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MINIREVIEW

Bacteriophage Control of Bacterial Virulence

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In 1930, Felix d'Herelle wrote ". . .the actions and reactions are not solely between these two beings, man and bacterium, for the bacteriophage also intervenes; a third living being and, hence, a third variable is introduced" (19). The contribution of bacteriophages to the pathogenicity of their bacterial hosts began to be uncovered as early as 1927, when Frobisher and Brown discovered that nontoxigenic streptococci exposed to filtered supernatants of toxigenic streptococcal cultures acquired the ability to produce scarlatinal toxin (28). We now know that these supernatants contained a bacteriophage encoding the scarlatinal toxin and that these investigators were describing transduction, i.e., the transfer of genetic material to a bacterial cell via phage infection. Although these early investigators lacked a mechanistic explanation for their observations, they postulated that the bacterium was "within certain limits, perhaps a matter of secondary importance and that toxicogenicity might be a property that could be acquired by different types of organisms." (28). Their hypothesis that bacteria acquire virulence properties has since gained widespread acceptance, as many virulence genes have been shown to undergo transfer among bacteria by phages (via transduction) and other mobile genetic elements such as plasmids (via conjugation).

Over time, a number of toxin genes were found to be phage encoded, and consideration of the role of phages in bacterial pathogenesis emphasized the dissemination of toxin genes among bacterial strains (10). However, it has become increasingly clear that toxin genes are only a subset of the diverse virulence factors encoded by bacteriophages. For example, some phages encode regulatory factors that increase expression of virulence genes not encoded by the phage (84), while others encode enzymes that alter bacterial components related to virulence (31, 58). Furthermore, phages have unique properties that enable them to contribute more directly to bacterial virulence than via transduction. Structural components of virion particles, for example, may be directly pathogenic (5, 6, 99). Additionally, phage-encoded genes frequently undergo replication and transcriptional activation following prophage induction, a process that was speculated to have a role in the production of diphtheria toxin by *Corynebacterium diphtheriae* as early as 1960 (3).

Since d'Herelle's time, his notion of the phage as a third variable in bacterial pathogenesis has proven correct. However, while d'Herelle emphasized the diminution of bacterial virulence by bacteriophages (19), we have instead come to learn that phages serve as a driving force in bacterial pathogenesis, acting not only in the evolution of bacterial pathogens through gene transfer, but also contributing directly to bacterial pathogenesis at the time of infection. This review provides a discussion of (i) the discovery of phage-encoded virulence factors, (ii) bacterial virulence properties altered by phages, (iii) regulation of phage-encoded virulence factors, and (iv) the role of in situ prophage induction in the control of bacterial virulence.

DISCOVERY OF PHAGE-ENCODED VIRULENCE FACTORS

Historically, the discovery that a virulence factor was phage encoded relied upon observation of transmissibility of the factor among bacterial strains, followed by demonstration, sometimes much later, that the relevant genes were located on a phage chromosome. The scarlatinal and diphtheria toxins, for example, were proven to be phage encoded many years after their production was initially reported to be transmissible (27, 28, 38, 44, 90, 102, 108). Later, genetic tools such as transposons Tn*phoA* and Tn*10d-bla* (4, 73) were developed to aid in the discovery of phage-encoded virulence factors. Since PhoA and Bla must be secreted in order to be active, screening fusion libraries for transducible PhoA or Bla activity allowed for the rapid identification of secreted, phage-encoded putative virulence factors, including the λ-encoded *lom* and *bor* (4) genes of *Escherichia coli* and the phage K139-encoded *glo* gene of *Vibrio cholerae* (73).

