Bacterial proteins that knock out cell defences: cruise missiles or cannonballs?
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Type III secretion systems:

Many bacterial pathogens are able to suppress and modulate host cell defences by "injecting" proteins known as "effectors" into the cytoplasm of host cells using a secretion apparatus known as a type III secretion system (TTSS) (or in a few cases a type IV secretion system). These systems are found in plant pathogens (e.g. *Xanthomonas campestris* pv. *campestris* – blackleg of cabbages) and animal pathogens (e.g. *Yersinia pestis* – the "black death" or plague).

The function of injected effectors is to inactivate and modulate cell defence mechanisms so that bacteria are able to grow as parasites on or in living infected cells, or to activate host cell death mechanisms so that the infected cell commits "suicide". The latter scenario saves bacteria from being "eaten" by amoebae and macrophages (animal cells that recognise and destroy invading bacteria) and/or provides nutrients to bacteria from the corpses of dead cells.

Reviews of type III secretion systems

Ghosh, P. (2004) Process of Protein Transport by the Type III Secretion System. *Microbiol. Mol. Biol. Rev.* **68**: 771-795.
Espinosa, A., and Alfano, J.R. (2004) Disabling surveillance: bacterial type III secretion system effectors that suppress innate immunity. *Cell Microbiol* **6**: 1027-1040.

The challenge – cruise missiles or cannonballs

Bacterial type III secretion systems inject a limited number of "effector proteins" into host cells. The effectors are structurally and functionally diverse proteins that act on a wide range of cellular targets. These include targets that are localized to different compartments within the cell such as the nucleus, outer membrane, cytoplasm and specific membrane-bound organelles. The effectors must interact with specific cell targets with sufficient efficiency to deactivate host defence mechanisms within minutes or hours. The injection needle has a pore of ~2-3nm diameter, which enables it to secrete proteins one by one. In some systems, proteins are injected through a tube attached to the needle (Figure 1).

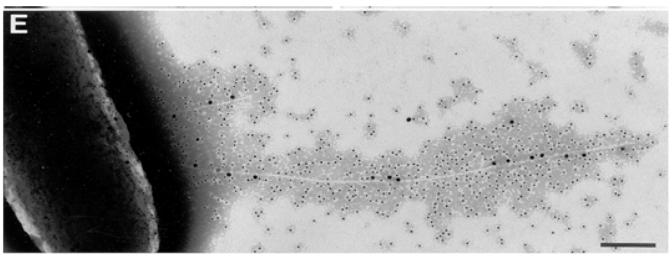

Figure 1: The *hrp* pilus is a narrow tube used to deliver partially unfolded effector proteins across the plant cell wall (proteins are secreted at the tip). Similar pili are found in some animal pathogenic bacteria, although some animal pathogens may exploit close cell-cell contact to inject proteins

