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Type III Secretion Systems (T3SSs), Structuring and Functions

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Abstract
Many bacterial species that cause diseases in plants and animals assemble large molecular machinery and the third secretion system (T3SS) is an important system that performs important and complex biological tasks such as the movement and transport of proteins from the bacterial cytoplasm through peri- and extracellular cells. They are used to infect cells or immune cells in the host body by injecting potent and toxic proteins directly into the host cell. In this review, we discuss our understanding of the structure, Assembly, Functions and architecture of type III secretion system (T3SS).

Keywords: T3SSs, Pathogens, Peptidoglycan

Introduction
Different bacterial pathogens employ the type three secretion system (T3SS), a macromolecular protein nano-syringe, to inject effectors into host cells. A 9-kDa protein polymerized into a needle-like filament makes up the extracellular portion of the syringe. This protein's structure and precise placement on the bacterial surface are two factors that are crucial for effective toxin injection. Different tactics have been evolved by bacterial pathogens to colonize, infiltrate, and destroy eukaryotic cells, T3SS is one of the most effective systems out there. It is present on the surface of many species that cause disease, such as *Salmonella*, *Shigella*, *Escherichia coli*, *Yersinia*, *Pseudomonas*, (Galan, J. E *et al*, 2014 [1]; Wagner, S. *et al*, 2018). The process of sensing calcium is still under discussion, whether it is from the inside by exporting machines or by the tip and needle from the outside (Shaulov *et al*. 2017) [3].

T3SSs Activities
Bacteria expressing the third secretion system have variable infection routes, However, their T3SSs are involved in the processing of many cellular processes such as host immune response, dynamic vesicle transport, signal transduction paathways, and cytoskeleton (Raymond B, *et al* 2013; Byttner D, 2016) [4, 11]. The effector toxins injected by the third secretion system are believed to be Exoenzyme (S, T, U, Y) (Hasannejad-Bibalan, M *et al*., 2021) [13] T3SSs are necessary for the invasion of non-phagocytic host cells and establishment of intracellular life cycles by intracellular pathogenic *Salmonella*, *Shigella*, and *Chlamydia* species. *Salmonella spp*. have two different T3SSs the (Salmonella pathogenicity island 1.T3SS) which is used to invade host cells, form the Salmonella-containing vacuole (SCV), and prevent host cell apoptosis, and the (Salmonella pathogenicity island 2 T3SS), which is used to control SCV trafficking and maturation and to encourage intracellular survival and replication (van der Heijden, J., and Finlay, B. B. 2012) [8]. In order to create an intravacuolar niche where they may differentiate, reproduce, and spread after invasion, Chlamydia species also employ a T3SS (Beeckman, D. S., and Vanrompay, D. C. 2010) [10], T3SSs are often used by external pathogens to influence host cytoskeleton and immune responses when an infection is occurring. The intestinal epithelium of the host is necessary for the attachment of enteropathogenic Escherichia coli (EPEC), enterohaemorrhagic E. coli (EHEC), and Citrobacter rodentium. Once attached, the T3SS causes cytoskeletal rearrangements and compromises the integrity of the epithelial barrier. (Santos, A. S., and Finlay, B. B. (2015) [7].

Assembling the T3SSs' Essential Parts
T3SSs are built in two steps: First, the basal body is generated by the general secretory route, and then the inner rod and needle are put together through a mechanism that depends on type III secretion. The inside-out model and the outside-in model are the two models available for the assembly of T3SSs (Fig 1).

