Phage as agents of lateral gene transfer

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When establishing lysogeny, temperate phages integrate their genome as a prophage into the bacterial chromosome. Prophages thus constitute in many bacteria a substantial part of laterally acquired DNA. Some prophages contribute lysogenic conversion genes that are of selective advantage to the bacterial host. Occasionally, phages are also involved in the lateral transfer of other mobile DNA elements or bacterial DNA. Recent advances in the field of genomics have revealed a major impact by phages on bacterial chromosome evolution.

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Abbreviation
LCG lysogenic conversion gene

Introduction

The study of viruses from bacteria (bacteriophages or short phages) was historically a major driving force in the development of molecular biology. Many general concepts of contemporary biology were derived from work with phages, including the first completely sequenced genomes. Phage research has since become the victim of its own success, the technical advances in biology now allows scientists to work with much more complicated organisms. The phage research community has contracted with only few exceptions, such as in dairy where phages are still a cause of important economical losses. Three recent trends have renewed the interest in phage research: phages influence the cycling of organic matter in the oceans, they are potential tools for the treatment of antibiotic-resistant bacterial pathogens and they have a major impact on bacterial genome evolution. More specifically, phages are important vectors for the lateral transfer of DNA between bacterial strains. In this review, we focus on important advances in this field over the past two years.

Lateral gene transfer

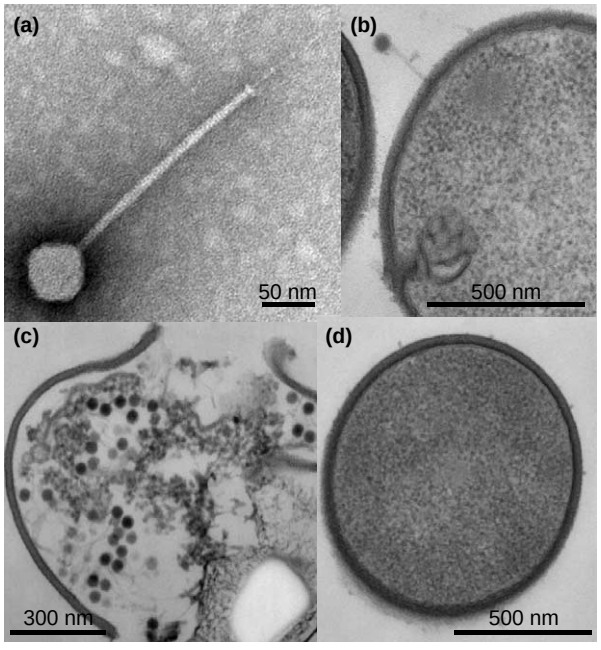
With about 100 sequenced genomes of bacteria in the public database and many more to come, genomics has changed our understanding of microbiology. In fact, the genomes of bacteria are remarkably fluid. A substantial part of the bacterial DNA is not transferred from the parental cell to its descendent ('vertical' transfer), but is acquired horizontally by transformation, conjugation or transduction ('lateral' transfer) [1••]. The replacement of a tree-like by a web-like representation of the phylogenetic relationship between bacteria is a visual expression of this change in perception of microbial evolution. An important element of mobile DNA is bacteriophages. Infection of a bacterial cell with a temperate phage (Figure 1a,b) can have two outcomes: multiplication of the phage with concomitant lysis of the bacterial host (Figure 1c) or lysogenization, (i.e. integration of the phage DNA into the bacterial chromosome as a prophage, Figure 1d). Bacterial genomics revealed that lysogeny is more the rule than the exception; many bacteria even contain multiple prophages (Figure 2a). Some temperate phages carry in their genomes extra genes that change the phenotype of the bacterial host ('lysogenic conversion genes', LCG) (Figure 2b). There is increasing evidence from bacterial pathogens that lysogeny is a motor of short-term bacterial evolution.

Phages as gene-transfer particles

Tailed phages are the most efficient gene-transfer particles developed in evolution. They represent densely compacted phage DNA [2] encased in a protective protein shell (the phage head) [3]. To this remarkable DNA storage device is added an equally efficient DNA transfer device, the phage tail and its associated fibres (Figure 1a). This structure assures both the specific recognition of the appropriate host cell and the guided injection of the phage DNA into the bacterial cell ([4,5], Figure 1b).

