Bringing host-cell takeover by pathogenic bacteria to center stage: Editorial

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Abstract

Intra-cellular pathogenic bacteria contrive processes in their host cell to create a niche for their own reproduction. One way that has emerged by which bacteria do that is delivery of secreted virulence factors, SVFs, to the cytoplasm of the host cells using the bacterial type IV secretion system, T4SS. These SVFs modulate the activity of their target host proteins, which in turn control key cellular processes. A major mechanism for the evolution of SVFs that modulate targets that do not exist in the bacterial kingdom is horizontal gene transfer. Recently, a number of bacterial SVFs were shown to act on two types of targets in host cells. First, a group of several SVFs modulate the activity and localization of one protein: Rab1 GTPase, a key regulator of intra-cellular trafficking. Second, ankyrin repeats-containing SVFs, referred to by microbiologists as Anks, interact with various binding proteins, which in turn regulate a myriad of cellular processes, including apoptosis. Modulation of trafficking and apoptosis are two examples of how invading bacteria takeover their host phagocyte, which instead of destroying the bacteria becomes a factory for its reproduction.

Introduction: Microbiologists and cell biologists rarely talk with each other; they publish most of their work in different journals and present it in separate meetings. One area in which their interests overlap is host-pathogen interaction. The question of how bacteria manipulate host cells to proliferate themselves involves both fields. Microbiologists identify bacterial factors that affect host cells, while cell biologists study processes targeted by these factors. Recently, a few takeover mechanisms have emerged when targets of virulence factors secreted by pathogenic bacteria like *Legionella*, were identified. So, what happens when microbiologists and cell biologists try to talk with each other?

First, there is a terminology barrier. When we asked cell biologists if they know of T4SSD, Dot/Icm, SidM, AnkX etc., they had no idea what we were talking about (Text Box 1). Cell biologists view *Legionella* as exotic bacteria that cause an esoteric disease, which affects a relatively small number of people (between 8,000-18,000 hospitalizations per year in the US: http://www.cdc.gov/legionella/patient_facts.htm). Compare it to the widespread heart, cancer and neurodegenerative diseases, and you can understand why most cell biologists who scan the literature, even the highly visible scientific magazines, ignore it. In addition, in the specific case of bacterial secreted virulence factors there is also a terminology clash, when both groups use the same term, “effectors”, for two different things. We propose a compromise,

which we are using in this editorial: using SVFs for the bacterial secreted virulence factors, and specifying the GTPase name with its downstream effector, e.g., Rab1-effector (see Text Box 2). A terminology conflict also happened in the case of the Ank SVFs (see below).

On the positive side, the two merging fields benefit from progress done in each discipline. Microbiologists, who identify virulence factors secreted by intra-cellular bacteria and their target host proteins, benefit from what is already known about these targets. Likewise, cell biologists frequently justify funding of their basic research by stating that it is relevant for human health. It can take a long time to see that happen. But, when it finally does happen, it is extremely satisfying.

To promote productive interactions between the two fields, we feature three reviews in the current issue of *Cellular Logistics*. These reviews summarize two examples of how knowledge accumulated on basic cell processes help to understand the way virulence factors secreted by pathogenic bacteria affect their host cells. In this editorial, we highlight the exciting field of host-cell invasion and takeover by pathogenic bacteria.

The process of cell takeover: Pathogenic bacteria, which replicate inside their host cells, cause various diseases in humans. Examples of these bacteria include *Legionella pneumophilia*, *Coxiella burnetii*, and *Anaplasma phagocytophilum*, which cause Legionnaires' disease, human Q fever, and anaplasmosis, respectively. These three diseases are not spread from person to person. For example, *Legionella* is transferred to humans through an intermediator, an amoebae that lives in water. The host cells of these bacteria are different types of phagocytes, cells that belong to the immune system, e.g., macrophages and neutrophils. Thus, the bacteria inhabit the exact same cells that are supposed to defend the body from their infection.

