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Control of gene expression by type III secretory activity

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Summary

The bacterial flagellum and the highly related injectisome (or needle complex) are among the most complicated multi-protein structures found in Gram-negative microorganisms. The assembly of both structures is dependent upon a type III secretion system. An interesting regulatory feature unique to these systems is the coordination of gene expression with type III secretory activity. This means of regulation ensures that secretion substrates are expressed only when required during the assembly process or upon completion of the fully functional structure. Prominent within the regulatory scheme are secreted proteins and type III secretion chaperones that exert effects on gene expression at the transcriptional and post-transcriptional levels. Although the major structural components of the flagellum and injectisome systems are highly conserved, recent studies reveal diversity in the mechanisms used by secretion substrates and chaperones to control gene expression.

Keywords

type III secretion; flagella; gene regulation; type III chaperone

Introduction

Gram-negative bacteria utilize at least six distinct secretion pathways to transport proteins across the inner and/or outer membranes of the cell envelope [1,2]. One of those pathways, the type III secretion system (T3SS), can be divided into two major classes, flagellar and non-flagellar. The flagellar T3SS is associated with the MS ring of the basal body and is responsible for secreting the extracytoplasmic components of the flagellum [3]. The non-flagellar T3SS is associated with the bacterial injectisome, which translocates effector proteins into the cytoplasm of eukaryotic host cells to promote the pathogenic/symbiotic lifestyle of the microorganism [4,5]. Both systems use secretion competency as a signal to coordinate gene expression [6]. In the case of the flagellum, coordinating gene expression with secretory activity ensures that structural components of the flagellum are expressed only when required during the different stages of flagellar assembly. In contrast, coupling gene expression to secretory activity in the injectisome systems provides a mechanism for sensing environmental stimuli such as contact of the bacterium with a eukaryotic target cell. The basic strategy involves a secretable protein and its cognate T3SS-specific chaperone. Secretion competency is detected by cells in one of two ways; (i) sensing a reduction in the cytoplasmic concentration of the secretable protein, (ii) sensing the presence of the newly released chaperone. The absence

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or presence of the secretable protein and/or chaperone influences gene expression at the transcriptional and post-transcriptional levels. In this review we discuss the variety of ways in which some secretion substrates and type III secretion chaperones couple gene expression to secretory activity.

Coupling secretory activity to flagellar assembly

The bacterial flagellum is assembled in a sequential manner involving more than 30 gene products and has been studied most extensively in *Salmonella enterica* serovar Typhimurium [3,7-9]. Flagellar genes are generally divided into early, middle, and late (expressed from class I, II, and III promoters, respectively) based upon their temporal expression patterns. The early genes (*flhDC*) encode the transcriptional activator for class II promoters. The middle genes encode the MS-, C-, P-, and L-rings, T3SS, rod, and hook proteins that together form the hook-basal body (HBB) complex spanning the inner membrane. Following assembly of the basal body, the rod and hook proteins are secreted by the T3SS and assemble at the distal end of the growing structure. Completion of the HBB complex triggers two related events. First, there is a switch in the substrate specificity of the T3SS from rod/hook proteins to the late secretion substrates (i.e., flagellin monomer, capping protein) which are required for the final stages of flagellar assembly [8]. The second event, a shift from middle to late gene expression, is mediated by the type III secretion chaperones FliT, FliA, and FlgN, and is intimately linked to secretion of their cognate secretion substrates.

Down-regulation of middle gene expression

FliT is the chaperone for the filament capping protein FliD [10]. Upon completion of the HBB complex FliD is secreted to the tip of the hook where it facilitates polymerizaton of the flagellar filament [11]. The depletion of FliD from the cytoplasm permits FliT to bind to FlhC, thereby inhibiting transcription of the middle genes whose products are no longer required for the assembly process (Fig. 1A-B) [12].

Up-regulation of late gene expression

Two additional middle genes are *fliA*, encoding the flagella-specific sigma factor (σ^{28}) required for activation of late gene transcription, and *flgM*, encoding the anti-σ^{28} factor FlgM, which is also expressed at a later stage from a class III promoter. In addition to its role in transcription, σ^{28} also functions as a chaperone that is required for FlgM secretion [13]. Prior to completion of the HBB complex, FlgM expressed from its class II promoter is retained in the cytoplasm where it directly binds to σ^{28} and inhibits expression of the late genes (Fig. 1A). Upon completion of the HBB complex, secretion of FlgM results in σ^{28}-dependent transcription of the late genes, allowing for final assembly of the flagellum (Fig. 1B). In addition, the reduction in cytoplasmic FlgM levels limits the duration of late gene expression by decreasing the half-life of free σ^{28} in the cytoplasm where it is degraded by proteases [14].

