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Review

Genetic distribution, characterization, and function of *Escherichia coli* type III secretion system 2 (ETT2)

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SUMMARY

Many Gram-negative bacteria use type III secretion system (T3SS) to inject effector proteins and subvert host signaling pathways, facilitating the growth, survival, and virulence. Notably, some bacteria harbor multiple distinct T3SSs with different functions. An extraordinary T3SS, the *Escherichia coli* Type III Secretion System 2 (ETT2), is widespread among *Escherichia coli* (*E. coli*) strains. Since many ETT2 carry genetic mutations or deletions, it is thought to be nonfunctional. However, increasing studies highlight ETT2 contributes to *E. coli* pathogenesis. Here, we present a comprehensive overview of genetic distribution and characterization of ETT2. Subsequently, we outline its functional potential, contending that an intact ETT2 may retain the capacity to translocate effector proteins and manipulate the host's innate immune response. Given the potential zoonotic implications associated with ETT2-carrying bacteria, further investigations into the structure, function and regulation of ETT2 are imperative for comprehensive understanding of *E. coli* pathogenicity and the development of effective control strategies.

INTRODUCTION

Type III secretion system (T3SS) is a complex nanomachine widely present in Gram-negative bacteria.[1] Recently, significant progress has been made in the understanding of T3SS structure, assembly, function, and regulation. T3SS comprises a needle-like complex injectisome that is inserted across the cell membrane.[2] Pathogens use T3SS to deliver effector proteins and subvert host cellular processes and immune responses, facilitating bacterial growth, survival, and virulence.[3] It has been shown that T3SSs are crucial for infections of various pathogenic bacteria, including pathogenic *E. coli*, *Salmonella*, *Shigella*, and *Yersinia.*[4] Several effector proteins have been identified and display a large repertoire of biochemical activities.[5] These effector proteins target essential cellular processes, including cell invasion, disruption of inflammatory responses, apoptosis, and intracellular trafficking.[6]

Except for the single T3SS locus, some pathogens harbor more than one T3SS, which function independently in different pathogenic processes. For example, *Salmonella* use *Salmonella* pathogenicity island 1 (SPI-1) encoded T3SS to invade host cells, while SPI-2 T3SS is responsible for intracellular survival.[7] Likewise, two distinct T3SSs have been identified in *E. coli*. The T3SS encoded by the locus of enterocyte effacement (LEE) containing LEE1-LEE5 operons is present in intestinal pathogenic *E. coli* subsets Enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC).[8] LEE is essential for the formation of attaching and effacing (A/E) lesions, which inject bacterial effector proteins into host cells and subvert cellular signaling pathways to the benefit of the pathogen.[9] In addition, a gene cluster sharing remarkable homology to *Salmonella* SPI-1 was identified as second T3SS in EHEC O157 strain Sakai by genome sequencing.[10] This gene cluster was named the *E. coli* type III secretion system 2 (ETT2) to distinguish it from the LEE-encoded T3SS.[11] ETT2 is prevalent in *E. coli* and *Escherichia albertii* (*E. albertii*).[12] This suggests that the ETT2 system may has evolved from a common ancestor and has been horizontally transferred between different bacterial pathogens. These findings have significantly broadened our understanding of ETT2 and its potential role in bacterial infections. Although most ETT2s have undergone widespread mutational attrition, they still play pivotal roles in infection and virulence of pathogenic *E. coli*. Therefore, understanding the unknown pathogenesis is of great significance for the prevention and treatment of pathogenic *E. coli*. In this regard, this review summarizes recent research regarding the genetic characteristics, distribution, and functions of ETT2, providing a more comprehensive and systematic understanding of ETT2.

VARIOUS ETT2 ISOFORMS ARE WILDLY DISTRIBUTED IN *E. COLI* AND *E. ALBERTII*

The ETT2 gene cluster consists of a 29.9 kb region, which is adjacent to the tRNA *glyU* locus in the *E. coli* chromosome. ETT2 isoforms are predicted to be acquired via horizontal gene transfer and are characterized by a lower G + C content (36.9% compared to 50.8% for the *E. coli*

