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Mobile effector proteins on phage genomes

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Bacteriophage genomes found in a range of bacterial pathogens encode a diverse array of virulence factors ranging from superantigens or pore forming lysins to numerous exotoxins. Recent studies have uncovered an entirely new class of bacterial virulence factors, called effector proteins or effector toxins, which are encoded within phage genomes that reside among several pathovars of *Escherichia coli* and *Salmonella enterica*. These effector proteins have multiple domains resulting in proteins that can be multifunctional. The effector proteins encoded within phage genomes are translocated directly from the bacterial cytosol into their eukaryotic target cells by specialized bacterial type three secretion systems (T3SSs). In this review, we will give an overview of the different types of effector proteins encoded within phage genomes and examine their roles in bacterial pathogenesis.

Introduction

Bacteriophage-encoded virulence genes can convert their bacterial host from a non-pathogenic strain to a virulent strain, or to a strain with increased virulence, by providing novel mechanisms to the bacterial cell that enable attachment, invasion and survival within their eukaryotic host. Sometimes the loss of a bacteriophage by a pathogen can render the bacterium non-pathogenic, however this is rare since many of the bacteriophages carrying bacterial virulence genes are temperate, forming stable lysogens within the host genome. Various phage-encoded exotoxins are found in many Gram-negative and Gram-positive bacteria, including *Corynebacterium diphtheriae*, *Escherichia coli*, *Shigella* spp, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Staphylococcus aureus* and *Streptococcus pyogenes*.[1,2] These exotoxins may be cytotoxic, enterotoxic or neurotoxic, causing an array of diseases ranging from mild gastrointestinal disease to life-threatening toxemia and sepsis. A recently discovered group of virulence factors to be included in the list of phage-encoded virulence factors are effector proteins or toxins (**Table 1**).[1–4] These are multi-domain multifunctional proteins that are secreted by specialized type three secretion systems (T3SSs) found in Gram-negative animal and plant pathogens. In this review, we begin with a brief discussion of the classical phage-encoded exotoxins including their distribution and function in bacterial pathogenesis. We will then explore the range of phage-encoded effector proteins that are described in pathovars of *E. coli* and *Salmonella enterica*.

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Classical Phage-Encoded Exotoxins

The effects of different phage-encoded exotoxins depend on the specificity of their target cell and target site within the cell. Three phage-encoded AB type exotoxins such as diphtheria toxin (DT), cholera toxin (CT) and Shiga toxin (Stx) are encoded by *C. diphtheria*, *V. cholerae* and *E. coli* and *Shigella* species, respectively. Each of them has very different cellular target sites and consequently diverse disease outcomes (**Fig. 1**).

DT is an AB type toxin made up of a single A and B subunit where A is the catalytic subunit and B is the receptor binding subunit on the eukaryotic target cell. The *tox* gene which encodes DT, is encoded by corynebacteriophage β and is located directly adjacent to the phage attachment site, *attR,* suggesting that this gene was acquired by imprecise excision from a donor host genome.[5] Expression of the *tox* gene is regulated by iron availability and is activated when iron levels are low.[6,7] DT is released from the bacterial cell and attaches to a receptor on the cell surface of respiratory epithelium cells. The toxin is internalized by pore formation and transported to its site of activity, the ribosome, where it ADP-ribosylates Elongation factor 2, thereby halting protein synthesis followed by subsequent cell death (**Fig. 1**). Little is known about the diversity of phages that encode the *tox* gene but the phages that have been examined appear to be highly similar.

Cholera toxin is the main cause of secretory diarrhea, a characteristic of cholera.[8] CT is an AB_5 toxin, consisting of a single A subunit and five B subunits. It is encoded within the genome of a filamentous phage named CTXphi, a 7 kb single-stranded DNA phage found in serogroup O1 and O139 isolates of *V. cholerae*.[9] The *ctxAB* genes are found directly adjacent to the phage attachment site, *attR*.[9] The location of these genes at the terminal region of the phage genome and the isolation of functional CTXphi genomes lacking these genes suggests that they were a later addition to the phage genome.[10] CTXphi can integrate into either of the host's two chromosomes and only at a single site, named *dif1* and *dif2*.[11–13] In *V. cholerae,* CT production is regulated by the bacterial encoded regulator ToxRS and its production increases under conditions encountered in vivo such as low pH, temperature and bile salt concentration.[14–16] CT is secreted from *V. cholerae* cells by the host Type II secretion system[17] and subsequently attaches to its target intestinal epithelium cell receptor, the GM1 ganglioside.[18] The B subunits are required for attachment and the toxin is taken up by endopinocytosis within a vacuole. The toxin is retrograde trafficked to the Golgi via early and late endosomes (**Fig. 1**). In the Golgi, the A subunit separates from the B subunits and enters the endoplasmic reticulum (ER) via coat protein I (COPI) vesicles.[19] The active A1 subunit is released into

Table 1. Phage-encoded effector proteins in *E. coli* and *S. enterica*

Phage	Effector protein	Function
	E. coli	
lambdoid	Cif	Cyclomodulin
lambdoid	EspJ	Transmission
SpLE3-like	EspL2	Actin remodeling
lambdoid	NleA	Golgi localized
lambdoid	NleG	Ubiquitin ligase
lambdoid	NleH	NF-kappaB activity
lambdoid	EspF (TccP)	Actin remodeling
	S. enterica	
Gifsy-1	GogB	Anti-inflammatory effector
Gifsy-1	GipA	Invasion
SopEphi	SopE	Guanine exchange factor mimic
Phage-like	SopE2	Guanine exchange factor mimic
Phage-like	SspH2	Inhibits actin polymerization
Phage-like	SseJ	Deacylase
ST64B	SseK3	Invasion
Gifsy-2	SspH1	Inhibits actin polymerization
Gifsy-3	SseI (GtgB)	E3 ubiquitin ligase

