significant economic losses. A variety of strategies (e.g. production conditions to minimize contamination and rotation of microbial cultures) are used to prevent and control phages in these industries.

Further Reading

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