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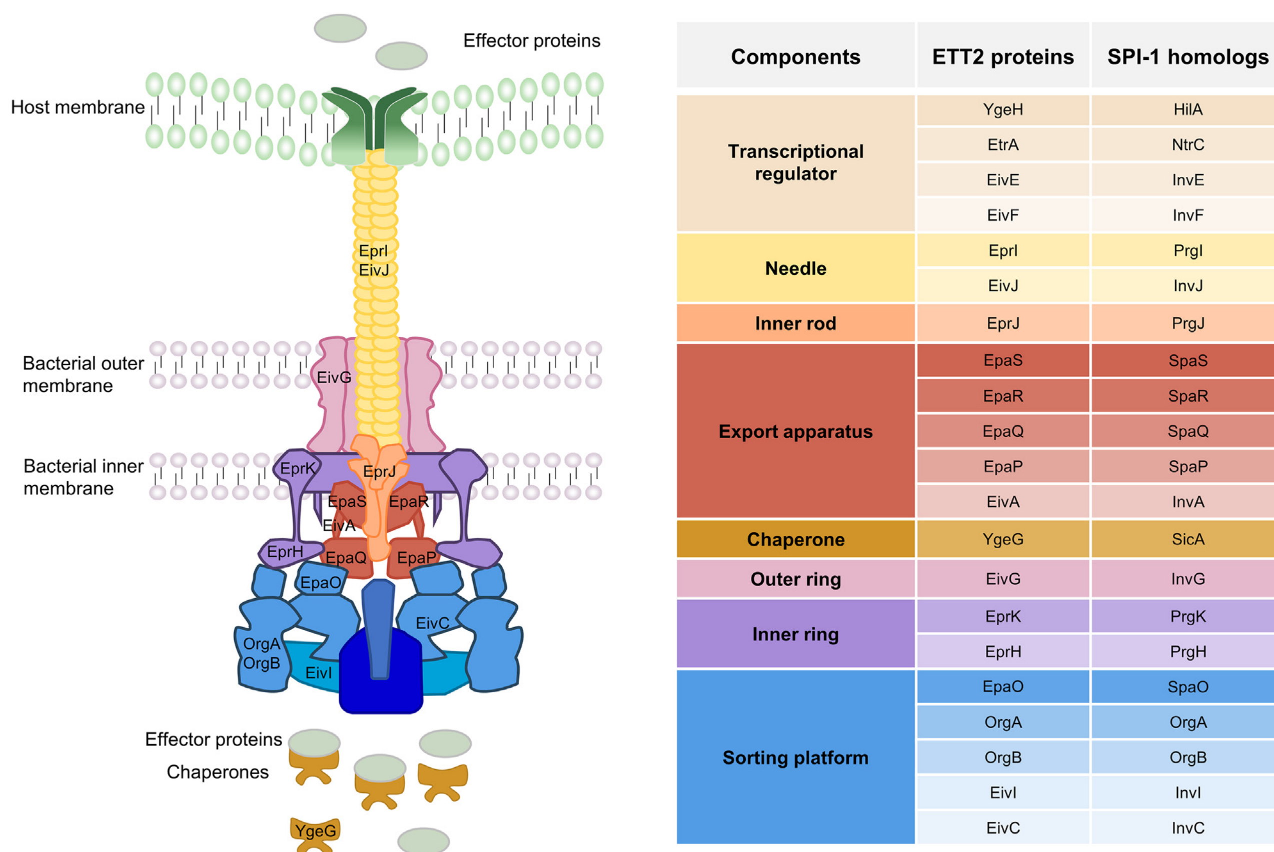


Components	ETT2 proteins	SPI-1 homologs
Transcriptional regulator	YgeH	HilA
	EtrA	NtrC
	EivE	InvE
	EivF	InvF
Needle	EprI	PrgI
	EivJ	InvJ
Inner rod	EprJ	PrgJ
Export apparatus	EpaS	SpaS
	EpaR	SpaR
	EpaQ	SpaQ
	EpaP	SpaP
	EivA	InvA
Chaperone	YgeG	SicA
Outer ring	EivG	InvG
Inner ring	EprK	PrgK
	EprH	PrgH
Sorting platform	EpaO	SpaO
	OrgA	OrgA
	OrgB	OrgB
	EivI	InvI
	EivC	InvC

Figure 1. Schematic diagram of a hypothetical ETT2 needle device

The intact ETT2 cluster harbors the necessary genes to assemble a functional secretory system. Among them, *epaO*, *orgA*, *orgB*, *eivI*, and *eivC* are responsible for encoding the sorting platform, and *epaS*, *epaR*, *epaQ*, *epaP*, and *eivA* encode the export apparatus. Meanwhile, EprK and EprH form the inner ring and EivG forms the outer ring. EprI and EivJ are the main components of the needle-like part. EprJ helps to form the inner rod, while additional genes encode transcriptional regulators and chaperones (YgeH, YgeG, EivE, EivF, and EtrA).

