Enteropathogenic *Escherichia coli*: cellular harassment

Rebekah DeVinney, Derek G Knoechel and B Brett Finlay*

The mechanisms by which enteropathogenic *Escherichia coli* (EPEC) mediates diarrhea remain a mystery. Recently a number of interesting and at times surprising results have come from studying EPEC interactions with host cells. Identification and characterization of bacterial factors, including Tir, EspA, EspB and EspD, and host responses have expanded our grasp of the diverse effects of EPEC on host cells.

Addresses
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada
*e-mail: bfinlay@unixg.ubc.ca

Current Opinion in Microbiology 1999, **2**:83–88

http://biomednet.com/elecref/1369527400200083

© Elsevier Science Ltd ISSN 1369-5274

Abbreviations

A/E	attaching and effacing
BFP	bundle-forming pilus
EPEC	enteropathogenic *Escherichia coli*
Esp	EPEC secreted protein
IL	interleukin
IP	inositol phopshate
LEE	locus of enterocyte effacement
PKC	protein kinase C
PLCγ	phospholipase Cγ
PMN	polymorphonuclear lymphocyte
REPEC	rabbit EPEC
TEER	transepithelial electrical resistance
Tir	translocated intimin receptor

Introduction

Enteropathogenic *Escherichia coli* (EPEC) is a major cause of diarrhea in the developing world [1••]. EPEC requires intimate attachment to the host epithelia for full virulence. Intimate attachment results in the formation of attaching and effacing (A/E) lesions, which are characterized by the degeneration of the epithelial cell brush border, and the formation of actin-rich pedestals within the host cell [2,3]. This requires a number of bacterial factors, including the EPEC secreted proteins (Esps), a type III secretory apparatus, and the outer membrane protein intimin. In the past year, much progress has been made identifying and characterizing bacterial factors necessary for A/E lesion formation both *in vitro* and *in vivo*. Additionally, investigators are making headway in determining the host signaling pathways modulated in response to EPEC infection. This review focuses on the recent major findings within these two areas. A model of EPEC pathogenesis incorporating some of these new findings is illustrated in Figure 1.

Bacterial factors

Hp90 unmasked as Tir

The EPEC outer membrane protein intimin had been shown to bind a 90 kDa protein (Hp90) found in host cell membranes that was tyrosine phosphorylated upon EPEC infection [4]. This receptor was recently identified as a bacterial protein that is translocated into the host cell and was renamed Tir (for translocated intimin receptor) [5••].

Tir is produced as an unphosphorylated 78 kDa protein within the bacterial cell and undergoes phosphorylation on one or more tyrosine residues in its carboxyl terminus (shifting the apparent molecular weight to 90 kDa) upon insertion into the host cell membrane [5••]. Tir has two proposed transmembrane domains and is thought to have at least three possible functions, the first being to bind intimin. The second function is to focus the cytoskeletal rearrangements induced by EPEC, which surprisingly does not involve the small GTP-binding proteins Rho, Rac, or Cdc42 [6]. Although adherence can occur in the absence of Tir, mutants deficient in Tir do not cause distinct actin accumulation or pedestal formation beneath adherent bacteria. The third function of Tir (possibly in conjunction with intimin) is to cause additional host signaling events, including the activation of phospholipase Cγ (PLCγ), once intimate attachment has occurred. The existence of Tir and its translocation was confirmed in a strain of enterohemmorhagic *E. coli* (another attaching and effacing pathogen) by Deibel and collaborators [7].

