Diarrhoeal disease caused by enteropathogenic *E. coli* (EPEC) is dependent on a delivery system that injects numerous bacterial ‘effector’ proteins directly into host cells. The best-described EPEC effectors are encoded together on the locus of enterocyte effacement (LEE) pathogenicity island and display high levels of multifunctionality and cooperativity within the host cell. More recently, effectors encoded outside the LEE (non-LEE effectors) have been discovered and their functions are beginning to be uncovered. The recent completion of the EPEC genome sequence suggests its effector repertoire consists of at least 21 effector proteins. Here, we describe the genomic location of effectors and discuss recent advances made on effector cellular function as well as their role in the infection process.

**Address**
Institute for Cell and Molecular Biosciences, Medical School, Newcastle University, Catherine Cookson Building, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

Corresponding author: Dean, Paul (p.dean@ncl.ac.uk) and Kenny, Brendan (brendan.kenny@ncl.ac.uk)

This review comes from a themed issue on Host-microbe interactions: Bacteria
Edited by Brendan Kenny and Raphael Valdivia

Available online 12th January 2009
1369-5274 © 2008 Elsevier Ltd. Open access under CC BY license.

DOI 10.1016/j.mib.2008.11.006

**Introduction**

Enteropathogenic *E. coli* (EPEC) is a human pathogen of the small intestine that causes severe watery diarrhoea, particularly among infants in developing countries [1]. EPEC is a member of a closely related family of pathogens that induce characteristic attaching and effacing (A/E) lesions on intestinal epithelial cells in humans (EPEC and EHEC — enterohaemorrhagic *E. coli*), ruminants (EHEC) and small animals including mice (*Citrobacter rodentium*) [2]. Hallmarks of EPEC disease are loss (effacement) of absorptive microvilli, induction of actin-rich pedestals underneath adherent bacteria, rapid watery diarrhoea, inhibition of nutrient/water transporter function, mitochondrial dysfunction, a weak inflammatory response and tight junction (TJ) disruption (see [1,3]). Upon initial contact with intestinal enterocytes, mediated in part by the bundle forming pilus (BFP), EPEC rapidly cause the effacement of microvilli and induce localised actin polymerisation that gives rise to a pedestal beneath the bacteria, to which the bacteria intimately attach. Mitochondrial dysfunction and disruption of nutrient transporters are also early events, whilst the disruption of TJ is only apparent later on during the infection process. EPEC is considered a non-invasive pathogen and relies upon on a type three secretion system (T3SS) to deliver effector proteins directly into host cells which subvert a myriad of host cellular functions, ultimately leading to disease [3]. The first EPEC effectors to be discovered are all encoded on a large genomic pathogenicity island called the locus of enterocyte effacement (LEE), which also carries EPEC’s only T3SS, with seven LEE effectors identified to date. More recently, effectors encoded outside the LEE region have been found in all A/E pathogens [4,5] which utilise the LEE T3SS for delivery into host cells and recent functional studies on these ‘non-LEE’ effectors have started to ascribe cellular functions to these proteins. The completion of the genome sequence of the prototypical EPEC strain E2348/69 (herein termed EPEC; www.sanger.ac.uk/Projects/Esherichia_Shigella) has confirmed the presence (or absence) of many non-LEE effector genes [6].

EPEC also possess a type two secretion system (T2SS), although little is known about its role in virulence or its protein substrates [6], and a type five secretion system (T5SS) which includes the enterotoxin EspC and other putative autotransporters [7,8**]. Interestingly, the entry of EspC into the host cells has been shown to be dependent on T3SS [8**] and it is likely that other autotransporters may also depend on the T3SS. In this review we will focus on the repertoire of type three secreted EPEC effector proteins and the important advances made on effector functions over the past few years. We redirect the reader to other reviews for information on other virulence factors and general mechanisms of EPEC pathogenesis [1,3,7].