chromosome). Thus, they may contribute to bacterial evolution. Our previous study also identified IS transposases in the ETT2 cluster, which may facilitate the transfer of ETT2 between different bacteria to generate new complex pathogenic phenotypes.[13] Various ETT2 isoforms have been identified in *E. coli* strains. The intact ETT2 gene cluster contains 35 open reading frames (ORFs), 21 of which are homologous to those of SPI-1.[14,15] Due to the high homology, these ETT2 components may assemble a complex injectisome. The *epr*, *epa*, and *eiv* operons, homologous to *prg*, *spa*, and *inv* in SPI-1, respectively, are predicted to encode secretion apparatuses (except *eivE* and *eivF*).[15,16] The sorting platform, which consists of EpaO, OrgA, OrgB, EivI, and EivC, determines the order of protein secretion in the ETT2.[17] The export apparatus encoded by *epaS*, *epaR*, *epaQ*, *epaP*, and *eivA*, the inner ring encoded by *eprK* and *eprH*, and the outer ring encoded by *eivG* constitute the needle complex base of the secretory apparatus.[18] EprI and EivJ are the main components of the needle-like part.[19] EprJ forms an inner rod to anchor the needle, which helps the needle connect the bacterial cytoplasm and host cell membrane. Additional genes present in these clusters encode transcriptional regulators and chaperones.[20] According to the homology of ETT2 to SPI-1, the putative needle-like structure of ETT2 has been mapped (Figure 1).

ETT2 has been found to be present in the majority of *E. coli* strains, including commensal strains.[21] Following the discovery of ETT2 in EHEC O157, the distribution of ETT2 was further investigated.[11,16] ETT2 was found to be ubiquitously distributed among intestinal *E. coli* strains, including EPEC, Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and Enterotoxigenic *E. coli* (ETEC).[16] The distribution of ETT2 genes was subsequently confirmed by Ren et al. However, in most strains, the ETT2 gene cluster undergone mutational attrition.[21] The ETT2 locus was also identified in Shiga toxin-producing *E. coli* (STEC).[22] Cheng et al. identified the ETT2 locus in pathogenic *E. coli* isolates from piglets with oedema and/or diarrhea and from dairy cows with mastitis, in which the prevalence of ETT2 among different pathotypes of porcine origin *E. coli* exceeded 70% in ETEC, STEC and STEC/ETEC isolates. Intact or deletion ETT2 isoforms were present in all *E. coli* isolates from pig or cow samples. Among them, ETT2 can be divided into 11 different isoforms, including intact type contained the whole 35 genes and the remaining lacked 3–13 genes in the left, middle, or right region of the ETT2 locus. The intact ETT2 is more prevalent in *E. coli* isolated from pigs and highly associated with *stx2e*.[23] The entire ETT2 island was present in all *E. coli* isolates from edema disease.[24] In addition, EAEC 042 and aEPEC F2_18C had the full complement of ETT2 genes.[21,25] Hartleib et al.

A **Isoforms** **Present**

kdu yqe yge epr epa eiv — EAEC NMEC APEC EDEC ETEC STEC *E. albertii*

4.99kb deletion
kdu yqe yge epr epa eiv — APEC

1.33kb insertion
4.99kb deletion
kdu yqe yge epr epa eiv — APEC

5.68kb deletion
kdu yqe yge epr epa eiv — APEC

8.74kb deletion
kdu yqe yge epr epa eiv — APEC ETEC STEC

deletion
deletion
kdu yqe yge epr epa eiv — ETEC

deletion
deletion
deletion
kdu yqe yge epr epa eiv — *E.coli* isolates from Mastitis in Cows

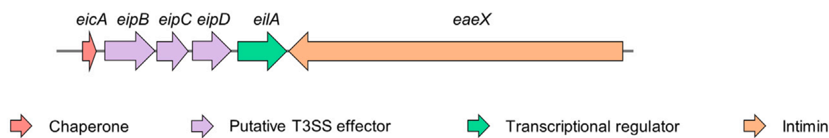
deletion
deletion
deletion
kdu yqe yge epr epa eiv — *E.coli* isolates from Mastitis in Cows

deletion
deletion
deletion
kdu yqe yge epr epa eiv — *E.coli* isolates from Mastitis in Cows

deletion
kdu yqe yge epr epa eiv — K12 MG1655

Sorting platform
Needle
Outer ring
Transcriptional regulator
Export apparatus
Inner rod
Inner ring
Chaperone