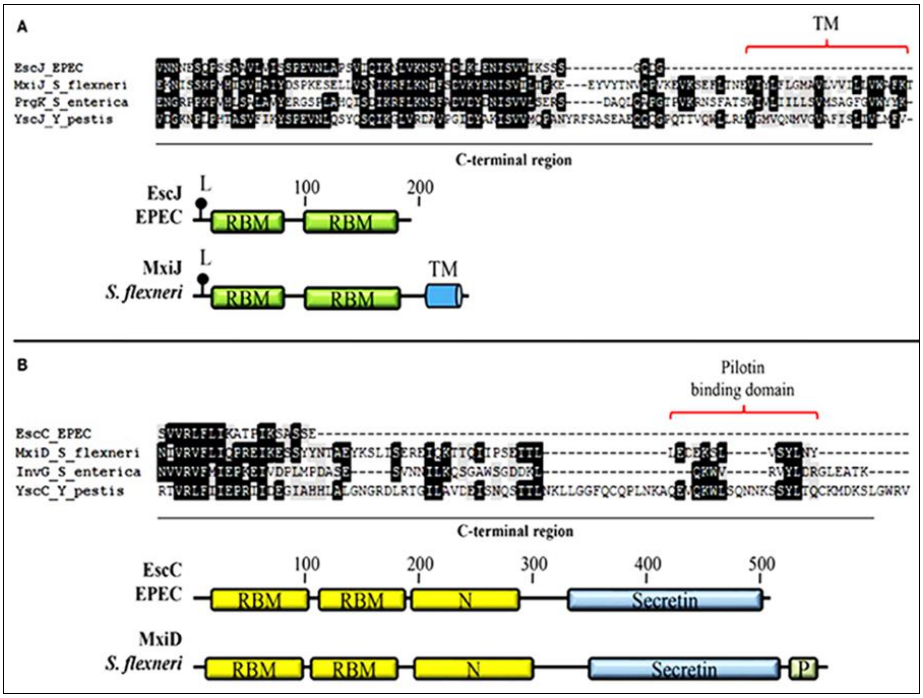

Fig 1: Basal body components of A/E pathogens possess distinctive features

The inside-out model suggests a method for injectisome construction that is simelar to that for assembly of flagelum. This ensures that machines that are capable of secretion and membrane translocation are effectively formed. (Diepold and Wagner 2014) [12], two stages of injection are distinguished in some assemblies the primary secretion-dependent machine assembly and the third type which is based on the secretion-dependent assembly of the needle thread, tip, and movable link. Assembly of the secretory core apparatus is constrained by two factors: the confinement of the fused compartments to the inner and outer membrane in two-dimensional space and the permeation of the peptidoglycan layer. Diebold and Wagner in 2014 proposed a bipolar model of aggregation stating that (1) the dependence of primary assembly on nucleation by components of the export apparatus ensures secretion efficiency (Fig 2a), and (2) the dependence of primary assembly on secretin formation ensures outer membrane translocation (Fig 2b).

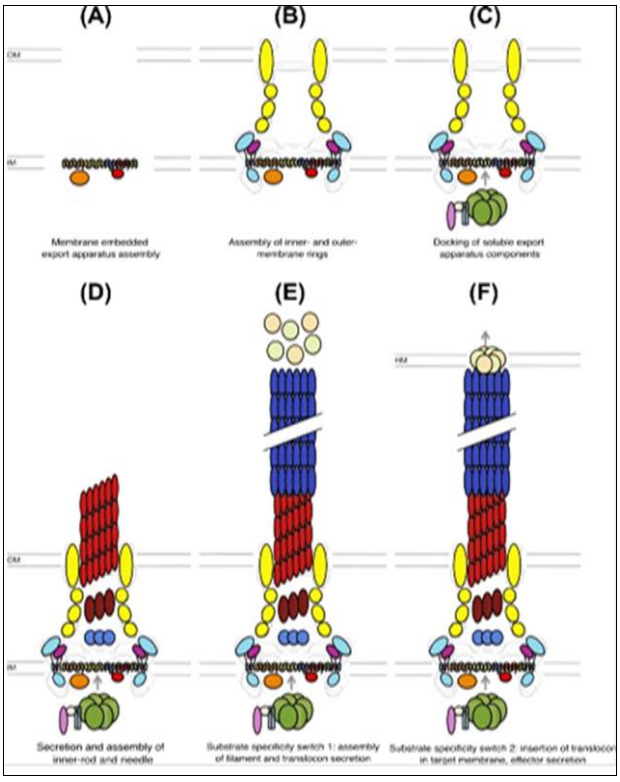

Fig 2

Assembly of the base is nucleated by prior assembly of the core export apparatus components SctR, SctS and SctT, as shown for the T3SS encoded by *Salmonella* pathogenicity island 1 (SPI-1) (Wagner *et al*. 2010). The SctR assembles independently into multimers that are stabilized by recruitment of SctT (Dietsche *et al*. 2016) [5].

