ELSEVIER

EPEC's weapons of mass subversion

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Enteropathogenic and enterohaemorrhagic *Escherichia coli* are closely related enteric pathogens whose ability to cause disease in humans is linked with a capacity to deliver bacterial 'effector' proteins into host epithelia to alter cellular physiology. Although the essential role of the locus of enterocyte effacement (LEE) pathogenicity island, which encodes effector proteins and the delivery machinery, has been established, more recent studies are uncovering additional layers of complexity. This is illustrated by the emerging multifunctional nature of the effectors and their ability to work together in redundant, synergistic and antagonistic relationships. Furthermore, new virulence-associated factors are continually being uncovered that are encoded outside the LEE pathogenicity island, some of which are not injected into host cells.

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Introduction

The family of 'attaching and effacing (A/E)' pathogens are enteric Gram-negative organisms that bind intimately to gut epithelial cells, leading to the localised effacement (loss) of absorptive microvilli and the accumulation of host cytoskeletal proteins beneath these adherent non-invasive bacteria. The human-specific A/E pathogen enteropathogenic *Escherichia coli* (EPEC) is a major cause of diarrhoea-related infant death in developing countries. Another A/E pathogen, enterohaemorrhagic *E. coli* (EHEC) is frequently carried asymptomatically by cattle but is highly infectious for humans, in whom it can induce a diarrhoeal disease often accompanied by complications that can be fatal (reviewed in [1,2]).

In this review, we focus on the latest studies that unearth the ever-increasing complexity of molecules and mechanisms deployed by A/E pathogens to manipulate host cell physiology to presumably enhance survival, replication and subsequent dissemination. Given the limitations of this review, we apologise in advance for focusing on the prototypic EPEC strain, not citing data from other relevant studies, and directing the reader to review articles for older studies.

The LEE – a fully equipped locus for bacterial attachment and cell surface remodelling

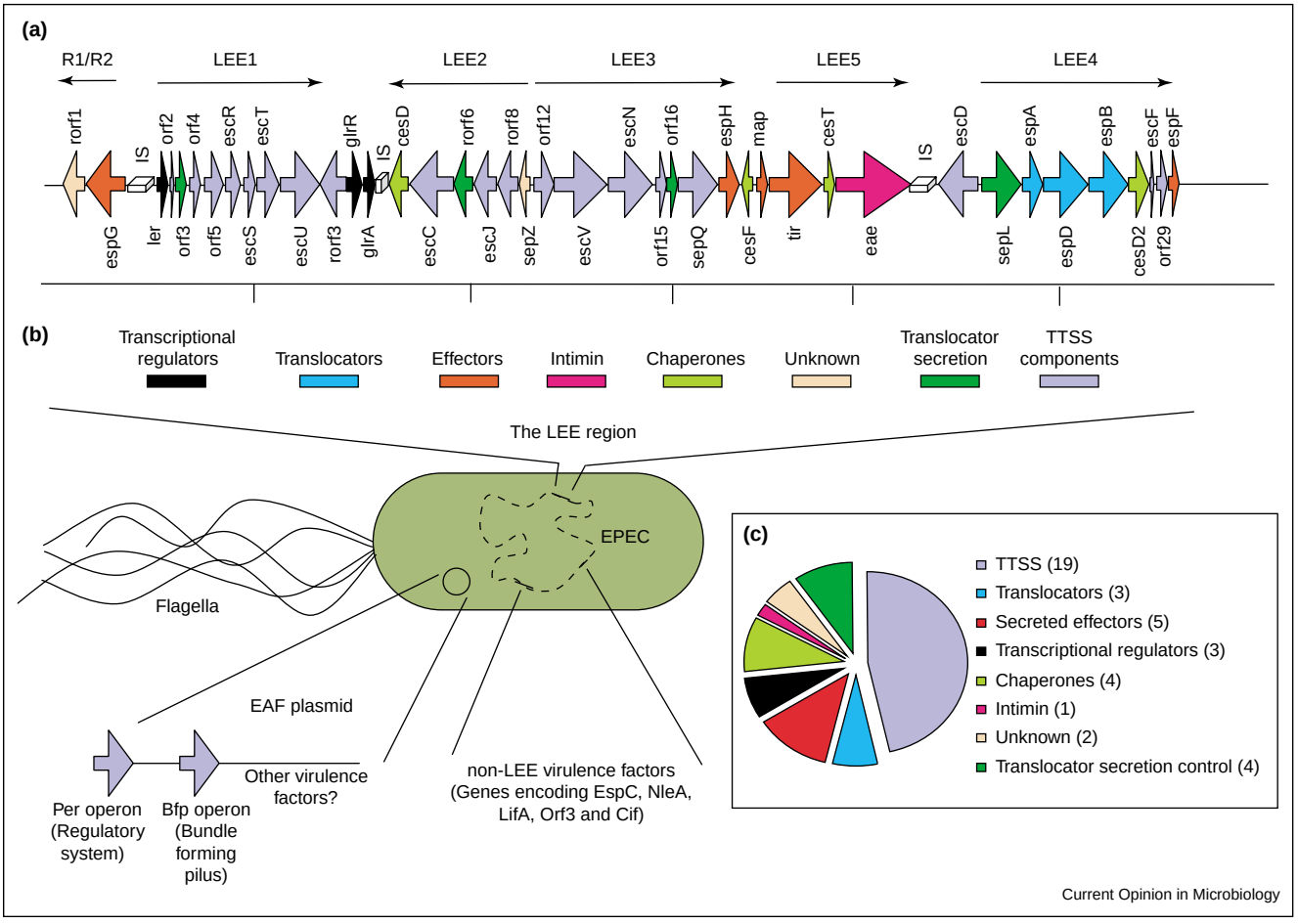
Although bacterial colonisation undoubtedly requires a capacity to respond to environmental changes and survive the hazardous journey through the gastrointestinal tract, it is EPEC's intimate interaction with small intestinal cells that appears to be the major determinant of disease, as mutants that adhere weakly are effectively avirulent (reviewed in [2,3]). EPEC attachment to host cells can be mediated in part by flagella and type IV pili, but the major adherence and disease-associated factors are encoded by the chromosomally located locus of enterocyte effacement (LEE) pathogenicity island (reviewed in [2–4]). The 35.5 Kb EPEC LEE region encodes proteins that fall into six main categories (see Figure 1) comprising transcriptional regulators, type III secretion system (TTSS) proteins, translocators, molecular chaperones, secreted effector proteins, and intimin (an outer membrane protein). The LEE pathogenicity island is present in all A/E pathogens and *in vivo* infection studies with their host species have demonstrated the essential nature of the TTSS in disease [5–7]. For example, a recent *in vivo* study used the mouse-specific A/E pathogen, *Citrobacter rodentium*, to elegantly demonstrate the requirement of each LEE-encoded protein for full virulence [8••].