Phage-encoded genes are not always transmissible, however, either due to technical limitations in detecting transduction or due to the fact that integrated prophages frequently become defective. In the present era, analysis of the genome sequences of bacterial pathogens can expeditiously reveal whether virulence factors are associated with phage-like DNA sequences regardless of whether they are transmissible. For example, the nontransmissible (86) *stx* genes in *Shigella dysenteriae* are adjacent to lambdoid phage-like sequences interrupted by numerous insertion sequences, suggesting that the toxin genes lie in a prophage that has been rendered defective by the insertion sequences (59). Moreover, transduction of a virulence gene is, in and of itself, insufficient evidence that the gene resides in a phage genome. Generalized transducing phages can package and transmit any chromosomal locus, and specific interactions between generalized transducing phages and chromosomal vir-

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ulence genes have been described. For example, the *Staphylococcus aureus* pathogenicity island SapI1, which contains the gene for toxic shock syndrome toxin (*tst*), is mobilized at high frequency by the generalized staphylococcal transducing phage 80α (76); a similar mechanism has not been ruled out for the transmission of the *V. cholerae* pathogenicity island (VPI), which was reported to correspond to the genome of a phage (49).

Currently, analysis of sequences surrounding virulence factor genes offers the most direct and sensitive method of determining whether virulence genes are associated with phage-like sequences. While this approach can reveal whether a virulence gene is associated with phage sequences, it cannot reveal whether the gene is part of a prophage capable of transducing it or of influencing its expression. Mutational analyses can complement sequence analysis in this respect, revealing, for example, the necessity of phage morphogenesis genes for transduction (100) or the necessity of phage regulatory sequences for virulence factor production (98).

BACTERIAL VIRULENCE PROPERTIES ALTERED BY PHAGES

Historically, exotoxin production has been the most widely recognized bacterial characteristic linked to bacteriophage infection (10). However, phage infection of bacteria is increasingly associated with additional effects on bacterial virulence. Phages can alter host bacterial properties relevant to all stages of the infectious process (Table 1), including bacterial adhesion, colonization, invasion, and spread through human tissues; resistance to immune defenses; exotoxin production; sensitivity to antibiotics; and transmissibility among humans.

Phages influence bacterial adhesion, colonization, and invasion. Early events during bacterial infection include adhesion, colonization, and sometimes, invasion. Phages have been shown to be involved in each of these steps in different bacterial pathogens. Phage-mediated adhesion is illustrated by *Streptococcus mitis*, a causative agent of infective endocarditis. Blood-borne streptococci adhere to platelets, fibrin, and exposed valvular matrix components, giving rise to the vegetations that damage heart valves and provide a source of bacteremia and septic emboli. Recently, Bensing et al. (7) used transposon mutagenesis to identify bacterial loci involved in platelet adherence. They identified a locus encoding two surface proteins, PblA and PblB, the gene sequences of which resembled phage capsid and tail fiber genes and were surrounded by other phage-like gene sequences (7). These genes reside on an inducible prophage, SM1 (8), and encode proteins present in the SM1 phage particle (8). Disruption of either *pblA* or *pblB* resulted in decreased platelet binding by *S. mitis*, although polar effects on other loci were not strictly ruled out (7). Whether and by what mechanisms the phage-particle-associated and/or the bacterial-membrane-bound fractions of PblA and PblB contribute directly to vegetation formation by *S. mitis* remain unknown.

Bacterial invasion of human tissues involves the elaboration of bacterial enzymes, including collagenases, hyaluronidases, and hemolysins. The group A streptococcal (GAS) hyaluronidase is phage encoded (43), and hyaluronidase activity is associated with phage particles themselves (5, 6). Since the capsule of GAS is composed solely of hyaluronic acid, the hyaluronidase presumably benefits the phage by aiding in capsule penetration during its infection of or release from streptococci. This phage-associated hyaluronidase activity may also aid the streptococci in their spread through human connective tissues. Although this hypothesis is untested, antibody to phage-encoded hyaluronidase is detectable in sera of patients infected by GAS (32).