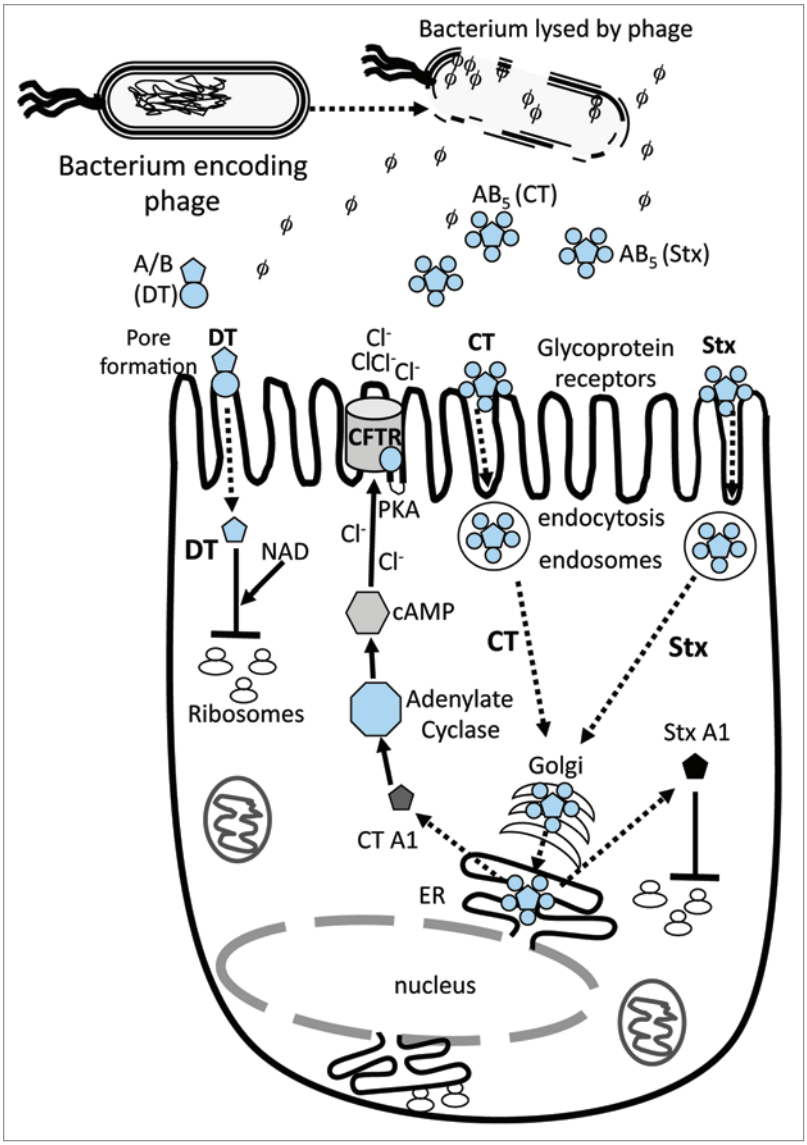

Figure 1. Schematic of toxins function in eukaryotic cells. Dotted arrows indicate steps in bacterial lysis with phage/toxin release, toxin uptake by host cell and target activity. CTXphi which encodes cholera toxin (CT) does not lyse its bacterial host cell. Black solid arrows or t-bars represent activation or blocking of specified bacterial function by toxin. AB denotes a two subunit protein, subunit A is the catalytic subunit and B is the receptor binding or docking subunit on target cell. AB_5 denotes a toxin that contains one A subunit and 5 B subunits. Three examples are given; the A/B diphtheria toxin (DT) and the AB_5 toxins CT and shiga toxin (Stx). DT gains direct entry into target cell by pore formation. The second method of toxin uptake is by receptor mediated endocytosis, a mechanism used by CT and Stx. Only the A subunit of DT enters the cell cytosol and along with NAD, ADP-ribosylates Elongation factor 2 blocking protein synthesis. Both CT and Stx are retrograde trafficked to the Golgi apparatus. In CT, the A subunit is dissociated from B subunits in the endoplasmic recticulum (ER) and released into the cytosol where it activates adenylate cyclase increasing cAMP which in turn activates PKA resulting in the CFTR membrane Cl^- channel activation. The A subunit of Stx ADP- ribosylates the 28S rRNA ribosomal subunit, thus blocking protein synthesis.

the cytosol where it stimulates adenylate cyclase, an important signaling molecule that increases cyclic AMP. Increased cAMP levels activate protein kinase A (PKA) permanently, thus opening the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel and ultimately causing chloride secretion from the cell (**Fig. 1**). CTXphi genomes are identical to each other in terms of gene arrangement, content and general sequence identity. The exception is the phage regulatory gene, *rstR*, which shows high sequence divergence.[20] The bacterial host receptor for CTXphi attachment has been identified as a Type IV pilus, named the toxin co-regulated pilus (TCP).[9]