How is it possible? Cell invasion occurs when phagocytes engulf bacteria into phagosomes. These pagosomes usually mature into lysosomes, where most bacteria are destroyed. But, some bacteria found a way to use phagosomes as an entryway to the cell. They either stall the maturation of phagosomes to lysosomes, or in some cases, have developed mechanisms to blossom in the hostile environment of the lysosome and call it home, e.g, *Coxiella* is resistant to the low PH of the lysosome.

Once inside the cell, the bacteria stay in the periphery and do not destroy the cell infrastructure. Instead, they modify and use it. The invading bacteria reproduce inside a replication niche surrounded by a membrane; e.g., *Legionella*-containing vacuole, LCV. The niche membrane is different from other cellular membranes and contains both bacterial and host proteins. To manipulate cellular processes, the bacteria secrete virulence factors through a type IV secretion system, T4SS (termed Dot/Icm in *Legionella*), which delivers these factors into the host

cytoplasm. T4SS is a sophisticated apparatus made of about two dozens different proteins, which spans the bacterial inner and outer membrane, its cell wall and the niche membrane [1, 2] (Figure 1).

What do SVFs do? The last count suggests that *Legionella* secretes 275 different SVFs through the T4SS system [3]. Deletion of a single SVF usually does not affect bacterial reproduction. However, collectively these SVFs subvert cellular processes to modify their niche and create a supportive environment for bacterial proliferation inside host cells [4].

How did bacteria acquire genes for regulation of proteins and processes that do not even exist in bacteria? Homologues of these genes are not present in non-intra-cellular bacterial pathogens. It seems that horizontal gene transfer from eukaryotes was a major mechanism in this co-evolution [5].

Two types of SVFs are currently in the spotlight (Figure 2): accessory factors of Rab GTPases and ankyrin repeat-containing proteins, Anks. Some SVFs have multiple domains, and at least one, AnkX, contains both a Rab-modifying and an Ank domain.

SVFs as regulators of Rab GTPase function: (Summarized by Tan and Luo, Machner and Chen; in this issue [6, 7]). "My all-time favorite protein is Rab1 GTPase! As a postdoctoral fellow, I showed that Ypt1 regulates endoplasmic reticulum (ER)-to-Golgi transport in yeast and that Ypt1 localization to the Golgi apparatus is conserved also for its mammalian homolog, Rab1 [8]. Twenty years later Rab1 was found on bacterial niche membrane and is the target of multiple SVFs", says Segev. What is so special about Rabs in general, and Rab1 in particular, that bacteria use it as a major target for manipulating their host cell? Rabs are key regulators of all membrane trafficking pathways in eukaryotic cells and Rab1 regulates the early phase of the secretory pathway [9]. Therefore, bacteria that acquired the ability to regulate Rabs can control these trafficking pathways.

How are Rabs regulated? Like all other GTPases, Rabs switch between the GTP-"on" and GDP-"off" forms. In addition, Rabs cycle between the cytoplasm and membranes. Upstream accessory factors help Rabs cycle between the "membrane-on" and "cytoplasm-off" states. In the cytoplasm, Ypt/Rab GTPases are "off" in a complex with the GDP-dissociation inhibitor, GDI. Two types of accessory factors help the activation of Ypt/Rab GTPases: GDI-displacement factors, GDFs, are membrane receptors that help GTPases get on membranes, and guanine-nucleotide exchange factors, GEFs, stimulate the exchange of GDP to GTP. In their GTP-bound form on membranes, Ypt/Rab GTPases interact with their downstream effectors to organize specific membrane domains. To de-activate Ypt/Rab GTPases, GTP-hydrolysis activation proteins, GAPs, stimulate GTP hydrolysis and the GTPases can then be extracted from membranes by GDI [10].

Bacterial SVFs change the activity and membrane localization of Rab1 in two completely different ways: nucleotide-membrane cycling and post-translational modification. One multifunctional SVF, SidM, can do both. In addition, at least one bacterial Rab effector is currently known (Text Box 1).