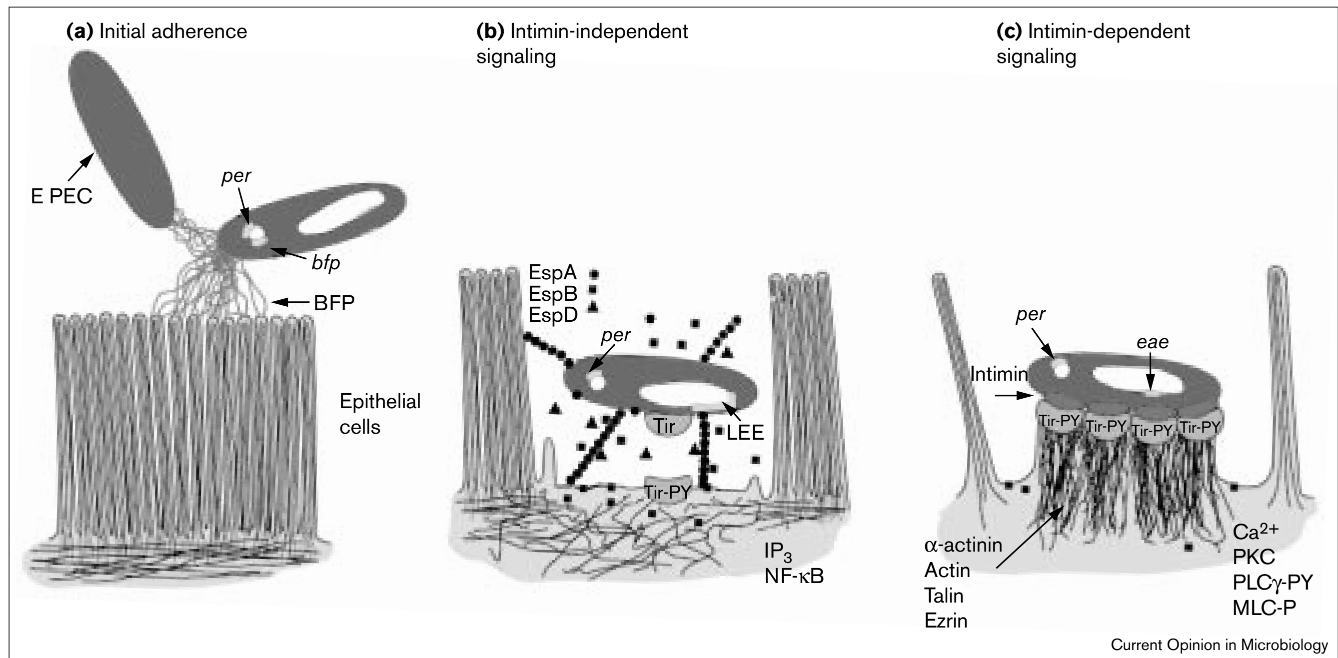
Secreted proteins

Six proteins have now been identified in EPEC tissue culture supernatants. Of these, EspA, EspB, EspD, and Tir are encoded within the EPEC pathogenicity island described below and are transported via a type III secretion system. All four are required for the formation of the characteristic A/E lesions [5,8–11], and their secretion, inducible under conditions similar to those found in the gastrointestinal tract, is regulated by temperature, pH, and osmolarity [12•]. The role of the other two secreted proteins, EspC and EspD, in EPEC pathogenesis is not known.

EspB is a 37 kDa protein that is produced and secreted upon contact with HeLa cells, and has been shown to be both translocated into the host cell and required for protein translocation during EPEC infection [13••]. Translocation of EspB was established using immunofluorescence, host cell fractionation, and an EspB–CyaA fusion protein. By measuring levels of cAMP production in the host cell the intracellular location and hence translocation of the EspB–CyaA fusion was confirmed. EspB was localized to both the cytoplasm and cell membrane of the host cell upon translocation [13••]. In addition, a 39 kDa secreted protein has recently been identified as EspD, although its sequence sheds little light on its possible function [11].

EspA has an apparent molecular weight of 25 kDa, and has been found within a novel filamentous organelle on

Figure 1



EPEC pathogenesis. **(a)** Initial adherence. Bacteria, individually or in aggregates, bind to host epitheial cells via the bundle-forming pilus (BFP), which is regulated by the plasmid-encoded *per* locus [1••]. This non-intimate attachment is referred to as localized adherence. **(b)** Protein secretion by EPEC leads to intimin-independent signaling events within the host cell. These events include inositol triphosphate (IP_3) fluxes and activation of the transcription factor NF-κB. Filamentous organelles containing EspA are present on the bacterial surface and Tir and EspB are translocated into the host cell. Tir becomes tyrosine-phosphorylated (Tir-PY) and acts as a receptor for the bacterial adhesin intimin, resulting in intimate attachment. The role of EspD is unknown. **(c)** The recruitment of cytoskeletal elements α-actinin, actin, talin, and ezrin are intimin-dependent signaling events [40]. Cytoskeletal reorganization into a pedestal is accompanied by an increase in $[Ca^{2+}]_{in}$, activation of protein kinase C (PKC) and phospholipase C-γ (PLCγ), and phosphorylation (P) of myosin light chain (MLC). Adapted with permission from [47].

the surface of EPEC [14••]. These organelles are present in the early stages of A/E lesion formation and form a bridge between the bacterium and the eukaryotic cell surface. EspA possesses a putative coiled-coil domain [15], and it has been proposed that other secreted proteins could be transported through the resulting pore [14••]. These EspA filaments are down-regulated upon A/E lesion formation, much like intimin (see below).