Shiga toxin (Stx) and the shiga-like toxin are also AB_5 toxins consisting of one active A-subunit and five identical B-subunits, similar to CT. Stx and Stx-like toxins are present on diverse lambdoid phages that insert at multiple locations in the genome of Shigella species and select *E. coli* pathovars.[21,22] Unlike the genes that encode DT and CT, the *stx* genes are not located adjacent to the phage attachment site but instead are present in the phage late gene region of the phage genome. The genome arrangement of the *stx* genes suggests that they were not picked up by imprecise prophage excision.[23] Moreover, it has now been shown that Stx production is tightly linked to prophage induction and the phage regulatory circuit rather than by host regulatory systems.[24,25] The Stx toxin is released from the bacterial cell upon phage lysis of the cell. The Stx B subunits bind to glycolipids on the host eukaryotic cell, specifically the cell surface receptor globotriosyl ceraminde (Gb3) (**Fig. 1**). Stx enters the host cell by a clathrin-dependent

pathway and is retrograde trafficked directly from early/recycling endosomes to the Golgi and then to the ER via a COPI-independent pathway.[26] The A1-subunit is released into the cytosol and inactivates the 60S ribosome subunit by N-glycosidic bond cleavage causing disruption of protein synthesis and cell death[27] (**Fig. 1**). The bacterial receptor for Stx phage binding and uptake is the highly conserved YaeT protein required for insertion of proteins into the outer membrane of Gram-negative bacteria.[28] Allison's group has demonstrated that 70% of Stx phages recognize the YaeT surface molecule via conserved tail spike proteins associated with a short tailed morphology.[28]

Shiga Toxin Encoding *E. coli* Pathovars

Escherichia coli is a commensal of the gastrointestinal tract of warm-blooded animals, the most common facultative anaerobe in the human intestines. In addition, many *E. coli* strains are pathogenic and cause enteric diseases ranging from severe watery diarrhea, dysentery and hemorrhagic colitis to extraintestinal infections such as cystitis, septicemia and meningitis. Diversity in the number and type of toxins produced by *E. coli*, as well as diversity in the bacteriophages involved, has enabled *E. coli* to become a versatile pathogen. To date, eight different *E. coli* pathovars have been identified; Enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), entrotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) and the extra-intestinal pathovars Neonatal Meningitis *E. coli* (NMEC) and Uropathogenic *E. coli* (UPEC).[29] EHEC, EPEC, ETEC and EAEC are all extracellular pathogens that cause secretory diarrhea, whereas EIEC is an intracellular pathogen that causes inflammatory diarrhea. EHEC, EIEC and EAEC are associated with infection of the large intestine whereas EPEC, ETEC, DAEC and EAEC are associated with infection of the small intestine. UPEC is associated with urinary tract infection.[29]

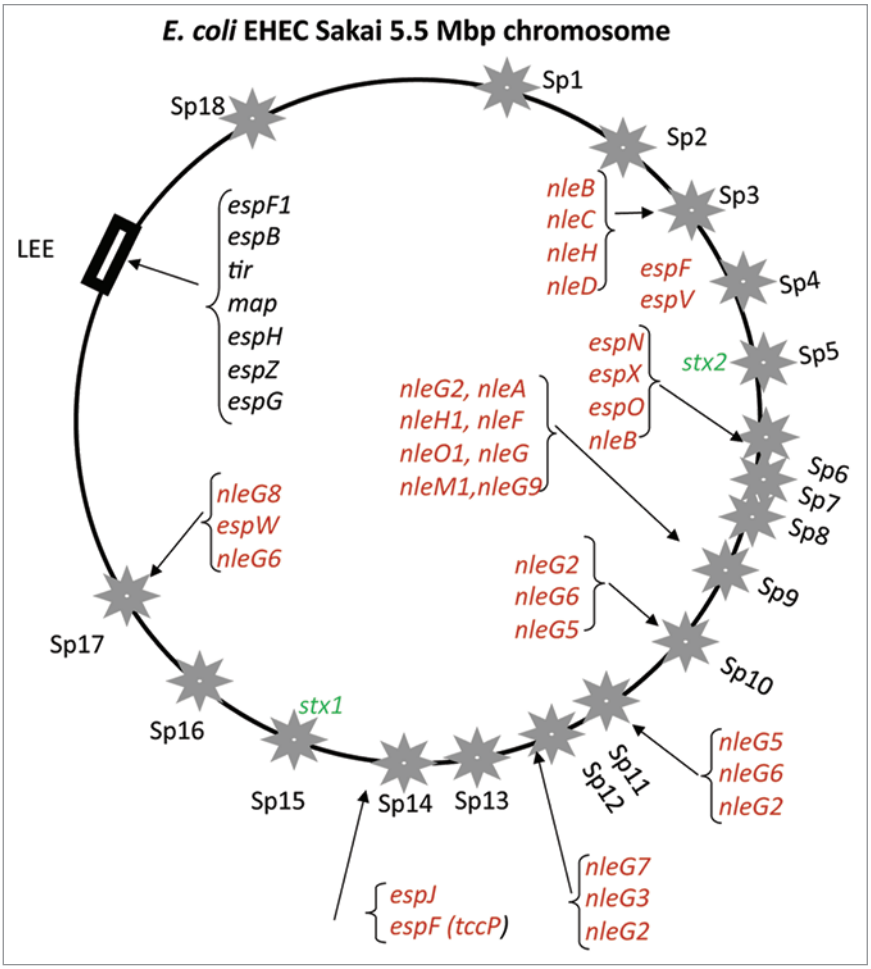

Figure 2. Prophage location on the *E. coli* O157:H7 strain Sakai genome map. Stars indicate prophages Sp1-Sp18 as named using the convention of.[38] Rectangular black box indicates the position of the LEE pathogenicity island. Four letter designations indicate effector genes (red) and toxins (green) identified within prophages and at the LEE island (black).[55]

