MINIREVIEW

Phage-Host Interaction: an Ecological Perspective

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Nearly 100 years ago, Felix d'Herelle, the codiscoverer of bacteriophages, used bacteria to control insect pests and used phages against bacterial disease. His approaches reflected ecological insights before this branch of biology became an established scientific discipline. In fact, one might have predicted that phage research would become the springboard for biotechnology and ecology. However, d'Herelle was ahead of his time, and the zeitgeist in the 1930s pushed physicists into the question "What is life?" Phages as the simplest biological systems were the logical choice for this question, and phage research became the cradle of molecular biology.

Now many researchers speak of a "new age of phage research." It is now realized that phages play an important role in ecology (e.g., phage impact on the cycling of organic matter in the biosphere at a global level) (27), that phages influence the evolution of bacterial genomes (most obviously in the development of bacterial pathogenicity) (7), and that phages might provide potential tools to face the antibiotic resistance crisis in medicine (59). With this new trend, we now see a clear shift from the reductionist approach, focusing on a handful of phages in carefully controlled laboratory conditions, towards the study of many different phages in the complexity of real-life situations.

In contrast to the molecular biology-oriented phage research where the interaction of molecules took center stage, ecology focuses on the interactions between organisms and their physical environment. Much of ecology is therefore about the evolution of biological diversity in space and time. In contrast to many branches of biology, ecology attributes a great importance to quantitative relationships and numbers and aims at a mathematical formulation of its observations. It is thus appropriate to start this review with an overview of phage titers encountered in the biosphere. Next, we ask how a parasite targets its host if the latter is scarce or not in an appropriate physiological state. Finally, we report on research that tries to bridge phage ecology and genomics and cell biology approaches. It is concluded that the integration of phages into complex networks of interacting biological systems, and analysis by molecular techniques, could give phage research a model character in biology again.

NUMBERS: PHAGE TITERS IN THE BIOSPHERE

Just 14 years ago, a Norwegian group surprised the scientific community with a report on the high concentration of phage-like particles in coastal water and the ocean and even higher concentrations in lakes (5). In eutrophic estuarine water, bacteria are found at a density of 10^6 cells/ml and viruses with a concentration of 10^7 particles/ml. These concentrations are estimates that vary with the seasons and the geographical location. In addition, these figures refer to physical and not viable entities. A popular model postulates about 10 to 50 different bacterial species and 100 to 300 different phage strains (70) in this environment. The higher number of phage species was justified by the fact that each bacterial species may be infected by 10 phage species. As one would expect, the production and distribution of marine phages are determined by the productivity and density of the host bacterial populations. This relationship is expressed by the virus-to-bacterium ratio that is frequently at 10 to 1. For a comprehensive review on viruses in water and an extensive reference list see reference 68. Substantial theoretical and experimental research efforts were undertaken to determine the quantitative degree of virus-mediated bacterial mortality and to assess the ratio of phage lysis versus grazing of bacteria by protists and multicellular organisms. The greatest impact of phage lysis was seen in oligotrophic environments, and the ratio of lysis versus grazing changed with water depth both in the ocean and in lakes (66). To summarize the extensive literature, different approaches in various environments yielded a remarkably constant rate of virus-mediated bacterioplankton mortality of about 15% per day (61). The rate seems to be higher for heterotrophic bacteria than for the autotrophic cyanobacteria (61). Ecologically even more important is the profound effect of phages on the relative proportions of different bacterial species or strains in a community.

All these figures have important consequence for our biological view of the world. If phages outnumber bacteria in the ocean, phages are likely to be numerically the most prominent biological systems on earth, with an estimated population size of $\geq 10^{30}$ phage particles. With these numbers, even rare phage-induced events manifest with high frequency. For example, transduction, the accidental packaging of bacterial host DNA into a phage particle, occurs under optimal laboratory conditions about once in every 10^8 phage infections. When calculated for the global marine phage population, it follows that gene transfer between organisms takes place about 20 million billion times per second in the oceans (16). The actual numbers will probably be lower due to smaller transduction efficiency and more rapid phage decay in the ocean than in the

laboratory. If only a small part of this DNA is traveling between different bacterial species, gene transfer via marine phages opens up enormous possibilities for horizontal DNA transfer between bacteria.

High concentrations of phages are not restricted to the ocean water. Counts of up to 10^9 phage per gram of marine sediment were recorded with bacterial counts higher than in the waters described above (21). Terrestrial ecosystems, e.g., soil associated with plant roots in sugar beet fields, revealed 10^7 viruses per gram by using transmission electron microscopy (1). Not all viruses will be viable, but hybridization experiments with *Serratia* and *Pseudomonas* spp. from the soil showed that about 5% of the bacteria are actually phage infected.

There is a dictum that phages are found where bacteria thrive. Thus, we should not be surprised that phages are found on us (skin) and within us (oral cavity, gut). In a survey of stool samples from 600 healthy adults, 34% of the subjects demonstrated coliphages (but only 1% showed high amounts) (28). Most of them were classified as temperate phages related to phage lambda. Ruminants that rely heavily on bacteria for cellulose digestion show intestinal phage concentrations in excess of 10^7 phages per gram of feces. Stool phages were mainly explored for their potential to trace fecal contamination in the environment and to monitor the intrusion of polluted surface waters into groundwater.