Locus of enterocyte effacement (LEE)

A recent study has shown that the 35 kb pathogenicity island known as the locus of enterocyte effacement (LEE) encodes EPEC's molecular apparatus both necessary and sufficient for attachment and effacement of epithelial cells [16••]. The entire LEE was cloned into *E. coli* K-12, conferring the attaching and effacing phenotype. Sequencing of the LEE region has recently been completed, showing a G + C content of 38.4% [17•], much lower than the 50.8% found within the *E. coli* chromosome [18], suggesting it came from a non-*E. coli* source.

It has been suggested that the LEE genes are separated into three functional domains [17•]. These include the region encoding intimate adherence (Tir and intimin), and the region encoding the secreted proteins (Esps) and their putative chaperones. The third region encodes a type III secretion system, a key genetic component of many bacterial pathogens, including *Yersinia*, which contains the prototype type III system (reviewed in [19]). A typical type III apparatus contains approximately 20 proteins, and at least 13 such proteins have been identified within the EPEC LEE on the basis of empirical evidence or sequence homology [17•]. Proteins secreted via this system are not processed at their amino terminus during secretion and do not contain a consensus secretion signal. It has been proposed that the signal lies within the 5′ region of the mRNA encoding these proteins (reviewed in [19]). Type III secretion systems differ from other bacterial secretory systems in that they appear to be devoted to the translocation of bacterial proteins directly into the host cell.

Chaperones

Many type III secreted proteins have unique chaperones that are required for successful translocation [19], and EPEC is probably no exception. A LEE-encoded protein CesD has been shown to interact with and to be necessary for the secretion of EspD [20]. A *cesD* mutant also demonstrated reduced secretion of EspB. CesD shares some sequence homology with other type III chaperones.

Interestingly, its pI is much more basic than those of other type III chaperones (7.1 as opposed to ~4.5). In addition, while all type III chaperones have been shown to be cytoplasmic, CesD has been localized to both the cytoplasm and the inner membrane. Tir may also have its own chaperone, OrfU. The similarity of OrfU to SycH, a *Yersinia* chaperone, along with location of its gene immediately downstream of *tir* make it a good candidate for this role [17•].

Intimin and the bundle-forming pilus (BFP)

Intimin is a 94 kDa outer membrane protein encoded by the *eae* locus within the LEE essential for intimate adherence and A/E lesion formation [21]. The amino terminus of intimin is well conserved among attaching and effacing pathogens, but the carboxyl terminus is quite divergent, reflecting its hypothesized role as the binding domain. The carboxy-terminal 280 amino acids bind to eukaryotic cells, and may involve β1 integrins as the receptor [22]. β1 integrins have not been reported on the apical surface of enterocytes, but have been reported on the apical surface of mouse Peyers patch M cells [23]. Interestingly, while an EPEC-like strain does bind to M cells in a rabbit model, it is able to resist ingestion [24]. Although intimin is necessary for A/E lesion formation, it seems to be down-regulated upon lesion formation, disappearing from the side opposite the site of intimate adherence [25].

Before intimate adherence and A/E lesion formation, EPEC adhere to epithelial cells *in vitro* in a distinctive pattern termed localized adherence (LA). This adherence is mediated by a type IV fimbria designated as the bundle-forming pilus (BFP) encoded within the EAF (for EPEC adherence factor) plasmid [26]. The *bfp* genes encode and regulate this extracellular structure. The *bfpA* gene, coding for the structural subunit of BFP, was shown to be required for full virulence in humans [27], and a putative nucleotide-binding protein encoded by *bfpF* seems to be involved in modulating EPEC aggregation and is also required for full virulence [27,28]. In contrast, recent work with pediatric small intestinal tissue suggests that BFP are not involved in initial adherence *in vivo* but are instead implicated in the formation of three-dimensional aggregates through inter-bacterial interactions [29•].