In enterohemorrhagic *E. coli* (EHEC) and Shiga toxin *E. coli* (STEC) strains responsible for hemorrhagic colitis and hemolytic uremic syndrome (HUS), the shiga-toxin (Stx) encoded by *stx1* or *stx2*, and enterohemolysin encoded by the *hly* locus are present within lambdoid phage genomes.[21,22,30–32] Since the first discovery of Stx in *E. coli* O157:H7 in 1983, which expresses the somatic (O) 157 and flagellar (H) 7 antigens, Stx has been identified in over 500 different *E. coli* serogroups.[33] The lambdoid phage (denoted as Sp15 in **Figure 2**) containing the *stx* operon encoding *stxA* and *stxB* genes in *E. coli* O157:H7 Sakai and EDL strains, is integrated at the *yehV* gene whereas the Stx2 encoding bacteriophage (denoted as Sp5 in **Figure 2**) is integrated at the *wrbA* gene. The majority of *E. coli* O157:H7 strains encode Stx2 and over 75% also encode Stx1. Among these Stx1 negative strains, a truncated prophage is always present at the *yehV* locus suggesting that these strains all contained the Stx1 phage at one point that was subsequently lost in a subset of strains.[34] In Stx1 negative strains, it was found that Stx2 was not at the *yehV* site, evidence that a diversity of phages are involved in Stx transfer.[34] Similarly, it was shown that different STEC isolates have varying bacteriophage titers and toxin production.[35] Structural analysis of bacteriophage-borne *stx1, stx2* and flanking sequences in *E. coli* O157, STEC and *Shigella dysenteriae* Type 1 strains demonstrated bacteriophage genomic variation indicating that unrelated bacteriophages encode shiga toxin genes.[31,32] In *S. dysenteriae* seroType 1 strains, Stx2 has only 56% identity to Stx1.

Some of the first studies to compare the whole genomic sequence of pathogenic *E. coli* strains examined O157 strains, a pathogen that emerged as a worldwide public health threat in the past three decades, and the laboratory-maintained commensal

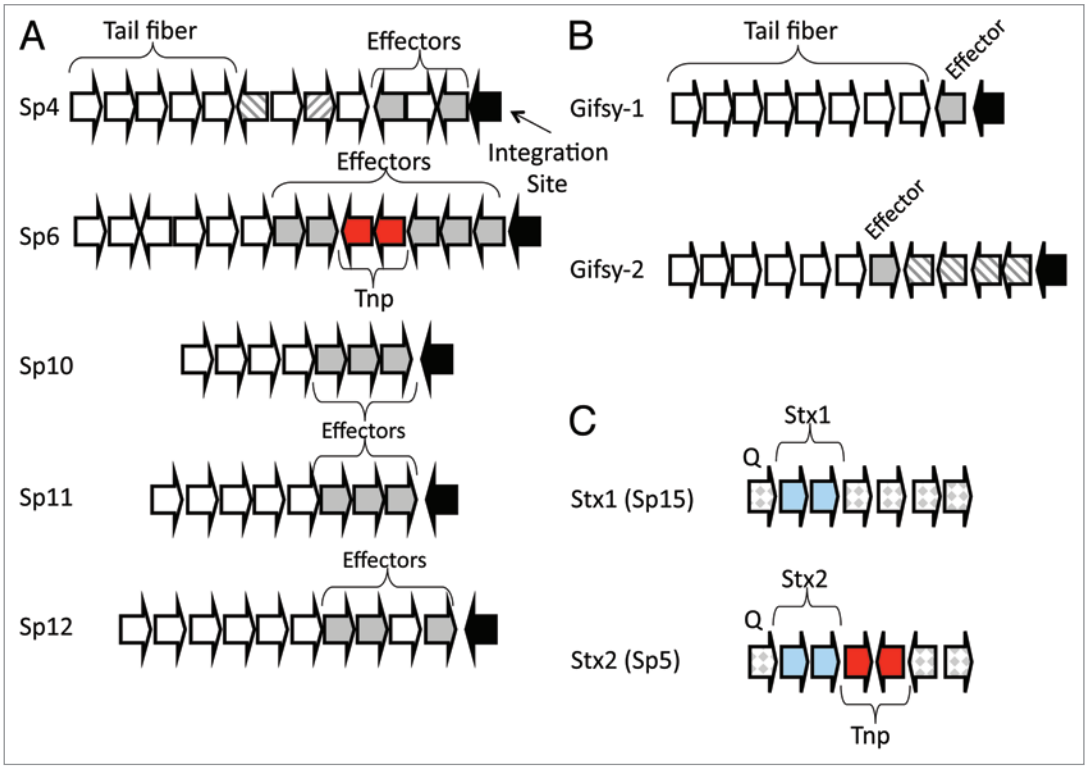

Figure 3. Location of effector genes and *stx* genes within prophages in *E. coli* and *S. enterica*. Partial prophage genomic maps are shown. (**A**) Terminal region of five lambdoid prophages from *E. coli* strain Sakai, (**B**) terminal region of *S. enterica* Gifsy-1 and Gifsy-2 prophages and (**C**) central region of the genome of Stx1 (Sp15) and Stx2 (Sp5) prophages encoding *stx1AB* and *stx2AB* genes, respectively, shown as blue arrows. Tail fiber genes are represented by white arrows and effector genes are shown as dark gray arrows. Putative additional virulence genes are shown as striped arrows. Black block arrows indicate the prophage site of genomic integration. Arrows in red represent transposase genes.