B



Chaperone
Putative T3SS effector
Transcriptional regulator
Intimin

Figure 2. ETT2 and *eip* locus structures

(A) The distribution of different isoforms of ETT2 in *E. coli* and *E. albertii*. Ten different ETT2 isoforms, including intact and mutant types were identified. The location and size of the gene deletion or insertion in the ETT2 isoforms are displayed. Homologous genes are vertically aligned. Dotted lines indicate gene deletions. Different colors represent the assumed functions of different genes in ETT2. The distributions of ETT2 isoforms in a variety of *E. coli* and *E. albertii* are shown.

(B) The structure of the *eip* locus. The name of each gene in the *eip* are displayed. Different colors represent the assumed functions of different genes in *eip* locus.

attempted to analyze the distribution of ETT2, and concluded that it was not present in any Extraintestinal pathogenic *E. coli* (ExPEC).[16] However, Newborn meningitis *E. coli* (NMEC) K1 strain EC10 was found to harbor all the genes needed to encode ETT2.[26] A lower rate of ETT2 was also present in 3% Uropathogenic *E. coli* (UPEC) strains.[27] ETT2 gene clusters were found in more than half of the Avian pathogenic *E. coli* (APEC) isolates. We previously identified five distinct ETT2 isoforms in APEC, including intact ETT2. Sequence analysis indicated that the mutational isoforms have 4.99, 5.68, and 8.74 kb gene deletions in the right end of the ETT2 island. There is also a 1.3 kb transposase insertion in the transcriptional regulation gene *ygeH*.[13] The wide distribution and multiple isoforms of ETT2 in APEC is an indication that APEC might be a potential risk to human health. Consequently, intact ETT2 was found in different pathogenic *E. coli*, with putative second type III secretion-associated locus (*eip* locus) positively. The *eip* locus, encodes homologues of SPI-1 translocators and T3SS-related proteins, might facility ETT2 to construct an active T3SS apparatus. In most *E. coli* strains, including EHEC O157:H7, ETT2 has undergone widespread mutational attrition.[21] Same ETT2 isoforms were found in *E. coli* from animal or human, indicating ETT2 could be horizontal transferred and potential risk to human health.

E. albertii is an emerging zoonotic foodborne pathogen associated with watery diarrhea, abdominal distention, vomiting, fever and even bacteremia in humans.[28] In addition, *E. albertii* has been implicated as an etiologic agent of disease in diverse domestic and migratory species of birds. *E. albertii* carries a large collection of virulence-related genes identical to EPEC and EHEC.[29] The ETT2 gene cluster has also been described in *E. albertii* strains.[30] Genome analysis of 482 *E albertii* strains revealed that 61.4% of the *E. albertii* strains had a nearly complete ETT2, and only 18.3% had 2-4 gene deletions.[12] Similarly, half of *E. albertii* strains isolated possessed intact ETT2.[31,32] It was found that 7 *E albertii* strains had either a complete absence of the *eivJ* gene or a 1 bp deletion or insertion to the same polyalanine site of the *eivJ* gene. Three strains had only *yqeF* and *yqeG* left in ETT2. Whereas, different mutational attritions at other ETT2 genes were identified in *E. albertii* strains.[32] Therefore, most *E. albertii* strains contain intact ETT2; only a small fraction of ETT2 have single-gene mutations at the same location. This phenomenon reveals a potential threat to human health. Studying the structure and distribution of ETT2 isoforms can help us further understand the evolution of pathogenic islands and the significance of ETT2 in public health (Figure 2A).