Host responses

Signal transduction

EPEC modulates several signal transduction pathways within the host cell. EPEC infection leads to inositol phosphate (IP) fluxes within the host cell that are dependent on an intact bacterial type III secretory system and EspB [10,30,31]. One consequence of IP fluxes is the release of Ca^{2+} from intracellular stores. Several groups have investigated the effect of EPEC on changes in intracellular calcium concentration ($[Ca^{2+}]_{in}$), and the results have been inconclusive. Initial studies using both population and single cell recording methods suggested that EPEC infection leads to an increase in $[Ca^{2+}]_{in}$, and that this increase is dependent on type III secretion and intimate attachment to the host cell via intimin [31,32]. Additionally, the increase in $[Ca^{2+}]_{in}$ could be blocked by the Ca^{2+} chelator BAPTA, which resulted in the inhibition of pedestal formation [32]. In contrast, in a recent report, Bain and colleagues [33] found no evidence for a change in $[Ca^{2+}]_{in}$ in response to EPEC infection, nor inhibition of pedestal formation by BAPTA. The authors hypothesize that the change in $[Ca^{2+}]_{in}$ observed by other groups is due to EPEC-induced cytotoxicity.

EPEC has been demonstrated to activate protein kinase C (PKC). The first suggestion for PKC activation came from studies examining the pattern of EPEC induced protein phosphorylation, which appeared identical to that induced by known activators of PKC [34]. More recently, Crane and Oh [35•] measured PKC activity directly, and found an enhancement in membrane-associated PKC in response to EPEC infection. PKC activation requires intimate adherence, because the EPEC intimin mutant CVD206 does not enhance PKC activity.

EPEC induced enhancement of IP fluxes, increase in concentration of $[Ca^{2+}]_{in}$, and PKC activation suggests the involvement of a phospholipase. Kenny and Finlay [36•] reported the appearance of a second group of proteins that are tyrosine phosphorylated in response to EPEC infection. One of these proteins was identified as PLCγ. PLCγ activation is dependent on intimin binding, but occurs at a later timepoint than Tir phosphorylation. The authors were unable to determine whether phosphorylated Tir played a role in PLCγ activation. PLCγ is probably not responsible for the IP_3 flux generated in response to EPEC infection, because this can occur in the absence of intimate adherence. Whether this PLCγ activation provides the secondary messengers needed for the intimin dependent PKC activation and Ca^{2+} fluxes has yet to be determined.

Cytokines

EPEC infection also affects cytokine release from host tissues. Hecht's laboratory demonstrated that EPEC induced the transmigration of polymorphonuclear lymphocytes (PMNs) in an interleukin (IL)-8 dependent manner [37]. In a recent paper from this laboratory, Savkovic *et al.* [38•] report that EPEC, unlike nonpathogenic *E. coli* strains, activates the transcription factor NF-κB, which leads to the initiation of IL-8 transcription and PMN transmigration. NF-κB activation requires EspB, but not intimate adherence. EPEC also inhibits host immune function; Malstrom and James [39] report that EPEC lysates inhibit IL-2 and IL-4 production from both intestinal and splenic lymphocytes. This effect is not dependent on either EspA or EspB, and the factors responsible for this effect have not been identified.

Diarrhea

How EPEC causes diarrhea is still unknown, but several recent studies have shed light on some possible mechanisms. One common mechanism used by other intestinal

pathogens is the secretion of Cl⁻ [40]. Initial studies demonstrated that EPEC infection led to depolarization of epithelial cell membranes, most likely due to changes in cellular electrolyte transport [41]. This was investigated further by Collington *et al.* [42•] who demonstrated that EPEC increases the short circuit current (Isc) in cultured epithelial cells, and that this increase is due in part to the stimulation of Cl⁻ secretion [42•]. EPEC has been demonstrated to alter transepithelial electrical resistance (TEER) in polarized monolayers [43]. Recent work shows that this decrease in TEER is due to disruption of epithelial tight junctions, resulting from the EPEC induced phosphorylation of myosin light chain [44•]. This alteration in barrier function may contribute to EPEC-associated diarrhea due to disruption of electrochemical gradients and increased intestinal permeability.