E. coli K12 strain.[36–38] These ground breaking studies determined, for the first time, the source and cause of the genome size variation previously found among *E. coli* strains based on pulsed-field gel electrophoresis.[39] These genomic studies were the first to show that *E. coli* strains contain a core-conserved sequence that is common to all genomes within the species as well as being interspersed with sequences unique to each strain. The presence of a core set of genes augmented with accessory genes has since been shown to be true for all free-living bacterial species. In one study, the *E. coli* O157 strain examined contained 1.3 million base pairs of strain-specific DNA, which included a bacteriophage containing Stx and a pathogenicity island carrying the locus of enterocyte effacement (LEE).[36] Genomic comparison of two *E. coli* O157 strains, Sakai and EDL933, also revealed large differences in their prophage content.[37,38] Strain EDL933 contained 12 prophage genomes whereas strain Sakai contained 18 prophage genomes (**Fig. 2**).[37,38] In silico genomic analysis of the two *E. coli* O157 strains identified numerous virulence factors encoded by these prophages.[37,38] The pathogenic potential of these two strains correlates with their bacteriophage-encoded virulence factors, demonstrating that acquisition of phages played a decisive role in the emergence of O157 as a foodborne pathogen. Whittam and colleagues demonstrated that the O157:H7 EHEC pathogen evolved in a stepwise process by the acquisition of prophages and conversion to a new serotype.[40–44] The stepwise evolutionary model proposed that the highly virulent O157:H7 EHEC strains emerged from an O55:H7 EPEC ancestor by sequential acquisition of virulence factors predominantly encoded on prophages. EPEC is a major cause of infant diarrhea in the developing world and is mainly distinguished from EHEC by the absence of the *stx* genes. EPEC contains the LEE island and the EPEC adherence factor (EAF) plasmid. Using whole genome microarray, Whittam and colleagues examined a number of O157:H7 strains and demonstrated that 85% of the genes that were variably absent/present were phage derived, in total 463 such phage-related genes were found.[41] Their analysis demonstrated the dominance of phages in the diversification of the *E. coli* O157:H7 genome.[41]

The 2011 Germany Outbreak *E. coli* O104:H4 Clone

A Stx2-producing *E. coli* O104:H4 clone emerged in the summer of 2011 in northern Germany and was responsible for one of the largest hemolytic uremic syndrome (HUS) outbreaks.[45,46] The Germany O104:H4 clone caused nearly 3,000 cases of diarrhea without HUS and nearly 900 cases with HUS leading to 50 combined deaths.[47] Unlike O157:H7, the O104:H4 serotype was not associated with animals prior to the outbreak and was rarely associated with humans in Germany. Using next generation sequencing technology, several groups sequenced multiple O104:H4 outbreak strains and determined that these strains carried genes present in enteroaggregative *E. coli* (EAEC) and EHEC pathovars.[48–50] The

Germany O104:H4 strains contained a plasmid carrying an aggregative adherence fimbria type (AAF/I) common to EAEC and a prophage carrying the Stx2 toxin inserted in chromosomal gene *wbrA* common to STEC. Phylogenetic analysis of 53 *E. coli* strains indicated that the O104:H4 strains from Germany formed a clade with O104:H4 strains from Africa, as well as with some EAEC strains.[50] The O104:H4 strains differed from the EAEC strains due to the presence of the Stx2 prophage as well as the antibiotic resistance genes, TEM-1 and CTX-M-15.[50] Similar to Stx containing prophage 933W from O157:H7, it was found that the *stxAB* genes in the O104:H4 prophage, were located between the antitermination Q gene and the S/R lysis genes of a lambdoid phage. In addition, there was a high degree of sequence identity between the O157:H7 and O104:H4 prophages suggesting recent horizontal gene transfer.[47] Thus, emergence of this highly virulent strain is a direct consequence of phage acquisition.

Phage-Encoded Effector Proteins

Effector proteins or toxins are large proteins contained within a range of bacterial species that are translocated directly from the bacterial cytosol into the target eukaryotic cell cytosol using contact dependent bacterial secretion systems. Within their eukaryotic host cell, these effector proteins cause a variety of effects and in many cases encode overlapping functions. Effector proteins contain several distinct domains or modules that appear to have arisen through recombination reassortment events.[51] Thus, the N-terminal region of one effector may show similar to the C-terminal region of another effector but lack any other homology. Most effector proteins also require a chaperone and thus have a specific chaperone-binding domain. Redundancy in functions encoded by these proteins is well documented.[52] Effector proteins function by targeting a range of structures within the eukaryotic cell such as actin filaments, microtubules, mitochrondria, lysosomes, the nucleus, the inner cell membrane and tight junctions, as well as disrupting many different cell signaling pathways such as MAPK, cyclic AMP and GTPases Rho, Rac and Cdc42.[52,53] Bacterial effector proteins function in many cases by molecular mimicry, hijacking eukaryotic cell function and signaling pathways for their own advantage. To date, effector proteins have only been identified on phage genomes in two species *E. coli* and *S. enterica* (**Table 1**).