Phages are also present in the food we eat. Many food products from our daily life are the result of fermentation processes by lactic acid bacteria. Cheese factories using *Lactococcus lactis* can be contaminated with high levels of phages; one study reported up to 10^9 phage per ml of whey and up to 10^5 phage per m^3 in the air (48). When the phage titers exceeded 10^3 PFU/ml, the yogurt fermentation process was delayed and came to a stop at higher phage titers, leading to important economical losses (unpublished observations).

CYCLES: DYNAMIC PHAGE-HOST RELATIONSHIPS

Time series experiments revealed the seasonality in marine phage titers, demonstrating a dynamic relationship between phages and their bacterial hosts (8). In fact, at any given moment the marine phage titer is the net result from two opposing processes: synthesis of new phage particles due to ongoing lytic phage infections, which is balanced by phage decay. Sunlight UV was identified as the major destructive factor (69), causing up to 5% phage infectivity loss per hour for surface waters due to thymine dimer formation.

Repeat samples from a Norwegian fjord demonstrated seasonality in the viral titers, with a low in the cold winter season and a high in the summer. As a general rule, bacterioplankton produces greater amounts of phages under environmental conditions favoring fast bacterial growth and productivity. Other phage cycles occur on shorter time scales, as predicted by the "killing the winner populations" hypothesis. This concept states that phages expand on the fastest growing host population in the given ecological setting (54). The phage epidemic ceases when the diminished host population no longer supports phage replication. There is strong ecological evidence that some bloom collapse is in fact mediated by phage lysis: in the prealpine Lake of Constance, a transient increase in bacterial abundance was closely followed by peaks in the frequency of infected bacteria and then free phage (32) (Fig. 1B). Variability in the ocean was shown even over half-hour time intervals, probably reflecting synchronized infection cycles (8).

Cyclic phage development was also seen in other environments. Two types of *Serratia* phages were associated with the soil surrounding sugar beets: temperate *Siphoviridae* with a long latent period and big burst size and virulent *Podoviridae* with a short latent period and a small burst size. Over a 6-month observation period, the initially predominant siphovirus population changed to one dominated by the podovirus. Apparently, the two phages are adapted to two quite different niches in the rhizosphere, where the availability and physiological status of bacteria and plants changes with time (2). Some reports indicate that phage-host interaction can be quite complicated in the soil. In streptomycetes, spatial heterogeneity in phage-host interaction and temporal changes in phage susceptibility defined bacterial escape strategies from phage lysis. It was revealed that germinating spores were more susceptible to phage infection than hyphae of developed mycelia. Mature, resistant mycelia adsorb most of the *Streptomyces*-specific soil phages and thus protect younger, susceptible hyphae from infection (15).

Fluctuating phage titers are also a common observation in the dairy factory. This reflects the buildup and ensuing disappearance of phages specific for a starter strain imposed by the starter strain rotation system. Phage dynamics were also documented in a large intervention trial in a cheese factory (14) (Fig. 1A). One starter combination was replaced by a second that was insensitive to the resident phages of the factory. The intervention resulted in a nearly immediate disappearance of the resident phages. However, 5 to 7 days after the intervention, the first phages infecting the new starters were detected. Restriction enzyme analysis of the phage DNA traced the origin of the new phages to the rare phages in the raw milk samples delivered to the factory during the intervention period. Phage titers increased rapidly to the former levels, and starter rotation had to be reintroduced. Persistence of the phage in the absence of a propagating starter strain was not observed in the factory (unpublished observations).

In the human gut, it was reported that phage types and titers differed when healthy adults and patients suffering from traveler's diarrhea were compared (28). The former excreted low titers of lambda-like phages, while the latter yielded higher numbers of T4-like phages. This change in the phage popula-