(1) Rab1 nucleotide-membrane cycling: SVFs secreted by *Legionella* act as Rab1 "on" and "off" regulators, using a mechanism similar to that used by the normal host Rab1 regulators. SidM acts both as a GDF and a GEF to activate Rab1 on LCV. Once on the LCV, Rab1 is thought to capture ER-derived vesicles to build the LCV membrane [4]. The bacteria also secret LepB, a SVF that acts as a Rab GAP to de-activate Rab1. Why would the bacteria secret factors that have opposite effects on the same protein? In this specific case the factors are secreted at different phases of the infection, turning Rab1 "on" early and "off" later.

(2) Post-translation modifications: Two types of Rab post-translational modifications catalyzed by bacterial SVFs are currently known: covalent attachment of adenosine monophosphate, AMPylation, or of phosphocholine, PCnation. These two mutually exclusive modifications occur on two adjacent amino acids that reside in the switch II domain of Rab1, Tyr77 and Ser76, respectively. Rab1 is the preferred substrate of the SVFs that catalyze these modifications, but other Rabs can serve as substrates too; notably Rab35, which regulates endosomal trafficking. While both reactions, AMPylation and PCnation, occur in eukaryotic cells, it is currently unknown whether they are used normally for Rab modulation.

What is the effect of Rab post-translational modifications on its interactions and function? Switch II is one of the two Rab domains whose three-dimensional structure changes when Rabs cycle between the "on" and "off" states, a change important for Rab interactions with GAPs and effectors. Currently, more is known about the effect of Rab AMPylation than about PCnation. AMPylation inhibits Rab1 interaction with both host and bacterial Rab1 GAPs, thus rendering a constitutively active Rab1. In addition, AMPylation inhibits the interaction of Rab1 with at least one host effector, MICAL3, without affecting its interaction with at least one bacterial SVF that acts as a Rab1 effector, LidA [11]. Rab AMPylation is reversible, with SidM catalyzing AMPylation and SidD catalyzing de-AMPylation, in the early and late phases of infection, respectively. Thus, it is possible that in the early phase of infection, AMP-Rab1 regulates ER-vesicle recruitment to the LCV, while removal of the AMP at a later phase allows the bacterial GAP LepB to turn the Rab off. Alternatively, it is possible that AMPylation regulates Rab function in a temporal fashion. Namely, it would allow Rab1 to interact with effectors only on the LCV but not in other compartments, thus inhibiting secretion.

PCnation is catalyzed by the SVF AnkX. It is still unknown what is the effect of PCnation on Rab function, whether it is reversible, and whether it regulates Rab function in a temporal or special manner. The fact that Rab35 is a reasonable substrate of AnkX raises the possibility that this modification affects endosomal trafficking, which is regulated by Rab35 [12].

(3) Rab effectors. LidA, a *Legionella* VF, was recently termed "Rab super-effector" because it interacts with Rabs in a very high affinity [13]. However, it is still not clear what is the effect of the Rab-LidA interaction. One possibility is that because of the high affinity of this interaction, interaction of the Rab with its normal effectors is inhibited. This would result in the inhibition of transport steps normally regulated by the Rab. However, LidA is enriched on the LCV and not on other cellular membranes, suggesting that the Rab-LidA interaction regulates trafficking into the LCV.

SVFs that contain ankyrin repeats (Summarized by Voth, in this issue [14]). Members of this group of SVFs are related through a common structural motif named after the protein in which they were first described. Each ankyrin repeat is about 33 amino acids long. A given protein might have dozens of these repeats, or as few as two, or apparently any number in between. While their discovery in SVFs does not point to a particular cellular process, it is truly remarkable that this protein motif has been used over and over again as an agent of virulence through type IV secretion. The implication appears to be that there are many different cellular targets that can enhance virulence. A socket wrench is a valuable tool to have in your toolbox, because it makes it possible to adjust many different sizes and shapes of nuts and bolts and screws. Likewise, each ankyrin-repeat protein may be like a different socket that can bind to one particular target protein and adjust its behavior in a way that favors virulence.