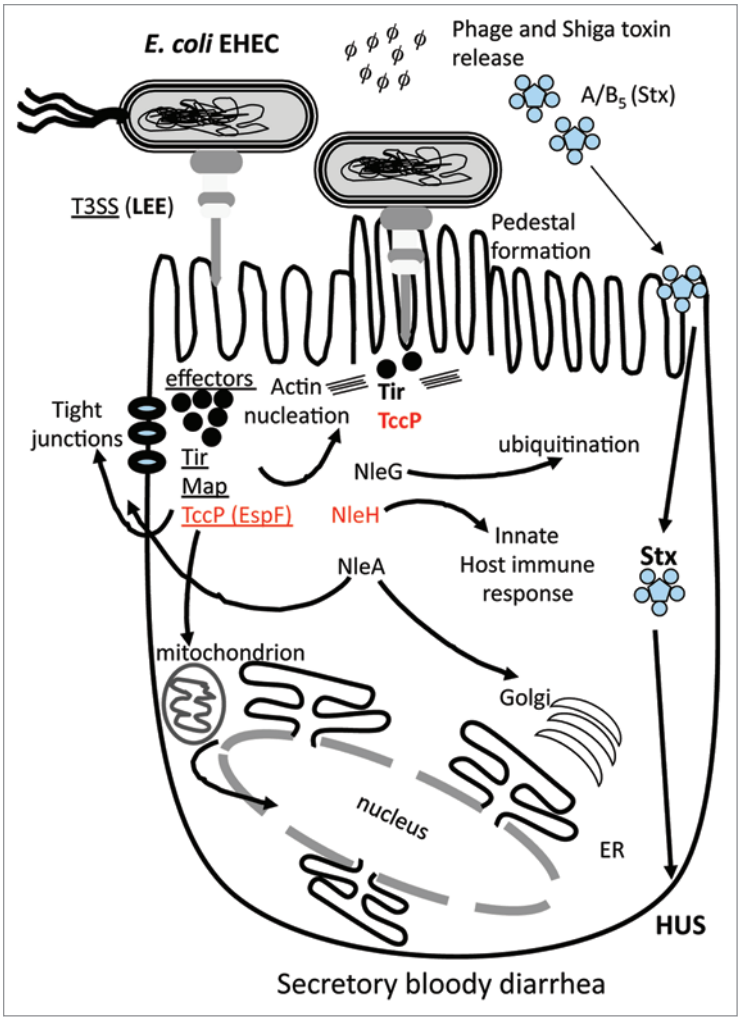

Figure 4. Schematic of the role of different effector proteins in EHEC colonization and pathogenesis. The LEE encoded T3SS is required for intimate binding and adherence of the bacterium to the eukaryotic cell surface which is mediated by the translocation of effector proteins Tir, Map and TccP (EspF). Tir and TccP are required for pedestal formation by directing actin rearrangement within the cell. The NleG family of effector proteins is the largest found in pathogenic *E. coli* and has been shown to be involved in protein ubiquitination. Many effector proteins are multi-functional such as TccP, which is also involved in cell tight junction disruption and interactions with the nucleus. Prophage-encoded effector genes are shown in red.

E. coli Phage-Encoded Effector Proteins

Nearly a hundred effector proteins have been identified among *E. coli* strains that are translocated via a common Type III secretion system (T3SS). Some of these effectors are encoded within prophages, some within pathogenicity islands and some elsewhere on the bacterial genome. As stated previously, EPEC and EHEC isolates are distinguished from each other mainly by the presence of the *stx* genes in EHEC strains. However, both EPEC and EHEC isolates along with ETEC isolates share the attaching and effacing (A/E) lesion phenotype. These isolates attach to the host cell surface resulting in cytoskeleton rearrangement and pedestal formation (**Fig. 3**). The factors involved in this host cell surface change are encoded within the LEE pathogenicity island. The canonical LEE island is a 35 kb region that encodes the Esc-Esp T3SS as well as effector proteins, their chaperones and regulators.[54] T3SSs are contact dependent secretion systems found in a large number of Gram-negative species. These secretion systems are multi-component structures that span the bacterial cell wall and protrude from the cell surface that can inject into a eukaryotic host cell to translocate effector proteins directly into the eukaryotic cell cytoplasm.

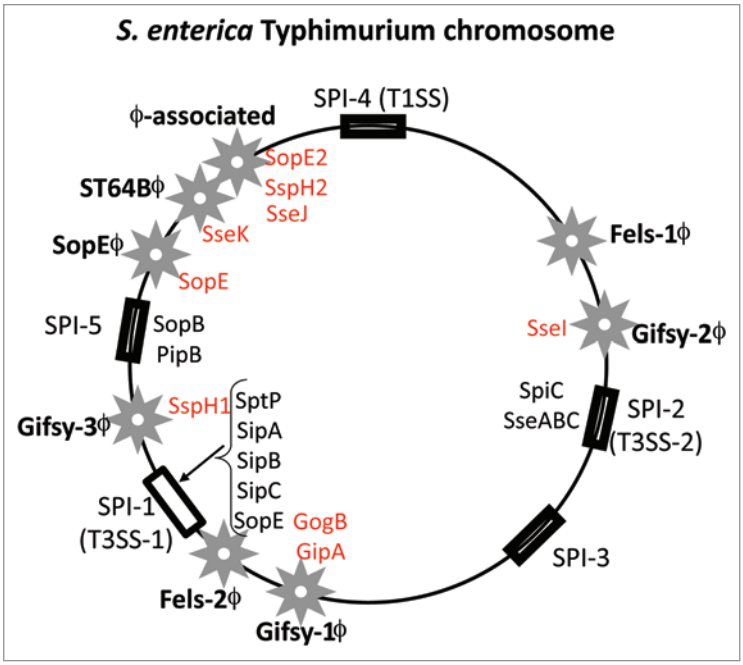

Figure 5. Prophage and pathogenicity island location on the *S. enterica* serovar Typhimurium genome map. Stars indicate prophages and rectangular black boxes indicate the position of *Salmonella* pathogenicity islands (SPIs). Four letter designations indicate effector genes identified within prophages (red) and SPIs (black).