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FIG. 1. Cycles: dynamic phage-host relationships. (A) Phages in the cheese factory. Cheese whey phage titers (*y* axis) observed on the starter strains B to P indicated on the *z* axis on a given day (*x* axis) before (negative days) and after the introduction of the new starter strain. Reprinted from reference 14 with permission. (B) Phages in a European lake. Chlorophyll a, bacterial abundance, bacteria with intracellular mature phage particles, free phages <100 nm in diameter, phage production, and burst size in the Lake of Constance during different seasons between late March and August of 1992. Error bars indicate standard deviations. Reprinted from reference 32 with permission.
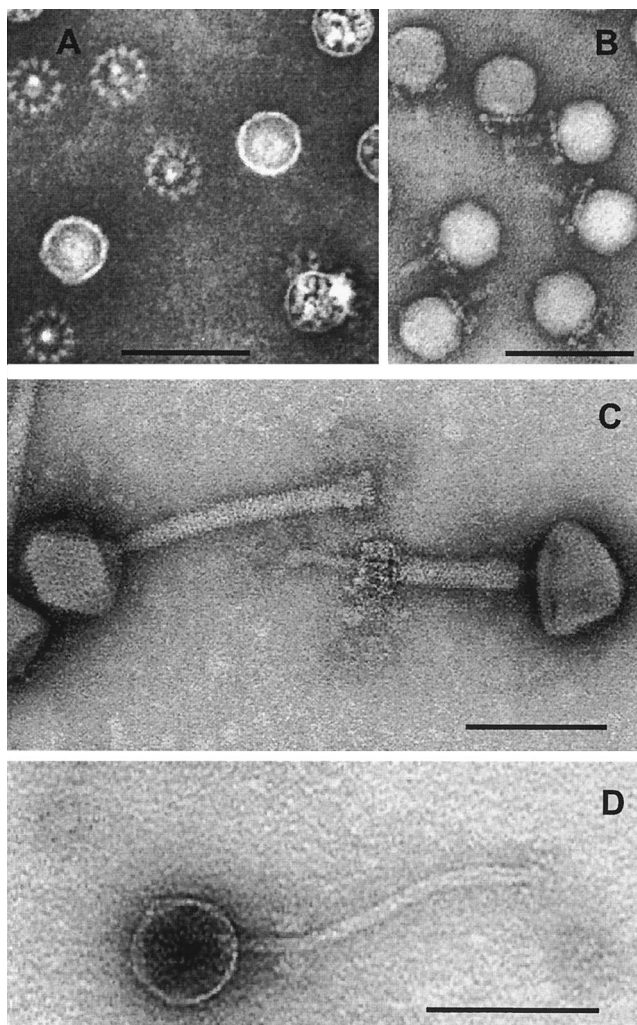
A

B
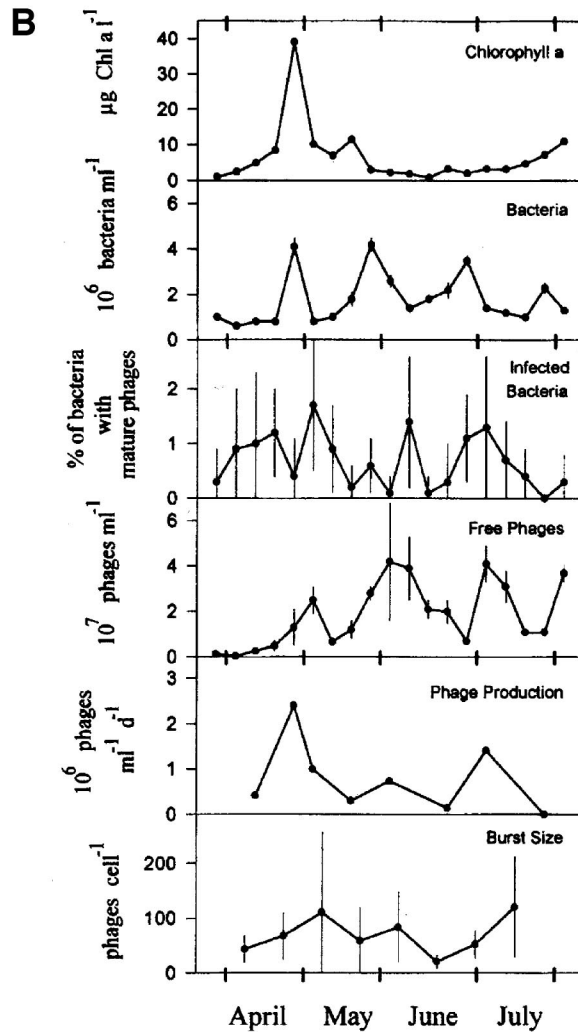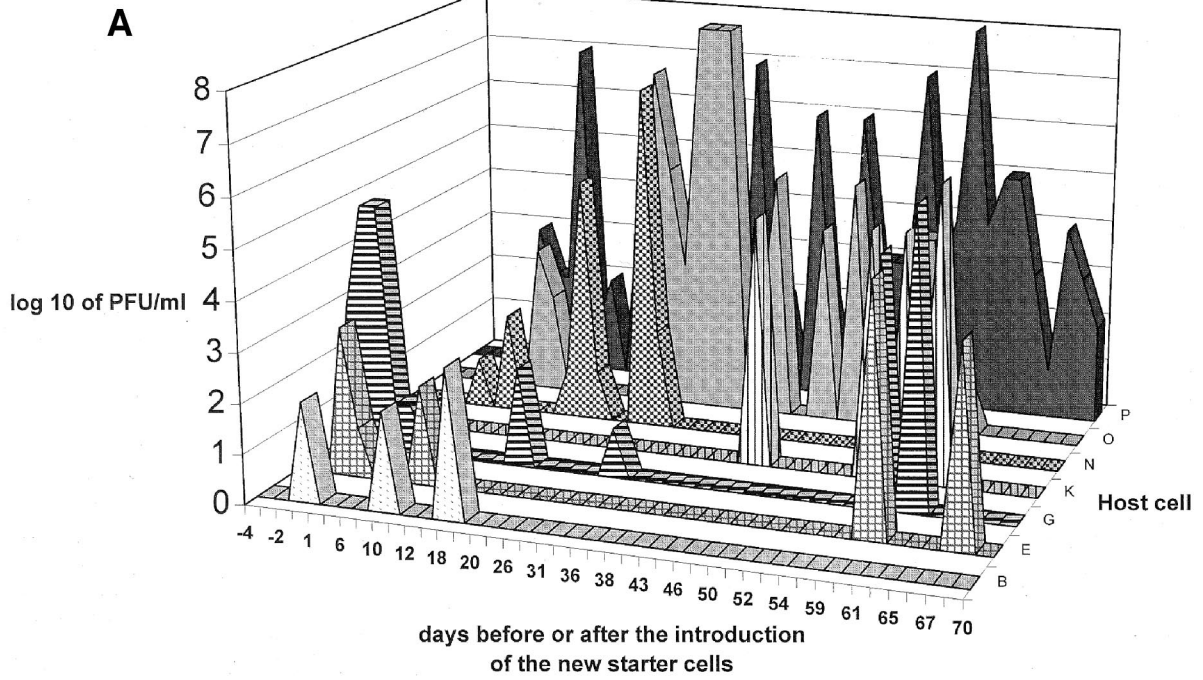
FIG. 2. Morphological diversity of phages isolated from food fermentation. The negative-stain electron microscopic pictures show a *Lactobacillus plantarum* myovirus LP65 (note the contracted tail in the right phage in panel C), a *L. plantarum* siphovirus LP45 (panel D), and a *Staphylococcus carnosus* stc1 or -2 podovirus (panel B, side view of stc2; panel A, stc1 [from beneath, the phage baseplate becomes visible as a wheel-like structure]). All phages were isolated from meat (salami) fermentation. The bars are 100 nm.

