Microreview

LEEways: tales of EPEC, ATEC and EHEC

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Summary

Intestinal pathogenic *Escherichia coli* are a major cause of worldwide morbidity and mortality. Currently seven intestinal pathovars are recognized causing a wide range of intestinal disorders that are sometimes associated with severe and even lethal complications. The arsenal of virulence factors is used to subvert cellular functions of the host thereby enhancing adaptation, virulence and pathogenicity. Virulence factor profiles are largely the result of the acquisition of mobile genetic elements such as prophages and pathogenicity islands. A group of highly adapted intestinal pathogenic *E. coli* that are characterized by the induction of 'attaching-and-effacing (A/E) lesions' have acquired a decisive pathogenicity island, the 'locus of enterocyte effacement – LEE' by horizontal gene transfer. This review focuses on recent advances in our understanding of A/E *E. coli*. It highlights novel functions of effector proteins, addresses the LEE flanking regions where additional genetic elements such as the LifA/Efa1 region have been identified, and points to implications for diagnostics and therapy due to the putative interconversion of A/E *E. coli* during infection.

Introduction

Escherichia coli is a remarkable versatile organism exhibiting astonishing genome plasticity (Croxen and Finlay, 2010; Tenaillon *et al.*, 2010). Even in the non-pathogenic *E. coli* K-12 strain used in many laboratories an estimated 17.6% of its coding sequence have been acquired from other species. The sequenced *E. coli* isolates exhibit a core genome of approximately 2200 genes and a pan-

genome of around 13 000 genes (Rasko *et al.*, 2008). Due to the acquisition of pathogenicity islands and other mobile genetic elements, the genomes of pathogenic *E. coli* can be up to 1 Mb larger than of their commensal relatives and could encode about 5000 genes. This leaves ample opportunity for substantial genetic diversity resulting in different virulence factor profiles. Recent genome analysis indicated that acquisition of genes as well as loss of genes has contributed to the emergence of the pathogroups in *E. coli* (Croxen and Finlay, 2010).

At present nine distinct pathovars have been recognized in *E. coli*. Further to the pathovars of uropathogenic (UPEC) and neonatal meningitis/sepsis-causing *E. coli* (NMEC), seven intestinal (diarrhoeagenic) pathovars – enteropathogenic *E. coli* (EPEC), atypical enteropathogenic *E. coli* (aEPEC or ATEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC) and diffusely adhering *E. coli* (DAEC) – have been described. In addition, there are other human pathovars such as adherent invasive *E. coli* (AIEC) that has been associated with Crohn's disease (Rolhion and Darfeuille-Michaud, 2007; Lapaquette *et al.*, 2009). Commonly, pathovars are defined based on specific virulence factor profiles and their characteristics and manifestations of pathogenicity in a given host. *E. coli* pathotypes tend to be clonal groups that express O (somatic) and H (flagellar) antigens that define their serogroup (O-type) or serotype (O- and H-type). Lately, intermediate strains have been described that based on their arsenal of virulence factors cannot be attributed to a recognized pathovar which exemplifies the ongoing changes in pathogenic *E. coli* strains (Müller *et al.*, 2007).

This review addresses recent advances in our understanding of the LEE-harbouring *E. coli* pathovars: EHEC, ATEC and EPEC. As by no means this can be comprehensive I do apologize to all colleagues whose excellent work could not be included due to space limitations.

Enteropathogenic *E. coli* (EPEC)