Fine tuning late gene expression levels

FlgK and FlgL are among the first proteins to be secreted following the switch to late secretion substrates. The chaperone for FlgK and FlgL is FlgN [10]. Following secretion of FlgK and FlgL, FlgN enhances translation of FlgM expressed from its class III promoter through a poorly understood mechanism (Fig. 1B) [15,16]. At this stage during assembly FlgM is thought to be unavailable to interact with σ^{28} until completion of the flagellar filament terminates further secretion and FlgM once again accumulates in the cytoplasm. It has been suggested that the FlgN-dependent enhancement of FlgM translation may allow cells to rapidly down-regulate σ^{28}- dependent genes following completion of the flagellar filament [16]. In addition to its role as an anti-sigma factor, FlgM may also regulate FliC (flagellin) expression at the post-transcriptional level [17].

Secretory activity as an inducing signal in the injectisome systems

The bacterial injectisome (or needle complex) is structurally similar to the flagellum and consists of a basal body-like structure, an associated T3SS, and a hollow needle-like filament that protrudes from the cell surface and serves as the conduit through which proteins are secreted [18]. Much like the flagellum, assembly of the injectisome is thought to proceed in a sequential manner starting with formation of a basal structure and ending with T3SS-dependent export of the needle protein to the distal tip [4]. The secretory activity of the fully assembled injectisome is tightly regulated and dependent upon specific activating signals, the most relevant being contact of the bacterium with eukaryotic targets cells. In most of the injectisome systems characterized from animal pathogens thus far, activation of the injectisome by host cell contact triggers three events: (i) secretion of the translocator proteins which form a pore in the target cell plasma membrane, (ii) translocation of the pre-formed effector proteins into the target cell, and (iii) increased expression of injectisome-related genes. In many of those pathogens the increase in gene expression is intimately coupled to secretion competency. It should be noted, however, that the secretion-dependent regulatory mechanisms described below are often times subject to additional levels of regulatory control that are beyond the scope of this review; for further information on the role of temperature, metabolic stress, and environmental signals we refer the reader to previous reviews of these topics [19-21].

Shigella flexneri

The injectisome of *Shigella flexneri* is expressed and assembled upon growth of the organism at 37°C. Contact of *Shigella* with host cells activates secretion by the injectisome and triggers transcription of ~15 effector genes [22]. Expression of the effector genes is controlled by MxiE, an AraC-like transcriptional activator. The transcriptional activity of MxiE is dependent upon binding its co-activator (IpgC) [23]. Furthermore, transcription from MxiE-dependent promoters in *E. coli* is dependent upon co-expression of IpgC and MxiE [24], highly suggestive of a direct interaction. IpgC also functions as a type III secretion chaperone for the translocator proteins IpaB and IpaC [25]. Prior to activation of the injectisome, MxiE-dependent transcription is inhibited through sequestration of IpgC by IpaB/IpaC and by a co-complex of the Spa15 co-anti-activator and the OspD1 anti-activator, which directly binds MxiE (Fig. 2A) [26]. Spa15 also serves as a secretion chaperone for OspD1, a secretable substrate. Upon activation of the injectisome, OspD1, IpaB, and IpaC are secreted from the cell thereby releasing MxiE and IpgC which are then thought to form the functional activator of transcription (Fig. 2B).

The injectisome regulon of *S. enterica* is highly homologous to that of *S. flexneri*. As mentioned above *S. enterica* has homologs of both MxiE (InvF) and IpgC (SicA) that fulfill the same regulatory role as described for *S. flexneri* [27]. An OspD1-like factor, however, appears to be absent from *S. enterica*.

Pseudomonas aeruginosa

In the absence of inducing conditions the T3SS regulon of *Pseudomonas aeruginosa* is expressed at a basal level possibly allowing for 1-2 injectisomes to be assembled per cell [28]. Unlike the *S. flexneri* and *S. enterica* systems, where secretory activity results in induction of only the effector genes, activation of the *P. aeruginosa* injectisome triggers increased expression of the entire system including genes encoding the effectors, translocators, regulators, chaperones, and injectisome structural proteins [21]. Each of these genes is under the transcriptional control of ExsA, an AraC-like family member. ExsA-dependent transcription is coupled to the secretory activity of the injectisome by a cascade of three interacting proteins (ExsC, ExsD, and ExsE). ExsD is an anti-activator that binds to and inhibits ExsA-dependent transcription (Fig. 2A) [29]. ExsC functions as an anti-anti-activator by

binding to and inhibiting the negative regulatory activity of ExsD [30]. ExsC also serves as a type III secretion chaperone for ExsE [31] [32]. Activation of the injectisome by host cell contact triggers translocation of ExsE resulting in a corresponding decrease in the cytoplasmic concentration of ExsE [33]. The reduced level of ExsE are though to liberate the ExsC chaperone, which then binds to and sequesters ExsD, thereby freeing ExsA to activate transcription of the entire system (Fig. 2B).