The invasive properties of *Salmonella enterica* serovar Typhimurium require the *Salmonella* pathogenicity island 1 (SPI1)-encoded type III secretion system, which injects effector proteins directly into the cytoplasm of host cells (40). Among the numerous effector proteins exported via this secretion system is SopE, which activates human Rho GTPases and contributes to efficient entry of *Salmonella* into tissue culture cells (33, 34, 105). The *sopE* gene is located on a temperate *Salmonella* phage, designated SopEϕ (62). Since effector proteins such as SopE interact intimately with mammalian intracellular components, lysogeny thus aids in the adaptation of *Salmonella* to its mammalian host. In this instance, two accessory genetic elements (SPI1 and SopEϕ) interact to enhance the virulence of a bacterial pathogen. Following epithelial translocation, *Salmonella* preferentially localizes to Peyer's patches within the bowel wall. Stanley et al. identified a *Salmonella* gene, *gipA*, encoded by phage Gifsy-1, that is expressed specifically in the Peyer's patch and is necessary for optimal survival in this environment (85). Therefore, two different phage-encoded genes facilitate two separate early steps during *Salmonella* infection, namely, uptake by intestinal epithelial cells (*sopE*) and survival in the Peyer's patch (*gipA*).

Phages enhance bacterial resistance to serum and phagocytes. During infection, bacterial pathogens encounter the serum- and phagocyte-mediated elements of the innate immune system. Staphylococci produce a number of proteins involved in phagocyte evasion, including a recently discovered chemotaxis inhibitory protein (CHIPS) that binds to and attenuates the activity of the neutrophil receptors for complement and formylated peptides (93; W. Van Wamel, A. Peshel, K. Van Kessel, and J. Van Strijp, Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001, abstr. B-98, p. 62, 2001). This function is proposed to protect *S. aureus* from neutrophil-mediated killing (93; Van Wamel et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.), an important host defense against staphylococci. The gene encoding CHIPS (*chp*) has been shown to reside on a functional phage that also transduces the staphylokinase (*sak*) and enterotoxin A (*sea*) genes and eliminates β-hemolysin production, presumably by insertional inactivation (Van Wamel et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001). Staphylococci also produce the phage-encoded Panton-Valentine leukocidin (PVL), a cytotoxin with direct activity against human phagocytes (48, 92). Thus, by inhibiting phagocytosis (CHIPS) and by directly attacking phagocytes (PVL), two different phage gene products counteract phagocyte-mediated destruction of their staphylococcal hosts.

Inside phagocytes, bacteria are subjected to oxidative stress within organelles that produce superoxide radical and hydrogen peroxide. Bacterial survival in the presence of these molecules is enhanced by enzymes such as superoxide dismutase, which catalyzes the conversion of superoxide ion into hydrogen peroxide and molecular oxygen (21, 23). Figueroa-Bossi and

TABLE 1. Bacterial virulence properties altered by bacteriophages

Bacterial property altered	Mechanism	Reference(s) or Source
Colonization/adhesion		
E. coli	The λ-encoded *lom* gene promotes adhesion to buccal epithelial cells.	4, 69, 72
P. aeruginosa	Phage FIZ15 promotes adhesion to buccal epithelial cells.	91
S. mitis	The SM1-encoded PblA and PblB surface proteins promote adhesion to platelets.	7, 8
V. cholerae	The toxin-coregulated pilus may be phage encoded.	49
Invasion		
S. enterica	Phage SopEϕ transduces a type III secretion system effector that promotes entry into epithelial cells.	62
	Phage Gifsy-1 encodes *gipA*, a gene that enhances survival in the Peyer's patch.	85
S. pyogenes	Hyaluronidase is phage encoded.	43
S. aureus	Fibrinolysin is phage encoded.	77
Resistance to serum/phagocytes		
E. coli	The λ-encoded *bor* gene confers a survival advantage in animal serum.	4
P. aeruginosa	Phages encode enzymes that alter the O antigen.	37
S. enterica	Phage Gifsy-2 encodes SodC, a superoxide dismutase.	24
	Phages encode enzymes that alter the O antigen.	74, 75, 106
S. dysenteriae	Phages encode enzymes that alter the O antigen.	31, 58
S. aureus	Phages encode CHIPS, a phagocytotoxin.	Van Wamel et al.[a]
	Phage ϕPVL encodes the Panton-Valentine leukocidin.	46
S. pyogenes	Lysogeny up-regulates the antiphagocytic M protein.	84
Exotoxin production		
B. avium	Pertussis toxin is phage encoded in *B. avium.*	van Horne et al.[b]
C. botulinum	Botulinum toxin is phage encoded.	29
C. diphtheriae	Diphtheria toxin is phage encoded.	38, 90
E. coli	The Shiga toxins are phage encoded.	39, 66, 103
P. aeruginosa	Pseudomonas cytotoxins are phage encoded.	35
S. dysenteriae	The Shiga toxin genes are associated with phage sequences, probably a defective prophage.	59
S. aureus	Staphylococcal enterotoxins are phage encoded. Staphylococcal exfoliative toxins are phage encoded. Toxic shock syndrome toxin is encoded by SapI, a mobile pathogenicity island transduced at high frequency by phage 80α.	9, 17, 18 107 55
S. pyogenes	Streptococcal pyrogenic (erythrogenic, scarlatinal) exotoxins are phage encoded.	30, 44, 102
V. cholerae	Cholera toxin is phage encoded.	100
Susceptibility to antibiotic		
S. aureus	Generalized transduction contributes to horizontal transmission of gram-positive antibiotic-resistance genes.	15, 26
S. pyogenes		
Transmission		
V. cholerae	Phage-encoded cholera toxin likely promotes transmission by stimulating copious amounts of watery diarrhea.	100

