



Genomic analysis of secretion systems

Mark J Pallen*, Roy R Chaudhuri and Ian R Henderson

Secretion of proteins into the extracellular environment is important to almost all bacteria, and in particular mediates interactions between pathogenic or symbiotic bacteria with their eukaryotic hosts. The accumulation of bacterial genome sequence data in the past few years has provided great insights into the distribution and function of these secretion systems. Three systems are responsible for secretion of proteins across the bacterial cytoplasmic membrane: Sec, SRP and Tat. Many novel examples of systems for transport across the Gram-negative bacterial cell envelope have been discovered through genome sequencing and surveys, including many novel type III secretion systems and autotransporters. Similarly, genomic data mining has revealed many new potential secretion substrates and identified unsuspected domains in secretion-associated proteins. Interestingly, genomic analyses have also hinted at the existence of a dedicated protein secretion system in Gram-positive bacteria, targeting members of the WXG100/ESAT-6 family of proteins, and have revealed an unexpectedly wide distribution of sortase-driven protein-targeting systems.

Addresses

Bacterial Pathogenesis and Genomics Unit, Division of Immunity and Infection, The Medical School, University of Birmingham, Vincent Drive, Birmingham B15 2TT, UK

*Correspondence: Mark J Pallen
e-mail: m.pallen@bham.ac.uk

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Abbreviations

ESAT-6	early secreted antigen target 6 kDa
ETT2	<i>Escherichia coli</i> type-III secretion system 2
FHA	forkhead-associated
PSI-BLAST	position-specific-iterative Basic local alignment search tool
sec	general secretory
Spi-1	<i>Salmonella</i> pathogenicity island 1
SRP	signal-recognition particle
Tat	twin-arginine translocation
TFSS	type-IV secretion system
TTSS	type-III secretion system

Introduction

Secretion of proteins into the extracellular environment is important to almost all bacteria, and in particular mediates interactions between pathogenic or symbiotic bac-

teria with their eukaryotic hosts. Secreted proteins must pass through one or more membranes and bacterial cells have evolved a variety of mechanisms for this purpose. The accumulation of bacterial genome sequence data in the past few years has provided great insights into the distribution and function of these secretion systems, such that the discussion of secretory pathways is now a standard feature of bacterial genome sequence publications. A summary of the findings of several recent genome papers is shown in Table 1. In this review, we summarise recent advances in the field of bacterial protein secretion, focusing in particular on novel insights obtained from the analysis of genome sequences.

Secretion across the cytoplasmic membrane

Three systems are responsible for secretion of proteins across the bacterial inner or cytoplasmic membrane: Sec (general secretory), SRP (signal-recognition particle) and Tat (twin-arginine translocation) pathways. Components of the Sec and SRP pathways are present in every organism (prokaryotic and eukaryotic) for which a genome has been completed [1]. Most organisms possess a single copy of each of the genes from each pathway. However, in rare cases, there are two *secA* homologues and recent evidence suggests that these paralogues do not function interchangeably but have distinct functions. In particular, *SecA2* in *Listeria monocytogenes* plays a distinct role in smooth-rough phase variation and in secretion of a subset of secreted proteins [2•], whereas *SecA2* of *Mycobacterium tuberculosis* is specifically required for *SodA* secretion [3•]. In both cases the second *SecA* protein has been implicated in the ability of the bacteria to cause disease.

The phylogenies of the Sec and SRP pathways closely resemble those inferred from the 16S rRNA from the same organisms, indicating no horizontal transfer of these genes between different organisms [1]. The universality of the Sec and SRP pathways coupled with the sequence diversity of the bacterial components compared with the archaeal and eukaryotic equivalents argues that this is the principal method of protein secretion in all cells and the most ancient protein secretion pathway. Interestingly, the SRP co-translational pathway has recently been implicated in the export of several large proteins secreted via the type V pathway, which possess unusual signal sequences [4••]. This is the first description of secreted proteins utilizing this pathway. However, our own analyses of signal sequences from protein sequences predicted from complete and incomplete genome sequences (IR Henderson, unpublished data) suggest that at least 80

such proteins from a diverse range of Gram-negative bacteria utilize this pathway, suggesting it is a common phenomenon.

The Tat system of protein export was first described for pH-dependent protein import into the thylakoid lumen and is the most recently described bacterial protein

Table 1

Summary of findings on secretion systems from a selection of recent bacterial genome sequencing projects.

