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Hijacking host ribosomes via tRNA mimicry

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The bacterial pathogen *Legionella pneumophila* uses effectors — toxins — to facilitate pathogenesis within host cells. A recent study identifies dual mechanisms of the effector SidI as an inhibitor of translation elongation. The N-terminal domain mimics tRNA, whereas the C-terminal domain glycosylates the ribosome.

Microorganisms utilize diverse strategies to seize control of the host translation machinery. In addition to virus-mediated subversion of host protein synthesis[1], many human bacterial pathogens are known to target the translation machinery. Perhaps the best-known example is the secretion of diphtheria toxin by *Corynebacterium diphtheriae*, which inhibits the activity of eukaryotic elongation factor 2 (eEF2) via ADP ribosylation[2]. Glycosylation of host proteins is another strategy used by many facultative intracellular bacteria to establish infection. The bacterial pathogen *L. pneumophila* deploys an arsenal of toxins to the cytoplasm of its host, affecting various cellular processes and signalling pathways[3]. Several *Legionella* effectors halt global protein synthesis by glycosylating elongation factors. For instance, the Lgt toxin family (Lgt1–3) inhibits the host elongation factor 1A (eEF1A)[4]. Toxins such as SidI and SidL also target translation elongation. SidI possesses a glycosyltransferease activity, but its molecular target remains unknown. SidI was initially proposed to bind to eEF1A and eEF1Bγ, but the binding effect does not correlate well with its cellular toxicity[5], suggesting the existence of other mechanisms.

In this issue of *Nature Cell Biology*, Subramanian et al.[6] investigate these mechanisms, first interrogating the redundancy of several *Legionella* toxins that are known to act as elongation inhibitors. Using cell-free in vitro translation and puromycin pulse–chase experiments in HEK293 cells, the authors identified SidI as the most prominent inhibitor of protein synthesis. Affinity purification–mass spectrometry experiments revealed that SidI interacts with many components of the translation machinery, including ribosomal proteins and translation factors. The authors then used cryo-electron microscopy to determine the structure of full-length SidI to gain mechanistic insights into its function. As predicted from the reported glycosyltransferase activity[7], the C-terminal segment of SidI adopted a glycosyl transferase fold and this function was confirmed by enzymatic assays. However, unlike Lgt2, which uses UDP-glucose as a precursor[4], SidI primarily utilizes GDP-mannose to glycosylate its substrates. The N-terminal domain of SidI has a unique inverted L-shaped structure, closely resembling a tRNA molecule. This molecular mimicry provides an explanation for the long list of SidI-interacting proteins revealed by affinity purification–mass spectrometry. Supporting this notion, the K-rich loop mutant of SidI did not bind to ribosomes, whereas mutants with a deficient glycosyl transferase domain maintained their ribosome-binding capacity. Intriguingly, both SidI mutants showed attenuated inhibitory effects on protein synthesis. Thus, SidI is a multidomain protein with two distinct functions: an N-terminal tRNA-mimicking region and a C-terminal glycosyl transferase. These domains are structurally independent but functionally intertwined, working together to inhibit protein synthesis in host cells.

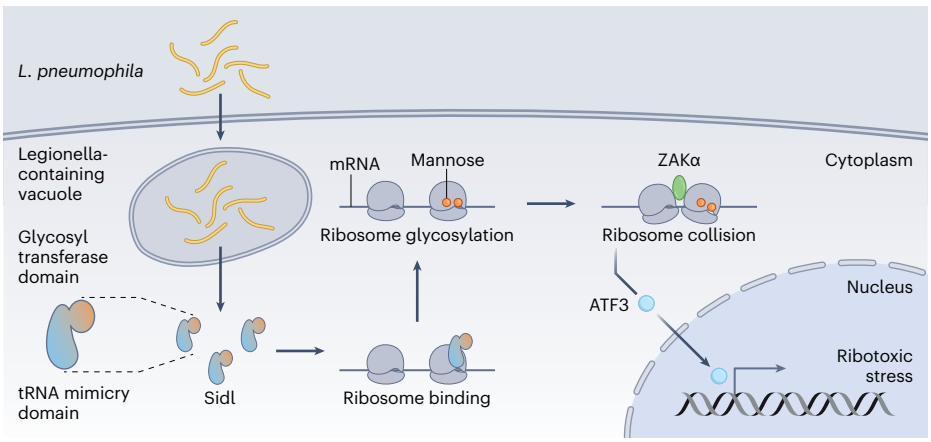

Fig. 1 | The *Legionella* toxin SidI induces ribosome collision via targeted glycosylation. Upon infection, *L. pneumophila* releases SidI into the cytoplasm of the host cell. SidI contains two functional domains: an N-terminal tRNA-mimicking domain and a C-terminal glycosyl transferase domain. Once inside the cell, SidI interacts with and glycosylates the elongating ribosomes, causing ribosomal stalling and collision. The colliding ribosomes trigger the activation of ZAKα, which is followed by p38 phosphorylation and subsequent induction of ATF3. The induction of ATF3 initiates the ribotoxic stress response in the cell, leading to cell death and bacterial dissemination. This series of events highlights the sophisticated strategies used by *Legionella* to hijack the host translation machinery, leading to pathogenesis and infection.