[a] Van Wamel et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001.
[b] S. J. van Horne, D. Bjornsen, P. Carpentier, and L. M. Temple, Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001, abstr. B-109, p. 64–65, 2001.

Bossi showed that a superoxide dismutase (SodC) of *S. enterica* serovar Typhimurium is encoded by a functional bacteriophage, Gifsy-2; isogenic *Salmonella* derivatives lacking Gifsy-2 were attenuated in a mouse infection model (24). Intriguingly, hydrogen peroxide is a highly effective chemical inducer of Gifsy-2, suggesting a possible relationship between SodC activity, which results in hydrogen peroxide production, and induction of the Gifsy-2 prophage (24).

Phages encode bacterial exotoxins. The most widely recognized examples of phage-encoded virulence factors are exotoxins. Exotoxin production is the major pathogenic mechanism of several bacterial pathogens, including *V. cholerae*, *C. diphtheriae*, and *Clostridium botulinum*. Phage-encoded exotoxins work by a variety of mechanisms that have been the subject of a previous review of the involvement of phages in bacterial pathogenesis (10).

Phages alter bacterial susceptibility to antibiotics. Most mobile antibiotic resistance genes are encoded on plasmids or transposons, and no examples of phage-encoded resistance genes are known. However, phages may play an important role, via transduction, in the mobility of these resistance plasmids among staphylococci (26) and streptococci (15). Streptococcal phages have transferred resistance to tetracycline, chloramphenicol, macrolides, lincomycin, and clindamycin (89) and streptomycin (42), probably via generalized transduction of non-phage-encoded resistance genes.

Phage-encoded products enhance transmission of bacterial pathogens. Cholera toxin up-regulates enterocyte adenylate cyclase activity, leading to profuse watery diarrhea (25) that is widely assumed to contribute significantly to the fecal-oral transmission of *V. cholerae* (61). Since the cholera toxin genes are phage encoded (100), this system is an example of a bacteriophage contributing to the transmission of its bacterial host among humans.

REGULATION OF PHAGE-ENCODED VIRULENCE FACTORS

While phages encode virulence genes that influence virtually all aspects of bacterial pathogenesis, phages have been thought to have little role in regulating the expression of these genes. Three main observations account for the predominance of this idea. First, virulence factors are often produced during lysogeny, when most phage-encoded genes are not efficiently transcribed. Some virulence genes, including the λ-encoded *bor* of *E. coli* and the phage-encoded *vir* of *Mycoplasma arthritidis* (94), lie on the noncoding strand relative to lytic phage genes, suggesting that phage transcriptional regulation has little, if any, influence over virulence factor production.