In the early 1940s *E. coli* has been implicated with human diarrhoeal disease and these strains (O111) have been termed 'enteropathogenic' *E. coli* (EPEC). In 1978, studies with human volunteers by Myron Levine using the
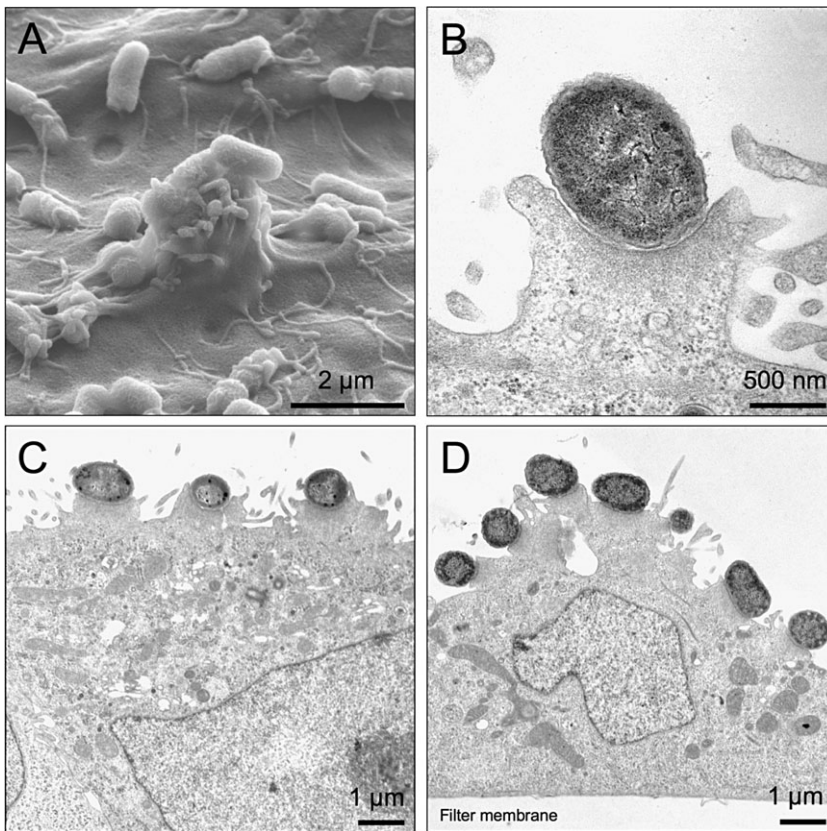
Fig. 1. Pedestal formation by atypical enteropathogenic *Escherichia coli* (ATEC) strains. Scanning (SEM) and transmission (TEM) electron microscopy of ATEC strains following HeLa or Int-407 cell infection (3 h).
A. SEM of pedestal formation by the ATEC strain 3431-4/86 (O8 : H⁻).
B. TEM of ATEC strain B6 (O26 : K60) 3 h of infection/HeLa cells.
C. TEM of ATEC strain B6 (O26 : K60) 6 h of infection/HeLa cells.
D. TEM of ATEC strain B6 (O26 : K60) 3 h of infection/INT-407 cells.
B–D. Polymerized actin is clearly visible underneath adherent bacteria.

EPEC O127 : H6 strain E2348/69 clearly demonstrated the intestinal pathogenic potential of EPEC strains. Based on these studies EPEC E2348/69 became the prototype EPEC strain. E2348/69 harbours the core 'locus of enterocyte effacement – LEE' whereas almost all other isolates investigated to date contain quite extended 5′ and 3′ flanking regions (e.g. Gärtner and Schmidt, 2004; Müller *et al.*, 2009). The typical 'attaching-and-effacing (A/E) lesions' in concert with pedestal formation that are characteristic for LEE-harbouring pathogens (Fig. 1) were only identified in subsequent investigations. A consensus definition of EPEC was accepted during the 2nd International Symposium on EPEC in São Paulo in 1995.

> EPEC are diarrheagenic *Escherichia coli* that produce characteristic histopathology known as attaching and effacing (A/E) on intestinal cells and that do not produce Shiga, Shiga-like, or verotoxins. Typical EPEC of human origin possess a virulence plasmid known as EAF (EPEC adherence factor) plasmid that encodes localized adherence on cultured epithelial cells mediated by the Bundle Forming Pilus (BFP), while atypical EPEC do not possess this plasmid. The majority of EPEC strains fall into certain well-recognized O : H serotypes such as O26; 55, 86, 111, 114, 119, 125, 126, 127, 128, 142 and 158.

Following these basic definitions various subgroups based on evolutionary considerations and the presence of

certain isotypes particularly of the *eae* gene product 'intimin' have been proposed (e.g. Ito *et al.*, 2007). Currently, 27 *eae* variants encoding distinct intimin types and subtypes have been established (e.g. Yamamoto *et al.*, 2009). The complete genome sequence of the EPEC E2348/69 prototype strain was reported in 2009 (Iguchi *et al.*, 2009) and – among other insights – provided a clear picture of the core LEE.