As is true for all injectisome systems the requirement for increased levels of the effector protein expression likely relates to the amount required to intoxicate host cells. The necessity for increased expression of the *P. aeruginosa* injectisome structural components is less obvious. One potential explanation might stem from the observation that *P. aeruginosa* likely uses the injectisome to protect itself in the environment from predators such as amoeba [34]. Low basal expression of the energetically expensive injectisome might be necessary to initiate intoxication but may not be sufficient for full intoxication unless additional injectisomes are assembled on the cell surface.

Yersinia sp.

The first observation of gene expression being coupled to secretory activity in the injectisome systems was made in *Yersinia pseudotuberculosis* [35]. Each of the three pathogenic species of *Yersinia* (*Y. entereocolitica, Y. pestis, Y. pseudotuberculosis*) utilize the injectisome to translocate Yop effector proteins into host cells. Growth of the organisms at 37°C induces transcription of the injectisome-related genes. Prior to activation of injectisome-dependent secretion by host cell contact, however, a complex consisting of LcrH (also called SycD) and YopD binds to the 5′ untranslated region of *yop* mRNAs and represses translation (Fig. 2A) [36,37]. LcrH also functions as a type III chaperone for YopB and YopD (secreted translocator proteins) [38,39]. For reasons that remain obscure, the negative regulatory activity of the LcrH-YopD complex is also dependent upon YscM1 (also called LcrQ) and YscM2 [36]. Neither protein is thought to directly interact with the LcrH-YopD complex. In the most simplistic model, activation of the injectisome by host cell contact triggers secretion of YopD and translocation of YscM1 and YscM2 into host cells (Fig. 2B). The corresponding decrease in the intracellular levels of these proteins then relieves the block on *yop* translation. In reality, however, the regulatory system is much more complicated with the potential for extensive cross-talk between type III secretion chaperones and secretion substrates (YscY, the chaperone for YscX which is essential for secretory activity, also interacts with LcrH; SycH, the chaperone for YscM1/M2 also interacts with YopH; SycO, the chaperone for YopO, also interacts with YscM1; and SycE, the chaperone for YopE also interacts with YscM1/M2) [40-42].

Contact of *Yersiniae* with host cells also results in increased *yop* transcription through an undefined mechanism [35]. Transcription in the *Yersinia* injectisome system is controlled by an AraC-like protein (LcrF/VirF). Unlike the *Shigella, Salmonella*, and *Pseudomonas* systems, however, where the transcriptional activity of the AraC homologs (MxiE and ExsA) is controlled by type III chaperones or chaperone-associated proteins that function as co-activators or anti-activators, there is no evidence that LcrF/VirF is controlled in such a manner.

Conclusions

The coupling of gene expression to secretion is a clever means of coordinating gene expression to coincide with stages in the assembly or completion of a complex secretory apparatus. In the flagellar systems this mechanism prevents expression of the late genes prior to completion of the HBB, serves as a way to conserve energetic resources, and prevents the late flagellin-like substrates from competing with rod/hook-like substrates for the secretion channel. In the injectisome systems, however, the coupling of gene expression to secretion serves as a mechanism to up-regulate gene expression in response to environmental signals that stimulate

the secretory activity of the injectisome. It is not yet clear in the inectisome systems whether there is hierarchical regulation whereby secretion of an early substrate might release a chaperone which either inhibits expression of specific early substrates or allows expression of secreted substrates required during a secondary phase in the infection process (or both).

One of the important findings in the injectisome systems is the diversity by which these regulatory systems function. This might be best illustrated by comparing the roles of the homologous chaperones (IpgC/SicA in *S. flexneri* and *S. enterica*, respectively; LcrH in *Yersinia sp.*, and PcrH in *P. aeruginosa*) for the translocator proteins. Whereas IpgC/SicA function as co-activators for transcription, LcrH functions as the post-transcriptional level to repress *yop* translation. In contrast, the LcrH homolog from *P. aeruginosa* (PcrH) lacks detectable regulatory activity [43,44]. This diversity likely reflects environmental pressures that have allowed each pathogen to fine tune gene expression patterns to meet the demands of specific niches.