tion was suggested to reflect disturbances of the intestinal microflora during a bout of diarrhea.

COMPLEXITY: PHAGE GENOMICS

Are phages genetically as diverse as they are numerous in the biosphere? Only a few phage groups have been intensively studied by sequencing projects (coliphages, mycobacteriophages, and dairy phages). These investigations demonstrated that the genetic relatedness between phages reflected more the phylogenetic relationships between their host bacteria than the possibilities of current gene transfer in a given ecological setting. For example, phages from dairy streptococci were closely related to phages from pathogenic streptococci living on the skin. *Lactococcus* phages are the prime cause of cheese fermentation failures. Due to this economic interest, their genetic

diversity was intensively explored. Different types of prolate-headed and large and small isometric-headed *Siphoviridae* (phages with long noncontractile tails) and rare *Podoviridae* (phages with short tails) were described. Interlaboratory phage comparisons led to the definition of 12 lactococcal phage species. In meat fermentation, you also find *Myoviridae* (phages with contractile tails) infecting *Lactobacillus* starters (see Fig. 2 for the basic tailed phage types). Mycobacteria are hosts to an amazingly diverse group of phages (51). Their morphology stimulated the researchers to use such fanciful names as Corn-dog phage. As variable as the morphologies are, their genomes are just as variable. Within the 14 sequenced mycobacteriophages, 10 lacked DNA sequence similarity with the other mycobacteriophages, and nearly half of the predicted proteins lacked any database matches. The workhorses of molecular bacteriology, *Escherichia coli* and *Bacillus subtilis*, are also hosts to about 10 phages with fundamentally different genome organization, not to mention substantial differences at the DNA sequence level within each group of phages. If these observations of 10 or more phages per bacterial species can be generalized, phages might indeed represent the largest unexplored reservoir of sequence information in the biosphere. Random sequencing efforts of viral DNA from two uncultured marine water samples (10) combined with a statistical analysis revealed between 400 and 7,000 different viral types in the two investigated 100-liter samples, with the most abundant type representing 3% of the total viral population. However, a different picture was obtained by the few complete marine phage genomes. This analysis led to the description of a new phage family (*Corticoviridae*), while other marine phages shared protein sequence similarity with coliphages T7, T4, lambda, P2, and dairy siphophages, suggesting a limited number of prototype phage genomes (18, 72) (Fig. 3).

The human gastrointestinal tract is rich in commensal life forms. About 10^{11} bacteria per ml of colonic content are normally found. The number of bacterial cells in the gut exceeds the total number of cells constituting the human body. Gut bacteria contain about 400 bacterial species, but only 40 species account for 99% of the total population. Also, shotgun cloning of the uncultured virus fraction of human feces underlined the genetic diversity of this fraction (9). Statistical calculations suggested between 160 and 1,200 viral genotypes in the intestine.

NUTRITION: INFECTING THE STARVED CELL

Scientists in the laboratory generally work with media maintaining optimal growth of their bacteria. Bacteria in the marine environment have to live with much less nutrients.