**EPEC effector nomenclature**

The naming of EPEC effectors has been based on three approaches and may be somewhat confusing to the lay reader. Traditionally, the term ‘Esp-’ was used to denote *EPEC* secreted protein and now includes the LEE effectors EspB/F/G/H. However, EspA and EspD, which have not been reported to possess effector activity, and the autotransporter EspC, also fall into this category, making effector designation a little ambiguous. A different approach was to name effectors to reflect their reported...
<table>
<thead>
<tr>
<th>EPEC effector</th>
<th>Island location</th>
<th>Cellular/physiological functions</th>
<th>Subcellular target sites</th>
<th>Proposed host partners</th>
<th>Functional motifs</th>
<th>Homologue family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tir</td>
<td>LEE</td>
<td>Actin polymerisation</td>
<td>Plasma membrane</td>
<td>IQGAP1</td>
<td>SH3-binding</td>
<td>None known</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TJ disruption&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cytoplasm</td>
<td>14-3-3-tau</td>
<td>GAP motifs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell detachment</td>
<td></td>
<td>Nck</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvilli effacement</td>
<td></td>
<td>α-Actinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGLT-1 inactivation</td>
<td></td>
<td>Talin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLCγ phosphorylation</td>
<td></td>
<td>Cortactin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regulating effector activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>Vinculin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Invasion non-polarised cells</td>
<td></td>
<td>Cytokeratin 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Map</td>
<td>LEE</td>
<td>TJ disruption</td>
<td>Mitochondria</td>
<td>EBP50/</td>
<td>PDZ1-binding</td>
<td>IpgB2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Filopodia formation</td>
<td>Cytoplasm</td>
<td>NHERF1</td>
<td>MTS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondrial dysfunction</td>
<td></td>
<td></td>
<td>WxxxE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvilli effacement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGLT-1 inactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition of NHE3 activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane remodelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aquaporin redistribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-WASP activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EspB</td>
<td>LEE</td>
<td>Anti-phagocytosis</td>
<td>Plasma membrane</td>
<td>Antitrypsin</td>
<td></td>
<td>YopD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvilli effacement</td>
<td>Cytoplasm</td>
<td>α-Catenin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Actin disruption</td>
<td></td>
<td>Myosin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pore formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EspF</td>
<td>LEE</td>
<td>Apoptosis</td>
<td>Mitochondria, cytoplasm</td>
<td>ABCF2</td>
<td>PRR</td>
<td>None known except other EspF variants such as EspF(U)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TJ disruption</td>
<td>Apical and lateral</td>
<td>Actin</td>
<td>SH3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>membranes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvilli effacement</td>
<td>TJ region&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ZO-1/ZO-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SGLT-1 inactivation</td>
<td></td>
<td>N-WASP and SNX9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pedestal maturation</td>
<td></td>
<td>binding domains</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microvilli elongation</td>
<td></td>
<td>MTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondrial dysfunction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pedestal maturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition of NHE3 activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane remodelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aquaporin redistribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-WASP activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EspH</td>