A central function of the LEE pathogenicity island is to form the TTSS — a multiprotein 'needle' spanning the bacterial envelope that directs the secretion of LEE-encoded translocator and effector proteins. One translocator, EspA (EPEC secreted protein A) forms a long hollow extension (up to 0.7 μm long) of the TTSS, through which the EspB and EspD translocators pass before inserting into the host plasma membrane [2–4]. This TTSS–Esp assembly then permits the direct injection of 'effector' proteins into the host cell to interfere with signalling processes. Although the LEE was believed to encode only five effector molecules [8••,9] (Tir, Map, EspF–H), a sixth effector (formerly *sepZ*, renamed *espZ*) has now been uncovered (K Kanack, J Kaper, personal communication).

LEE-encoded effectors – few but able

The workhorses of the LEE pathogenicity island are undoubtedly its effector molecules, which, after delivery

Figure 1



Schematic representation of known EPEC virulence factors encoded on the chromosome or EAF plasmid. **(a)** The chromosomally located LEE (locus of enterocyte effacement) pathogenicity island is essential for type III secretion and the formation of A/E lesions on the host cell surface. The LEE comprises five main operons (LEE1–5; plus R1/R2) and 41 genes, of which the function of almost all are known allowing the genes to be grouped based on function is shown in **(b)**. The functional grouping of the LEE gene products is also shown in **(c)** revealing a high proportion of LEE genes involved in the formation of the type III secretion system while others encode effectors, chaperones and transcriptional regulators (numbers in brackets indicate number of gene products in each category). Also depicted in **(b)** are non-LEE encoded virulence factors that include the bundle forming pilus encoded on the EAF plasmid and genes found in several pathogenicity islands on the chromosome such as the those encoding EspC, Orf3, LifA and Cif.

into the host cell, can interfere with many host cellular processes (reviewed in [2,10] and see Table 1). The most extensively studied LEE effector is Tir (translocated intimin receptor), owing to its early discovery and unique nature as a plasma membrane receptor. Remarkably, Tir interaction with the bacterial outer membrane protein intimin not only mediates intimate adherence but also triggers host cell signalling events that lead to cytoskeletal protein accumulation beneath the adherent bacteria (reviewed in [4]). This latter property of Tir is dependent on its undergoing tyrosine phosphorylation within the host cell (reviewed in [4,11]), with the kinases mediating this event recently identified [12–14]. Although such modifications play an essential role in bacterial-mediated actin nucleation *in vitro*, this event may not be crucial *in vivo* ([15]; A Phillips, personal communication).

Multifunctionality of LEE effectors

The extraordinary multifunctional nature of Tir is becoming increasingly evident, given the recent findings that Tir–intimin interaction not only triggers intimate attachment and actin nucleation but also tyrosine phosphorylation of the host phospholipase, PLC-γ1 (reviewed in [4]), in addition to facilitating Cdc42-dependent invasion of non-phagocytic cells [16•] and downregulation of EPEC-mediated filopodia formation [17•]. Indeed, multifunctionality is an emerging theme of LEE effectors, as the Map (mitochondrial-associated protein) effector not only targets host cell mitochondria, where it interferes