Organism	Lifestyle characteristics	Genome characteristics	Secretion systems	References
<i>Bifidobacterium longum</i>	Inhabits human gastrointestinal tract (GIT). Suggested to have beneficial health effects.	2.26 Mb; 60% G+C. Shares a high percentage of genes with phylogenetically unrelated co-inhabitants of the GIT.	200 proteins with Sec-type signal peptides, including a homologue of mammalian serine protease inhibitors (serpins) that may play a role in evasion of host immune system.	[52]
<i>Chlamydomonas reinhardtii</i>	Obligate intracellular pathogen. Cause of guinea pig inclusion conjunctivitis. Important model of human <i>Chlamydia trachomatis</i> infections.	1.17 Mb genome, plus 7966 nt plasmid. 3/4 of genes conserved in other sequenced members of the <i>Chlamydiaceae</i> . Remainder of genome encodes niche-specific functions.	Has TTSS. Secreted proteins include <i>tox</i> genes — members of the YopT family of type III secreted cysteine proteases. These might play a role in remodelling the host actin cytoskeleton during invasion.	[53]
<i>Clostridium tetani</i>	Causative agent of tetanus. Tetanus toxin blocks release of neurotransmitters from presynaptic membrane leading to continuous muscle contractions.	2.8 Mb genome plus 74 kb plasmid. 28.6% G+C. Tetanus toxin is plasmid encoded.	Sec-dependent signal peptides found in 419 genes. Sec appears to be the major protein translocation apparatus. SecB and SecE are absent, suggesting a modified Sec pathway. No Sec-independent TAT system identified. Several ORFs show homology with the type II secretion system from <i>V. cholerae</i> .	[54]
<i>Escherichia coli</i> CFT073	Uropathogenic <i>E. coli</i> . Causes acute cystitis and pyelonephritis.	5.23 Mb genome with similar organisation to <i>E. coli</i> K12 and O15. No plasmids. Only 39.2% of the combined non-redundant protein set of the three genomes is present in all three strains.	Absence of TTSS and other virulence genes found in O15. Contains type II secretion system conserved in K12 but absent from O157 chromosome (although a type II secretion system is encoded by the plasmid pO157). CFT073 genome encodes at least seven putative autotransporters including Pic, which is encoded on a 100 kb PAI that also contains <i>upxBDA</i> , which encode a member of the type I RTX-like secretion system. This system lacks a <i>upxC</i> gene, suggesting a novel class of RTX-like secretion system.	[42]
<i>Fusobacterium nucleatum</i>	Dominant oral bacterium. 'Bridge-pathogen' — not a pathogen in its own right but facilitates the aggregation of other pathogenic species.	2.17 Mb; 27% G+C.	Secretes very few proteins. Lacks type II, III and IV secretion systems. Has a Sec system, but missing SecB. No Sec-independent TAT system was identified.	[55]
<i>Pseudomonas putida</i> KT2440	Metabolically versatile saprophytic soil bacterium. Certified biosafety host for the cloning of foreign genes.	6.18 Mb; 61.6% G+C. High level of genome conservation with the pathogen <i>P. aeruginosa</i> .	There are 281 genes from <i>P. aeruginosa</i> that are absent from <i>P. putida</i> . These include genes encoding a TTSS.	[56]
<i>Ralstonia solanacearum</i>	Soil-borne plant pathogen with wide host range.	Bipartite genome structure consisting of a 3.7 Mb chromosome plus 2.1 Mb megaplasmid. 67% G+C.	Contains a TTSS that is essential for virulence. Genome encodes a large number of type III effector proteins (40, as opposed to the 25 encoded in <i>Shigella</i>). This may correlate with the wide host range. TTSS genes do not have an atypical G+C content, suggesting that they might represent ancestral pathogenicity genes. Conversely, many of the type III effectors do have atypical G+C contents.	[57]