We are just beginning to elucidate the bacterial factors necessary for disease. Recently two EPEC proteins have been shown to be required for the development of disease *in vivo* [45••]. Using the closely related rabbit pathogen, REPEC O103, Abe and colleagues [45••] demonstrated that the bacterial proteins EspA and EspB are essential for both A/E lesion formation and diarrheal disease. Rabbits infected with REPEC strains containing deletions in either EspA or EspB were unable to form A/E lesions, but retained the ability to adhere nonintimately. No symptoms of disease were evident after infection with either mutant strain. EspA and EspB join intimin [46] in the growing list of identified EPEC virulence factors, and emphasizes the requirement for A/E lesion formation in disease.

Conclusions

The secreted proteins represent EPEC's molecular arsenal during pathogenesis, and understanding their effects is crucial to mounting a counterattack. Discovering functional roles for Tir and EspA within the past year represent a significant advance. Finding evidence of the translocation of both Tir and EspB was also important. But while some questions have been answered, many more remain. The functions of EspB and EspD, as well as the possibility of EspA and EspD translocation, are important areas to probe in the future. Current research has shown that EPEC infection has a modulating effect on a number of signaling pathways within the host cell, although the significance of each event is not yet clear. The key determinants and mechanism of diarrhea are a major goal of these studies. Further investigation of the interactions between EPEC's secreted proteins and host cell signaling pathways should hopefully bring us closer to this goal.

Acknowledgments

R DeVinney and DG Knoechel contributed equally to this work. Work in BBF's laboratory is supported by a grant from the Medical Research Council (MRC) of Canada and he is a Howard Hughes International Research Scholar and an MRC Scientist. DGK is supported by a Natural Sciences and Engineering Research Council (NSERC) Postgraduate Scholarship.

References and recommended reading

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

• of special interest
•• of outstanding interest

1. Nataro J, Kaper J: **Diarrheagenic *Escherichia coli*.** *Clin Microbiol Rev* •• 1998, **11**:142-201.
This is a comprehensive review covering all six classes of diarrheagenic *E. coli*. The pathogenesis, epidemiology, detection, and clinical considerations for each pathogen are described in detail.

2. Knutton S, Baldwin T, Williams P, McNeish A: **Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*.** *Infect Immun* 1989, **57**:1290-1298.

3. Moon H, Whipp S, Argenzio R, Levine M, Giannella R: **Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestine.** *Infect Immun* 1983, **41**:1340-1351.

4. Rosenshine I, Ruschkowski S, Stein M, Reinscheid DJ, Mills SD, Finlay BB: **A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation.** *EMBO J* 1996, **15**:2613-2624.

5. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB: •• **Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells.** *Cell* 1997, **91**:511-520.
In this paper, the authors discover the identity of Hp90, the intimin receptor. They describe the transfer of Tir to the host cell membrane via EPEC's type III secretion system, where it becomes phosphorylated and acts as a receptor for intimin. This is the first documented instance of a pathogenic bacterium inserting its own receptor into a host cell membrane.

6. Ben-Ami G, Ozeri V, Hanski E, Hofmann F, Aktories K, Hahn KM, Bokoch GM, Rosenshine I: **Agents that inhibit Rho, Rac, and Cdc42 do not block formation of actin pedestals in HeLa cells infected with enteropathogenic *Escherichia coli*.** *Infect Immun* 1998, **66**:1755-1758.

7. Deibel C, Kramer S, Chakraborty T, Ebel F: **EspE, a novel secreted protein of attaching and effacing bacteria, is directly translocated into infected host cells, where it appears as a tyrosine-phosphorylated 90 kDa protein.** *Mol Microbiol* 1998, **28**:463-474.

8. Kenny B, Lai LC, Finlay BB, Donnenberg MS: **EspA, a protein secreted by enteropathogenic *Escherichia coli*, is required to induce signals in epithelial cells.** *Mol Microbiol* 1996, **20**:313-323.

9. Donnenberg MS, Yu J, Kaper JB: **A second chromosomal gene necessary for intimate attachment of enteropathogenic *Escherichia coli* to epithelial cells.** *J Bacteriol* 1993, **175**:4670-4680.

10. Foubister V, Rosenshine I, Donnenberg MS, Finlay BB: **The *eaeB* gene of enteropathogenic *Escherichia coli* is necessary for signal transduction in epithelial cells.** *Infect Immun* 1994, **62**:3038-3040.