Of the effector proteins identified in *E. coli*, 60 have been identified within prophages in the EHEC Sakai strain, 39 of which were experimentally shown to be translocated into eukaryotic cells[55] (**Fig. 2**). Of the 13 lambdoid prophages present within the genome, nine of these prophages encode effector proteins[55] (**Fig. 2**). The two shiga toxin-encoding phages Sp5 and Sp15 in strain Sakai do not encode any effector genes. Similar to many phage-encoded toxin genes, with the exception of *stx* genes, the majority of effector toxins in prophage genomes are found in the terminal region of the genome, after the tail fiber genes, and adjacent to the phage attachment site[1,55] (**Fig. 3A and C**). The location of multiple effector genes at the end of phage genomes suggests acquisition by imprecise excision from a previous host genome (**Fig. 3**). Similar to the *ctxAB* genes that encode CT, the percent guanine-cytosine of the effector genes differs significant from the rest of the phage genome again suggesting that they are not true phage genes per se.[55] The *E. coli* effectors identified were named following the esp (E. *coli* secreted protein) and nle (non-lee encoded effector) nomenclature (**Fig. 3**).

The effectors identified in the *E. coli* Sakai strain fell into greater than 20 protein families, the largest of which is NleG and has 14 representatives in this strain.[55] Savchenko and colleagues recently identified an ubiquitin E3 ligase function in the C-terminal region of NleG.[56] Ubiquitination is an essential eukaryotic function that involves attachment of an ubiquitin polypeptide to a lysine residue of a target protein. The targeted protein is either destined for degradation by the host proteasome or its function is regulated. It has now been demonstrated that the NleG family function in vitro as E3 ubiquitin ligases by acting as a scaffold between E2 enzyme and the target protein in ubiquitination.[56] The target protein(s) as well as the role in pathogenesis of NleG effectors have yet to be identified. The few previous studies on effectors with E3 ubiquitin ligase function suggest they are involved in suppression of the host immune response by targeting immune-related host proteins to proteasome degradation.[57–59]

Of the many different effector genes that have been identified within pathovars of *E. coli* only a handful have known host cell target sites and to date, most have unknown functions.[52,60] Both Tir and the phage encoded EspF (aka TccP) effector contain mitochondrial targeted sequences (MTS) within their N-terminal regions[52,60] and a recent study suggested that EspF targets and disrupts nucleolus function by a mitochondrial based mechanism (**Fig. 4**). EspF as well as Map have also been shown to disrupt tight junctions resulting in induction of apoptosis. The phage-encoded NleA and its homologs were shown to target the cell tight junctions (disrupting cellular barrier function) as well as mediate trafficking to the Golgi[52,60] (**Fig. 4**).

Salmonella enterica Phage-Encoded Effector Proteins

Salmonella enterica can cause acute infections, ranging from gastroenteritis (*S. enterica* serovar Typhimurium) to enteric fever (*S. enterica* serovar Typhi) depending on the serovar and strain examined.[61] *Salmonella enterica* is a facultative intracellular food-borne pathogen that causes morbidity and mortality worldwide as well as economic losses due to food recalls.[62] This bacterium can colonize birds, reptiles and mammals and is subdivided into seven subspecies: I, II, IIIa, IIIb, IV, VI and VII.[63–66] Infections in warm-blooded animals such as humans are generally caused by subspecies I isolates, which encompass more than 2,300 different serovars.[64,66] *S. enterica* serovar Typhimurium is one of the most common causes of food-borne infections causing inflammatory diarrhea.

The key virulence genes of *S. enterica* serovar Typhimurium are encoded on mobile and integrative genetic elements that were acquired by horizontal gene transfer (**Fig. 5**).[67–71] Numerous prophages and pathogenicity islands have been identified and characterized from *S. enterica* Typhimurium genome sequences (**Fig. 5**). Five pathogenicity islands named Salmonella pathogenicity island 1 (SPI-1) to SPI-5 have been identified within the genome (**Fig. 5**).[70,72–78] Two of these pathogenicity islands, SPI-1 and SPI-2, contain T3SSs and effector proteins and are present in all *S. enterica* strains. Eight different phages or phage-like region have also been characterized and shown to carry effector proteins and are variably present among strains (**Fig. 5**).[69,70,79–81]

Pathogenicity Islands (PAIs) are a group of integrative elements which encode multiple virulence factors that confer an advantage to the bacterium within its target eukaryotic host and were first described in an *E. coli* uropathogenic strain 536.[82–85] PAIs integrate into host genome usually at tRNA sites by site-specific recombination mediated by the PAI-encoded integrase. In *E. coli*, PAI-encoded integrases have been shown to be unrelated

to integrases present in phages indicating that phages and PAIs are diverse elements not closely related to one another.[86,87] SPI-1 and SPI-2 encodes multiple virulence genes involved in invasion and enteropathogenesis including T3SSs and several effector proteins (**Fig. 5**).[72,88,89] SPI-3 and SPI-4 are much smaller regions and appear to have a less significant role in pathogenesis. The SPI-5 region carries a number of effector proteins that are secreted by both T3SSs encoded on SPI-1 and SPI-2 (**Fig. 6**).[78]

Among *S. enterica* strains, approximately 30 different effector proteins have been identified, most of which are encoded within the same region that contains their cognate T3SS (SPI-1 and SPI-2).[90] As was the case for *E. coli,* the *S. enterica* phage-encoded effector genes are found at the terminal region of the phage genome but generally only occur as a single gene within the phage genome (**Fig. 3B**).[3] Serovar Typhimurium effectors secreted by T3SS-1 trigger membrane ruffling and internalization within a vacuole and are required for host cell invasion (**Fig. 6**). These effectors activate cytoskeleton rearrangement by causing actin nucleation, which disrupts the microvilli on the apical cell surface and aid in bacterial uptake, creating a Salmonella containing vacuole (SCV) within the host cell cytosol (**Fig. 6**). Salmonella then secretes effector proteins into the cytosol to modulate intracellular survival, first by preventing lysosome fusion with the SCV (**Fig. 6**).[91] Both T3SSs are involved in translocating proteins from the SCV into the host cell cytosol, many of which are required for modulation of the host immune response.[90,91] Among *S. enterica* strains examined nine effector proteins are found on prophage genomes, SseI within Gifsy-2ϕ, GogB and GipA within Gifsy-1ϕ, a homology of SspH1 on Gifsy-3ϕ, SseK3 on phage ST64B, SopE within SpoEϕ, and SopE2, SspH2 and SseJ on a phage-like region.[3] T3SS-1 translocated effectors SopE and SopE2 and T3SS-2 translocated effectors SseI and SspH1 are required for modulation of the host immune response.[90] SopE and SopE2 are also involved in activating Rho GTPases by mimicking guanine exchange factors.[90]