Some bacteria have learned to use phages for their own purposes. In *Pseudomonas aeruginosa*, two phage-tail gene-clusters have developed into bacteriocins [6]. The defective *Bacillus subtilis* prophage PBSX has maintained the capacity to build a size-reduced phage head into which 13 kb fragments of random bacterial DNA are packaged. A prophage remnant of *Rhodobacter capsulatus* acts as a gene-transfer agent for random 4.5 kb fragments of bacterial DNA in bacteria-controlled DNA exchange between cells in the stationary phase [7]. Prophage-like elements from *Mycobacterium tuberculosis* encode active integration/excision systems [8].

Figure 1



Streptococcus thermophilus phage Sfi21: lytic phage infection versus lysogeny. **(a)** Phage Sfi21, a typical tailed phage of the *Siphoviridae* family, after negative staining in the electron microscope (EM). Phage head, a non-contractile tail and a single tail fibre are clearly visible. **(b)** An EM thin section shows how phage Sfi21 adsorbs to its bacterial host. **(c)** Infection results in the multiplication of the phage and the lysis of the cell. The heads of progeny phages can be seen inside a cell with disrupted cell wall which has lost most of its cytoplasm. **(d)** Alternatively, phage Sfi21 integrates its DNA into a tRNA gene of the bacterial chromosome resulting in a lysogenic cell with normal morphology and growth properties. The entire phage genome is transcribed in a programmed way during lytic infection [53] whereas only small segments of the prophage genome near both prophage attachment sites were transcribed in the lysogenic cell [15] (see Figure 3b below). The Sie prophage protein protects the lysogenic cell against superinfection with virulent phages.

A particularly interesting case is the 15 kb-long pathogenicity island SaPI1 from *Staphylococcus aureus* encoding the toxin Tst involved in toxic shock. In cells infected with *S. aureus* phage 80α, SaPI1 is excised from the chromosome, it replicates autonomously and interferes with phage growth by directing the encapsidation of its own DNA into specially tailored small phage 80α heads commensurate with its size. Upon phage-mediated transfer to a recipient organism, SaPI1 integrates by means of its own integrase [9••].

Specialised transduction

Resolvase-type integrases from phages of Gram-positive bacteria have no requirements for cofactors facilitating their integration into heterologous hosts [10]. If a prophage is imprecisely excised from the heterologous host, small segments of flanking bacterial DNA can be co-packaged with the phage DNA and transferred to the original host ('specialized transduction'). In accordance with this model, prophages from low GC content Gram-positive bacteria frequently contain extra genes in the vicinity of *attR*, the right attachment site (Figure 2b). Sometimes these genes differ in GC-content from the surrounding DNA and suggest a phage-mediated gene transfer from a rare heterologous host differing in GC content ([11], Figure 3a). In the case of pathogenic bacteria, these extra genes frequently encoded important virulence factors like bacterial toxins [12•,13••,14••] (Figure 2b). These extra genes were also observed in commensals and free-living bacteria and belonged to the few prophage genes expressed in the lysogenic state ([15], Figure 3b); only in a few cases did database matches suggest a physiological role for these extra genes (Figure 3c).