ETT2 CONTRIBUTES TO BACTERIAL VIRULENCE OF *E. COLI*

Although most ETT2s have undergone widespread mutational attrition, they still play pivotal roles in infection and virulence of pathogenic *E. coli*. Study of ETT2 and bacterial pathogenicity has been reported more frequently in ExPEC than in intestinal pathogenic *E. coli*. There are several transcriptional regulators located in the ETT2 virulence gene cluster, which has been shown to alter adhesion, motility, biofilm formation, serum bactericidal resistance, and intracellular survival of bacteria.[33]

The infection of host cells by pathogens is classically considered a multistep process, in which adhesion is the first step. Bacteria use fimbriae to enhance the adhesion between themselves and host cells, thereby enhancing pathogenicity.[34] In APEC, deletion of *ygeG* can enhance the adherence to and invasion of DF-1 cells.[35] Similarly, both *eivC* and *etrA* gene deletion strains can promote upregulation of fimbriae genes, while the adherence to, and invasion of, DF-1 cells do not differ significantly among the wild-type, mutant, and complementation strains.[36,37] The deletion of the ETT2 or *eivA* gene led to reduced ability of invasion and intracellular survival in human brain microvascular endothelial cells (HBMECs) compared to the parental NMEC K1 strain.[38] The regulator YqeI has also been shown to play a role in the adhesion of APEC.[39] The Δ*etrA* and Δ*eivF* mutants are considerably more adherent to intestinal epithelial cells than the parent strain of EHEC O157.[40] Similarly, deletion of the *eivF* gene also enhances the colonization ability of APEC in chicks.[41] Flagella is a motor device that helps bacteria reach colonization sites and spread after infection.[42] In APEC, deletion of *eivC* significantly downregulated the expression of *flgB*, *flgD*, and *flgF* genes, resulting in impaired flagella and reduced bacterial motility.[37] Compared with wild-type APEC, Δ*yqeH*, Δ*yqeI*, and Δ*ygeK* mutants also have fewer and damaged flagella, resulting in significantly reduced motility.[39,43,44] A deletion of the ETT2 gene cluster affects the synthesis of flagellar proteins in *E. coli*, which is associated with urinary tract infections and septicemia.[33]

Biofilm formation is also associated with bacterial pathogenicity. Deletion of the *yqeH* gene decreases expression levels of genes related to AI-2 and c-di-GMP synthesis, resulting in significantly less biofilm formation.[44] Inactivation of *ygeK* resulted in reduced ability of APEC to form biofilms.[45] However, the biofilm formation of the ΔETT2 mutant was significantly increased compared to the wild-type strain APEC40.[46] There is a correlation between resistance to serum bactericidal effects and the virulence of *E. coli* strains.[47] Deletion of the *eivC* gene significantly reduces transcription of virulence genes involved in serum resistance, causing impaired serum resistance, and ultimately reduced colonization and proliferation capacities *in vivo*.[37] Similarly, the inner membrane ring (Δ*eprHIJK*) mutant of EHEC or APEC are completely serum sensitive and significantly attenuated in virulence.[48,49] Recent studies have also found that the deletion of *ygeK* or *yqeI* gene decreased the bacterial sensitivity to serum bactericidal.[39,43] However, ΔETT2 mutant is more resistant to serum bactericidal than the wild-type strain, which may due to the enhanced LPS and biofilm formation capacity.[50]

INTACT ETT2 MAY SUBVERT THE HOST INNATE IMMUNE RESPONSE THROUGH EFFECTOR PROTEINS

Due to the widespread gene deletion, gene mutation, and frameshift of ETT2, it is often thought that ETT2 does not encode a functional secretion system and does not have secretion activity. However, an increasing number of studies have shown that an intact ETT2 and *eip* gene cluster is widely present in different bacteria, suggesting it may be able to deliver effector proteins; some ETT2 effector proteins have even been shown to subvert the host innate immune response.[51] Intact ETT2 in the EAEC 042 strain has been proposed as an active system that may possess potential effector proteins and the ability to inject them into host cells.[52] The NMEC K1 strain EC10 may utilize intact ETT2 to invade and survival in HBMECs.[38] The complete ETT2 is present in all identified *E. coli* isolates from edema disease, whereas ETT2 carried by *E. coli* strains from non-edema disease contains deletions.[24] Similarly, ETT2 is complete in EHEC capable of causing severe clinical symptoms, while other STEC variants causing mild symptoms contain deleted variants.[15,16] This phenomenon may also illustrate the importance of intact ETT2 for pathogenic *E. coli* to exert virulence during host infection, although the exact mechanism of action is unknown. During infection, bacteria and their components can interfere with various immune response processes within host cells. Interleukin (IL)-1β and IL-8 expression levels were significantly increased in HD-11 cells infected with the Δ*eivC* and Δ*etrA* strains, compared with those in HD-11 cells infected with the wild-type strain.[36,37] EspE3 is a recently identified ETT2 secreted protein that may function as an E3 ubiquitin ligase. *espE3* is located outside of the ETT2 gene cluster and its transcriptional level is positively regulated by the ETT2 transcriptional regulator and chaperone YqeH and YgeG, suggesting that YqeH and YgeG may accelerate the pathogenic process of APEC during infection by regulating *espE3* transcription. EspE3 protein not only promotes APEC adhesion and invasion, but also influences the transcription of host cell inflammatory factors (such as IL-6, IL-8 and TNF-α) during bacterial infections, suggesting that EspE3 is involved in the interaction between APEC and host cells. APEC may deliver the effector E3 ubiquitin ligase EspE3 into host cells and interfere with host ubiquitin-related innate immune pathway, which facilitates the bacterial survival and virulence.[51] Moreover, the inner rod protein EprJ and needle protein EprI of ETT2 can activate the NLRC4 inflammasome in mouse primary bone marrow-derived macrophages (BMDMs), triggering activation of Caspase-1, which cleaves Gasdermin D (GSDMD) and promotes the release of IL-1β, eventually inducing macrophage pyroptosis and host death.[53–55] Taken together, ETT2 may involve in pathogen-host interactions.