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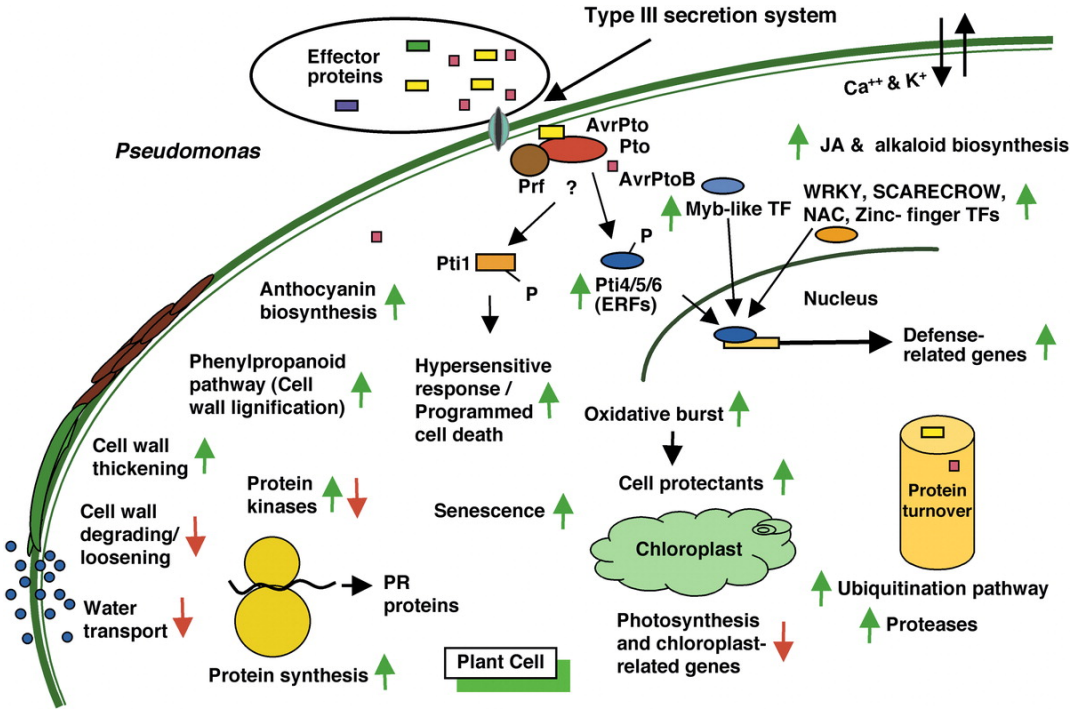
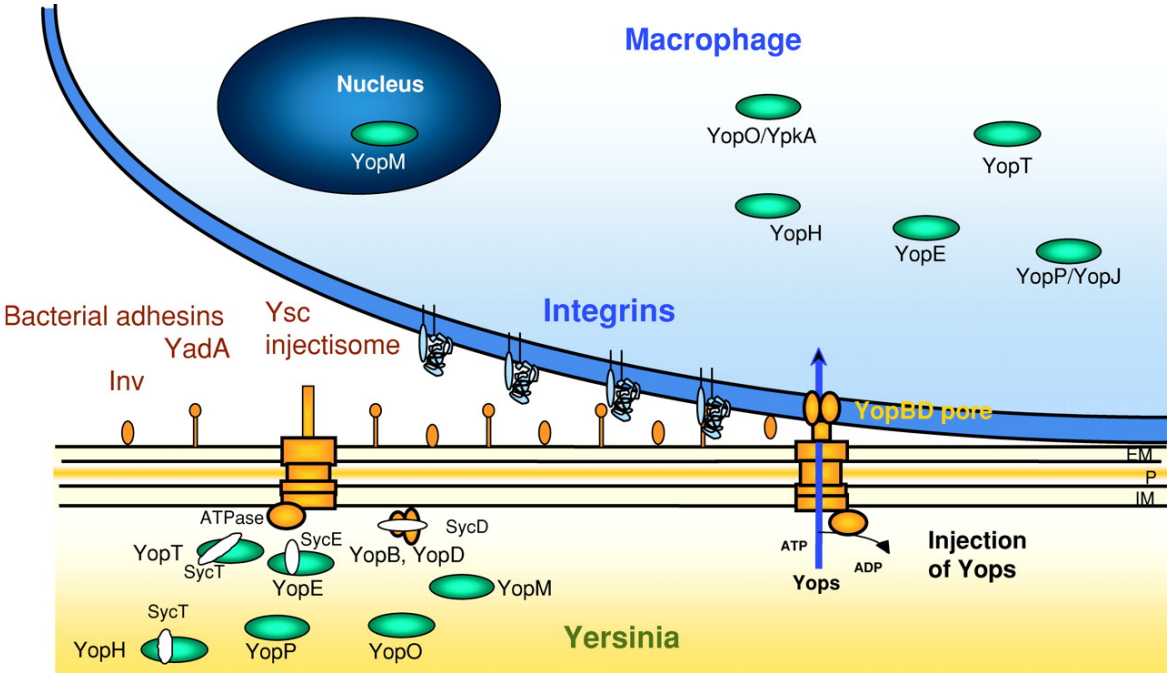
Real time imaging of effector delivery by *Salmonella enterica* showed that injection of the SipA effector protein began 10-90 seconds after docking and proceeded for 100-600 seconds until the SipA pool of $6x10^3$ molecules/bacterial cell was exhausted (Schlumberger *et al.* 2005). Current evidence suggests that effectors are probably delivered in numbers ranging from 10-10,000 molecules per bacterial cell, depending on the effector. Effectors range in size from 10-150kD (~2-25nm diameter).