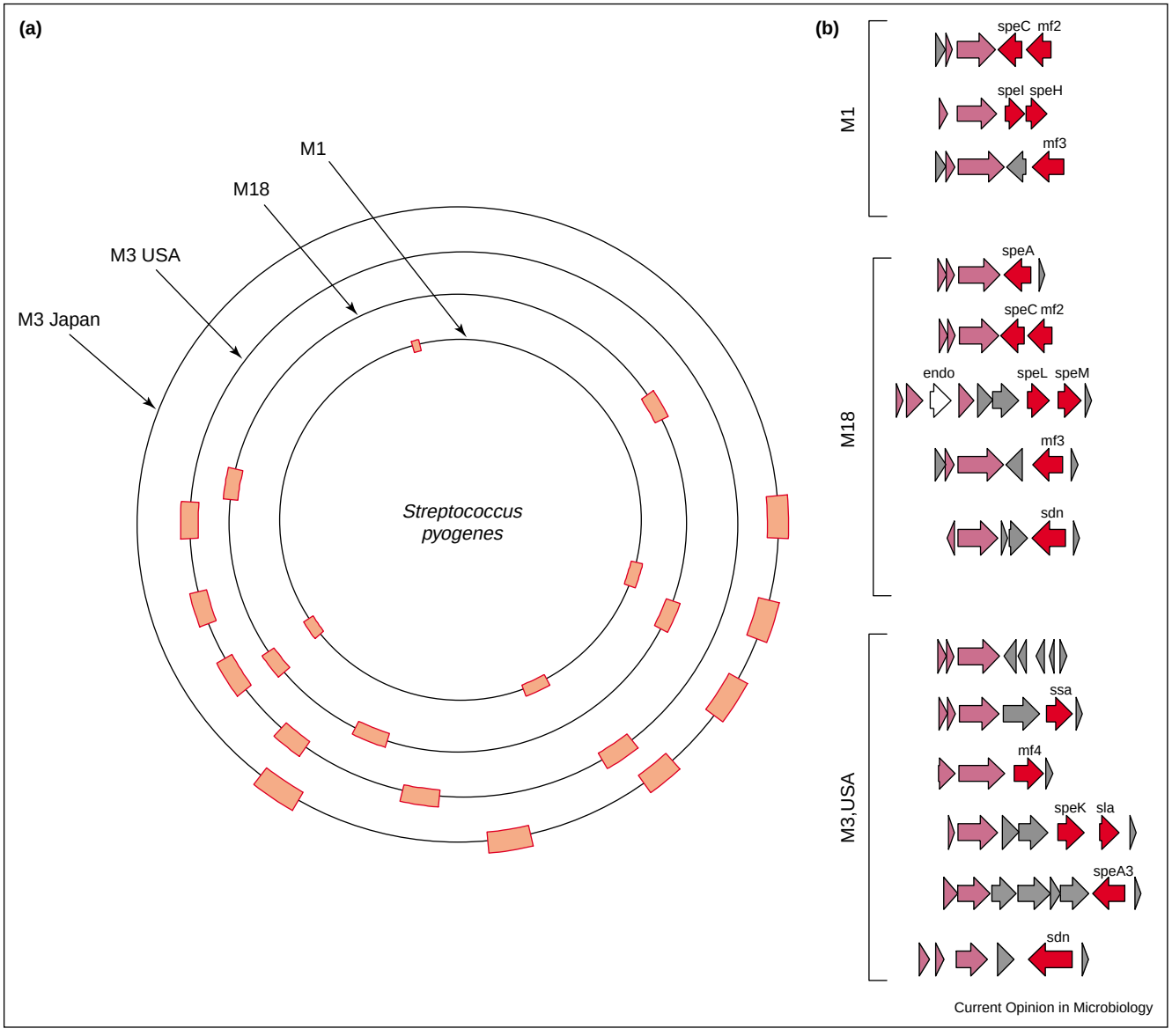
LCG were also identified in prophages from Gram-negative bacteria (Figure 4a). Some of them were located at the prophage genome ends (e.g. O serotype-converting enzymes were found near *attL*, the left attachment site, in several prophages [16,17•]). However, the majority of the extra genes or 'morons' (for more DNA) were detected in the centre of the prophage genomes. Preferred insertion sites for LCG were located downstream of the Q antiterminator, the lysis and the N antiterminator genes [18]. They tend to represent transcription units with their own promoters and terminators that are regulated independently from the rest of the prophage [19,20]. Some of the LCG were shown to respond to environmental cues [21,22]. In fact, when bacteria were grown under conditions that mimicked pathological conditions [23], or when they were grown in infected animals [24], prophage genes belonged to the most prominent genes of the entire bacterial chromosome that changed the expression level.

Generalised transduction

Phages such as *Salmonella* phage P22 or coliphage Mu occasionally commit the error to package even a headfull of bacterial DNA instead of phage DNA. Upon infection of the next host, this bacterial DNA can be incorporated into the bacterial chromosome ('generalised transduction'). Despite the interest in gene flux in the environment, sparked by the discussion of the risks associated with the release of genetically modified microorganisms, only a few recent reports have investigated generalised transducing phages in terrestrial habitats (in *Streptomyces* and *Listeria*) [25,26]. One technical report addressed the problem of PCR-detection of phage-encapsidated bacterial DNA when working with uncultivatable bacteria and their phages [27].