Sometimes a new conversation between strangers (in this case microbiologists and cell biologists) can produce an awkward moment. That's the case with the nomenclature of the ankyrin-repeat containing SVFs. The ankyrin repeat was first recognized as a prominent feature of the metazoan cytoskeleton protein ankyrin [15]. However, today ankyrin repeats are recognized as a widely used structural module in many otherwise unrelated proteins [16]. To avoid confusion, the name ankyrin and the abbreviation Ank have been reserved by cell biologists for the family of spectrin-binding cytoskeleton proteins where the repeat motif was first named. Other proteins that possess the repeat domain, including numerous transcription factors, cell cycle regulators and signal transduction proteins, are given a more cumbersome designation as ankyrin repeat-containing proteins, AnkRP. The need for this distinction is not as obvious in the microbial world and consequently a nomenclature dilemma has emerged in the literature where the "ank" shorthand has been used to describe all ankyrin repeat containing proteins. As a result the AnkG and AnkB virulence factors share the same name as two major isoforms of ankyrin found in mammalian cells [17]. Imagine the confusion if the mammalian ankyrins turn out to be downstream

effectors of the prokaryotic “Ank” virulence factors. Perhaps the bacterial proteins could be given a slightly different name such as AnkRP-G or AnkRP-B to indicate that they are *ankyrin-repeat proteins* rather than bona fide ankyrins.

It is hard to know at this point why this structural motif appears so often as a T4SS substrate, as there are still many unanswered questions about ankyrins and ankyrin repeats. By convention, the ankyrin repeat domain is thought of as a module that mediates specific protein interactions, as opposed to having a catalytic activity. Sequence variation at the surface of the module makes it possible for ankyrin repeats in different proteins, sharing the same core structure, to participate in a seemingly limitless array of distinct protein binding activities. For example, the large number of different ankyrin repeats in mammalian ankyrins [17] allows them to interact with an array of different integral plasma-membrane proteins. Along these lines, the prokaryotic ankyrin repeat-containing proteins appear to function at a variety of subcellular locations (nucleus, cytoplasm, vacuole membrane) where they bind to and alter other proteins. These interactions, in turn, lead to changes in gene expression as well as changes in protein activities and post-translational modifications that ultimately promote virulence. Knowing the structural parameters underlying the diversity of ankyrin repeat protein interactions should help to streamline elucidation of the mechanisms responsible for their downstream effects.

The downstream effect of one bacterial AnkRP is to modulate apoptosis. Organisms can halt bacterial takeover by either waging an immune response or by apoptosis of infected cells; multiple signaling pathways control both processes. *Legionella* does not stop NFκB-induced immune response, and currently, it is not clear why the bacteria are not affected by an immune response [18]. However, *Legionella* does block the apoptotic response, which would be expected to limit infection by interfering with the bacteria’s ability to reproduce, through the activity of the SVFs, SdhA and SidF [4]. Likewise, a *Coxiella* SVF, AnkG, is believed to function by inhibiting cell death to allow continued bacterial infection [19].

Perspectives: Currently, the field of host-pathogen interaction is bursting with new information about bacterial SVFs and their host targets. These findings raise new questions about the effect of the SVF-target interaction on cellular processes. In most cases, while the biochemical activity of the target protein is known, the role of the SVF-target interaction is not clear. Therefore, the field is now ripe for cell biologists to join the battle.

The discovery of multiple SVFs that modulate Rab1 function raises the obvious question: Why Rab1? Is it because it regulates the first step of the secretory pathway, or because of its recently identified role in autophagy [20]? Is modulation of other Rabs, notably Rab35, important for bacterial proliferation? Are the post-translation modifications used normally to regulate Rab function in cells, or is it specific to bacteria? Very little is known about the newest Rab posttranslational

modification, PCnation: Is it reversible? How does attachment of PC affect Rab function?

The discovery of ankyrin repeat containing SVFs raises the same kinds of questions and more. The ankyrin repeat VFs appear to be targeted to multiple intracellular destinations following type IV secretion. Do these SVFs function synergistically or independently? Are they all essential to successful reproduction, or do they make additive contributions at their respective sites to enhance bacterial survival. What are the binding partners recognized by the ankyrin repeat proteins? Do the ankyrin repeat proteins act by mimicking similar activities in host cells, or do they produce novel gain of function effects on host systems? Can drugs that block their interactions with target proteins act as antibiotics by blocking pathogenesis?