The *eip* locus comprises of six genes inserted between the *E. coli* backbone genes *yicM* and *nlpA*, which encodes homologues of SPI-1 translocators and T3SS-related proteins (Figure 2B). Three components EipB, EipC, and EipD exhibit sequence identity with *Shigella* T3SS effector proteins IpaB, IpaC, and IpaD.[56] In addition, the *eip* locus contains genes encoding for putative type III effector chaperone (*eicA*), a novel HilA-like transcriptional regulator (*eilA*), and a homolog of outer membrane adhesion/invasion protein intimin (*eaeX*).[21,57] It was demonstrated that the distribution of *eip* locus is closely related to the intact ETT2 gene cluster.[13,21,26,57] Thus, the *eip* components may cooperate with ETT2 to construct an active T3SS apparatus, so that a complete ETT2 transport unidentified effector proteins into host cells to interfere with host defenses and promote bacterial infection and colonization.[21] A number of recent studies have provided evidence that intact ETT2 possesses a function to manipulate host cellular processes and immune responses, and since T3SSs are generally activated by interactions with host cells, which may explain the difficulty in identifying effector proteins. However, the effector proteins secreted by ETT2 during bacterial pathogenesis and the exact mechanism by which ETT2 affects the inflammatory response are still unknown although the secretory properties of an intact ETT2 have been experimentally demonstrated.[51] Further studies are needed to help elucidate the mechanisms underlying these secretion systems and to prevent bacterial diseases and potential human infections.

CROSS TALK BETWEEN ETT2 AND OTHER VIRULENCE FACTORS

Pathogenic bacteria can perceive environmental changes and promote their survival and infection by altering gene expression. Regulators encoded in ETT2 could influence the expression of genes within and outside of ETT2 gene cluster. In contrast, the expression of ETT2 might also be affected by other regulators and quorum sensing system.

Several regulators were identified within the ETT2 gene cluster, including YgeH, YqeI, EtrA, EivF, and YgeK/EtrB. Previous studies have shown that these regulators contributed to the bacterial pathogenicity through regulating the expression of ETT2 and other virulence genes. The transcriptional activator HilA directly regulates the transcription of SPI1 apparatus genes in *Salmonella*.[58] The YgeH protein in EAEC 042 exhibited 29% similarity to HilA, which also play important roles in the upregulation of ETT2 genes. However, the YgeH protein of EHEC O157 appeared to be non-functional.[59] YqeI affects the expression of the genes involved in bacterial localization, locomotion and biological adhesion, which contributes to the pathogenicity of APEC.[39] It has been found that the regulators EtrA and EivF leads to decreased secretion of proteins encoded by the LEE in EHEC.[40] Whereas, the autoregulatory transcription factor EtrB/YgeK could activate LEE expression not only by repressing expression of *etrA* and *eivF* but also by directly interacting with the *ler* regulatory region to activate LEE expression in EHEC.[60,61]

