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Commentary

Bacteriophages in the evolution of pathogen–host interactions

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Theterm "emerging infectious diseases" has recently been popularized as a way to describe the introduction of new infectious agents to human populations (1). Bacterial infectious diseases can "emerge" by different methods. Emergence may involve the discovery that a disease of unknown etiology has a microbial etiology, such as peptic ulcers caused by infection with *Helicobacter pylori* bacteria (2). Infectious diseases can also emerge as a result of exposure of specific human populations to microorganisms that are new to those populations. Such strains could emerge as new epidemics or pandemics, as was the case with the spread of the bacterial cause of cholera, *Vibrio cholerae,* from Asia to South America (3). In addition to these well appreciated mechanisms, advances in the understanding of the molecular basis of microbial pathogenesis have led to the hypothesis that more virulent bacterial strains could emerge through recent acquisition of virulence factors. The current worldwide epidemic of the Gram-negative enterobacteriaciae *Salmonella* has significant public health implications (4) and may provide an important example to illustrate this principle.

Salmonellae are intimately associated with all animal species. Some salmonellae such as *Salmonella typhi* are host range-specific. *S. typhi* cause the systemic illness enteric fever only in humans and chimpanzees. Other salmonellae such as *Salmonella typhimurium* have a broad host-range and cause gastroenteritis, an acute intestinal illness characterized by diarrhea, in a variety of animals including cattle, sheep, horses, and humans. The increase in salmonellosis over the last decade is largely attributed to an increase in transmission of broad host-range organisms to humans from infected food products contaminated in centralized food-processing facilities (5). Interestingly, recent outbreaks of *S. typhimurium* have been attributed to a multiply antibiotic-resistant phage type, DT104, which appears to have increased virulence for humans and cattle (6). This clinical example indicates that differences exist between highly similar salmonellae within animal populations and that more virulent organisms may be selected and expanded. In this issue of the *Proceedings*, Mirold *et al.* (7) describe a mechanism by which *S. typhimurium* could evolve to a more pathogenic state by bacteriophage lysogenic conversion and therefore provide a potential molecular basis for these clinical and epidemiologic observations.

Recent advances in understanding the molecular basis of host–pathogen interactions highlight the role that microbial evolution could play in the emergence of bacterial pathogens, including *Salmonella*. A variety of bacterial virulence factors are encoded on DNA elements that show evidence of more recent acquisition when compared with genes encoding highly conserved essential metabolic functions. In some cases, this horizontal gene transfer is obvious because the virulence factors are encoded within highly mobile genetic elements such as transposons, plasmids, and bacteriophages. In other cases, the site of chromosomal insertion and/or the nucleotide composition of virulence factors indicates that they were acquired by horizontal gene transfer without evidence of currently functional transfer machinery (8). Such elements, termed pathogenicity islands, can contain >40 kilobases of DNA. Thus, bacterial virulence properties can evolve in quantum leaps through horizontal gene transfer of blocks of genetic material rather than accumulation of single-nucleotide mutations (9).

Among the mechanisms for transfer of DNA, lysogenic conversion by bacteriophages appears to be advantageous. Lysogenic transformation is efficient, and in contrast to conjugative transfer of plasmids, does not require intimate contact between bacteria. Bacteriophages can carry large blocks of DNA and can survive harsh conditions that eliminate bacterial populations. Therefore, DNA important to a population can be preserved until a host for lysogenic conversion is reintroduced into an environmental niche. Bacteriophages can also spread DNA directly to an entire population of bacteria, eliminating the need for clonal expansion of a specific population.

Virulence factors encoded on bacteriophages may allow the bacterium to enlarge its host range and increase its fitness in an environmental niche by promoting evasion of host immune defenses or providing mechanisms to breach host structural barriers. In support of this concept, a variety of major bacterial toxins associated with important epidemics are carried on bacteriophages (Table 1), including the diphtheria (10) and cholera toxins (11). An example of recent emergence of a pathogen with a bacteriophage encoded toxin is *Escherichia coli* serotype O157:H7, which causes hemolytic–uremic syndrome in children (12). Virulence factors other than toxins can also be carried on bacteriophages. Figueroa-Bossi and Bossi recently have shown (31) that several *S. typhimurium* isolates are lysogenic for the *Gifsy*-2 prophage and that this prophage can activate and lysogenize strains lacking *Gifsy*-2. This prophage encodes a superoxide dismutase gene, *sodC*, that is involved in virulence. Bacteriophage λ and its relatives carry outer membrane proteins that are important for bacterial resistance to innate immune factors such as serum complement (13). Interestingly, recent evidence indicates that the VPIΦ *Vibrio cholerae* bacteriophage encodes a pilus that functions as a colonization factor for the human intestine as well as the receptor for the cholera-toxin encoding CTXΦ bacteriophage (14).

Recent work with a variety of animal and plant Gram-negative bacterial pathogens indicates that a major virulence mechanism is the ability to directly transfer bacterial proteins to eukaryotic cells through a contact-dependent secretion mechanism. These systems, termed type III secretion systems (TTSS), are encoded on plasmids and large chromosomal pathogenicity islands (15). This mechanism has been likened to a bacterial syringe for simultaneous injection of multiple effector proteins that modify host-cell physiology. This system provides the advantage that a large number of effector proteins can be transferred rapidly and simultaneously into host cells. TTSS may permit pathogens more flexibility in evolving within eukaryotic hosts because new translocated proteins can be

The companion to this Commentary begins on page 9845.