Bacteria are able to use effectors to disable a cell that is 100-1000x their own size. This raises the question of how a limited number of bacterial proteins injected at a specific location in the periphery of the cell are able to encounter target proteins with sufficient speed and efficiency to disable surveillance and defence mechanisms that are activated within minutes of contact between the invading bacterium and the cell under attack. The level of protein injected is so low that in most cases we are unable to detect the injected protein using microscopy-based techniques, and must infer delivery from the physiological effects of these proteins on plant cells (as illustrated in figure 2 below). In studies where proteins are artificially expressed at high levels we observe that some effectors are localized to particular cellular compartments, such as the nucleus, chloroplast or plant membrane.



Figures 2 and 3 (below): Injection of effector proteins (AvrPto/AvrPtoB and Yops) by type III secretion systems into plant and animal cells. Most figures tend to exaggerate the size of the bacterial cell relative to the plant cell.



In order to relate these artificial studies to "natural" interactions we need to take into account the impact of scale, number and targeting mechanisms on the likehood of injected proteins "finding" a cellular partner.

We can identify at least five potential strategies bacteria can use to deliver proteins to their targets:
1) Point of entry = point of attack. The target of the injected protein is immediately adjacent to the point of injection.
2) Diffusion 1: cannonballs. Bacterial proteins reach distant targets by diffusion through the cell matrix (cytoplasm). The frequency of protein-protein interactions between host proteins has been modeled by Batada et. al. (2004). Clearly the success of a diffusion based approach will be affected by the frequency and location of targets.
3) Diffusion 2: shrapnel. Bacterial proteins generate a small, rapidly diffusing signal that interacts with distant targets.
4) Membrane assisted transport – the conveyor belt mechanism. Bacterial proteins target host proteins that are present in the cell membrane. They can attach themselves to the inner face of the membrane and diffuse in two dimensions until they interact with a host target.
5) Active targeting: cruise missiles. Eukaryotic cells have already evolved mechanisms for trafficking proteins to specific locations in the host cell. Bacterial proteins may be able to mimic signals present on host cell protein to ensure they are "delivered" to the correct target.

Batada et al. estimate that active trafficking is needed for proteins to interact with each other in cells >50μm radius.

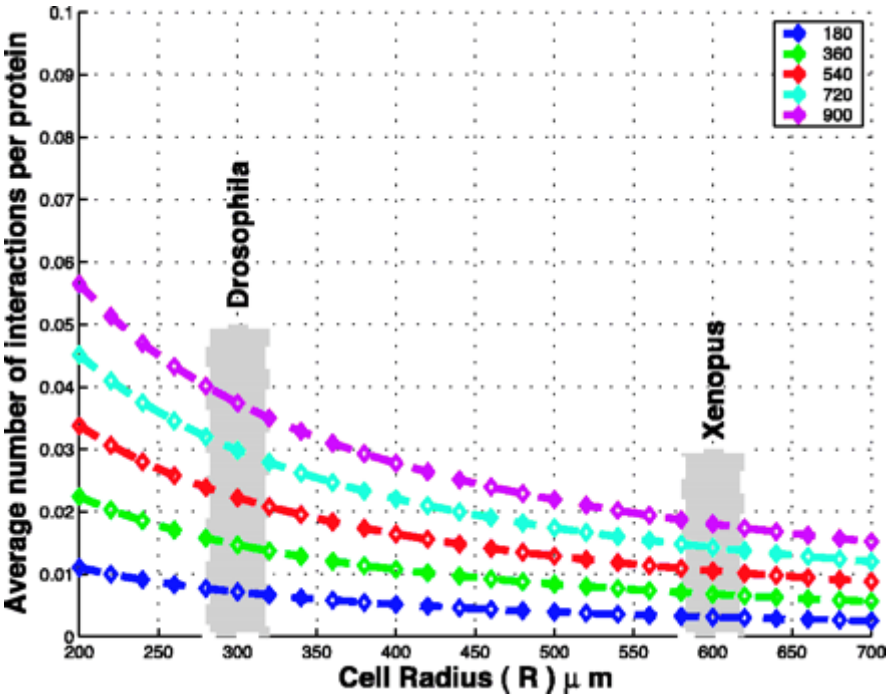


Fig. 4. Expected rate of interactions over all possible separations of mRNA sources as a function of half-life. The following parameters were used in Eq. **5**: a protein synthesis rate (β_1 and β_2) of one per 2 min, number of mRNAs of type I and II (n_1 and n_2) of four each, a diffusion coefficient (D) of 10^{-8} cm^2/s for both types, an interaction distance (ε) of 100 Å, and five values of half-life (δ), which are shown in the legend in minutes.