Consequently, in situ burst size (the number of phages produced per infected cell) is generally smaller in the marine environment than in the laboratory, where bacteria grow to larger sizes. It was predicted that the normal state of a marine bacterium corresponds to the nutritional state of a laboratory bacterium under stationary phase. In the laboratory, bacteria cannot be productively infected with phages in the stationary phase. In general, infected stationary-phase cells release progeny phage only after resumption of cell growth. This observation could explain the persistence of virulent phages within populations of nongrowing cells. However, there are reports of *Pseudomonas* phages that productively infect host cells main-
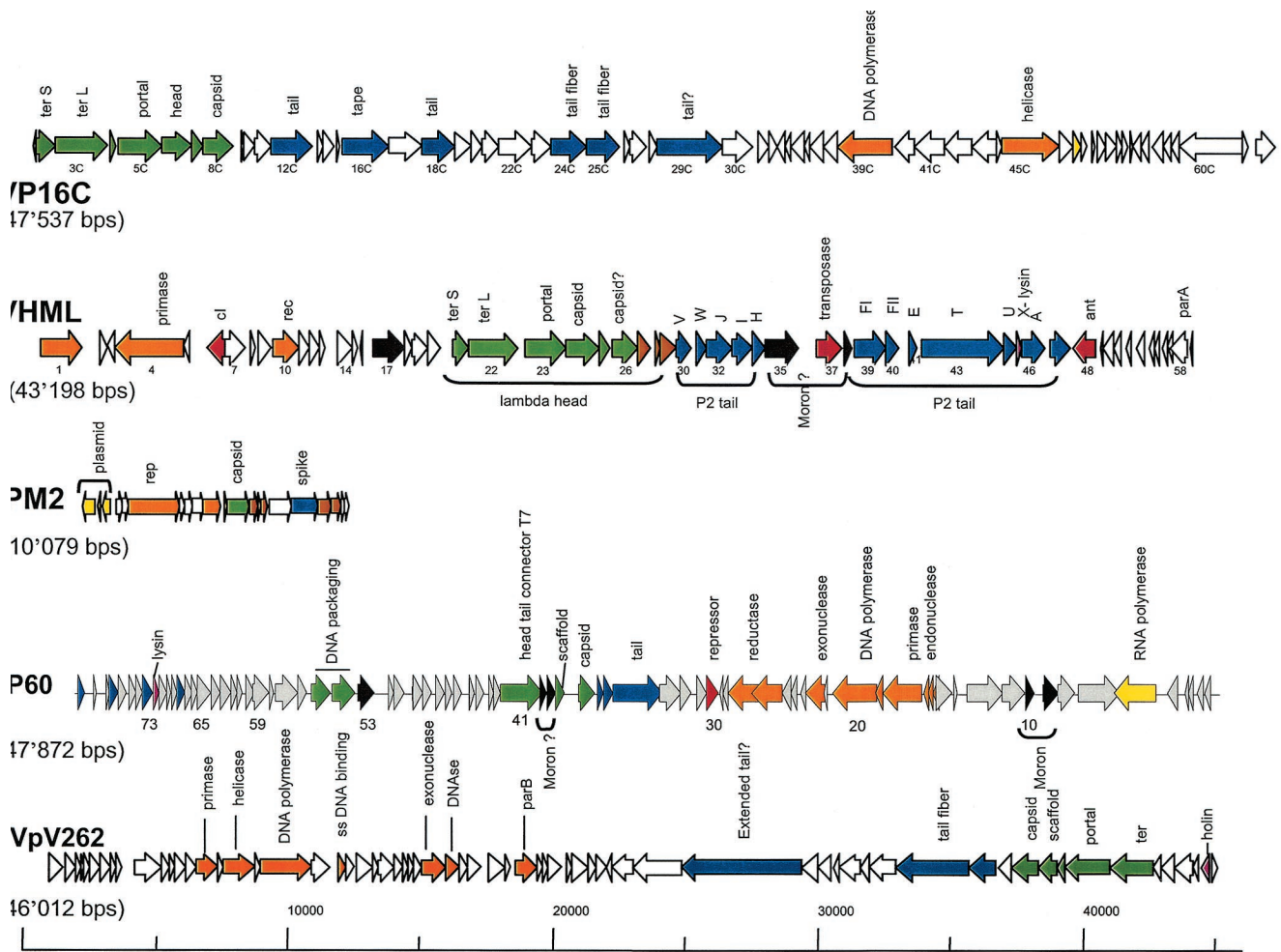
FIG. 3. Genomic diversity of phages isolated from the ocean. The genome maps of the currently sequenced marine phages are shown. From top to bottom: *Vibrio parahaemolyticus* phage VP16C resembling dairy *Siphoviridae* over the structural genes (GenBank accession no. AY328853; AY328852 for the related phage VP16T); *Vibrio harveyi* phage VHML resembling *E. coli* siphophage lambda over the head and myophage P2 over the tail genes (no. AY133112); *Alteromonas* phage PM2, the first lipid-containing bacterial virus attributed to the new phage family *Corticoviridae* (no. NC_000867); cyanophage P60 infecting *Synechococcus* sp., which shows a distant *E. coli* phage T7-like genome organization (no. NC_003390); *V. parahaemolyticus* phage VpV262, another marine phage with a T7-like genome organization (no. NC_003907). Not shown are a *Roseobacter* phage SIO1 (no. NC_002519), since it only distantly resembled T7 phages and otherwise nothing else in the database. Also not shown is the broad-host-range vibriophage KVP40 (no. NC_005083) because its 244,835-bp genome is larger than all depicted phage genomes together. KVP40 resembles *E. coli* myovirus T4 closely over the structural and DNA replication modules but differs by large insertions of DNA lacking database matches. The likely functions of the genes are color coded: green, head; blue, tail; brown, head-to-tail genes (in VP16C) and transmembrane structural genes (in PM2); orange, DNA replication; yellow, transcription; red, lysogeny and recombination; mauve, lysis; black, lysogenic conversion genes.

tained under starvation conditions (57). In this case, the latent period was lengthened and the burst size greatly reduced when compared to those of logarithmic-phase infection (71). In fact, mycobacteriophages have found a way to replicate in slow-growing cells (51).