Atypical enteropathogenic *E. coli* (ATEC)

Atypical enteropathogenic *E. coli* (aEPEC, ATEC) by definition are EPEC that lack the EAF plasmid including the EAF-encoded bundle-forming pili and the *per* (*plasmid-encoded regulator*)-mediated regulation of T3SS-dependent virulence factors. This results in a distinct adherence phenotype (ATEC: mostly diffuse adherence versus EPEC: localized adherence) and a moderate pathogenicity. As ATEC strains have been isolated from diarrhoea patients as well as healthy individuals their pathogenic potential has long been controversial. However, ATEC strains harbour the LEE and secrete and inject effector proteins into target cells (Hernandes *et al.*, 2009). More recent epidemiology clearly demonstrated that (some) ATEC strains can cause prolonged diarrhoea and are a quite frequent

cause of diarrhoea in children (Afset *et al.*, 2006; Scaletsky *et al.*, 2009; Spano *et al.*, 2009). The heterogeneity of ATEC strains is reflected by the more than 200 O-serogroups that have been identified so far (Tennant *et al.*, 2009). Interestingly, most do not belong to the classical EPEC O-serogroups and many have been designated Ont (non-typeable). The most common O-serogroups are O51>O145>O26, O55 and O111. These observations suggest that ATEC represent most likely not a subgroup of EPEC generated by the putative loss of the EAF plasmid.

Enterohaemorrhagic *E. coli* (EHEC)

In a seminal discovery, Riley *et al.* (1983) identified the – at the time – rare *E. coli* O157 : H7 as the causative agent in an outbreak of bloody diarrhoea. Other researchers demonstrated the production of verotoxin (Shiga toxin) (see Johannes and Römer, 2010) and Karmali *et al.* reported the link between O157 strains and the haemolytic-uraemic syndrome (HUS) (Karmali *et al.*, 2010). O'Brien and colleagues demonstrated the presence of homologues of the *Shigella dysenteriae* 1 toxin in these *E. coli* strains (O'Brien *et al.*, 1984). These strains have been denoted enterohaemorrhagic *E. coli* (EHEC) and are predominantly distinguished from EPEC strains by the presence of *stx* genes. EHEC are further subgrouped in sorbitol-fermenting SF-EHEC and non-fermenting or typical strains. Among the more than 160 serogroups identified in EHEC in addition to the classic O157 serogroup, O26, O111, O103 and O145 have been recognized as important causes of enterohaemorrhagic colitis and HUS.

The locus of enterocyte effacement – LEE

EPEC, ATEC and EHEC share the A/E lesion formation as one of the most characteristic traits of pathogenicity (Croxen and Finlay, 2010). These bacteria bind intimately to intestinal epithelial cells, which leads to a localized effacement of absorptive microvilli and the accumulation of host cytoskeletal proteins just beneath the attached bacteria. This results in the development of 'pedestals' (Fig. 1). The capacity for A/E lesion formation is encoded mainly on the LEE, a pathogenicity island of non-*E. coli* origin that had been identified in 1995 (McDaniel *et al.*, 1995; Elliott *et al.*, 1998). The core LEE harbours the genes for a type III secretion system (T3SS), regulators, chaperones and effector proteins. Reminiscent of a 'molecular syringe', effector proteins are injected through the T3SS needle directly into the cytoplasma of the target cell where they subvert the cells cytoskeletal and signalling machinery. Apparently, T3SSs are very successful pathogenicity modules as besides *E. coli* they have been found in many Gram-negative animal and plant pathogens including *Salmonella*, *Yersinia*, *Shigella*, *Pseudomonas*, *Xanthomonas*, etc. T3SSs are fascinating molecular machines and have been studied structurally and mechanistically in great detail by many laboratories (addressed recently, e.g. by Müller *et al.*, 2008; Enninga and Rosenshine, 2009). Genome sequencing of the prototype O157 : H7 strain identified a secondary T3SS (*E. coli* type III secretion system 2 – ETT2) present in almost all commensal and pathogenic *E. coli* strains. The ETTS gene cluster harbours homologues of genes found in *Salmonella* Spi-1, -2 and -3 pathogenicity islands. However, in almost all *E. coli* strains the ETT2 T3SS is non-functional due to varying degrees of mutational attrition (Makino *et al.*, 2003; Ren *et al.*, 2004).