<td>LEE</td>
<td>Modulating actin dynamics</td>
<td>Pedestals</td>
<td>Actin</td>
<td>SH3</td>
<td>None known</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytoskeleton disruption</td>
<td>Plasma membrane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EspZ</td>
<td>LEE</td>
<td>Unknown</td>
<td>Pedestals</td>
<td></td>
<td></td>
<td>None known</td>
</tr>
<tr>
<td>EspG</td>
<td>LEE</td>
<td>Microtubule disruption</td>
<td>Microtubule colocalisation</td>
<td>Tubulin</td>
<td>VirA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TJ disruption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paracellular permeability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aquaporin redistribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DRA transporter inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NleH1</td>
<td>PP2</td>
<td>Pro-inflammatory</td>
<td>Golgi</td>
<td>Sec24</td>
<td>PDZ1</td>
<td>OspG</td>
</tr>
<tr>
<td>NleJ</td>
<td>PP2</td>
<td>Anti-phagocytosis</td>
<td></td>
<td></td>
<td></td>
<td>None known</td>
</tr>
<tr>
<td>NleB2</td>
<td>PP4</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>None known</td>
</tr>
<tr>
<td>NleG</td>
<td>PP4</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>AIP56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NleD</td>
<td>PP4</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>HopAP1, HopH1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>NleG</td>
<td>PP4</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>NleH2</td>
<td>PP6</td>
<td>Pro-inflammatory</td>
<td></td>
<td></td>
<td></td>
<td>OspG</td>
</tr>
<tr>
<td>NleF</td>
<td>PP6</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>NleA</td>
<td>PP6</td>
<td>Inhibition of protein secretion</td>
<td>Golgi</td>
<td>Sec24</td>
<td>PDZ1</td>
<td>None known</td>
</tr>
<tr>
<td></td>
<td></td>
<td>by interference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>with COPII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(EspI)</td>
<td>PP6</td>
<td></td>
<td></td>
<td>PDZK11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SNX27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MAIS3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TCOF1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> TJs = tight junctions, Actin = actin filament, α-Catenin = α-catenin, Myosin = myosin, N-WASP = N-Wiskott-Aldrich syndrome protein, SNX9 = sorting nexin 9, MTS = membrane targeting signal, 14-3-3 = 14-3-3 protein, GAP = GTPase activating protein, SH3 = src homology domain 3, NHE3 = sodium-hydrogen exchanger 3, COPII = coat protein complex II, PDZK11 = PDZ and麒麟like protein 11, SNX27 = sorting nexin 27, MAIS3 = MAP interacting protein 3, TCOF1 = transcriptional co-activator with PDZ and knot domains 1, OspG = outer surface protein G, HopAP1, HopH1 = Hoppap1, Hoph1.
The predicted set of EPEC effectors comprises 21 full-length genes and at least 5 identified pseudogenes (i.e. genes truncated by stop codons, missing start codons, containing frameshift mutations). Where more than one copy of an effector exists, genes are numbered in accordance with sequence comparison to known EHEC homologues. For example, the full-length gene EspL is more similar to EHEC EspL2, whilst the EspL pseudogene corresponds with EspL1 and is named accordingly. All effectors are found on the chromosome in pathogenicity islands specified in [6] and illustrated in Figure 3. We have attempted to include all the known and documented effector functions and the known subcellular locations from published sources. ‘Homologue family’ gives an example of a known homologue from an effector family; where indicated as ‘none known’ this does not discount similarities with unassigned hypothetical proteins. Island location is illustrated in Figure 3 in accordance with [6]. All other references can be found in the text or in [3]. Phos, phosphorylation; MTS, mitochondrial targeting sequence; PRR, proline-rich repeat; SH3, src homology domains; PMN, polymorphonuclear; TJ, tight junction; IQGAP, IQ motif containing GTPase activating protein; EBP50/NHERF1, Na+/H+ exchanger regulating factor 1; ezrin–radixin–moesin, ERM-binding phosphoprotein of 50 kDa.