11. Lai LC, Wainwright LA, Stone KD, Donnenberg MS: **A third secreted protein that is encoded by the enteropathogenic *Escherichia coli* pathogenicity island is required for transduction of signals and for attaching and effacing activities in host cells.** *Infect Immun* 1997, **65**:2211-2217.

12. Kenny B, Abe A, Stein M, Finlay BB: **Enteropathogenic *Escherichia* • *coli* protein secretion is induced in response to conditions similar to those in the gastrointestinal tract.** *Infect Immun* 1997, **65**:2606-2612.
The induction of enterophathogenic *E. coli* (EPEC) protein secretion is characterized in terms of temperature, pH, and osmolarity dependence. Previously described dependence of secretion upon carbon dioxide levels was explained by using different buffering systems. The authors also postulate how induction of protein secretion within the intestine and subsequent inhibition in the colon might occur based on their findings.

13. Wolff C, Nisan I, Hanski E, Frankel G, Rosenshine I: **Protein** •• **translocation into host epithelial cells by infecting enteropathogenic *Escherichia coli*.** *Mol Microbiol* 1998, **28**:143-155.
This work is significant in that it demonstrates that a second enteropathogenic *E. coli* protein, EspB, is translocated into the host cell. The authors use a variety of techniques to confirm translocation. EspB is

localized to both cytoplasmic and membrane fractions of the host cell. This work also suggests that EspB is required for its own translocation. The intracellular function of EspB remains an intriguing question.

14. Knutton S, Rosenshine I, Pallen MJ, Nisan I, Neves BC, Bain C,
•• Wolff C, Dougan G, Frankel G: **A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells.** *EMBO J* 1998, **17**:2166-2176.

The authors propose a function for secreted protein, EspA. Using gentler washing techniques, these investigators isolated a filamentous structure containing EspA on the surface of enteropathogenic *E. coli* (EPEC). This organelle may be EPEC's molecular conduit to the host cell. If so, other type III secreting pathogens may possess similar structures. The authors also present a revised four-step model of EPEC adherence incorporating the EspA filamentous structure.

15. Pallen MJ, Dougan G, Frankel G: **Coiled-coil domains in proteins secreted by type III secretion systems [letter].** *Mol Microbiol* 1997, **25**:423-425.

16. McDaniel TK, Kaper JB: **A cloned pathogenicity island from**
•• **enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K-12.** *Mol Microbiol* 1997, **23**:399-407.

The cloning of the LEE pathogenicity island into K-12 and the resulting attaching and effacing histopathology provides unequivocal evidence that this pathogenicity island is both necessary and sufficient for virulence. Beyond this, it reinforces the notion that avirulent bacteria can be transformed into host-damaging pathogens by the transfer of a single virulence cassette.

17. Elliott SJ, Wainwright LA, McDaniel TK, Jarvis KG, Deng YK, Lai LC,
• McNamara BP, Donnenberg MS, Kaper JB: **The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69.** *Mol Microbiol* 1998, **28**:1-4.

Having completed the sequencing of the LEE, the authors adopt a standardized nomenclature system for many of the proteins encoded within on the basis of *Yersinia* homologues. A table comparing all 41 ORFs greater than 50 amino acids with similar proteins in other bacteria establishes a framework for the LEE genes.

18. Blattner FR, Plunkett G 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF *et al.*: **The complete genome sequence of *Escherichia coli* K-12 [comment].** *Science* 1997, **277**:1453-1474.

19. Hueck CJ: **Type III protein secretion systems in bacterial pathogens of animals and plants.** *Microbiol Mol Biol Rev* 1998, **62**:379-433.

20. Wainwright LA, Kaper JB: **EspB and EspD require a specific chaperone for proper secretion from enteropathogenic *Escherichia coli*.** *Mol Microbiol* 1998, **27**:1247-1260.

21. Jerse AE, Yu J, Tall BD, Kaper JB: **A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells.** *Proc Natl Acad Sci USA* 1990, **87**:7839-7843.

22. Frankel G, Lider O, Hershkoviz R, Mould AP, Kachalsky SG, Candy DCA, Cahalon L, Humphries MJ, Dougan G: **The cell-binding domain of intimin from enteropathogenic *Escherichia coli* binds to beta1 integrins.** *J Biol Chem* 1996, **271**:20359-20364.