The genes encoding structural proteins of the T3SS are largely conserved whereas genes encoding effector proteins show substantial variability (Müller *et al.*, 2009). Castillo *et al.* (2005) pointed out that the conserved T3SS gene cluster in the LEE appears to have been acquired by horizontal gene transfer while genes encoding secreted proteins are more diverse and might have been obtained by distinct events. The LEE is integrated in the chromosome at either one of the tRNA sites of *selC*, *pheU* or *pheV* and one further site that has not been identified yet.

LEE flanking regions

The prototype EPEC E2348/69 strain harbours the core LEE of 35.4 kb whereas subsequent studies showed that the size of the LEE due to the varying size of its flanking sequences might reach up to 110 kb in other EPEC, ATEC and EHEC isolates (Gärtner and Schmidt, 2004; Müller *et al.*, 2009). Examples of genetic elements integrated in the flanking regions include IS elements (e.g. IS*2*, IS*3*, IS*629* and IS*630* homologues), prophages (e.g. CP4-44, 933L), novel effector genes (*rorf0/ibe*), etc. (Fig. 2). Several strains have been found to harbour IS elements on both sides of the core LEE (Müller *et al.*, 2009). The newly identified effector protein Ibe (Buss *et al.*, 2009) is encoded 5′ of the LEE core region. In the 3′ LEE flanking regions the *lifA/efa* gene encoding lymphostatin was identified regularly indicating that these regions might contribute to pathogenicity, e.g. by facilitating colonization of intestinal sites (Klapproth *et al.*, 2005; Babbin *et al.*, 2009; Deacon *et al.*, 2010). The *lifA* region of several strains contains *ent*, *nleA* and *nleB* genes in the same order. The G+C content of the *lifA* region (ranging from 42.9% to 44.4%) differs from the *E. coli* chromosome (50.8%) and the LEE core region (38.4%). Therefore, a simultaneous integration of the LEE and various flanking regions as one genetic element appears rather unlikely (Müller *et al.*, 2009).

Fig. 2. Schematic overview of flanking regions associated with LEE pathogenicity islands of EPEC, ATEC and STEC strains. This scheme depicts the varying sizes of flanking regions associated with the core LEE in human and animal pathogenic EPEC, ATEC and EHEC strains. The positions of IS elements, prophage genes, the LifA/Efa1 regions and the rorf0 (*ibe*) genes are indicated. The three known chromosomal insertion loci are given. (Modified with permission from Müller *et al.*, 2009.). BSTEC: bovine STEC; REPEC: rabbit EPEC.

Pedestal formation

Actin reorganization leading to pedestal formation is regarded as one of the hallmarks of A/E *E. coli* pathogenicity. In contrast to intracellular pathogens A/E *E. coli* are unique in subverting the host actin cytoskeleton from the outside making infection with EPEC a welcomed tool for cell biologists. In EPEC this is a dynamic process resulting in considerable movement of the bacteria on the surface of epithelial cells. Many laboratories have focused on unravelling the signals and mechanisms leading to actin polymerization and pedestal formation and many host factors involved in actin assembly have been localized to pedestals (Bhavsar *et al.*, 2007).

However, in triggering the actin assembly EPEC and EHEC show interesting differences despite the homology between the translocated intimin receptor (Tir) proteins that are essential for signal induction (Sal-Man *et al.*, 2009). Tir is a T3SS-injected protein that following injection inserts into the cytoplasmic membrane as a 'hairpin' leaving its N- and C-terminus in the cytoplasma. The central part of Tir interacts with intimin thereby mediating intimate attachment of the bacteria. Interestingly, even isolated (recombinant) Tir protein is able to functionally integrate into the target cell membrane – without the need for adherent bacteria. This unexpected property of Tir might broaden the range of affected cells and potentially also enhances intimin-mediated attachment (Michgehl *et al.*, 2006). EPEC mediate actin polymerization by triggering Arp2/3-mediated nucleation through members of the Wiskott–Aldrich Syndrome Protein family (WASP).