By contrast, phage ecology and phage-mediated DNA transfer became a focus in marine microbiology [28]. Researchers realised that viruses (most of them probably

Figure 2



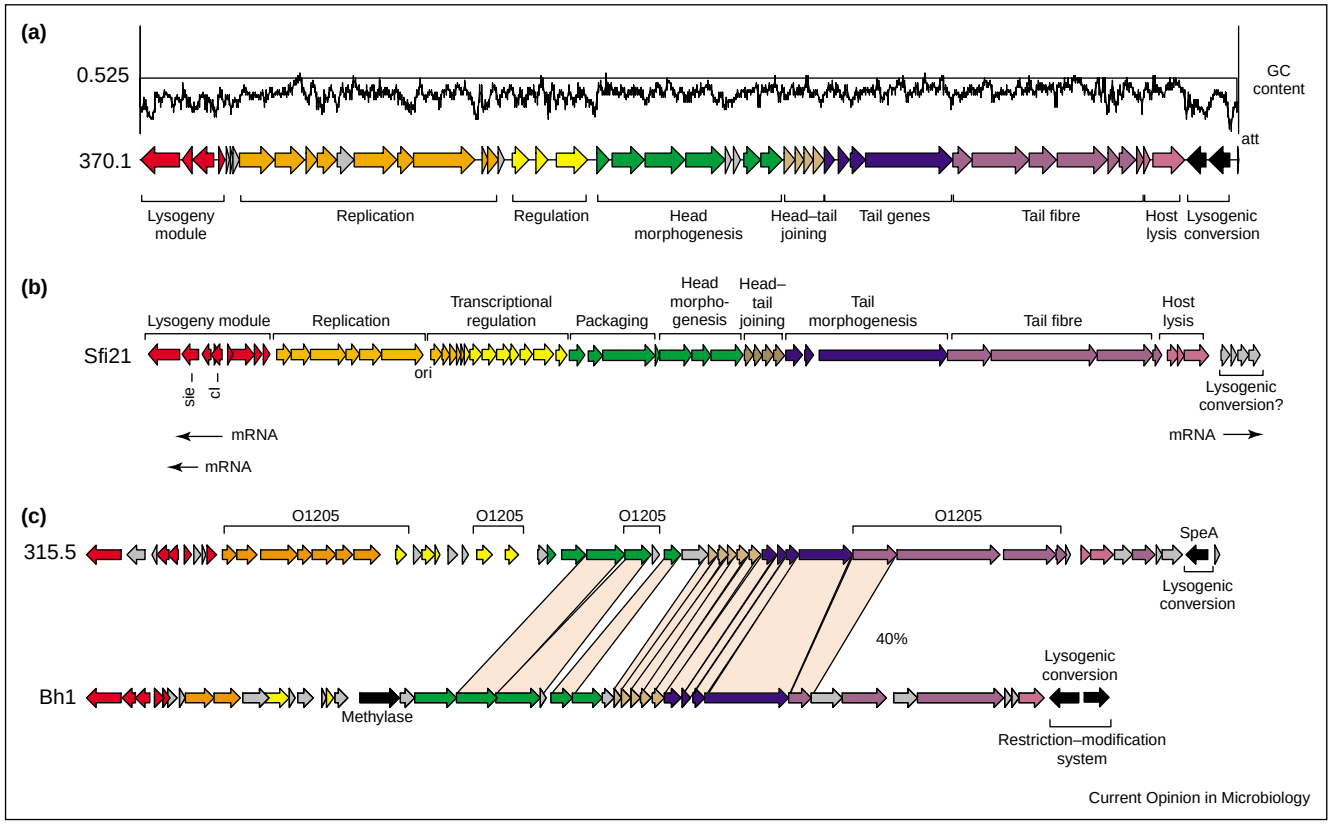
Prophages from *Streptococcus pyogenes* encode many potential virulence factors. **(a)** Prophages are visualised as red boxes on the circular genome maps of four sequenced *S. pyogenes* strains representing three different M types. **(b)** Partial gene maps of the indicated *S. pyogenes* prophages covering the genome region between the lysis module (violet) to the right attachment site *attR*. Grey arrows represent genes of undetermined function. The prophages are noted in clockwise order as they appear in the indicated genome. Candidate lysogenic conversion genes are marked in red and are annotated: mf, mitogenic factors; sdn, streptodornase; sla, streptococcal phospholipase A_2, spe, streptococcal pyrogenic exotoxins; ssa, streptococcal superantigen.

phages) outnumber bacteria in the open ocean by a factor of ten [29]. In view of the large volume of the world's oceans and the high titre of phage particles of 10^7/ml of seawater, phages particles are the most abundant biological entities on earth [30]. If one anticipates a transduction frequency of 10^{-8} per plaque forming unit for marine phages [31], it was calculated that phage-mediated gene transfer takes place at the incredible rate of about 20 million billion times per second in the oceans [1••]. However, the genomics of the predominant marine bacteria and their phages is still in its infancy. Only a handful of marine phages have been sequenced [32] from the 400–7000 viral types estimated in 100 litre water samples [33].