Second, many phage-encoded virulence genes are located near the attachment (*att*) sites of their respective phages, the portion of the integrated phage adjacent to the host chromosome. This finding suggests that they were acquired from the ancestral bacterial chromosome via aberrant phage excision events, perhaps as independently regulated operons. This property is true of all of the gram-positive exotoxin-encoding bacteriophages in which the *att* site is known, including diphtheria toxin (52), staphylococcal enterotoxin A (18), staphylokinase (18), PVL (47), staphylococcal exfoliative toxin (107), CHIPS (Van Wamel et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol. 2001), streptococcal erythrogenic (scarlatinal) toxin (45), and streptococcal pyrogenic exotoxin C (30). Interestingly, many phage-encoded virulence genes in gram-negative organisms are not located near the *att* site, including the Shiga toxin genes of *E. coli* (64, 71), the cytotoxin gene of *Pseudomonas aeruginosa* (35), and the SodC (24) and SopE (62) genes of *Salmonella*.

Third, many phage-encoded virulence genes are regulated by chromosome-encoded transcription factors. For example, the iron-dependent transcriptional repressor DtxR, encoded on the *C. diphtheriae* chromosome, represses diphtheria toxin production by binding to an operator overlapping the toxin gene promoter (12, 81, 83). Since DtxR is known to have numerous chromosomal target genes involved in iron acquisition (54, 78–80, 82), diphtheria toxin production is apparently regulated as part of a global cellular response to environmental stimuli. Similarly, the cholera toxin genes (*ctx*) are up-regulated by the *V. cholerae* virulence transcriptional activators ToxR and ToxT (22, 60). While *toxR* appears to be a component of the ancestral *V. cholerae* genome, *toxT* is encoded on VPI (36). Thus, the CTXϕ-encoded *ctx* genes are regulated both by a chromosomal locus (*toxR*) and by a locus encoded on another presumedly mobile genetic element (*toxT*). Likewise, phage-encoded staphylococcal toxins are regulated by the chromosomal accessory gene regulatory locus (*agr*), which coordinates gene expression during the post-exponential growth phase in culture (67). Since these examples of phage-encoded virulence factors are coordinately regulated with other genes not encoded on phages and not solely involved in pathogenesis per se, they are consequently not thought to be regulated in concert with other phage-encoded genes.

In contrast to these examples in which phages encode virulence factors but are thought to play little role in their regulation, our recent work on the Shiga toxin-encoding phages of *E. coli* has established the principle that the bacteriophage life cycle can exert control over virulence factor production by bacterial pathogens. The Shiga toxins (Stx1 and Stx2) are the principal virulence factors of enterohemorrhagic *E. coli* (EHEC), accounting for the most-severe consequences of EHEC infection, including hemorrhagic colitis and hemolytic uremic syndrome (11, 68, 95). The *stx* genes in EHEC strains are located within prophages related to λ. Like λ, these prophages contain transcriptional units for various functions, such as replication, morphogenesis, lysis, etc., that are coordinately expressed during specific intervals following prophage induction due to the regulatory influence of phage promoters, phage repressor, transcriptional terminators, and transcriptional antiterminators (Fig. 1). The *stx* genes were found within the late operons of the Stx-encoding phages, suggesting that they could be transcribed from the late phage promoter (p_R') along with lysis and morphogenesis genes following prophage induction (Fig. 1) (64, 71). Indeed, phage-inducing agents such as mitomycin C have been shown to increase Stx production by *E. coli* (2, 41; A. E. Hull, D. W. K. Acheson, A. Donohue-Rolfe, G. T. Keusch, and P. Echeverria, Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991, abstr., p. 390, 1991), and subsequently, it was demonstrated that overexpression of *Q*, a gene that encodes an antiterminator necessary for efficient late gene expression in λ-like phages, resulted in increased Stx production by *E. coli* strains harboring Stx-encoding phages (65).

We characterized the mechanisms by which prophage induction augments Stx production by constructing a mutant derivative of the Stx2-encoding phage ϕ361 in which the p_R' promoter and a portion of the *Q* antiterminator gene necessary for efficient transcription from p_R' were deleted. A lysogen of the mutant phage was drastically impaired in Stx2 production both in vitro and in a mouse model of intestinal infection (98), implying that the primary regulatory element in Stx2 production is the late phage promoter p_R' and that Stx2 production relies ultimately upon prophage induction. The previously characterized p_{Stx2} promoter adjacent to stx_2 (87) is apparently not a dominant regulatory element in Stx2 production.