23. Clark MA, Hirst BH, Jepson MA: **M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells.** *Infect Immun* 1998, **66**:1237-1243.

24. Inman L, Cantey J: **Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer's patch in *Escherichia coli* diarrhea in the rabbit.** *J Clin Invest* 1983, **71**:1-8.

25. Knutton S, Adu-Bobie J, Bain C, Phillips AD, Dougan G, Frankel G: **Down regulation of intimin expression during attaching and effacing enteropathogenic *Escherichia coli* adhesion.** *Infect Immun* 1997, **65**:1644-1652.

26. Donnenberg MS, Giron JA, Nataro JP, Kaper JB: **A plasmid-encoded type IV fimbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence.** *Mol Microbiol* 1992, **6**:3427-3437.

27. Bieber D, Ramer SW, Wu CY, Murray WJ, Tobe T, Fernandez R, Schoolnik GK: **Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*.** *Science* 1998, **280**:2114-2118.

28. Anantha RP, Stone KD, Donnenberg MS: **Role of BfpF, a member of the PilT family of putative nucleotide-binding proteins, in type IV pilus biogenesis and in interactions between enteropathogenic *Escherichia coli* and host cells.** *Infect Immun* 1998, **66**:122-131.

29. Hicks S, Frankel G, Kaper JB, Dougan G, Phillips AD: **Role of intimin**
• **and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue *in vitro*.** *Infect Immun* 1998, **66**:1570-1578.

Most scientific studies of enteropathogenic *E. coli* (EPEC) pathogenesis to date have been with human epithelial cultured cell lines. The need to shift our investigations toward more applicable models is underscored by these results from *in vitro* organ culture experiments. While emphasizing the importance of intimin in adherence to intestinal tissue, the authors question the role of bundle-forming pili (BFP) in non-intimate attachment. When put in the context of previous reports underlining the importance of BFP in EPEC pathogenesis, it should stimulate further work in this area.

30. Foubister V, Rosenshine I, Finlay BB: **A diarrheal pathogen, enteropathogenic *Escherichia coli* (EPEC), triggers a flux of inositol phosphates in infected epithelial cells.** *J Exp Med* 1994, **179**:993-998.

31. Dytoc M, Fedorko L, Sherman PM: **Signal transduction in human epithelial cells infected with attaching and effacing *Escherichia coli in vitro*.** *Gastroenterology* 1994, **106**:1150-1161.

32. Baldwin TJ, Ward W, Aitken A, Knutton S, Williams PH: **Elevation of intracellular free calcium levels in HEp-2 cells infected with enteropathogenic *Escherichia coli*.** *Infect Immun* 1991, **59**:1599-1604.

33. Bain C, Keller R, Collington G, Trabulsi L, Knutton S: **Increased levels of intracellular calcium are not required for the formation of attaching and effacing lesions by enteropathogenic and enterohemorrhagic *Escherichia coli*.** *Infect Immun* 1998, **66**:3900-3908.

34. Manjarrez-Hernandez HA, Baldwin TJ, Aitken A, Knutton S, Williams PH: **Intestinal epithelial cell protein phosphorylation in enteropathogenic *Escherichia coli* diarrhoea.** *Lancet* 1992, **339**:521-523.

35. Crane J, Oh J: **Activation of host cell protein kinase C by**
• **enteropathogenic *Escherichia coli*.** *Infect Immun* 1998, **65**:3277-3285.

This paper shows, for the first time, direct activation of protein kinase C (PKC) by enteropathogenic *E. coli* (EPEC). The authors measured PKC translocation from the cytosol to the membrane, and found an increase in membrane-associated activity in response to EPEC infection.

36. Kenny B, Finlay B: **Intimin-dependent binding of enteropathogenic**
• ***Escherichia coli* to host cells triggers novel signalling events, including tyrosine phosphorylation of phospholipase C-γ^1.** *Infect Immun* 1997, **65**:2528-2536.

In this report, the authors describe a second wave of signaling events in epithelial cells in response to enteropathogenic *E. coli* (EPEC) infection. One of these events is the tyrosine phosphorylation and activation of phospholipase C-γ^1. Phospholipase C-γ^1 activation is dependent upon intimate adherence to the host cell, via the bacterial protein intimin.