New insights from prophage genomics

The impact of phage-mediated lateral gene transfer can easily be read from the published bacterial genome

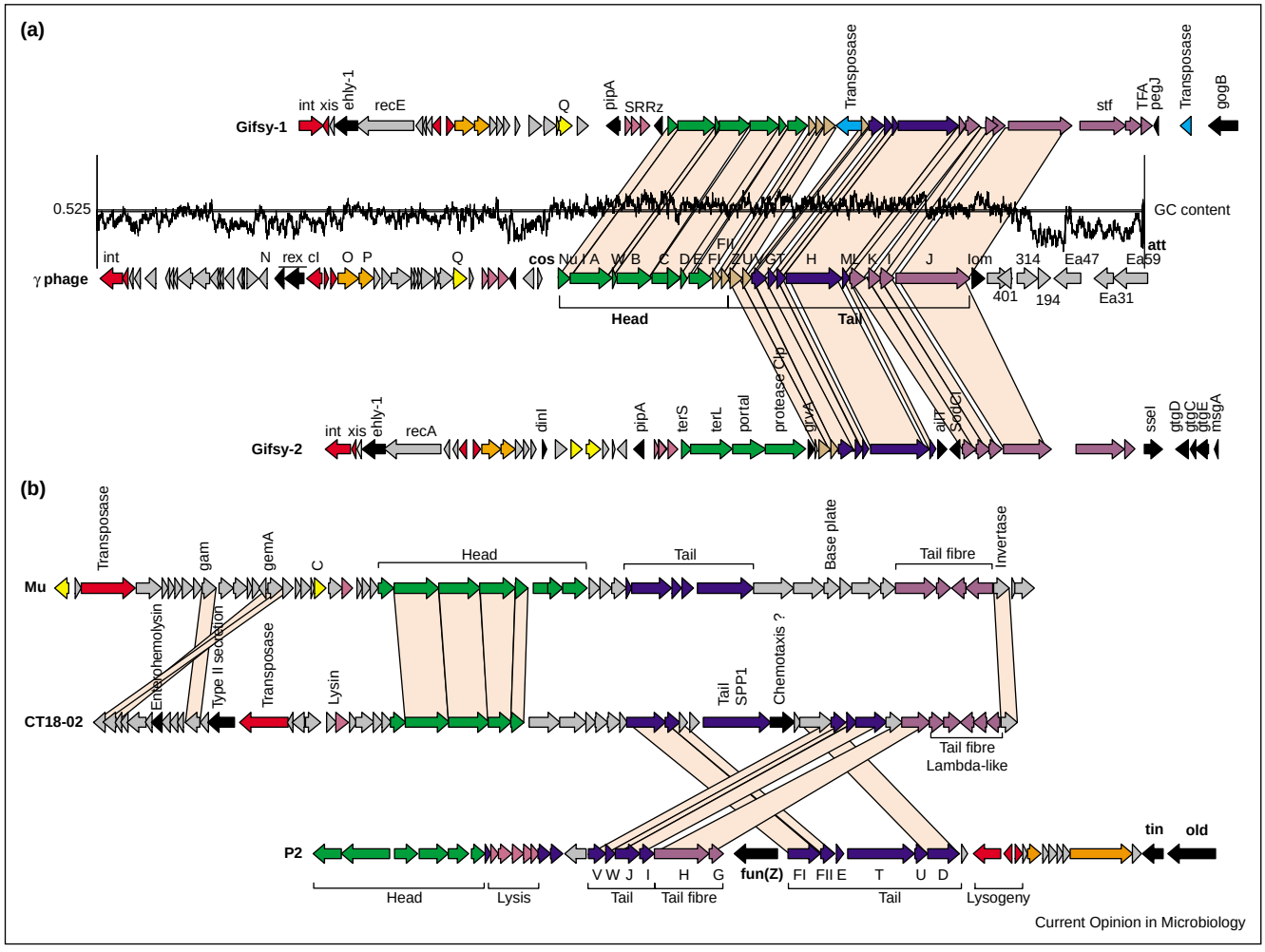
Figure 3



Genome maps of prophages from pathogenic and dairy streptococci and *Bacillus halodurans*. **(a)** Gene map of the *Streptococcus pyogenes* prophage 370.1, a member of the proposed genus of Sfi11-like *pac*-site *Siphoviridae*. Note the distinct drop in GC content of the lysogenic conversion genes near the *attR* site encoding virulence factors. The vertical bar gives 100% GC content (top) and 0% GC content (bottom); the horizontal line marks 52.5% GC. The modular structure of the phage genome is indicated by colour coded arrows and explained in the brackets under the map; for example, the lysogenic conversion genes are indicated by the black arrows. Grey arrows represent genes without database matches. **(b)** Gene map of the *Streptococcus thermophilus* prophage Sfi21, the type strain of the proposed Sfi21-like genus of *cos*-site *Siphoviridae*. Note the comparable modular structure with 370.1 and the restriction of transcription (arrows under the prophage map) to both prophage ends (cI, immunity repressor, sie, superinfection exclusion). The genes to the right of the host lysis cassette lack database matches. **(c)** Alignment of the *Bacillus halodurans* prophage Bh1 with *S. pyogenes* prophage 315.5. Genes sharing amino acid sequence relatedness are connected by pink shading. The amino acid identity was 40% (range: 25–69%) At the map position where 315.5 encodes a candidate virulence factor (SpeA3, marked in black), Bh1 encodes *Hae*III-like restriction/modification genes. The brackets above the 315.5 map annotated with O1205 identify regions of protein sequence sharing with *Streptococcus thermophilus* phage O1205.

sequences [34•]: two-thirds of the sequenced low GC Gram-positive bacteria and γ-*Proteobacteria* (Gram-negative bacteria) contained identifiable prophages. Many bacteria were polylysogenic (contained multiple prophages, Figure 2a). Prophage DNA represented up to 16% of the chromosomal DNA (*Escherichia coli* O157 strain Sakai with 18 prophages) [35•]. Theoretical reasoning on the basis of Darwinian evolution predicted aspects of an arms race and of mutualism in the genetic interaction of phage and bacterial genomes [36•,37•]. Cooperation (mutualism) was demonstrated by the observation of many virulence factors encoded by prophages from pathogens [18], including prominent examples such as the cholera toxin from *Vibrio cholerae* or the shiga-like toxin from enterohaemorrhagic *E. coli* (see also Update). The arms race aspect of prophage genomics was also documented: most prophages from sequenced