Table 1 Continued

Organism	Lifestyle characteristics	Genome characteristics	Secretion systems	References
<i>Shigella flexneri</i> 2a 2457T	Human pathogen. Cause of the majority of cases of endemic bacillary dysentery in developing nations.	4.6 Mb; 50.9% G+C. Also contains large virulence plasmid. <i>Shigella</i> is phylogenetically indistinguishable from <i>E. coli</i> . There are many chromosomal rearrangements relative to <i>E. coli</i> K12, because of the presence of many IS elements. Also many pseudogenes (8.1% of genome).	Virulence plasmid encodes a TTSS. Type III effectors include IpaH, which aids escape from macrophage vacuoles. Multiple copies of <i>ipaH</i> genes on both plasmid and chromosome. Chromosomal copies are associated with prophage-like islands.	[58]
<i>Streptococcus agalactiae</i>	Emerging human pathogen. Causes bacterial sepsis, pneumonia and meningitis in neonates.	2.16 Mb; 35.7% G+C.	650 membrane-associated or secreted proteins, including several potential virulence factors such as Sip. 177 proteins carry a signal peptide.	[59]

secretion pathway. In *Escherichia coli* the Tat export system consists of four integral membrane proteins: three are encoded in a single operon (*tatABC*) and one is encoded by a gene (*tatE*) located distantly on the genome [5]. Genome screening has revealed that the standard Tat pathway consists of one TatC molecule and two sequence-divergent TatA homologues [5]. A few species possess multiple TatC homologues but this is not mirrored by an equivalent multiplicity of TatA and TatB homologues. *Bacillus subtilis* possesses two *tatAC* gene clusters that are differentially regulated, suggesting two parallel Tat translocation routes, although a single route composed of paralogous components is also a possibility [6]. Surprisingly, homologues of the Tat family are not ubiquitous among bacteria, nor are they found in yeast or animals [5]. Signal peptides of Tat-translocated peptides possess twin-arginine motifs surrounded by several moderately conserved amino acids. This motif has led to the development of TATFIND 1.2 programme to predict substrates for the Tat system from genome sequences [7]. Use of this programme revealed that the number of proteins secreted via the Tat pathway varies greatly among different organisms: *Streptomyces coelicolor* possesses 145 Tat substrates, whereas some bacterial species possess none.

Type II secretion

Pseudomonas aeruginosa produces toxins and hydrolytic enzymes that are secreted via a type II secretory pathway using the Xcp machinery. Analysis of the genome sequence of *P. aeruginosa* strain PAO1 revealed the presence of a second type II secretion system encoded by the *hxc* (for 'homologous to *xcp*') gene cluster. This system exports an alkaline phosphatase and is regulated in response to phosphate levels [8[•]]. *P. aeruginosa* represents the first example of two functioning type II systems in the same cell. However, recent evidence from experimental studies (using representational difference analysis) and from analysis of genome sequences has

uncovered two distinct type II secretion systems within the species *Yersinia enterocolitica*: one system, *yts2*, is ubiquitous in the species, whereas the other, *yts1*, is confined to the high pathogenicity strains and is important in mouse virulence [9].

Type III secretion

The world of bacteriology was surprised nearly a decade ago by the discovery of two type-III secretion systems in the same bacterium [10] — the so-called Spi-1 (*Salmonella* pathogenicity island 1) and Spi-2 (*Salmonella* pathogenicity island 1) systems within *Salmonella enterica*, each of which has a distinct function (Spi-1 mediates enterocyte invasion while Spi-2 influences survival within macrophages).

However, the discovery of unsuspected novel type-III secretion systems (TTSSs) through genome sequencing has been a recurrent theme of the past few years, as has the identification of more than one such system in the same cell (Table 2). *Vibrio parahaemolyticus* is a Gram-negative marine bacterium that causes food-borne gastroenteritis. However, unlike its close relative *V. cholerae*, which lacks any TTSS, this pathogen possesses two separate TTSSs, suggesting important differences between virulence mechanisms in the two species. One of the systems is encoded by genes within a pathogenicity island on the *V. parahaemolyticus* chromosome 2 and is limited to strains pathogenic to humans, whereas the other TTSS is encoded within chromosome 1 and appears to be common to all strains within the species [11[•]].

There is an even greater multiplicity of TTSSs in *Burkholderia pseudomallei* and related species, where two systems, TTS1 and TTS2, most closely resemble TTSSs from plant pathogens, whereas a third system, TTS3 or Bsa, is similar to those from animal pathogens (e.g. Spi-1 from *Salmonella enterica*) [12^{••},13]. TTS2 and TTS3 occur

Table 2

Genomes containing type III secretion systems identified since the 1998 review by Hueck [60].