With modifications this pathway is exploited by several pathogens (such as EPEC, *Vaccinia* virus, *Shigella flexneri*, *Listeria monocytogenes*, *Rickettsia*) (Bhavsar *et al.*, 2007; Sal-Man *et al.*, 2009). EPEC activate the Nck pathway that is triggered by phosphorylation of Tir-Y^{474} by host kinases (Bommarius *et al.*, 2007). This creates a docking site for Nck that in turn mediates activation of WASP proteins. The Src-family kinase c-Fyn was found to be essential for Tir^{Y474} phosphorylation following its transient activation induced by intimin–Tir clustering in lipid rafts (Hayward *et al.*, 2009). Recently, it has been reported by the Frankel group that – prior to pedestal formation – the WxxxE effector Map (Kenny *et al.*, 2002; Bulgin *et al.*, 2010) induces transient filopodia formation before robust actin polymerization is triggered (Berger *et al.*, 2009). A second Nck-independent pathway requires Y^{454} (in EPEC E2348/69) to be phosphorylated (Campellone and Leong, 2005). This facilitates binding to a second adaptor protein that, however, remains elusive and needs to be identified.

Despite the homology between EHEC and EPEC Tir, the EHEC-Tir protein is not interchangeable with EPEC-Tir. For pedestal formation in EHEC the non-LEE-encoded effector protein $EspF_u$ (*E. coli* secreted protein F-like protein from prophage U, also known as TccP) is essential. A 12-amino-acid sequence in the C-terminus of EHEC-Tir recruits $EspF_u$ to the sites of EHEC adherence (Allen-Vercoe *et al.*, 2006; Campellone *et al.*, 2006). $EspF_u$ belongs to the growing family of effector proteins that are encoded outside the LEE (non-LEE-encoded effector proteins – NLEs) but nevertheless are injected

into target cells by the T3SS of the LEE. Apparently, EspF$_u$ activates N-WASP by direct binding to the autoinhibitory segment of the GTPase-binding domain (GBD) of N-WASP (Sallee *et al.*, 2008; Campellone, 2010). Members of the IRSp53 family – involved in membrane and actin dynamics – directly interact with EspF$_u$ and the Tir 454-463 domain which is essential for EspF$_u$-dependent actin assembly (Weiss *et al.*, 2009). Genetic and functional loss of IRSp53 blocks actin assembly and show this protein to be the missing host factor linking Tir and EspF$_u$ in EHEC pedestal formation. This is a novel mechanism of activation restricted to the activation of N-WASP with thus far no counterpart in the host cell. Whether IRSp53 is actually the missing link in the Nck-independent second pathway in EPEC infection needs to be seen. Furthermore, Whale *et al.* (2006) identified several strains with non-O157 EHEC, EPEC and ATEC serogroups, which simultaneously use the TccP and the Nck pathway for induction of actin remodelling – again emphasizing the versatility of these pathogens.

There has been remarkable progress in recent years in our understanding of the intricate molecular mechanisms evolved by A/E *E. coli* to subvert the host cell cytoskeleton by specifically inducing actin polymerization from their extracellular location. However, the physiological significance of pedestal formation and subversion of the actin network in infection are not really understood. How these perturbations might serve for the benefit of the pathogen has not been resolved besides more general statements that disturbing the cytoskeleton of the target cells affect cell shape, motility and signalling and thereby enhances pathogenicity. Indeed, several reports demonstrated no direct correlation between actin polymerization, adhesion, pedestal formation, effacement of brush border villi, pathogenicity and bacterial colonization *in vivo* (Bai *et al.*, 2008; Sal-Man *et al.*, 2009). Nevertheless, investigating pedestal formation by A/E *E. coli* has certainly generated new insights into mechanisms of actin polymerization.