In addition, ETT2 expression is modulated by other regulators and quorum sensing system. H-NS plays a central role as a modulator of gene expression in response to various environmental changes. Remarkably, H-NS binds to the *ygeH* regulatory region and represses *ygeH* and other ETT2 genes expression.[59] EAEC 042 EilA encoded by the *eip* locus, a homologue of the HilA regulator, affects biofilm formation and epithelial cell adhesion by regulating the expression of *eip* locus genes *eipB*, *eipC*, *eipD*, *eicA*, and *eaeX*, as well as ETT2 genes *eivF* and *eivA*.[56] Moreover, the LuxR family transcriptional regulator DctR has been demonstrated to act as a negative regulator for the expression of the LEE locus in EHEC O157:H7. In contrast, DctR positively regulates the expression of ETT2 core genes, which may contribute to the survival and successful infection of APEC.[62,63] Additionally, transcription factor QseA positively regulates both LEE and ETT2 by directly activating the *ler* promoter and *etrB* expression.[60] The quorum sensing system signaling molecule AI-3 can also promote the expression of LEE by inhibiting expression of *eivF* and *etrA* in EHEC.[64] Expression of the ETT2 gene has also been shown to be repressed by the small regulatory RNA Esr055. The expression of Esr055 is positively affected by DeoR regulator and exogenous DNA. When bacteria pass from the

small intestine to the large intestine, the low DNA concentration in the large intestine inhibits Esr055 expression, leading to up-regulate the ETT2-related genes. Upregulation of genes may contribute to EHEC O157 colonization, which promotes further bacterial infection.[65]

In intestinal pathogenic *E. coli*, ETT2 and other virulence factors are closely related. The ETT2 virulence island and Stx2e virulence factor are frequently present in both EHEC and STEC.[15] Of the 215 ETT2-positive *E. coli* isolated from piglets with diarrhea, 53.5% also carried other virulence factors such as enterotoxins, fimbriae and α-hemolysin.[66] In other pathogenic *E. coli* of different serotypes, ETT2 islands are independent of the presence of some pathogenic virulence islands, such as LEE, the high pathogenicity island (HPI), and the locus of proteolytic activity (LPA).[16] In ExPEC, however, there is no correlation between ETT2 and 15 APEC virulence factors.[13] The correlation between ETT2 and other virulence factors is closely related to bacterial pathogenicity; it will be important to confirm whether these virulence factors cross talk with the expression of ETT2 islands (Figure 3).

CONCLUSION AND FUTURE PERSPECTIVES

We have summarized the characterization and function of ETT2 based on the available research reports, which further suggests the importance of ETT2 for *E. coli* and the fact that there are still many aspects of ETT2 worth exploring. ETT2 is widely distributed as intact or deletion mutant in many pathogenic *E. coli* and *E. albertii*. On the one hand, *E. coli* carrying the ETT2 gene cluster cause human and livestock diseases (including diarrhea, edema and various inflammatory conditions) to varying degrees, which are hazardous to health, and on the other hand, ETT2-positive *E. coli* can also act as a reservoir of ETT2 virulence island transfers, which can give more strains the opportunity to acquire horizontally transferred genes, thus contributing to the formation of new and complex pathotypes and increasing the risk of disease.

Although most ETT2 gene clusters are incomplete, an increasing number of studies have shown that many of the genes in ETT2 are capable of influencing the biology of *E. coli* and thus the virulence and pathogenicity. ETT2 has traditionally been considered non-functional due to its incompleteness. We propose that intact ETT2 has the potential to function by secreting potential effector proteins and helping the bacteria to subvert host cellular processes and pathways during host infection. The regulators within the ETT2 gene cluster also contribute to the *E. coli* pathogenicity by affecting various virulence gene expression.

The findings indicate that bacteria carrying ETT2 virulence islands represent potential risks to human health, and it will be important to study them in depth. However, our current understanding of the ETT2 virulence island is limited. Investigating whether ETT2 exists in bacteria other than *E. coli* is an important avenue to explore, as it could shed light on the prevalence and potential impact of this virulence island across different bacterial species. Understanding whether ETT2 secretes effector proteins and the roles of unstudied ETT2 effector proteins are also critical for unraveling the mechanisms underlying bacterial virulence. Furthermore, delving into the specific mechanisms by which these effector proteins assist bacteria in expressing virulence factors is essential for grasping the pathogenic potential of these bacteria. Further research aimed at elucidating the structure, function, and regulation of ETT2 will not only enhance our understanding of bacterial pathogenicity but also aid in identifying new targets for vaccine development.