Species	References
<i>Aeromonas salmonicida</i>	[17*]
<i>Bordetella bronchiseptica</i>	[61]
<i>Bordetella parapertussis</i>	[61]
<i>Bordetella pertussis</i>	[61]
<i>Bradyrhizobium japonicum</i>	[62]
<i>Burkholderia cepacia</i> genomovar III	[63]
<i>Burkholderia cepacia</i> LB400	(RR Chaudhuri, unpublished data.)
<i>Burkholderia mallei</i> ATCC23344	[13]
<i>Burkholderia pseudomallei</i>	[64]
<i>Citrobacter rodentium</i>	[65]
<i>Erwinia carotovora</i>	[66]
<i>Erwinia chrysanthemi</i>	[67]
<i>Escherichia coli</i> 042	(RR Chaudhuri, unpublished data.)
<i>Escherichia coli</i> O157:H7 EDL933	[68]
<i>Mesorhizobium loti</i>	[69]
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	[70]
<i>Pseudomonas fluorescens</i> SBW25	[71]
<i>Salmonella bongori</i>	(RR Chaudhuri, unpublished data.)
<i>Salmonella paratyphi</i> A	(RR Chaudhuri, unpublished data.)
<i>Salmonella typhi</i>	[72]
<i>Shigella dysenteriae</i> M131649	(RR Chaudhuri, unpublished data.)
<i>Xanthomonas axonopodis</i> pv. <i>citri</i> str. 306	[73]
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	[74]

Our unpublished observations were based on a PSI-BLAST search of complete and incomplete genome sequence data held in the ViruloGenome database (<http://www.vge.ac.uk>), using the YscC protein from *Yersinia pestis* as the query sequence.

in both *B. pseudomallei* and in *B. mallei*, whereas TTS1 is specific to the more pathogenic *B. pseudomallei* strains [13]. Initial functional characterisation of Bsa/TTS3 suggests that it plays an essential role in modulating the intracellular behaviour of *B. pseudomallei* [12**].

The situation within the genus *Yersinia* is also complex, in that while the well-characterized plasmid-encoded Ysc–Yop system is present in all three pathogenic species, the genome sequence of the *Y. pestis* chromosome revealed an as yet uncharacterised second Spi-2-like TTSS, which is also encoded in the *Y. pseudotuberculosis* genome (MJ Pallen, unpublished data). Furthermore, Biotype IB strains of *Y. enterocolitica* possess a Spi-1-like chromosomally encoded TTSS that has been termed Ysa (for *Yersinia* secretion apparatus) [14]. There has been considerable progress in characterizing the Ysa system in the past year, with several secretion substrates now identified, and cross-talk and functional overlaps with the Ysc system demonstrated [15,16].

A novel type III secretion system has been identified in the fish pathogen *Aeromonas salmonicida* subsp. *salmonicida*

using a broadly hybridising *yscV* probe. This TTSS has been shown to secrete proteins, including an ADP-ribosyltransferase, and to contribute to virulence [17*]. The same probe appeared to hybridise to genomic DNA from two strains of *Campylobacter jejuni*, raising the exciting possibility that some strains from this species might harbour a TTSS [18].

Another important conceptual advance is the realization that type-III secretion systems are not restricted to pathogens, but are also found in commensals and symbionts. The first clue to this came from the discovery of a TTSS encoded on a mega-plasmid from *Rhizobium* sp. NGR234 that endows this bacterium with the ability to associate symbiotically with leguminous plants [19]. Similar systems were subsequently discovered in other rhizobia (recently reviewed in [20]). Now, three endosymbionts of animals have been shown to possess TTSSs: first, *Photobacterium luminescens*, an endosymbiont of nematodes pathogenic to insects, which possesses a Ysc–Yop-like system [21], second, *Sodalis glossinidius*, an endosymbiont of the tsetse fly *Glossina* spp., which uses a Spi-1-like system for cell invasion [22], and third, the primary endosymbiont of the grain weevil *Sitophilus zeamais*, which also possesses a Spi-1-like system [23*]. Furthermore, it is clear from our own analyses that TTSS-associated genes from the ETT2 gene cluster (encoding a second *E. coli* TTSS, of unknown function that was discovered from analysis of the genome sequences of *E. coli* O157) are present in the majority of commensal strains within the well-characterised *E. coli* reference (ECOR) collection (<http://foodsafety.msu.edu/whittam/ccor>) (C-P Ren and MJ Pallen, unpublished data).

Another recent development at the intersection of genomics and type III secretion has been data mining of genome sequence and experimental data to identify novel type-III effectors. This approach has been employed most extensively in the plant pathogen *Pseudomonas syringae* pv. tomato DC3000, where 36 TTSS-secreted proteins have been identified in this strain, with many more candidate effectors [24**–26**].