* We have attempted to include all the known and documented effector functions and the known subcellular locations from unpublished sources.

* Functional motifs and ‘proposed host partners’ correspond to those that have been proved to have functional significance, although in some cases, data from yeast two-hybrid protein–protein interactions are included as in [49,50].

* Photobacterium syringae effector proteins.

* Also has Shigella enterotoxin homology.

The LEE effectors — highly interdependent and multifunctional

Historically, the LEE effectors were the first to be identified in EPEC and to date a total of seven LEE-encoded proteins delivered into the host cell have been discovered, namely Tir, Map, EspF, EspG, EspZ (previously SepZ), EspH and EspB (which is also a translocator and essential for the delivery of effectors into the host cell) (see Table 1 and [3]). Also present on the LEE is the outer membrane protein Intimin which, whilst not delivered into host cells, causes numerous host cell responses directly through its bacterial-encoded receptor Tir or through various host cell receptors [1,3]. All the LEE effectors, except EspZ [12], have proven deleterious effects on the host cell (Table 1 and see [3]) and it is likely that EspZ has important effector functions as it is delivered early in the infection process and at similarly high levels as the essential virulence determinant, Tir [13]. The importance of the LEE effectors in the disease process is unclear but animal infections using Citrobacter and EHEC (as EPEC lacks a suitable animal model) indicate that Tir is essential, linked to its role in bacterial attachment, whilst the other LEE effectors have a smaller but additive contribution to virulence [2,10,14,15].

The functions of the LEE effectors are highly varied and Table 1 gives an up-to-date and comprehensive list of reported effector functions. An emerging theme for the LEE effectors, consistent with findings in other T3SS-pathogens, is their multiple and overlapping functions (termed functional redundancy) and their interdependence and cooperativity in subverting host cell activities (Table 1 and Figure 1). For example, Map and EspF synergise [16] whilst EspG and EspG2 function redundantly [17] in the disruption of epithelial TJ, with the Map/EspF TJ-disrupting activity proven in vivo [14,18]. Tir is essential for actin-pedestal formation following binding to Intimin but is also involved in TJ disruption, independent and dependent of Intimin (Dean and Kenny, unpublished). Tir also downregulates Map-induced filamentation (see [3]) and coordinates with EspF, Intimin and Map to cause microvilli effacement [19*]. Indeed, Knutton and colleagues have also reported overlapping roles for LEE effectors in microvilli effacement ex vivo, using human intestinal material [20]. In addition, Map and EspF both target mitochondria to alter organelle shape and cause dysfunction — an activity that occurs in vivo and proven to be important in disease
Such effector cooperativity appears to be just the ‘tip of the iceberg’ as a systematic genetic study in which the LEE effectors were deleted in many different combinations eludes to an unprecedented level of functional cooperativity between effectors (Kenny et al., unpublished). To this end, all the major reported hallmarks of EPEC disease can be attributed to the cooperative efforts of the LEE effectors (Figure 1).

In addition to their cooperative nature, the LEE effectors are strikingly multifunctional (Table 1) binding a large number of eukaryotic proteins and targeting various host cell compartments (Table 1 and Figure 1). This is best exemplified by EspF which localises to multiple cellular compartments (including cytoplasm, mitochondria, apical and lateral membranes) and interacts with at least 12 reported host proteins, with its delivery linked to mitochondrial dysfunction, microvilli effacement, TJ disruption, apoptosis, epithelial transporter inhibition, anti-phagocytosis, membrane remodelling and actin-pedestal maturation [15,16,18–22,26,51–53]. Like other LEE effectors, the modular construction of EspF facilitates its multifunctional behaviour (Figure 2), with specific motifs inducing distinct cellular responses, such as an N-terminal mitochondrial targeting sequence (MTS) linked to mitochondria dysfunction and apoptosis.

The complexity of EPEC effector function. The multifunctional and overlapping properties of the EPEC effectors are depicted here by grouping effector functions together. Three effectors have anti-phagocytic activities (shown here using the phagocytic-like gut-associated antigen presenting M-cells) whilst at least five effectors act on microvilli and four inhibit SGLT-1 and other transporter activity, four disrupt tight junctions and three are involved in pedestal and filopodia formation. At least three Nle effectors are also involved in inflammatory pathways. Microtubule and Golgi/ER disruption appears to be specific to EspG/Orf3 and NleA, respectively. Also shown are effectors which have known actin-modulating properties. TJ, tight junctions; MT, microtubules; AQP, aquaporins; NHE3, sodium hydrogen exchanger; Cl/OH, Cl−/OH− transporter; SGLT-1, sodium glucose cotransporter-1; ER, endoplasmic reticulum; MV, microvilli.
[15,21], whilst proline-rich repeats, that include src homology 3 (SH3)-binding domains, recruit sorting nexin 9 (SNX9) causing membrane remodelling ([22]; Table 1 and Figure 2). EspF, like its EHEC homologues EspF and EspF/U/Tccp, recruits N-WASP (a key regulator of actin polymerisation), with studies on EspFU revealing it specifically activates N-WASP by mimicking an internal regulatory element [23,24]. Finally, although EspF plays an essential role in anti-phagocytosis, EspB–myosin interaction has recently been reported to inhibit both phagocytosis and microvilli effacement [25]. However, as both of these bacterial processes can occur with EspB/EspF-positive but not EspB-positive/EspF-negative strains [19*,26]; this suggests that EspB is neither solely responsible nor sufficient.