The nutritional considerations are also relevant for applied phage research like the therapeutic use of phages against pathogenic bacteria. Empirical phage therapy was conducted in the United States during the 1930s and in the Soviet Union until quite recently, where substantial success against bacterial diarrhea and skin infections was reported (59). Notably, oral T4-like phages survived the gastrointestinal passage in mice (19) and humans (unpublished results). However, the presence of a host cell and a corresponding phage is not sufficient to lead

to a phage infection in the intestine. The host cell must be in an appropriate physiological state to allow productive infection, and the cell must be in an accessible anatomical location for the phage. Mouse experiments demonstrated that the resident *E. coli* flora was protected against oral phage exposure, although the majority of the fecally excreted *E. coli* strains were infected by the same phage in vitro (19). At first glance, one would expect that the gut is a nutritionally privileged anatomical site. However, *E. coli* within the lumen of the colon is nutritionally deprived and nonreplicating (53) and is thus a poor target for phages. The metabolically active intestinal *E. coli* cells are found in microcolonies associated with the mucus layer of the gut mucosa (37, 52), where they are probably physically shielded from luminal phages. In contrast, *E. coli*

strains freshly introduced into the mouse infection model were fully susceptible to oral phage exposure (19). Apparently, these new *E. coli* cells were actively replicating but not yet associated with the mucus layer of the gut mucosa. This example illustrates that we need a detailed knowledge of the ecology of target bacteria and the therapeutic phages in the gut of the mammalian host if phage therapy is to be successful (38). Similar considerations apply for the application of phages to other body sites, e.g., the skin. The Soviet experience suggested the use of phages for the treatment of wound infections (59), and food microbiologists explore the use of phages to decontaminate the skin of slaughtered chicken from *Salmonella* and *Campylobacter* spp. (30) and *E. coli* O157 for beef (38). The potential medical and food safety applications of phages are favored by the availability of broad-host-range *Myoviridae* for *Staphylococcus aureus* (50) and *Listeria monocytogenes* (41).

POLYVALENCY: FINDING THE RIGHT CELL

In near coastal water, the bacterial population consists of 10^6 cells per ml distributed over 100 different bacterial host species (68). Less than 10^4 cells per ml therefore constitute the average target population for a species-specific phage. *Streptococcus thermophilus* phages are found with titers of 200 infectious phages per ml of raw milk (14), while its target cell is only detected in raw milk after enrichment. How can phage infection cycles be maintained under these conditions? Laboratory experiments with three phages, including T4, showed that phage production did not occur below 10^4 cells/ml (67). Statistical analysis and back-calculations in natural marine environments predicted 10^5 cells/ml as minimal bacterioplankton concentrations for successful virus production. However, some marine viruses replicated efficiently down to 10^3 specific host cells/ml (62). The determination of the variations of host cell concentration over time allowed the approximation that cyanophage replication still occurred when the host cell concentration fell to 10^2 cells/ml (65).

Broad-host-range (polyvalent) phages could be a solution to this dilemma. Indeed, some observations suggest that phages isolated from nutrient-poor marine environments showed a trend towards increased polyvalency, possibly representing an adaptation to low host cell concentrations.

For most phages investigated in the laboratory, host species specificity is the rule. The polyvalent phages infecting different genera in the *Enterobacteriaceae* must be regarded with some caution because this family is such a closely related bacterial group. Even data on marine phages indicate that most of them are host species specific, many even demonstrate strain specificity. Polyvalence was more prevalent in cyanophages, but fluorescence-labeled cyanophages (33) demonstrated that they attached specifically only to their host and not other bacteria of the natural consortium. Data from the ocean showed that polyvalency was correlated with phage morphology. Phages isolated from high-light-adapted *Prochlorococcus* hosts yielded exclusively *Podoviridae* that were strain specific (60). In contrast, low-light-adapted *Prochlorococcus* hosts yielded *Myoviridae* that also infected *Synechococcus* spp., a phylogenetically related cyanobacterium. Similarly, *Synechococcus*-infecting *Myoviridae* also cross-infected *Prochlorococcus* spp., lending some support to the polyvalency concept in the marine envi-

ronment. Also, in other environments, *Myoviridae* showed a broader host range than *Siphoviridae* and *Podoviridae*.

Polyvalency was not described in dairy phages: the analysis of hundreds of phage isolates from cheese factories showed a very narrow host range. We are only aware of exceptions for *Lactobacillus* phages appearing in sauerkraut fermentation: one phage isolate could infect two ecologically related *Lactobacillus* species (42). Furthermore, about 30% of lactobacilli constituting the major commensals in the vagina of healthy women were lysogenic. Many lysogens could be induced by mitomycin C, and some phages could infect up to five different *Lactobacillus* species, which dominate the vaginal flora (36).