We also studied the Stx1-encoding phage H-19B. Like the stx_2 genes in ϕ361, the stx_1 genes in H-19B are located between the late promoter p_R' and the lysis genes. However, the p_R' promoter is not necessary for Stx1 production by lysogens of H-19B (97). Instead, multiple promoters contribute to stx_1 transcription, including the adjacent iron-regulated promoter p_{Stx1} (14), as well as upstream phage promoters (p_R and p_R') that are regulated by prophage induction. Other phage-related mechanisms besides control of stx_1 transcription contribute to Stx1 production following H-19B induction. Replication of the H-19B genome, with the associated increase in stx_1 copy num-

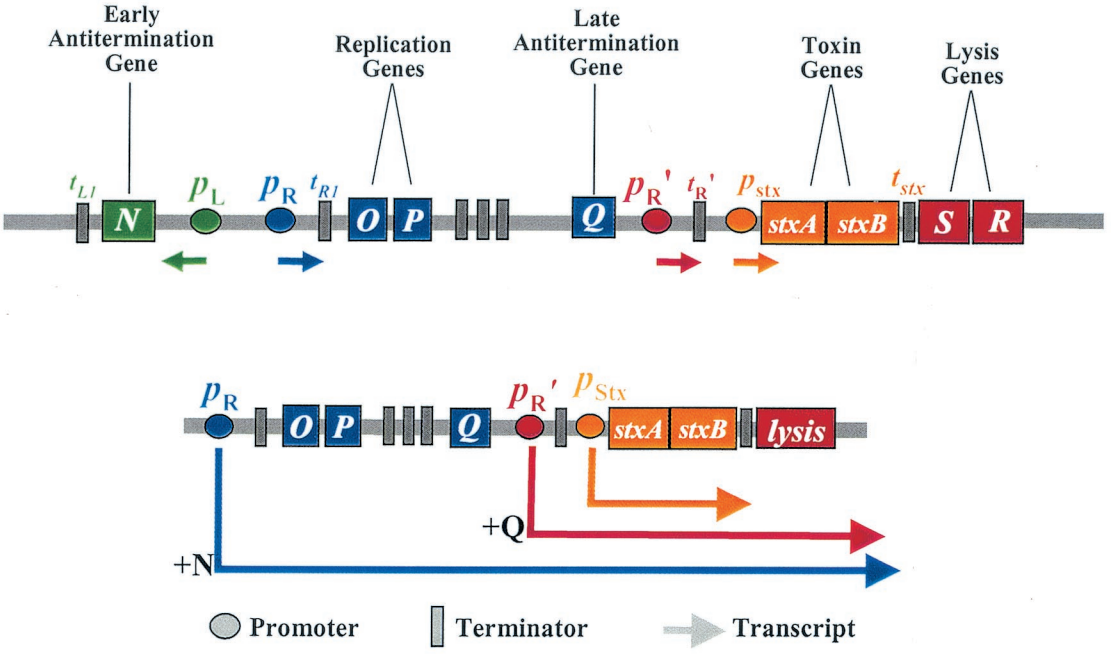

FIG. 1. Phage control of Stx production and release by *E. coli*. (Top) A partial map (not to scale) of an integrated Stx-encoding prophage is shown. Transcription from phage promoters is limited both by direct phage repressor activity at p_L and p_R and by transcriptional terminators (downstream of p_L, p_R, and p_R'). Following prophage induction, repressor cleavage allows for increased transcription from p_L and p_R and production of antiterminators N and Q. These proteins facilitate transcription initiated at p_R and p_R', respectively, by allowing readthrough at several terminators, including t_L, t_{R1}, and t_R'. (Bottom) Both the p_R and p_R' promoters, as well as the toxin-associated promoter (p_{Stx}) can contribute directly to *stx* transcription following prophage induction. Additionally, the phage replication gene products (O and P) contribute to Stx production by amplifying *stx* copy numbers. The phage lysis gene products set a limit on the duration of Stx production and provide a mechanism of toxin release from the cell (97).