LEE- and non-LEE-encoded effectors

Among the 41 open reading frames of the LEE (EPEC E2348/69) (Elliott *et al.*, 1998; Iguchi *et al.*, 2009) only three are genes encoding translocator proteins (EspA, EspB, EspD) and seven encode effector proteins (Tir, Map, EspB, EspF, EspH, EspZ and EspG). In other A/E *E. coli* strains, additional LEE-encoded effectors have been identified such as the 'IQGAP1-binding effector' protein Ibe (rOrf0). *Ibe* could be identified mostly in EHEC, less frequently in ATEC followed by EPEC and appears to enhance ruffle formation in target cells (Buss *et al.*, 2009). The classic LEE-encoded effectors have been reviewed recently (e.g. by Kenny *et al.*, 2002; Dean and Kenny, 2009).

Recent studies have shown that the effector repertoire of A/E *E. coli* pathogens is much larger than previously thought and by no means restricted to LEE-encoded proteins. Following the first non-LEE-encoded effector NleA/EspI in 2004 many additional NLEs have been identified (for review see Dean and Kenny, 2009). In fact, a recent study addressing the T3SS-dependent secretome in the A/E model organism *Citrobacter rodentium* by employing SILAC (stable isotope labelling with amino acids in tissue culture) identified all previously known effectors and several novel ones. Addressing the effector repertoire of the O157 : H7 (Sakai) EHEC strain, > 60 putative effector genes were identified and 39 proteins were confirmed as translocated effectors (Tobe *et al.*, 2006). These studies exemplified the vast repertoire of injected factors in A/E *E. coli* that are directly involved in manipulating the host cell.

Functional studies on the effects of translocated effector proteins in A/E *E. coli* have demonstrated that (probably all) effectors are multifunctional proteins that each manipulate the host cell in several ways. EspB was the first effector shown to be multifunctional as it doubles as translocator being part of the injection pore and as an effector protein in the cytoplasma of the target cell (e.g. Hamada *et al.*, 2010). Interestingly, many translocated effectors show overlapping functions and team-up for cooperative activities – a theme that has been summarized as 'functional redundancy' (Dean and Kenny, 2009). Well-described examples are EspF and Map or NleA that affect cellular junctions and induce apoptosis, or the effacement of microvilli that depends on activities of Map, Tir and EspF (Dean *et al.*, 2006; Guttman *et al.*, 2006; Thanabalasuriar *et al.*, 2009). EspH and EspJ team-up to antagonize phagocytosis and opsono-phagocytosis of EPEC by macrophages (Marchès *et al.*, 2008; Dong *et al.*, 2010).

Even though some effectors have been well studied there are still surprises. Just recently, EspF – previously associated with tight-junction disruption and apoptosis – was shown to be the first bacterial protein to target and disrupt the nucleolus through a mitochondria-based mechanism that is effective late in EPEC infection (Dean *et al.*, 2010a). NleE – previously associated with polymorphonuclear transepithelial migration – and NleB – associated with *C. rodentium* colonization of murine intestine – were found to interfere with innate immune responses by inhibiting NF-κB activation by blocking the transfer of NF-κBp65 into the nucleus (Nadler *et al.*, 2010; Newton *et al.*, 2010).

The activities of the effector proteins investigated thus far clearly show that A/E *E. coli* modulate a plethora of host cell functions. Intriguingly, there are already numerous examples that these pathogenic bacteria simultaneously co-inject effector proteins to moderate and

counterbalance exceedingly destructive activities. If effectors such as EspF induce apoptosis (Dean *et al.*, 2006), another effector such as NleH inhibits apoptosis (Hemrajani *et al.*, 2010). Likewise, Tir induces pedestal formation while EspM inhibits pedestal formation. However, EspM induces quite dramatic changes in the cytoskeletal architecture of epithelial cells and disorients tight junctions. Surprisingly, this does not affect barrier function and survival of the cells (Simovitch *et al.*, 2010). This 'balanced pathogenic impact' of A/E *E. coli* is further illustrated by recent studies addressing the functions of EspGs and EspZ. The two EPEC effectors EspG and EspG2 that induce a detaching phenotype in host cells by activating the host cell protease calpain were found to be kept in check by the co-delivered Tir protein. Lack of Tir protein results in rapid host cell loss and necrosis indicating that this activity of the Tir protein is needed to maintain epithelial integrity. This study not only identifies an additional function of the Tir protein but also provides a further example of balanced pathogenicity (Dean *et al.*, 2010b). Recently, the host binding partners and function for the LEE-encoded effector EspZ/SepZ were identified (Shames *et al.*, 2010). These authors could show that EspZ enhances the phosphorylation of focal adhesion kinase (FAK) and AKT during EPEC infection thereby contributing to host cell survival mechanisms.