Even if polyvalency is not the rule, many phages have developed efficient methods to change their host range by elegant genetic tricks. A classical case is phage Mu, containing a recombinase that inverses the orientation of the receptor-interacting gene leading to the synthesis of a new receptor recognition specificity. A coliphage possessed two different tail fiber proteins and showed the combined host range of phages containing either one or the other tail gene (56). The similarities in the tail fiber genes of coliphages belonging to different phage families (P2, T4, lambda) provide evidence that illegitimate recombination resulting in domain exchanges occurs at previously unappreciated levels (31). Similarly, phages infecting lactic streptococci can alter their host range by exchanging variable domains flanked by conserved collagen-like repeats that serve as target sites for homologous recombination (23). A fundamentally different method of host range changes was recently described for *Bordetella* phages (40). At one genome end, the *mtd* (major tropism determinant) gene encodes the phage protein responsible for host cell recognition. Its C-terminal end varies between isolates differing in host range. Directly adjacent to *mtd* is a second nonidentical copy of the variable end of *mtd* called TR (for template repeat), followed by a reverse transcriptase gene. Tropism switching is the result of a TR-dependent reverse transcriptase-mediated process that introduces base pair substitutions leading to amino acid changes at about 20 defined positions in the variable part of *mtd*. The authors proposed a mechanism for this process analogous to the site-specific retrohoming ability of group II introns.

LYSOGENY: A SURVIVAL STRATEGY FOR BOTH PHAGE AND BACTERIA

Theory predicts that lysogeny becomes the preferred strategy when the cell density falls below the lower limit necessary for maintenance of the phage density by repeated cycles of lytic infections. The argument is that the production of temperate phages is independent of host cell density. Indeed, two marine surveys revealed 40% mitomycin C-inducible cells, and similar proportions of lysogens were identified in *Pseudomonas* colonies from lakes. In contrast, UV or sunlight was not a good inducer of prophages in water samples. The surveys showed a trend for lysogeny to be more prevalent in oligotrophic environments (35). This observation fits with theory, since this setting is dominated by the low density of slow-growing bacteria. Other data contradict this interpretation. Surveys in estuarine waters showed a seasonal development of lysogeny with highs in the summer months when eutrophic conditions were prevalent and lows in the winter months when cells were at

their minimum (20, 44). There are further contradictions with expectations. First, spontaneous induction of prophages is generally low (10^{-2} to 10^{-5} phage per bacterium per generation). This release can only account for far less than 1% of the phage concentrations in the ocean (35). Second, large phage surveys in the North Sea revealed that only 10% of the phage isolates are temperate. In contrast, the genome maps of the major virulent *S. thermophilus* phages isolated from dairies still betray their origin from temperate parental phages. The preponderance of virulent phages in dairy collections might therefore represent a secondary character and an adaptation to the abundance of host cells in the dairy environment. In fact, serial passage of a temperate *S. thermophilus* phage resulted in its replacement by a virulent derivative deletion mutant after only a few days.

However, lysogeny is a survival strategy for phage as well as for bacteria (17). Lysogens frequently outcompete the nonlysogenic congeners, possibly due to the selective advantage conferred by lysogenic conversion genes of the prophages. Some of them are relatively universal, such as immunity functions and superinfection exclusion genes. Other prophages contribute genes that make the lysogen competitive under special ecological situations (serum resistance conferred to the lysogen by the phage lambda *bor* gene during blood growth of *E. coli* [3]). This phenomenon seems to be widespread in bacterial pathogens where many virulence factors are encoded by prophages. However, even the laboratory phages P1, P2, lambda, and Mu confer to the *E. coli* lysogen a higher metabolic activity and faster and longer growth than the nonlysogens (25, 39).

Shiga-toxin producing *E. coli* (STEC) strains represent a spectacular case of lysogeny. These strains are commonly found in the intestines of asymptomatic cattle, while in humans the STEC O157:H7 strains are dangerous food pathogens. The available evidence suggests that they are derived over the last 50 years from the enteropathogenic *E. coli* strain O55:H7 by the acquisition of two prophages encoding the Shiga toxins Stx1 and Stx2, the major pathogenicity factor of STEC (58). In fact, the two sequenced O157:H7 isolates contained 16 to 18 prophages, including many closely related lambda-like prophages. Whole-genome PCR scanning of eight distinct O157 strains revealed a high degree of genomic diversity, mainly due to extensive structural and positional diversity of the prophages, implying that prophages are the major factor in generating genomic diversity in the O157 lineage (49).

Toxin production differed in human and bovine STEC isolates (54). The lysogen expresses Stx in case of low iron concentration (a typical growth-limiting factor for intestinal bacteria) (64), leading to intestinal hemorrhage (liberating iron from red blood cells leading to resumed bacterial growth and Stx downregulation). Notably, *stx* is under the control of the Fur repressor and thus part of a large bacterial iron-controlled regulon. Similarly, the diphtheria toxin encoded by a corynephage is under the control of the DTxR, the master repressor of an iron regulon in this gram-positive bacterium. Stx has no physiological secretion pathway and is only released by lysing cells. As antibiotics induce the prophage, chemotherapy can result in an aggravation of the clinical condition. In a fascinating illustration of the selfish gene concept, STEC recruits bystander intestinal *E. coli* cells via infection with the released Stx phage for an amplification of the suicidal Stx production (29).