ber, was the most quantitatively important mechanism of increased Stx1 production. Phage-mediated lysis regulates the quantity of Stx1 produced by limiting the duration of Stx1 accumulation following prophage induction and provides a mechanism for toxin release (97). In summary, by amplifying *stx* copy number, by contributing to *stx* transcription, and by allowing for Stx release, the Stx-encoding phages are intimately involved in the regulation of Stx production and thereby the pathogenicity of EHEC.

The Stx-encoding prophages are not the only prophages implicated in the regulation of virulence factor production. Some 40 years ago, Barksdale et al. discovered that UV light, a potent prophage-inducing agent, greatly enhanced diphtheria toxin production, suggesting that prophage induction and replication could amplify toxin production (3). Similarly, UV light enhances phage and streptococcal pyrogenic exotoxin A (scarlatinal toxin) production by GAS (108), as well as production of the phage-encoded platelet-binding proteins of *S. mitis* (7, 8). Mitomycin C increases production of CTXϕ virions and cholera toxin by *V. cholerae* (B. Davis and M. Waldor, unpublished observations), and ToxR-independent cholera toxin production has been detected from the replicative form of CTXϕ, indicating the possible activity of alternative induction-related phage promoters for *ctx* transcription (53). Although mutational analyses have not been carried out in these cases, the results are highly suggestive that, as for Stx1 and Stx2, the production of other virulence factors may be controlled, in part, by prophage induction.

ROLE OF IN SITU PROPHAGE INDUCTION IN BACTERIAL VIRULENCE

Although prophage induction has been shown to contribute to the production of several virulence factors in vitro, we have little knowledge regarding the contribution of the phage life cycle to the regulation of virulence factor production during infection. It is conceivable that certain bacterial pathogens exist in a less virulent state until they encounter a stimulus for prophage induction within the human body, at which time production of a virulence factor, regulated as part of the phage life cycle, could contribute to the pathogenesis of the bacterium. We propose that this in situ prophage induction could help to explain when and where certain virulence factors are produced during the course of bacterial infection. Moreover, virion production and release within the human body, with subsequent phage infection of other resident bacteria, could contribute to pathogenesis by amplifying the number of virulence gene-encoding organisms in the body during infection (1).

What are the stimuli for in situ prophage induction? Many prophages are induced by environmental conditions that lead to bacterial DNA damage and, in the case of *E. coli*, activation of RecA, which in turn catalyzes cleavage of phage repressors (56). Such conditions include exposure to agents produced by human cells, including the reactive oxygen species generated and released by leukocytes, and to exogenous agents, such as antibiotics. Many antibiotics commonly used to treat diarrhea,

for example, are known to induce Stx-encoding phages and therefore to promote toxin production by EHEC (51, 57, 101, 109). Perhaps not coincidentally, numerous epidemiological studies have detected an association between increased severity of EHEC infection and treatment with antibiotics (13, 16, 50, 70, 104). This observation has obvious clinical ramifications, in that antibiotics may actually worsen the clinical course of infection by bacteria such as EHEC.

Phage-inducing agents derived from human cells have been implicated in the pathogenesis of at least three organisms. We showed in EHEC that H_2O_2 (a known inducer of λ-like phages) and neutrophils, an endogenous source of H_2O_2, can induce Stx-encoding prophages, which enhances toxin production by EHEC cells (96). The superoxide dismutase (SodC)-encoding *Salmonella* phage Gifsy-2 (described above) is also induced by H_2O_2 (24). H_2O_2 encountered by *Salmonella* in the phagosome could result in prophage induction and subsequent up-regulation of SodC production. In this way, prophage induction could serve as an adaptive response by coupling production of the protective SodC enzyme with exposure to reactive oxygen species. In a third example, Broudy et al. discovered a soluble phage-inducing factor (SPIF), elaborated by human pharyngeal epithelial cells, which induced the streptococcal pyrogenic exotoxin C (SpeC)-encoding phage of GAS and subsequent toxin production by this pathogen (12a). Although the SPIF molecule and its mechanism of prophage induction remain to be characterized, these observations may reflect an important role for an epithelium-derived prophage-inducing factor in the pathogenesis of GAS.