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* of special interest

** of outstanding interest

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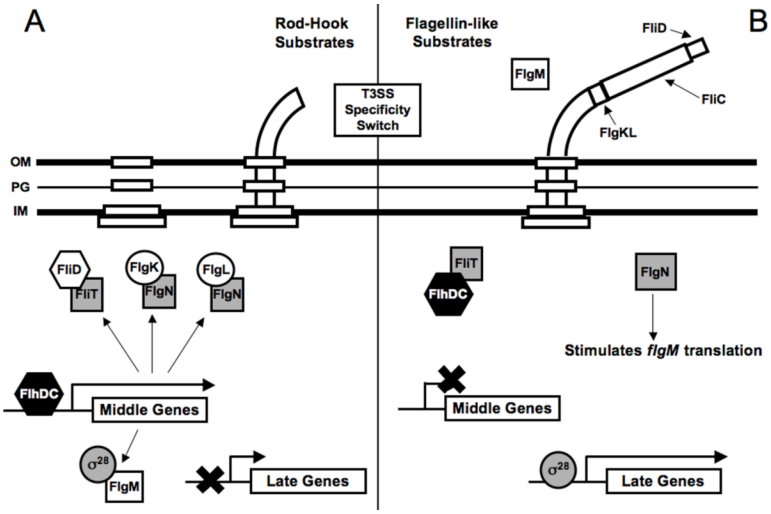

Figure 1.

Schematic representation of the steps in flagellar gene regulation that are coupled to secretory activity in *Salmonella enterica*. Type III secretion chaperones are represented by shaded squares. **(A)** Hook-basal-body (HBB) assembly. During HBB assembly the hook-associated proteins (FlgK and FlgL), filament capping protein (FliD) and the FlgM anti-σ^{28} factor are expressed in an FlhDC dependant manner. Prior to switch in the substrate specificity of the T3SS, however, each of these proteins accumulates in the cytoplasm in association with their respective chaperones (FliT, FlgN, FlgN, and FliA [σ^{28}]). The binding of FlgM to σ^{28} prevents transcription of the late genes. **(B)** Upon completion of the HBB the specificity of the T3SS switches to late substrates and FliD, FlgK, FlgL, and FlgM are secreted thereby releasing their respective chaperones. FliT binds to and sequesters FlhC resulting in the inhibtion of middle gene expression. σ^{28} directs transcription of the lates genes including the flagellin monomer (*fliC*). FlgN positively regulates FlgM translation from its class III promoter to allow for rapid down-regulation of late gene expression upon cessation of secretion.

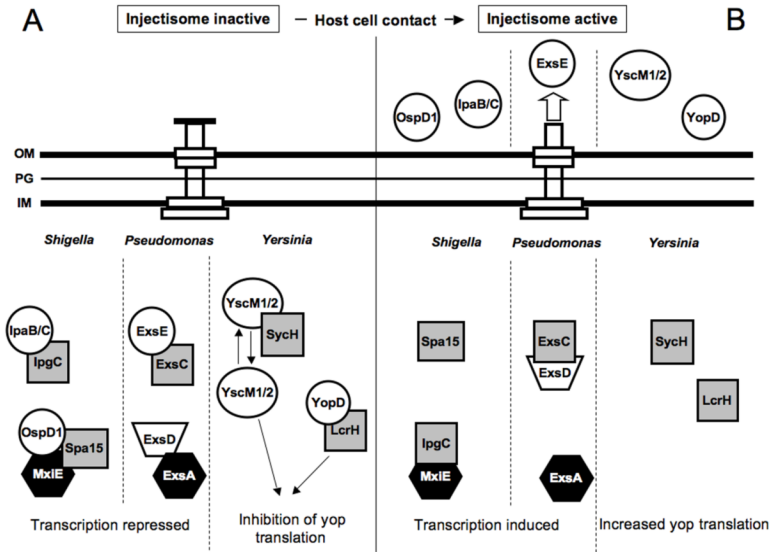

Figure 2.

Schematic representation of injectisome regulatory systems that couple gene expression to secretory activity in *Shigella flexneri*, *Pseudomonas aeruginosa*, *Yersinia sp.* Type III secretion chaperones are represented by shaded squares, black hexagons represent transcriptional activators, and white circles represent secretion substrates. **(A)** Prior to activation of the injectisome regulatory proteins accumulate in the cytoplasm and inhibit gene expression. In *S. flexneri* the complex of OspD1/Spa15 binds to and inhibits MxiE-dependent transcripton. In *P. aeruginosa* ExsD inhibits ExsA-dependent transcription. In the *Yersiniae* YopD, LcrH, and YscM1/M2 cooperate to inhibit *yop* translation. **(B)** Activation of the injectisome following contact of the pathogen withhost cells triggers secretion and an increase in gene expression. In *S. flexneri* secretion of OspD1 disrupts the negative regulatory activity of the OspD1/Spa15 complex. In addition, secretion of IpaB/C releases IpgC, which functions as a co-activator for MxiE-dependent transcription of the type III effector genes. Secretion of ExsE in *P. aeruginosa* allows ExsC to sequester ExsD thereby freeing ExsA to activate transcription. In *Yersinia sp.* secretion of YopD and YscM1/2 relieves the block on *yop* translation.