Experiments with mice demonstrated that the resistance or susceptibility pattern of the intestinal flora towards the released Stx phage exerts either a protective or an enhancing effect on the severity of STEC infections. Clinically, this observation could explain the variability of the disease symptoms in different O157-infected patients.

The observation that pathogenic bacteria convert environmental and commensal bystander bacteria via lysogenization into toxin-producing bacteria is not restricted to STEC (55) but was also found in *Vibrio* spp. (26) and *Streptococcus pyogenes* (11).

PHAGE RESISTANCE

Phages are a nuisance in the dairy industry, where they interfere with industrial milk fermentation when they attack starter bacteria. Therefore, food microbiologists have intensively studied phage-host interactions. Lactic acid bacteria were under substantial evolutionary pressure to develop antiphage strategies, as demonstrated by numerous phage resistance systems naturally found in lactococci. These mechanisms range from the blocking of phage DNA injection (45) to abortive infection systems (*abiA* to *abiU*) interfering with phage DNA replication, RNA transcription, phage development, and morphogenesis. The evolutionary arms race between bacteria and their phages has been well documented by dairy microbiologists. For example, against bacterial restriction systems, phages used different escape strategies covering either the loss of restriction sites (47) or the gain of a methylase gene (34). Acquisition of chromosomal DNA by lactococcal phages confronted with *abi* mechanisms has been repetitively demonstrated (6, 24). The high number of prophages in *Lactococcus lactis* offers in fact a large supply of new genetic material to superinfecting phages which is accessible via homologous recombination. Interestingly, the rich knowledge on phage resistance mechanisms acquired in the dairy field could also contribute to the understanding of the coexistence of phages and bacteria in the marine environment or the management of phage infections in humans (e.g., the cigarette smoke-induced prophage propagation purportedly linked to a decline of the protective *Lactobacillus* flora in the vagina which might open the way to vaginosis and candida superinfections) (36).

THE LOOK AHEAD: INTEGRATIVE BIOLOGY WITH PHAGES

Over the last 10 years, genome sequencing has fundamentally changed microbiology. However, a genomic sequence is fundamentally static. True biological processes are defined by the dynamic interaction of biological systems. Phage and bacterium interaction is conceptually the simplest case of such genome interaction. The infection process provides a precise kinetics for this interaction. Microarray analysis of phage transcription throughout the infection cycle is still limited to phage T4-infected *E. coli* cells (43). In the next logical step, microarray analysis from the invading phage must be integrated with that of the infected bacterium, especially when it concerns such a well-investigated organism as *E. coli*.

Phage infection is a highly dynamic process, probably inducing bacterial gene expression. Some of these events might even

FIG. 4. Phage-host interaction. Thin-section electron microscopy of myophage LP65 infecting an oral commensal *Lactobacillus plantarum* strain. Note the conspicuous membrane system underlying the phage adsorption site. The phage is the same as the myovirus shown in a negative-staining preparation in the central panel of Fig. 2. The bar is 100 nm.

be visible in thin-section electron microscopy (Fig. 4). If the genomes of both the phage and the bacterium were sequenced, transcription of both genomes could be analyzed on microarrays at different time points after phage infection. If this analysis is combined with thin-section immune electron microscopy and proteomics, one could combine cell biology with gene and protein expression studies to examine the consequences of two genomes confronting each other in a hostile encounter.

However, the analysis must not stop here, since this corresponds still to the test tube situation that has dominated phage biology in the past. The phage research community should immediately envision the next level of complexity with interacting systems. An attractive test system is provided by streptococcal lysogens. Many prophages from low-GC-content, gram-positive bacteria encode candidate lysogenic conversion genes located between the phage lysin gene and the right attachment site (4). In lactic streptococci and in lactobacillus commensals, these prophage genes are expressed during broth culture (63). In contrast, the expression level of the corresponding prophage genes in pathogenic streptococci is low. Interestingly, when *Streptococcus pyogenes* comes in contact with pharyngeal cells (the natural target cells in streptococcal angina), the expression of the lysogenic conversion gene is upregulated (12). Broudy and coworkers have identified a low-molecular-weight compound released by the pharyngeal cell that induces the expression of this prophage gene (13). In this cell culture system, one has the chance to study by microarray analysis the interaction of a phage, a bacterium, and a human cell in a system that is directly relevant for the disease process. The mammalian host not only promotes prophage induction in

S. pyogenes, but it also favors the lysogenization of nontoxigenic bystander bacteria resulting in the generation of new toxin-producing cells (11). Within the mammalian host, bacteria apparently alter not only their gene expression (46) but their genomes themselves.

There are still other suitable model systems to study phage-host interaction in a relevant ecological context. A lysogenic avian *E. coli* pathogen, when injected into a chicken, showed an upregulation of prophage gene expression (22). This and similar whole-animal experimental systems allow the analysis of genome interactions at the next complexity level. As the data analysis for such complex genome interactions will represent a substantial challenge, the old virtue of phages, i.e., "small is beautiful," will again become an asset in the postgenomic era.

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