We believe that the examples described here demonstrate the need for a systematic evaluation of the extent to which the induction of prophages influences expression of the virulence factors that they encode. For example, consider the pathogenesis of endocarditis caused by *S. mitis*. It is important to learn whether the organisms encounter prophage-inducing agents in the bloodstream or in the endocardium that lead to increased expression of the phage-encoded platelet-binding factors. Such information could have important clinical implications in the treatment of bacterial diseases. For instance, patients suffering from or at risk for diseases in which prophage induction plays a pathogenic role might benefit from anti-inflammatory agents that reduce the generation of prophage-inducing antibacterial factors by human cells and from avoiding the use of prophage-inducing antibiotics.

EVOLUTIONARY CONSIDERATIONS AND CONCLUSIONS

Bacteriophages are intimately associated with bacterial virulence genes. What factors account for this association? This relationship likely reflects the mobility that virulence genes derive from being phage encoded, allowing for their widespread dissemination among bacterial populations. Furthermore, virulence genes encoded by phages may withstand environmental exposure better than those encoded by bacteria. For example, Muniesa et al. found that Stx2-encoding phages persisted in river water longer and were more resistant to chlorination and pasteurization than Stx2-encoding bacteria, suggesting that phage particles serve as a more durable environmental reservoir of the *stx*$_2$ genes than bacteria (63). Thus, enhanced mobility and persistence in the environment may provide a selective advantage to virulence genes that are phage encoded.

This relationship may be of benefit to phages as well. For example, the hyaluronidase encoded by and incorporated into the particles of *S. aureus* phages may facilitate phage penetration of the *S. aureus* capsule during phage infection or release. Similarly, O-antigen alteration by phages of certain gram-negative pathogens, which may aid in bacterial evasion of immunity, presumably benefits the phage by conferring superinfection resistance on the bacterial host. Moreover, in some cases, virulence factors may actually be integral components of the phage particle. The CTXϕ-encoded Ace protein, reported to account in part for *V. cholerae* enterotoxicity (88), is thought to be a minor coat protein of the CTXϕ virion (100). Thus, these putative virulence factors may ultimately be phage attributes that incidentally contribute to the pathogenicity of the bacterial host. Other virulence factors may bestow upon phages unknown advantages, a possibility that merits future investigation.

One paradoxical implication of in situ prophage induction in bacterial pathogenesis is that virulence factor production could be linked to the death of the bacterial cell via phage-mediated lysis. This consideration would not apply to pathogens such as *V. cholerae*, since production of the filamentous CTXϕ does not destroy its host bacterial cell. In fact, CTXϕ and cholera toxin share a mechanism of nonlethal secretion from the cell (20). In contrast, we have learned that in *E. coli* lysogens of phage ϕ361, Stx2 is apparently produced only when the late phage promoter, responsible for lysis gene transcription, is active (98). This finding implies that toxin production involves simultaneous and obligatory phage-mediated cell death. One rationale for the tight linkage between Stx2 production and phage induction is that production of Stx2 by a small number of doomed cells contributes in some way to the survival of the remaining lysogenic population whose prophages were not induced (98). Alternatively, if the unit of selection is ϕ361 or the *stx*$_2$ genes themselves, death of the host bacterium could be irrelevant.

In summary, bacteriophages are intimately involved in bacterial pathogenesis, including adhesion and invasion, evasion of immunity, and exotoxin production, largely by transducing a diverse set of virulence genes. In some cases, the expression of these genes may be driven in part by induction of the prophages that encode them. Since prophage induction contributes to virulence gene expression and since the human body is replete with prophage-inducing chemicals, prophage induction in the human body may be an important, underrecognized mechanism of bacterial pathogenesis.

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