Effector multifunctionality is further demonstrated by Map and Tir which like EspF, possess motifs that mediate a broad array of functions ([3]; Table 1 and Figure 2). All of Tir’s reported activities depend on its extracellular domain (Figure 2) binding to Intimin, following Tir insertion into the host plasma membrane. Tir’s N-terminal and C-terminal domains remain intracellular and interact with numerous signalling, adapter and cytoskeletal proteins with Tir function dependent on its phosphorylation sites, a GTPase activating protein (GAP)-like motif and a polyproline region (Figure 2) [27–29]. Interestingly, although tyrosine phosphorylation of Tir is essential for actin-pedestal formation in immortalised cell lines, human biopsy material has revealed that this crucial event is independent of tyrosine phosphorylation ex vivo, suggesting care should be taken when using cell lines to elucidate effector function [30**]. Map on the other hand is reported to mimic the active form of Cdc42 — a small GTPase — to induce filopodia formation [31], in contrast to an earlier report demonstrating a depen-
The six identified non-LEE effector encoding pathogenicity islands of EPEC E2348/69. Predicted effector genes were identified by mining the EPEC genome using over 400 known/predicted effector sequences. The identified effectors and genomic islands support the genome sequence published data (see text), from which the genomic island names were obtained. Only those genomic regions encoding the effectors and with low %GC content (graph above each island) are shown with most prophage-related genes surrounding these regions omitted. Genes and strand direction are shown by individual arrows which are drawn to scale within each island and colour coded (see inset). Multiple copies of genes are numbered according to close homologues in EHEC as explained in the legend to Table 1. Pseudogene key: (a) Cif; C-terminally truncated protein not produced or secreted in this EPEC strain [48]; (b) NleH3; C-terminal truncated; (c) NleO; no start codon; (d) EspL1; stop codon in middle of gene; (e) NleB3; N-terminal truncated.

The six identified non-LEE effector encoding pathogenicity islands of EPEC E2348/69. Predicted effector genes were identified by mining the EPEC genome using over 400 known/predicted effector sequences. The identified effectors and genomic islands support the genome sequence published data (see text), from which the genomic island names were obtained. Only those genomic regions encoding the effectors and with low %GC content (graph above each island) are shown with most prophage-related genes surrounding these regions omitted. Genes and strand direction are shown by individual arrows which are drawn to scale within each island and colour coded (see inset). Multiple copies of genes are numbered according to close homologues in EHEC as explained in the legend to Table 1. Pseudogene key: (a) Cif; C-terminally truncated protein not produced or secreted in this EPEC strain [48]; (b) NleH3; C-terminal truncated; (c) NleO; no start codon; (d) EspL1; stop codon in middle of gene; (e) NleB3; N-terminal truncated.
surrounding the WxxxE effector family has been elo-
quently discussed in a recent review which provides a
compelling argument to suggest these effectors may not
be GTPase mimics after all [34**]. Finally, Map possesses
an N-terminal MTS and targets the mitochondria where it
is imported via the classical TOM/Hsp70 import system and
causes mitochondrial dysfunction [35] (see Figures 1
and 2).