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AUTHOR CONTRIBUTIONS

S.W.: Conceptualization, editing, project administration and funding acquisition. X.W.: Writing and revising. H.Z.: Draft writing. J.H., B.Z., W.G., Z.W., D.W., J.Q., M.T., and Y.B.: Discussion and help for revising. F.S. Review and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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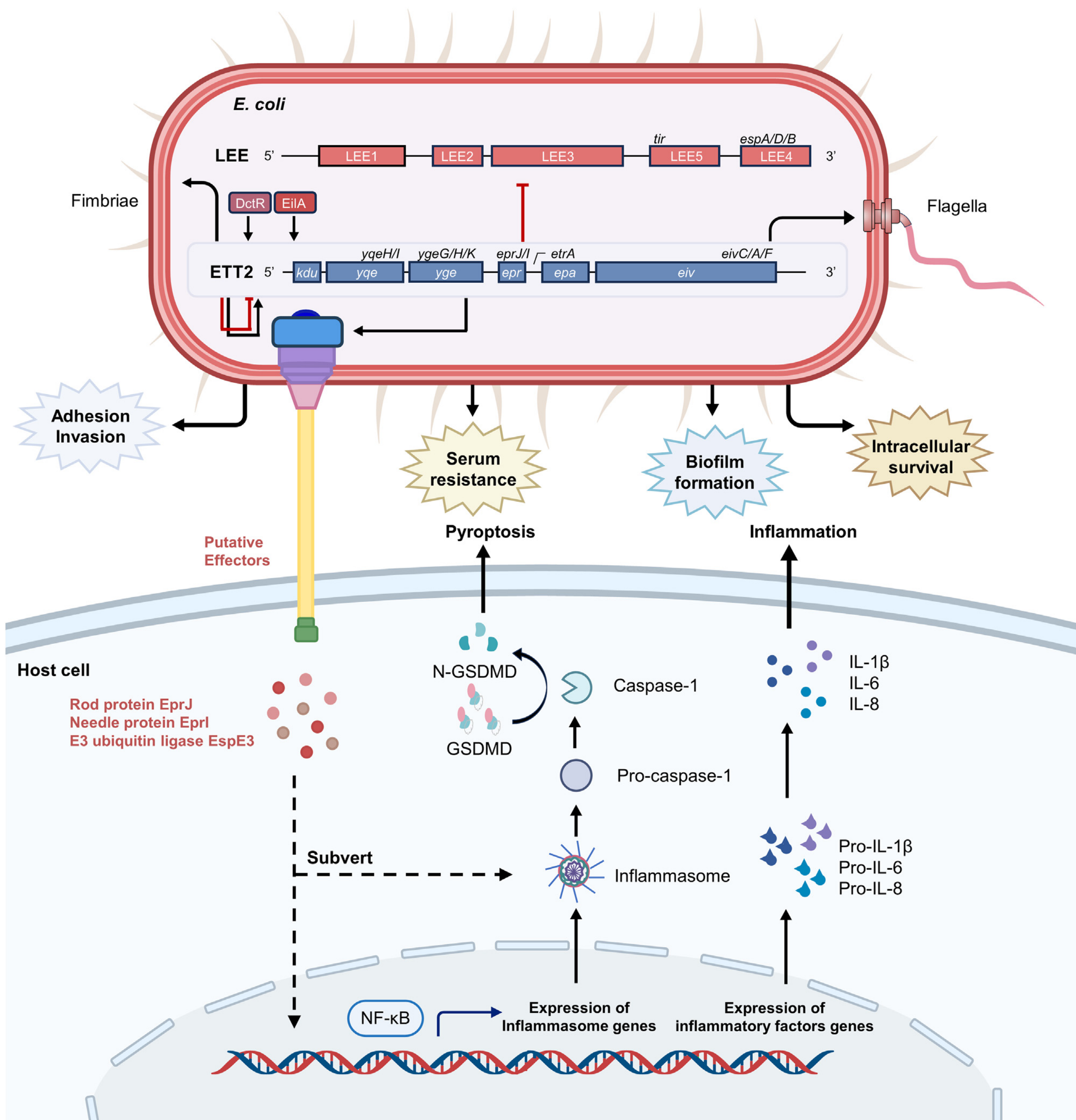

Figure 3. Scheme of the ETT2 function in *E. coli*

ETT2 plays pivotal roles in the infection and virulence of pathogenic *E. coli*. ETT2 can regulate the motility, biofilm formation, serum resistance, adhesion, invasion and intracellular survival, and contribute to the bacterial virulence. In addition, ETT2 and its component can interfere with host immunity response, eventually inducing host cell death. The black arrows indicate activation, and the red lines with flat ends represent inhibition.

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