37. Savkovic S, Koutsouris A, Hecht G: **Attachment of a noninvasive enteric pathogen, enteropathogenic *Escherichia coli*, to cultured human intestinal epithelial monolayers induces transmigration of neutrophils.** *Infect Immun* 1996, **64**:4480-4487.

38. Savkovic S, Koutsouris A, Hecht G: **Activation of NF-κB in intestinal**
• **epithelial cells by enteropathogenic *Escherichia coli*.** *Am J Physiol* 1998, **273**:C1160-C1167.

The authors report the transcription factor NF-κB is activated in response to enteropathogenic *E. coli* (EPEC) infection. This requires active bacterial signaling, and an EspB mutant strain had no effect on NF-κB activity. NF-κB activation leads to an increase in IL-8 transcription, resulting in the EPEC-induced neutrophil transmigration reported earlier by these authors.

39. Malstrom C, James S: **Inhibition of murine splenic and mucosal lymphocyte function by enteric bacterial products.** *Infect Immun* 1998, **66**:3120-3127.

40. Finlay BB, Falkow S: **Common themes in microbial pathogenicity. II.** *Mol Biol Microbiol Rev* 1997, **61**:136-169.

41. Stein MA, Mathers DA, Yan H, Baimbridge KG, Finlay BB: **Enteropathogenic *Escherichia coli* markedly decreases the resting membrane potential of Caco-2 and HeLa human epithelial cells.** *Infect Immun* 1996, **64**:4820-4825.

42. Collington G, Booth I, Knutton S: **Rapid modulation of electrolyte**
• **transport in Caco-2 cell monolayers by enteropathogenic** ***Escherichia coli*** **(EPEC) infection.** *Gut* 1998, **42**:200-207.
In this report, the authors demonstrate that enteropathogenic *E. coli* (EPEC) infection alters epithelial cell electrolyte balance, by increasing short cirucuit current (Isc). This increase in Isc coincided with pedestal formation, and requires EPEC secreted Esp proteins. The initial rise in Isc was in part dependent on chloride, with the balance due to either influx of sodium or amino acids.

43. Canil C, Rosenshine I, Ruschkowski S, Donnenberg MS, Kaper JB, Finlay BB: **Enteropathogenic** ***Escherichia coli*** **decreases the transepithelial electrical resistance of polarized epithelial monolayers.** *Infect Immun* 1993, **61**:2755-2762.

44. Yuhan R, Koutsouris A, Savkovic S, Hecht G: **Enteropathogenic**
• ***Esherichia coli*****-induced myosin light chain phosphorylation alters intestinal epithelial permeability.** *Gastroenterology* 1998, **113**:1873-1882.
The authors report that enteropathogenic *E. coli* (EPEC) infection perturbs epithelial cell tight junctions. This effect is due to myosin light chain phosphorylation. Myosin light chain phosphorylation leads to a decrease in transepithelial cell electrical resistance (TEER), resulting from disruption of tight junctions. Treatment with inhibitors of myosin light chain kinase (MLCK), but not inhibitors of protein kinase C or tyrosine kinases protect epithelial cell junctions from the decrease in TEER, suggesting an involvement for MLCK.

45. Abe A, Heczko U, Hegele R, Finlay B: **Two enteropathogenic**
•• ***Escherichia coli*** **type III secreted proteins, EspA and EspB, are virulence factors.** *J Exp Med* 1998, **188**:1907-1916.
The authors investigated the role of EspA and EspB in virulence, using the closely related rabbit pathogen REPEC O103. Attaching and effacing lesion formation was not present in rabbits infected with the EspA or EspB deletion strains, although nonintimate adhesion was not effected. The rabbits infected with either mutant strain showed no evidence of disease. This is the first report identifying secreted proteins as virulence factors.

46. Donnenberg MS, Tacket CO, James SP, Losonsky G, Nataro JP, Wasserman SS, Kaper JB, Levine MM: **Role of the eaeA gene in experimental enteropathogenic** ***Escherichia coli*** **infection.** *J Clin Invest* 1993, **92**:1412-1417.

47. Donnenberg MS, Finlay BB, Kaper JB: **Interactions between enteropathogenic** ***Escherichia coli*** **and host epithelial cells.** *Trends Microbiol* 1997, **5**:109-114.