They conclude that:

"Even if the cellular environment were not crowded and did not contain spatial barriers (21–23), diffusion alone would not be sufficient for carrying out cellular processes at a significant rate in cells >50 μm in radius. The limitations of the short-range nature of diffusion may not afflict small secondary messengers (such as cAMP, inositol 1,4,5-trisphosphate, or Ca^{2+}) (24), which can diffuse many times faster than proteins; however, the cell must use some mechanism to enrich the local concentration of short-lived interacting proteins. Anchor (8) and scaffold proteins (25) are some of the ways cells surmount the limitations of diffusion. Design of a reliable signaling system using intermediates that freely diffuse is a challenging engineering problem that evolution has solved by spatially constraining the positions of slowly diffusing intermediates while using faster diffusing intermediates (i.e., secondary messengers) to propagate signal over a larger distance. This design significantly increases the reliability and timing of individual signaling links within a signal transduction pathway, which otherwise would be plagued by undesirable large fluctuations in timing of time-critical cellular functions. The estimate of the average rate of protein interactions derived in this article shows clearly that, in large cells with a low number of mRNA transcripts (2, 3), unlocalized proteins with short half-lives most likely will not interact sufficiently nor rapidly enough to transmit a biologically meaningful signal. Thus, we are led to conclude that the role of localization is not only to prevent cross-talk between different signaling pathways but also to increase the probability of interactions of proteins that are within the same pathway."

We would like to model the parameters affecting different strategies for type III effector delivery and compare the conclusions against known features of type III delivery.

At present we have evidence that:
1) Proteins are delivered through a hollow needle with an inner diameter of 2–3 nm. Thus transport seems to require substrates which are essentially unfolded, and each needle will deliver single proteins sequentially. These proteins must refold in the host cell before interacting with host targets.
2) Many effector proteins become attached to the inner face of the cell membrane after secretion and interact with membrane localized targets
3) Other proteins interact with targets in the cytoplasm or discrete cellular compartments such as the nucleus or mitochondrion.
4) Real time imaging of effector delivery by *Salmonella enterica* showed that injection of the SipA effector protein began 10-90 seconds after docking and proceeded for 100-600 seconds until the SipA pool of $6x10^3$ molecules/bacterial cell was exhausted (Schlumberger *et al.* 2005).
5) Proteins need to reach their targets within minutes-hours after injection in order to effectively disable host defences
6) Many bacteria use a belt and braces approach where multiple effectors target the same protein or process
7) Structural models are available for some secreted effectors
8) Some secreted proteins, such as YopM can associate to form dimers and multimers

The MMSG investigation will examine the effect of cell architecture, spatial distribution and concentration of effectors and targets, and passive vs. active transport processes on the likelihood of effector-target contact in order to address the following questions.

- Do bacteria need to exploit active transport processes within plant cells if injected proteins are to reach distant targets?
- Do the characteristics of the type III secretion system impose constraints on injected proteins. For example, is there a high probability that injected proteins can interact after secretion to form pairs, triplets and higher order structures.
- What is/are the most efficient means of attack for a TTSS-dependent bacterium?

References for effector trafficking and localization

Batada, N.N., Shepp, L.A., and Siegmund, D.O. (2004) Stochastic model of protein-protein interaction: Why signaling proteins need to be colocalized. *PNAS* **101**: 6445-6449.
Schlumberger, M.C., Muller, A.J., Ehrbar, K., Winnen, B., Duss, I., Stecher, B., and Hardt, W.-D. (2005) Real-time imaging of type III secretion: Salmonella SipA injection into host cells. *PNAS* **102**: 12548-12553.