Our own ‘domain-hunting’ surveys have identified fork-head-associated (FHA) domains (which usually bind phospho-serine or phospho-threonine peptides) in TTSS proteins [27*]. More specifically, the gene for a chlamydial FHA-domain protein (CT664 from *Chlamydia trachomatis* and its orthologues) lies within a TTSS cluster that also encodes a serine–threonine protein kinase (CT673 in *C. trachomatis*). This suggests a role for phosphorylation-dependent protein–protein interactions in the chlamydial TTSS. A more divergent FHA domain also occurs in the SctD family of proteins (a group of membrane-associated proteins of unknown function, but required for type-III secretion, that includes YscD from *Yersinia* and HrpQ from *Erwinia amylovora*), perhaps

hinting at a broader role for serine–threonine phosphorylation in type III secretion.

Using PSI-BLAST searches and multiple alignments we have shown that tetratricopeptide repeats — a class of 34 amino acid all- α -helical imperfect repeats commonly implicated in protein–protein interactions — delineate the LrcH-like class of type III secretion ‘chaperones of the translocators’, providing a new conceptual framework for defining the mechanisms and determinants of specificity of chaperone–substrate interactions [28**]. In addition, a bioinformatics study has discovered a glucoamylase-like domain in a candidate ETT2 effector, implicating this protein in glycogen metabolism, perhaps within host cells [29*].

Type IV secretion

The type IV protein secretion system (TFSS) is ancestrally related to the bacterial conjugation machinery. This is a multi-component system that, like the type III systems, forms a pilus-like structure that can span both membranes and interact with eukaryotic cells delivering bacterial proteins directly to the host cytosol. Recent investigations have utilized high-resolution yeast two-hybrid assays to identify interactions between the components of the TFSS thus suggesting a model for assembly of the TFSS pathway [30]. Several type IV systems have recently been identified from bacterial genome sequences and from more traditional methods of investigation [31–34]. Despite their obvious relationship to the conjugal machinery, this mechanism is not as widespread among the proteobacteria as one would imagine from a supposed primitive history. Indeed, phylogenetic analyses suggest the TFSSs have evolved from a common ancestral system with virtually no shuffling of constituents even between sequence-divergent systems [35]. Genome comparison of several phytopathogenic bacteria has revealed the presence of TFSSs in *Xanthomonas* spp. and *Xylella fastidiosa* spp. [36]. However, *Ralstonia solanacearum* possessed only those genes encoding proteins associated with the inner membrane. Interestingly, analyses of both the *P. aeruginosa* and *Pseudomonas fluorescens* genomes did not reveal the presence of members of this family [37], indicating that the TFSS is not as widely distributed among Gram-negative bacteria as the other protein secretion systems.

Type V secretion (autotransporters)

Five families of protein translocating outer membrane porins are currently recognized in the Gram-negative bacteria. Two of these families, the autotransporter and two-partner secretion systems, are included under the umbrella of type V protein secretion. Genome sequencing has revealed that autotransporters are almost ubiquitous among the Proteobacteria. Yen *et al.* [38] recently identified 120 autotransporters from 20 bacterial genera across the breadth of the Proteobacteria and provided an

evolutionary tree of the conserved outer-membrane translocating units. Comparison with 16S RNA trees suggests the autotransporter domains arose almost exclusively by speciation and late gene duplication events within a single organism, with horizontal transfer of genes between distant organisms occurring rarely. Our own PSI-BLAST analyses of the current databases (IR Henderson, unpublished data) suggest that the autotransporter family possesses more than 700 members and as such represents the largest protein secretion pathway in Gram-negative bacteria. Several reports have described the existence of autotransporters outside the Proteobacteria, in particular a large family of conserved polymorphic proteins within *Chlamydia* spp. [39–41]. We also note the existence of at least one putative cyanobacterial autotransporter (IR Henderson, unpublished data).