A/E *E. coli* pathogens use their portfolio of LEE-encoded and non-LEE-encoded effector proteins to subvert and modulate cellular and barrier properties of the host in a well-controlled manner. In addition to the well-documented hierarchy in the regulation of LEE-encoded gene expression (Mellies *et al.*, 2007; Humphries *et al.*, 2010; Wang *et al.*, 2010) there is now growing evidence that – following injection – there appears to be a second level of control as these processes might even be functionally equilibrated among the co-injected effectors in the host cell cytoplasma to ensure balanced pathogenicity. The interactions between the expanding repertoire of injected virulence factors will provide fascinating insights into the subtle balance in the interaction with target cells, will provide additional tools to study signalling processes in the host cell, and, furthermore, might identify putative targets for intervention.

Pathogroup discrimination and putative implications for diagnostics and therapy

The relationship and putative interconversion of the three A/E *E. coli* pathogroups has been studied by several laboratories (e.g. Mellmann *et al.*, 2009). There are many differences between EPEC and EHEC isolates beyond the expression of phage-borne Stx and its subtypes, such as the presence of pathogenicity islands, the adaptation to different hosts, and their complex interactions with host

barriers resulting in either asymptomatic or overt infections. ATEC strains represent a rather heterogenous group and therefore conclusive characteristics beyond the lack of the EAF plasmid distinguishing ATEC strains from classical EPEC and the lack of *stx* genes to set them apart from EHEC/Shiga toxin-producing *E. coli* (STEC) are missing. Indeed, some ATEC O26 strains have been isolated that carry virulence and fitness modules of EHEC (Bielaszewska *et al.*, 2007). A recent study describing *stx⁻*/EAF⁻ A/E *E. coli* strains isolated from patients with bloody diarrhoea based on multi-locus sequence typing (MLST), serotype and virulence profiles identified these isolates as EHEC (Bielaszewska *et al.*, 2008) that have lost the *stx* genes during infection. These strains were consequently termed 'EHEC-LST'. The closer relatedness of (at least a fair number of) ATEC strains to EHEC rather than to EPEC is further emphasized by studies of strains isolated from cattle and sheep – the common host of EHEC (Cookson *et al.*, 2010). Loss and gain of genes during *E. coli* infections is a common event in the human intestine (Mellmann *et al.*, 2009) and may result in the interconversion of EHEC to apparent ATEC strains. In addition, transfer of virulence factors through mobile elements and horizontal gene transfer further complicates the characterization of distinct intestinal *E. coli* pathogroups. This has been exemplified by the identification of 'intermediate strains' expressing a profile of indicator virulence factors that does not allow for a reliable assignment of a distinct pathovar (Müller *et al.*, 2007). Therefore, for reliably assessing the virulence potential of a given isolate, the identification of a few 'indicator virulence factors' is surely not enough. Here, a considerable extension of the tested matrix of 'diagnostic' genes might be a first improvement. In addition, in ATEC strains the serogroup should be determined as well. Identification of a common EHEC serotype could be indicative of a potential risk for the development of HUS.

In summary, recent studies addressing A/E *E. coli* have underlined the enormous plasticity of the *E. coli* genome and have again clearly shown that bacterial chromosomes are not static and fixed entities. Moreover, the detailed studies on the molecular mechanisms of effector interactions with host cells have led to fascinating insights into the intricate subversion strategies developed by these seemingly common organisms resulting in a well-balanced pathogenicity.

Acknowledgements

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