**EPEC non-LEE effectors and non-LEE pathogenicity islands**

Whilst mining the EHEC (O157:H7 Sakai strain) genome
sequence with over 200 known/predicted T3SS-depend-
ent effector proteins, Pallen and colleagues identified 49
putative effectors [5]. At least 39 of these predicted
proteins, of which many are homologues, were confirmed
as secreted effectors in EHEC and include NleA-H
(notig 12 NleG homologues) and newly described
EspJ-O, EspR and EspV-Y effectors [5]. The recent
completion of the EPEC genome sequence (strain E2348/69)
enabled a similar ‘effector mining’ approach using an expanded list of over 400 known/predicted
effector sequences and identified only 21 putative effec-
tors (Dean and Kenny, unpublished; Table 1 and
Figure 3). Thus, as recently reported [6*], EPEC appears
to have a much smaller non-LEE effector repertoire than
EHEC, encoding NleA-H (two homologues of B, E, H
and only 1 of NleG), EspJ and EspL2, Orf3 (EspG2;
which is the only EPEC effector so far identified that is
not present in EHEC) and pseudogenes for NleH, EspO,
NleB, EspL and Cif (Table 1 and Figure 3). Thus, whilst
the EEE effector repertoire is well conserved, the set of
non-LEE effectors is apparently flexible as EPEC strains
B171-8 and E22 (rabbit-EPEC) possess 28 and 40 effec-
tors, respectively, compared to 21 for the prototypical
strain [6*].

The non-LEE effector genes are clustered in six patho-
genicity islands (Figure 3) scattered throughout the gen-
ome, usually with a low G+C% content in regions
corresponding to effector genes. Interestingly, the non-
LEE effector genes are surrounded by phage-related and/
or transposase-like genes implying that, like most patho-
genicity islands, they were acquired through horizontal
acquisition [6*]. Whilst EPEC E2348/69 carries at least 21
effector-encoding genes, it is not known whether they are
all expressed but undoubtedly other effectors remain to
be identified. Indeed, there are many hypothetical genes
in the non-LEE islands that are likely candidates as
effectors because of their low %GC content and close
proximity to known effector genes (Figure 3).

Because of the more recent discovery of non-LEE effec-
tors, relatively little is known about their cellular function
(summarised in Table 1). But NleA is reported to inhibit
protein secretion [36*], EspJ inhibits phagocytosis [37],
whilst NleE [38] and NleH [39] activate innate immune
responses. Studies with the mouse model suggest that
EspJ, NleB, NleE, NleF and NleH play a role in colonisation
and full virulence [40-43] whilst NleC and D have no
detectable function [40,41]. Although NleA (also called
EspI) was reported to be a key virulence factor [44],
another study using the same mouse model, reported that
it only contributes to full virulence — as with most other
effectors [10]. Interestingly, despite NleE and NleH
reportedly inducing innate immune responses, EPEC
T3SS-dependent function has been demonstrated to inhib-
it, not activate, such responses in small intestinal cells and
mouse studies [45*,46]. Importantly, the reported inhibi-
tory mechanism was not dependent on LEE effectors,
implicating non-LEE effectors in the process [45*]
although a LEE effector was recently identified that
inhibits NF-κB activation (Kenny et al., unpublished)— revealing yet another overlapping role for
LEE and non-LEE effectors. Therefore, a discrepancy
exists as NleE and NleH appear to induce inflammatory
responses, whilst EPEC’s overall effect on host/host cells is
anti-inflammatory. It is possible that EPEC transiently
induces pro-inflammatory responses but rapidly inactivates
this response mechanism before the disruption of TJ’s, that
is associated with onset of inflammatory cascades [45*].
Thus, like the activities of many other EPEC effectors, a
delicate balance likely exists between pro-inflammatory
and anti-inflammatory signalling mechanisms [47].