Structure of the Type III Secretion System

The T3SS, also known as the injectsome, is an intricate, 3.5 MDa nanomolecular device made up of over 20 proteins. Across several bacterial species, (Erthardt *et al*., 2010), The third secretory apparatus is made up of three annular structures embedded in the inner and outer bacterial membranes, connected by a circumferential inner rod, and a syringe-shaped structure that protrudes above the bacterial surface with a central channel that is 2-3 nm in diameter (Burkinshaw and Strynadka, 2014) [6] Although the fundamental makeup of the so-called needle complex is relatively similar in all of the bacteria examined to date, extracellular filaments are built on top of the needle in the supramolecular structures of Enteropathogenic *E. coli* and T3SS (Daniel *et al.*, 2001; Sekiya *et al*., 2001 [14]). According to the ultrastructures that build them up, from the outside in, researchers Gaytán and colleagues divided them into extracellular appendages, basal bodies, and cytoplasmic components. The picture below displays numerous solved protein structures together with a schematic depiction of T3SS in A/E pathogens (Gaytan, M. O *et al*., 2016) [15].

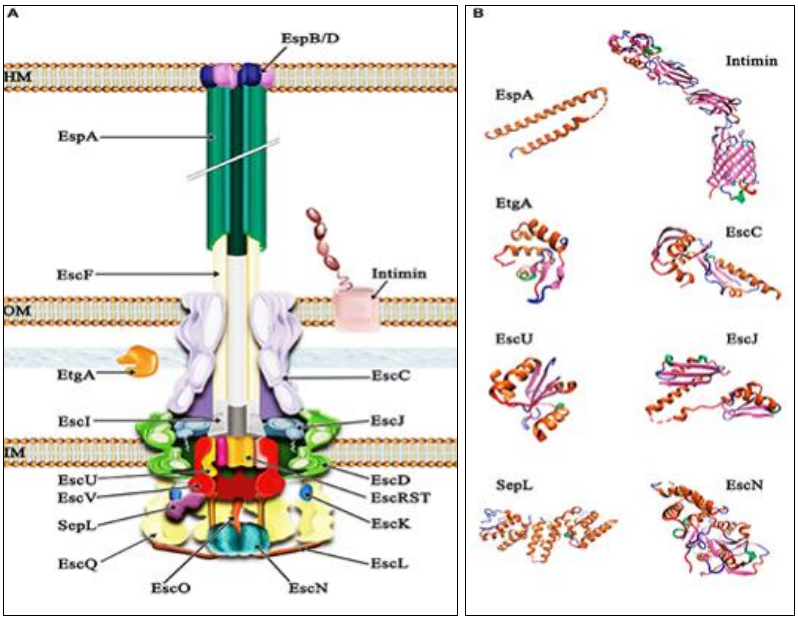

Fig 3: Schematic representation of the type III secreation system of A/E pathogens

A multi-ring basal structure buried in both the inner and outer bacterial membranes, with its proximal end connected to an export apparatus (EA) and an ATPase complex in the cytosol, makes up the core of both flagellar- and NF-T3SSs. The T3SS is joined at its distal end to a flagellar hook or a needle to transfer virulence proteins into the host directly or to polymerize an extracellular filament used for movement (Erhardt M *et al*., 2010, and Buttner D., 2012) [21, 20].