Examination of genome sequences demonstrates a multiplicity of autotransporter proteins in most genomes including *Brucella abortus* [31], *Bartonella* spp. [32], *Pseudomonas* spp. [37] and *E. coli* [42]. Comparative analyses of the *P. aeruginosa* and *P. fluorescens* genomes reveals the presence of three and nine autotransporter proteins, respectively, and comparative analyses of several phytopathogenic bacterial genomes reveal the distribution of multiple autotransporters similar to each other and to autotransporters from human pathogens, suggesting the need for different repertoires of genes in different ecological niches [36,37]. For example, the Tsh autotransporter has only been found in association with invasive isolates of *E. coli*, including recently sequenced pyelonephritis isolate CFT073 [42] and the neonatal meningitis strain, RS218 (IR Henderson, unpublished data), suggesting that this protein play a role in survival in the blood stream. *In silico* analyses have identified several autotransporters that have been subsequently characterized [43,44], and used in reverse vaccinology studies, in which vaccine targets are selected through iterative experimental sieving from a large number of candidates identified via genome sequencing [45]. Recent analysis of the HecA/HecB two-partner system from *Erwinia chrysanthemi* reveal that members of this pathway appear to be unique to Gram-negative bacterial pathogens and universally conserved in necrogenic plant pathogens [46].

Gram-positive protein secretion and targeting

While protein secretion systems in Gram-negative pathogens have hogged the limelight, protein secretion and targeting in Gram-positives has attracted less, but growing, attention. ESAT-6 (early secreted antigen target 6 kDa) is a small protein of unknown function from *Mycobacterium tuberculosis* that is secreted into the supernatant even though it lacks a typical Sec-dependent signal sequence. It plays a crucial role in virulence in this organism and is an attractive target for immunodiagnosis [47,48**]. A survey of genome sequences revealed

that ESAT-6 has over 20 homologues in *M. tuberculosis*, ten of which lie in five large conserved gene clusters that also encode large membrane-bound ATPases similar to YukA from *B. subtilis* [47]. Similar clusters are also found in other mycobacteria, corynebacteria and *S. coelicolor*, raising the possibility that these ATPases might represent a dedicated secretion system for the ESAT-6-like proteins [47]. We have recently identified distant ESAT-6 homologues in Gram-positive bacteria outside the high G+C group, including *B. subtilis*, *Clostridium acetobutylicum*, *Staphylococcus aureus* and *Listeria monocytogenes* [48**]. In each case the genes for the ESAT-6-like proteins cluster with a gene for a YukA-like protein. This 'guilt-by-association' adds weight to the idea that the YukA-like proteins form a novel Gram-positive secretion system for the ESAT-6-like proteins — a compelling hypothesis in need of experimental verification.

In many Gram-positive bacteria, proteins are targeted to the cell wall and tethered to peptidoglycan through the action of a membrane-bound transpeptidase, sortase, which recognizes a characteristic targeting motif LPXTG (or similar sequences). A genomic survey of sortases and their substrates [49] revealed several unexpected findings: first, in most genomes, unlike in the archetypical *S. aureus*, sortase genes tend to cluster with those of their substrates, and second, contrary to expectations, sortases are found in spore-forming bacteria, in *Streptomyces* and even in one archaeon, *Methanobacterium thermoautotrophicum*. There are even sortase/substrate pairs encoded in the genomes of some Gram-negative bacteria, for example *Shewanella* sp. and *Pseudomonas syringae* [49] (MJ Pallen, unpublished data). Subsequent laboratory-based analyses have confirmed that some of the novel sortase substrates identified in the *S. aureus* genomes are expressed *in vivo* and surface-located [50]. However, a warning that not every protein can be identified by scrutiny of genome sequences comes from a recent study in which a second enzyme targeting the LPXTG motif, termed LPXTGase, was isolated from *Streptococcus pyogenes* and *S. aureus*, but an absence of aromatic amino acids and an abundance of alanine and of uncommon amino acids suggests a non-ribosomal origin of this intriguing protein [51**].

Conclusions

Genome sequencing has brought an explosion in our knowledge of the distribution of secretion systems. However, in most cases this has not yet been matched by progress in our understanding of the mechanisms of targeting and secretion. The growing list of examples where more than one secretion system of a particular class is encoded within the same genome provokes several questions. Are substrates specifically targeted to just one system, or is there redundancy? If specificity occurs, what is its molecular basis? Is more than one system active at the same time? If not, how is gene expression regulated to

ensure that simultaneous deployment of two or more systems is avoided? How much cross-talk is there between different systems? Given that the gene clusters encoding secretion systems often include regulatory genes, how is regulation of the secretion apparatus integrated into the cell's global regulatory networks and how far do regulators that we traditionally think of as regulators of secretion influence transcription of genes that have nothing to do with this process?

One thing is clear — the genome-sequencing revolution has revealed an abundance of secretion systems and associated compelling questions that bacteriologists interested in protein secretion have plenty of work for the coming decades!

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