bacterial genomes showed inactivating point mutations, inactivating DNA insertion (often transposases) or progressive DNA deletion leading to defective prophages, prophage remnants and isolated prophage genes in bacterial genomes. A recurring observation was isolated phage integrase genes in bacterial genomes suggesting that these phage recombination genes involved in lateral gene transfer are of selective value to the bacterial host. Notably, several pathogenicity islands were flanked by direct repeats, the presence of phage integrase and integration into tRNA genes [38]. It is tempting to speculate that some pathogenicity islands have recruited the integration system from decaying prophages to achieve mobility.

Figure 4



The chimeric nature of some prophage genomes suggests that they are the likely product of lateral gene transfer. **(a)** The gene maps of *Salmonella enterica* serovar Typhimurium prophages Gifsy-1 and Gifsy-2 were aligned with *E. coli* phage lambda. Sequence-related genes were linked by pink shading. The GC scan of phage lambda suggests distinct origins for the left and the right arm of its chromosome. Genes are colour-coded with the convention established in Figure 3. Grey arrows represent genes that do not fit into the scheme for the temperate phages from low GC content Gram-positive bacteria. **(b)** Alignment of the *Salmonella enterica* serovar Typhimurium prophage CT18-02 with phage Mu and P2. Genes sharing sequence relatedness were linked by pink shading. CT18-02 combines genes from two different genera of Myoviridae (Mu and P2) and tail-fibre genes from phage lambda.

Selected examples: *Streptococcus*, *Salmonella* and *Xylella*

Streptococcus pyogenes strains belonging to different M serotypes and associated with different pathologies (M1, wound infection; M3, toxic shock; M18, rheumatic fever) were closely related at the DNA sequence level [11,13••,14••]. The alignment of the genomes showed only a few gaps and, remarkably, prophages accounted for the majority of the variation in gene content between the strains. The two M3 strains differed from each other only by genome translocations and inversions. Some of them were flanked by prophages. The *S. pyogenes* prophages belonged to Sfi21-, Sfi11- and r1t-like *Siphoviridae* that represent the majority of prophages currently described in low GC-content Gram-positive bacteria [37•]. It was proposed that the recent emergence of highly virulent strains was the result of the sequential acquisition of three prophages with their specific LCG (superantigens, toxins and secreted enzymes) over the past decades. Microarray hybridization showed that prophage DNA represented a major part of the *S. pyogenes* strain-specific DNA.