Basal Body

Three membrane rings are linked by a periplasmic inner rod to form the basal body. In the inner membrane (IM), the proteins EscD and EscJ make two concentric rings, while the protein EscC creates the ring in the outer- membrane (OM) (Fig 3). According to Sekiya *et al*. (2001) [14], the exterior diameters of the outer- membrane and inner membrane rings are calculated to be 16.7 1.9 nm and 18.1 2.5 nm, respectively.Similar in height and likely large enough to pass through bacterial membranes and the peptidoglycan layer are the basal bodies of EPEC (31.4 4.3 nm) and *Shigella* (31.6 0.3 nm) (Sekiya *et al*., 2001) [14]. The needle complex consists of a multi-ring basal body and a needle, made up of approximately 25 different proteins. The basal body spans the inner membrane, the peptidoglycan layer, and the outer membrane, and anchors the needle-like structure into the bacterial membrane (Cornelis, G.R., 2006) [18] According to Tosi, T. *et al*. (2014) [19], the basal body is made up of an outer membrane ring formed of oligomerized secretin PscC and an inner membrane ring made of the lipoprotein PscJ Burns.

Export Apparatus

The export apparatus is made up of a central, nonameric, ring-shaped inner membrane protein called the export gate, also known as CdsV in Chlamydia species and SctV in universal nomenclature. The export gate engages additional inner membrane proteins SctR, SctS, SctT, and SctU to form the export apparatus. (Jensen, J. L *et al*., 2020) [22].

Extracellular Appendages

The Needle

The main structure of the T3SS is made up of the needle and the basal body, together known as the nedle complex (NC). Different bacterial species have different numbers of NCs in each cell. *Salmonella typhimurium*, for example, assembles 10–100 NCs per cell (Kubore *et al*., 1998), While *Pseudomonas aeruginosa's* surface only has a small number of (NCs), (Perdu *et al*., 2015) [24] EscF is a membrane ring protein that associates with EscC, EscD, and EscJ as well with the filament protein EsPA to form a continuous channel that connects the bacterial cytoplasm with the host cell. This continuous channel is known as the needle and is made up

of multiple copies of EscF, which is essential for the secretion of all T3 substrates and, consequently, for virulence (Fig 1). EscE and EscG, two chaperones that inhibit premature polymerization and are necessary for its assembly, connect with it in the cytoplasm as well. (Wilson *et al*., 2001, Sal-Man *et al*., 2012) [25, 26].

The Filament

An accessory in extracellular T3SS systems of A/E pathogens it acts as a transducer between the needle and the transport hole generated in the host cell membrane (also present in Brodeyell's spp) (Medhekar *et al*., 2009) [31].

The Translocation Pore

The creation of the transloconpore on the host cell membrane is the last stage in the construction of the T3ss needle apparatus, this then enables the direct delivery of effecter protein into the host cytoplasm, two hydrophobic membrane proteins, known as the minor translocon protein and the major translocon protein based on their relative size to one another, and together to form the translocon pore (Dey, S., *et al* 2019) [32].

Strategies Possessed by the T3SSs Device to Target Host Cells

Acute invasive infections frequently involve the T3SS. T3SS interferes with signal transduction once it has been triggered by interaction with eukaryotic cells via pilins or flagella, leading to cytotoxicity directly mediated by effectors and changes in immunological responses (Anantharajah, *et al*., 2016) [16].

T3SS's Mechanism of Action

From a variety of bacterial pathogens, hundreds of effector proteins have been identified as possible T3SS substrates. To get beyond the host defense system, pathogenic T3SS effector proteins can change signal transduction pathways. According to Gu. L. *et al*. (2019), certain bacterial effectors can penetrate host cells, compromise their immune systems, and eventually cause host cell death.

Conclusion

The essential role of the **T3SSs** apparatus in virulence is a major driver for understanding its structure, assembly, and major transporter protein. Antibiotics have contributed to the treatment of bacterial infections with high success, but their over-prescribing and their bad uses led to an excessive increase in the emergence of severe resistance by bacteria, which has become a major life-threatening problem, and it was found that the third secretion system (T3SS) is responsible for millions of infections annually.

Due to the complex nature of T3SS, there are several ways in which it can be targeted and eliminated bacteria (Hotinger *et al*., 2021).

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