Finally, the function of only a dozen or two of the 275 *Legionella* SVFs is currently known. Interesting new insights are likely to emerge as the functions of the rest of the SVFs are unraveled in future studies. Individually, the known SVFs may not be essential for a successful bacterial proliferation. In terms of designing therapeutic strategies against intra-cellular pathogens, it would be worthwhile to identify SVFs that are essential for host cell takeover and to focus attention on those.

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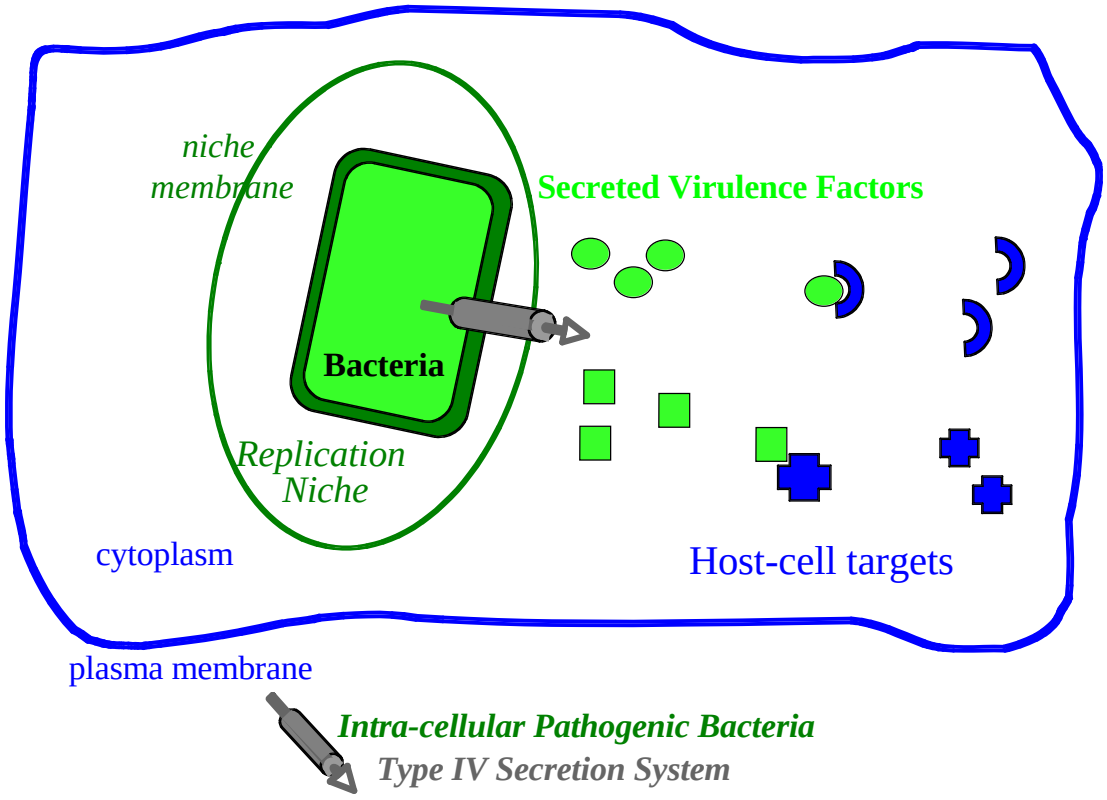

Figure 1. The use of type IV secretion system, T4SS, for host-cell takeover by bacteria. Pathogenic bacteria create a membrane-bound niche inside the host cell in which they replicate. Using the T4SS conduit (Dot/Icm system in *L. pneumophilia*), bacterial secreted virulence factors (SVFs) are delivered to the cytoplasm of the host cell through the bacterial inner and outer membranes and cell wall, as well as through the membrane of the niche in which they replicate. In the cytoplasm, various SVFs (green) interact with host target proteins (blue) to modify their function and/or localization.