**Conclusions**

‘Multifunctional, cooperative and redundant’ are three
overriding themes that describe EPEC effector beha-
vour and are becoming increasingly accepted for various
T3SS-pathogen effectors. Interestingly, the LEE effec-
tors appear to subvert many ‘core’ epithelial cell pro-
cesses and consequently, all of the major EPEC disease-
related hallmarks have been attributed to the LEE
effectors, suggesting the non-LEE effectors may func-
tion mainly as accessory/efficiency factors. However, it is
worth pointing out that individual non-LEE effectors are
more highly conserved between A/E pathogens than
LEE effectors [6*], possibly reflecting the targeting of
well conserved processes, whilst LEE-effector variability
may reflect different host ranges. The high level of
functional interdependence between the LEE effectors
possibly reflects their continued co-evolution within the
LEE pathogenicity island and this sets an exciting pre-
cedent that other effectors co-inherited together may
display similarly complex levels of interplay. A future
challenge will be to define the contribution of all effec-
tors in each disease process by identifying the effector
domains and motifs responsible. Only then the contribu-
tion of the particular effector function to the disease
process can be assessed in animal models. Nonetheless
the expanded repertoire of effectors provides fascinating
opportunities to understand how pathogens subvert cel-
lular processes and increase our understanding of vital
eukaryotic processes.
Acknowledgements
We apologise in advance to the authors of the published work that is not included in this review because of space constraints. Work within our laboratory is funded by the Wellcome Trust.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


7. The published EPEC genome sequence revealed the prototypical EPEC strain appears to have a comparatively small repertoire of type three secreted effectors in addition to a host of other virulence factors. This paper is of particular interest given that most functional studies performed on A/E pathogen effectors have used this prototypical strain.


10. A paper with far-reaching implications as it is the first to show the cooperation between these two secretion systems with the internalisation of the EspC autotransporter protein into host cells dependent on the type three secretion system. Potentially, it opens the door to other secreted proteins, particularly autotransporters, being delivered into host cells by this mechanism.


21. Dean P, Maresca M, Schuller S, Phillips AD, Kenny B: Potent diarreagenic mechanism mediated by the cooperative action of three enteropathogenic Escherichia coli-injected effector proteins. Proc Natl Acad Sci U S A 2006, 103:1976-1981. This paper identified the effectors that underpin EPEC-mediated of microvilli effacement and reveal effacement is the result of the concerted action of at least three effectors. In addition, a potent mechanism was uncovered to explain the rapid onset of watery diarrhoea relating to the inhibition of the major intestinal water pump SGLT-1.


is an SH2/3 ligand that recruits and activates tyrosine kinases required for pedestal formation. Mol Microbiol 2007, 63:1748-1768.


A important study which identifies that effector function using cell lines is different to that which likely occurs in vivo. Specifically, it reveals that Tir tyrosine phosphorylation which is essential for pedestal formation in cell culture models, is not important within *in vivo* differentiated epithelia. This cautions on the need to use or at least verify new finding in the most appropriate model.


An excellent and concise review article that puts forward a robust and convincing argument to suggest that the WxxxE containing effectors may not be mimics of small GTPase and that the WxxxE motif itself may be convincing argument to suggest that the WxxxE containing effectors may not be mimics of small GTPase and that the WxxxE motif itself may be


This paper provides a plausible explanation about why EPEC infection is linked with a weak inflammatory response. EPEC was shown to inhibit innate immune responses (IKK, MAP kinase and PI-3 kinase pathways through unidentified effector(s)). Crucially, this inhibition occurred before tight junctions were disrupted, preventing elicitors such as flagellin stimulating inflammatory responses in the basolateral domain.


The first comprehensive and detailed study into the cellular function of a non-LEE effector, NleA and the first EPEC effector shown to inhibit protein secretion. The authors go to great lengths to characterize NleA function and reveal the protein binding partners and a molecular mechanism to explain the inhibition of protein secretion.