Salmonella enterica serovars Typhimurium and Typhi share a closely related genome, although they differ substantially with respect to their prophages (Figure 4). Most of the larger gaps in the alignment of the two chromosomes were explained by prophage insertion. Microarray analysis [39] and further genome sequences

[40•] demonstrated that prophages are also a major contributor to the genetic differences between *Salmonella* strains belonging to the same serovar. The Typhimurium prophages belonged to the P2-like genus of *Myoviridae* and the lambda-like genus of *Siphoviridae* (Figure 4a). The Typhi prophages were distant lambda relatives and also a hybrid Mu/P2 prophage was observed (Figure 4b). Animal experiments with *Salmonella* deletion mutants demonstrated that prophages are not ephemeral selfish DNA that litters the bacterial chromosome, but contributors of numerous virulence factors (Figure 4a). In fact, the variable assortment of prophages was interpreted as a transferable repertoire of pathogenicity determinants in *Salmonella* [41].

The alignment of the genomes from two different pathovars of the plant pathogen *Xylella fastidiosa* revealed three chromosomal regions that were translocated and inverted, but otherwise they shared 98 per cent of the genes [42••]. The Temecula strain contained six prophages none of which shared sequence relatedness with the five prophages from the 9a5c strain. One prophage in each strain resembled filamentous phages. Prophage genes were again the major contributor to the strain-specific genes. The three chromosomal rearrangements were all flanked at one border by a phage integrase gene (see also Update).

Lateral gene transfer between phages

Virulence genes were apparently transferred between phages belonging to different phage groups [43] or infecting different bacterial species [37•] thereby increasing the lateral spread of these genes in bacteria. Sequencing data from coliphages and dairy phages also demonstrated that large phage gene clusters were transferred between distinct groups of phages [44] confirming tenets of the classical modular theory of phage evolution. The strikingly different GC-content of the left and right arm of phage lambda suggests the heterologous origin of this reference phage (Figure 4a). The mosaic character of phages was greater in Gram-negative than in Gram-positive bacteria [45]. In some lambdoid coliphages short conserved sequences were identified at the boundaries of functional modules. This suggested homologous recombination as the driving force for lateral gene transfer between phages [46]. However, the comparison of other lambdoid coliphage genomes suggested that non-homologous recombination occurs everywhere and the observed order in phage genome organisation is the consequence of selection forces eliminating all non-viable recombinants [47]. Recent sequencing data identified hybrids between phage genera (Figure 4b), phage families [17,48] and even temperate and virulent phages [34•]. This abundant lateral gene transfer between previously well-defined phage groups now poses a major dilemma for phage taxonomy and ideas on phage evolution [49].

Conclusions and outlook

Prophages contribute a substantial share of the mobile DNA of their bacterial hosts and seem to influence the short-term evolution of pathogenic bacteria. Automated methods for systematic investigation of prophages and other mobile DNA elements in the available 100 bacterial genome sequences will be necessary to understand their role in bacterial genome evolution. In the past, phages were mainly investigated as the simplest model systems in molecular biology. Now it is increasingly realised that phage research will be instrumental in the understanding of bacterial abundance in the environment. One can predict that phage research will impact diverse areas such as geochemistry and medicine. Success will largely depend on integrative multidisciplinary approaches in a field that has, until recently, been dominated by reductionist thinking.

Update

Recent work has demonstrated that a prophage from a *Lactobacillus* oral-cavity commensal contains candidate lysogenic conversion genes near both prophage genome ends which are sequence-related to mf2 and mf4 from *S. pyogenes* prophages (see Figure 3b) [50]. In addition, microarray analysis demonstrated that 50% of the strain-specific DNA from *Lactobacillus* gut commensal is represented by prophage DNA [51].

In *E. coli* O157, induction of the prophage is required for toxin synthesis and release. Toxin synthesis is secondarily amplified by phage infection of non-toxigenic intestinal *E. coli* commensals, representing a new strategy of bacterial pathogenesis [52].

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- • of special interest
- •• of outstanding interest

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