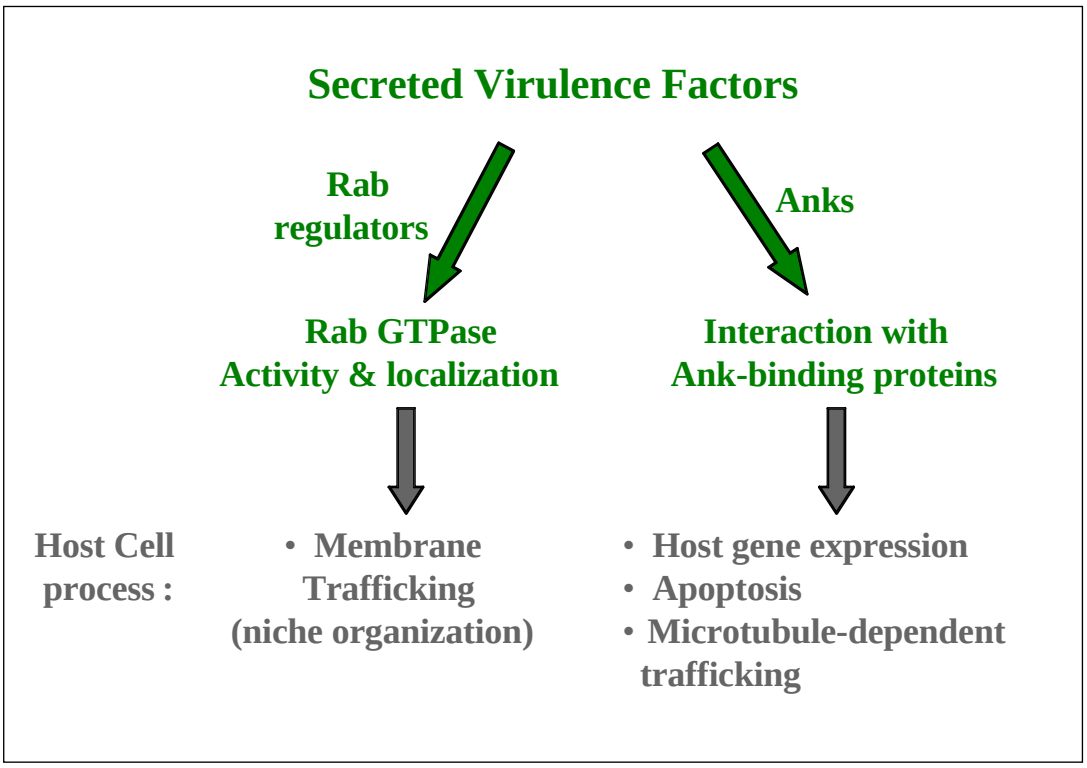

Figure 2. Two mechanisms by which bacterial secreted virulence factors, SVFs, manipulate processes in host cells. One type of SVFs modulates the function of Rab GTPases, which regulate membrane trafficking, including transport into the bacterial replication niche. A second type of SVFs contains ankyrin repeats and affects host protein interactions that depend on such domains. Known interactors of these SVFs include proteins that regulate gene expression, apoptosis, and microtubule-dependent trafficking.

Text Box 1:

Meet the Players

Legionella SVFs*		Effect on Rab1
SidM/DrrA:	GDF GEF AMPylation	Rab1 activation and Membrane recruitment
LepB:	GAP	Rab1 deactivation
SidD:	deAMPylation	
LidA:	Coiled –coil protein	Rab1-effector
AnkX:	PC	unknown

* Look at the text for acronym definition

Text Box 2:

Terminology clash:

Microbiologists have termed virulence factors secreted by bacteria: “effectors”.

Cell biologists have termed proteins that interact with GTPases in their GTP-bound form and function downstream of the GTPases: “effectors”

Because here we are dealing with virulence factors that modulate GTPase function, I suggest:

Keep secreted virulence factors, “SVFs”, for the bacterial proteins.

Specify the type of GTPase for the GTPase effector, e.g., “Rab1 effector”.

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