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Table 1. Examples of lysogenic bacteriophage-encoded virulence determinants

Virulence determinant	Bacteria	Bacteriophage	Ref.
SopE	*S. typhimurium*	SopEΦ	7
SodC	*S. typhimurium*	*Gifsy*-2	31
Cholera toxin	*V. cholerae*	CTXΦ	11
Toxin-coregulated pilus and CTXΦ receptor	*V. cholerae*	VPIΦ	14
Diphtheria toxin	*Corynybacterium diphtheriae*	Converting β-phage	10
Shiga-like toxin-I and -II	Enterotoxigenic *E. coli*	Shiga-like toxin converting phages	12
Serum resistance	*E. coli*	Lambda	13, 28
Enterotoxin A and staphylokinase	*Staph. aureus*	Φ 13	29
Streptococcal pyrogenic exotoxins A and C	Group A *Streptococci*	T12 and 3GL16	30

acquired independent from the delivery mechanism. Because the secretion/translocation apparatus is capable of recognizing a secretion signal that is highly flexible in its amino acid sequence, this provides an advantage in the generation of a wide variety of new effector genes. Those genes that provide selective advantage can then be maintained and transmitted throughout the population through mobile genetic elements. A pathogen therefore may be able to fine-tune or acquire a new method of interaction with its host more efficiently than by less sophisticated secretion mechanisms. Mirold *et al.* (7) provide evidence that this idea could in fact be a reality for pathogenic salmonellae.

TTSS are major virulence factors for salmonellae. Most salmonellae have two distinct virulence-associated TTSS encoded within *Salmonella* pathogenicity islands 1 and 2 (SPI1 and SPI2) (16, 17). The SPI2 TTSS is expressed in bacteria when they reside intracellularly, and it is required for systemic virulence in inbred mice, which is a model system for enteric fever (18). In contrast, the SPI1 TTSS is involved in interactions between salmonellae and epithelial surfaces, playing a role in epithelial cell invasion by induction of macropinocytosis and other phenotypes related to intestinal inflammation and gastroenteritis. For the SPI1 TTSS, several effector proteins important to gastrointestinal illness are encoded within SPI1, whereas others, such as *sopE*, are encoded elsewhere in the chromosome.

The paper by Mirold *et al*. (7) describes the important result that *sopE*, an effector of the SPI1 TTSS encoded outside SPI1, is a component of a bacteriophage (SopEΦ). The *sopE* gene product appears to be involved in the induction of *in vitro* epithelial-cell invasion by induction of membrane ruffling as well as the stimulation of gastrointestinal inflammation and fluid secretion in cattle (19, 20). SopE, when expressed in cultured epithelial cells, can stimulate membrane ruffling by stimulating guanine nucleotide exchange on Rho GTPases, including CDC42 and Rac (21). Several *Salmonella* proteins likely act in concert with SopE to produce the morphological and clinical phenotypes associated with mammalian cell infection, including SipA, an actin-binding protein that stimulates actin polymerization (22); SptP, a tyrosine phosphatase that disrupts the cytoskeleton (23); and SopB, which displays inositol phosphate phosphatase activity and contributes to gastrointestinal fluid secretion (24, 25).

Mirold *et al*. (7) also demonstrated activation and subsequent horizontal gene transfer by SopEΦ into different *Salmonella* strains by lysogenic conversion. SopEΦ lysogens were found to have a limited distribution among *S. typhimurium* isolates and appear to be associated with strains that have caused recent outbreaks in Germany. If SopEΦ is a recent acquisition, and if it confers on its host strain a selective advantage, then the prevalence of these strains should increase over time. Thus, a new *Salmonella* strain with increased fitness and different virulence properties could emerge. Because not all pathogenic salmonellae express *sopE* and because *sopE*-null mutants still have intact virulence-associated phenotypes as measured by using *in vitro* assays (7, 19, 20), it is possible that SopE increases the bacteria's success in a specific animal host or that it provides a small incremental increase in virulence not measured by these assays. Although the presence of *sopE* has not been examined in the more virulent *S. typhimurium* DT104 isolates, it is interesting to speculate that DT104 acquired new TTSS effectors by a similar bacteriophage mechanism.

This mechanism may be common among bacterial pathogens. Recently, it has been observed that *Pseudomonas aeruginosa* responsible for human disease variably express ExoS and ExoU, two TTSS effectors (26, 27). Our laboratory has also recently identified a TTSS effector (SspH1) uniquely present in an *S. typhimurium* strain isolated from a cow with systemic disease (unpublished data). The discovery of Mirold *et al.* may define an important mechanism for evolution of bacteria with unique virulence properties. Their discovery should result in more investigations that examine the presence of *sopE* and other TTSS effector genes in various bacterial pathogens isolated from natural populations. In the future, TTSS effectors could be important markers for epidemiological monitoring of broad host-range bacterial pathogens that cause outbreaks in humans.

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