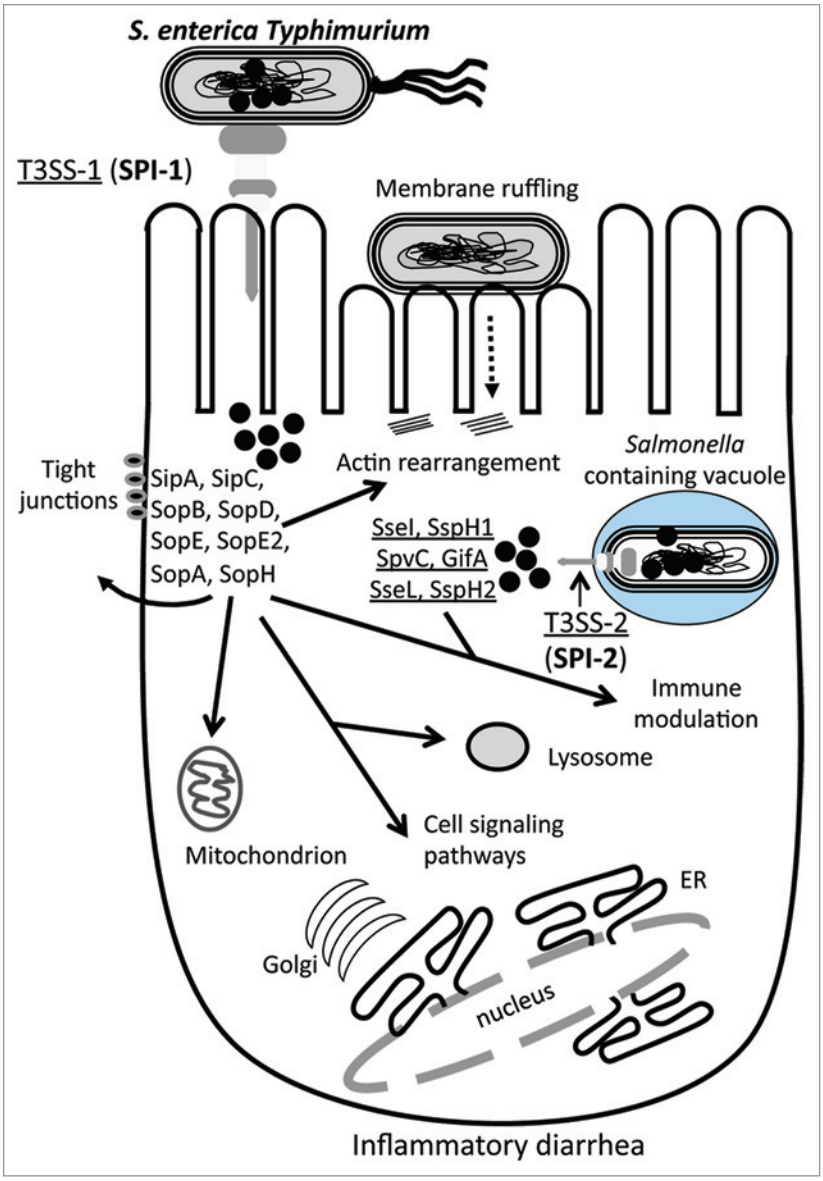

Figure 6. Schematic of the role of different effector protein functions and targets in *S. enterica*. *Salmonella enterica* encodes two T3SSs, each within a Salmonella pathogenicity island, SPI-1 and SPI-2. Similar to EHEC effector proteins, a number of effectors are required for intimate binding to the host cell surface, which leads to the uptake into a Salmonella containing vacuole (SCV). Within the SCV, the T3SSs translocates a range of effector proteins into the cytosol that allow for intracellular survival. The *S. enterica* effector proteins target the tight junctions, mitochondria and lysosomes, as well as several cell signaling pathways. Prophage-encoded effectors are shown in red. See text for details.

Conclusion

Key to effector protein function is their delivery into their target cell. Bacteria encode three contact-dependent secretion systems named T3SS, T4SS and T6SS that translocate effector proteins directly from the bacterial cytosol into the eukaryotic cytosol. Interestingly the evolutionary origin of these three systems is very different as are the effector proteins translocated by each. The T3SS shows homology to the bacterial flagellar system, the T4SS shows homology to the bacterial conjugation system and the T6SS shows homology to a contractile phage tail-like structure. It was shown that this structure contains an exterior sheath wrapped around an interior tube, similar to that in phage T4. However, the structural similarities do not translate into significant primary sequence homology and it remains to be determined whether functional similarity exists.[92] The T6SSs are a recent

discovery and only a handful of effector proteins have been identified and one could speculate that some of their effector proteins may be phage encoded. Although effector proteins identified to date number nearly 150, the function(s) of the vast majority of these proteins remains unknown. In addition as more species and strains are sequenced the number of phage-encoded effector proteins is sure to increase and with it our knowledge of how phages encoding cargo genes interact with their host genomes and the host regulatory systems.

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