More evidence for secretion signals within the mRNA of Type 3 secreted effectors

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Many Gram-negative bacteria, both commensals and pathogens, use Type 3 Secretion Systems (T3SS) to manipulate the behavior of eukaryotic cells. The targeted cells can be as diverse as those of single-cell eukaryotes or the cells of plants and animals (1). T3SS are distantly related to flagella and both systems consist of protein complexes that span the inner and outer membranes (2). The flagellar system has a curved hollow tube called the hook extruding from the outer membrane while the T3SS have a needle-like appendage in that position. The flagellar system exports the flagellin protein across both membranes through the secretory complex and hook where the flagellin polymerizes into a flagellum. Other regulatory and structural components are also exported (3, 4). The T3SS exports proteins that polymerize to form a needle and then proteins that form a pore in the host cell membrane, the translocon. Finally, proteins called Type 3 effectors are translocated directly from the cytoplasm of the bacterium into the host cell.

It is known that within the first 30 amino acids of a Type 3 effector, there is a signal sequence that is necessary, and often sufficient, for targeting the effector to the T3SS (5, 6). However, many bacteria encode more than one T3SS (and a flagella) and some effectors are exported only by a particular T3SS. Additionally, the T3SS is able to export proteins in a hierarchical fashion, with proteins that form the translocon being exported before the effectors. Chaperones appear to provide the information for sorting proteins into the appropriate hierarchy and to the appropriate T3SS (7, 8). The chaperone-binding domain of the effector is downstream of the N-terminal signal sequence, usually between residues 30 and 140.
The nature of the N-terminal signal sequence of Type 3 effectors has been a matter of controversy. The genes encoding some effector proteins are tolerant of frame-shift mutations in the signal sequence, suggesting that the amino acid sequence is not important for targeting. This led to the hypothesis that the signal may be in the mRNA rather than in the protein (9-14). However, the opposite approach of changing the nucleotides without changing the amino acid sequence by using synonymous codons revealed that the amino acid sequence is important, not the nucleotide sequence (15). Since then, more evidence in support of a mRNA signal was obtained when it was reported that just the 5' untranslated region (UTR) is sufficient for fliC export by the flagellar system of E. coli (16). Additionally, a C-terminal region of Y. enterocolitica yopR is required for export and this region has nucleotide, but not amino acid, sequence requirements (17).

In this issue of the Journal of Bacteriology, Niemann et al., screened 25 nucleotides of the 5' UTR from each of 42 Salmonella Type 3 effectors for their ability to direct translocation into host cells when fused to a cya reporter (18). Five of the UTRs were sufficient to direct translocation: gtgA, cigR, gogB, sseL, and steD. One trivial explanation could be that the start codons are not correctly annotated for these genes and that the putative UTRs are actually translated sequences. However, this possibility appears to have been eliminated by using mass spectrometry to search for peptides corresponding to the UTR sequence in cell lysates. None were found.

Salmonella enterica serovar Typhimurium encodes flagella and two discrete T3SS encoded by Salmonella Pathogenicity Islands 1 and 2 (SPI1 and SPI2). The five effectors with apparent export signals in their UTRs are all SPI2 effectors. The UTR of gtgA provided the strongest translocation signal so it was chosen for further studies. This UTR was not sufficient to direct the translocation of all proteins indicating that sequence
downstream of the UTR also plays a role. Only seven nucleotides of the *gtgA* UTR were sufficient to direct translocation.

An affinity purification approach identified Hfq as binding to the *gtgA* 5’UTR. Furthermore, an *hfq* mutation was unable to translocate any of the five UTR-Cya fusions. This was not a general T3SS defect because the T3SS remained intact in the mutant and continued to secrete a non-*hfq*-dependent control, SpvD-Cya. The lack of translocation was also not due to a decrease in protein expression or transcript stability in the *hfq* mutant. However, when fusing Cya to full-length effector proteins, the *hfq* mutant was able to translocate two of the five, suggesting additional targeting mechanisms for these two effectors (CigR and GogB).

The additional signal complexity revealed in this work reinforces just how challenging the prediction of secretion can be, in either the same organism or across organisms. This challenge has necessitated analytical (e.g. (19, 20)) and machine learning approaches to aid in the discovery of secreted proteins (6, 21, 22). This intriguing paper will certainly stimulate discussion and re-invigorate the quest to define the signals within Type 3 effectors that target them for secretion.

References