It is conceivable that the tRNA-mimicking domain of SidI targets the ribosome as the substrate for its glycosyl transferase activity by 'proximity mannosylation'. Indeed, using in vitro glycosyltransferase assays, Subramanian et al.[6] showed that SidI mannosylated at least 25 ribosomal proteins, nearly half of which were located near the decoding centre. Ribosomal proteins are known to undergo various post-translational modifications, including phosphorylation, methylation, acetylation, ubiquitination and ADP-ribosylation[8]. The physiological consequence of ribosome glycosylation in the form of mannose remains a fundamental question. The authors observed ribosome stalling and collision, as evidenced by the presence of RNase-A resistant disomes – complexes containing two ribosomes – in sucrose density gradients, as well as eS10 ubiquitination. Building on these findings, the authors examined the transcriptome of HEK293 cells infected with wild-type *Legionella* or a mutant that did not secrete any toxins. Among the differentially expressed genes in response to *Legionella* infection, the gene encoding activating transcription factor 3 (*ATF3*) showed the most prominent upregulation. ATF3 is a stress-induced transcription factor that regulates diverse genes involved in metabolism, oncogenesis and immunity[9]. The induction of ATF3 is also a sign of the ribotoxic stress response, and is initiated by autophosphorylation of the leucine zipper as well as ZAKα, leading to activation of the stress-activated protein kinases p38 and JNK[10]. Indeed, depletion of ZAKα and p38 prevented ATF3 induction in response to *Legionella* challenge. As well as the ribotoxic stress response, ribosome pausing also triggers the integrated stress response, which suppresses translation initiation to mitigate ribosome collision by reducing ribosome loading[11]. However, the integrated stress response was barely activated in the presence of SidI, probably owing to the more severe ribotoxic stress induced by *Legionella* infection. In line with this notion, ATF3 orchestrates a transcriptional programme that ultimately leads to cell death. These findings shed light on the complex interplay between bacterial toxins, ribosome modifications and cellular stress responses, providing valuable insights into the mechanisms underlying pathogenesis during *Legionella* infection.

The discovery of dual functional domains in SidI by Subramanian et al.[6] expands the repertoire of *Legionella* weaponry and enriches our understanding of the host–pathogen relationship. The findings of tRNA mimicry and ribosome modification by SidI lead to further questions. First, what is the mechanism by which mannose modification leads to ribosome stalling? Further, given the clustering near the decoding centre, does the addition of mannose affect mRNA decoding or ribosome translocation? Given the wide range of codon optimality, ribosome pausing is likely to occur at specific codons, whereas ribosome translocation could cause elongation slowdown across the entire coding region. A previous study using high doses of anisomycin showed that complete ribosome stalling does not lead to ribosome collision[10]. Following the same line of reasoning, it is possible that SidI-mediated ribosome mannosylation results in slowing, rather than complete blockage of elongation. Alternatively, tRNA mimicry by SidI might exhibit codon preferences, leading to codon-specific ribosome modification and stalling. A high-resolution map of ribosome occupancy across the transcriptome via ribosome profiling would help comprehensively address this question.

Despite the global inhibition of protein synthesis in the presence of SidI, the translation of ATF3 was selectively upregulated. Preferential translation of stress-associated mRNAs is a common strategy for cells to deal with adverse conditions[12]. This is exemplified by ATF4, a transcription factor of the integrated stress response; many stress-related genes rely on *cis*-regulatory elements such as upstream open reading frames (uORFs) to control the translation of main coding regions[13]. Indeed, the 5′ untranslated region of *ATF3* mRNA contains an overlapping uORF, which is crucial for selective *ATF3* translation under ribosome collision. However, in a similar reporter assay, the uORFs of *ATF4* do not seem to respond to ribosome collision induced by anisomycin or SidI. This suggests that the uORF number, length, position and other features might be critical for the regulatory effects. In addition to the regulatory uORF, it is unclear whether ATF3 translation is immune to ribosome collision, and if so, how the ribosome collision is circumvented. The molecular details of selective ATF3 translation in the presence of SidI remains to be deciphered.

tRNAs are essential components of the protein synthesis machinery, making them prime targets for effector toxins used by pathogenic microorganisms. Many of these tRNA-targeting toxins are endonucleases that are capable of cleaving specific tRNAs, effectively blocking protein synthesis[14]; tRNA cleavage elicits ribosome pausing and subsequent ribotoxic stress in host cells. A recent study reported that human SAMD9 functions as a virus-activatable nuclease that cleaves the anticodon of phenylalanine tRNAs[15]. Upon SAMD9 activation, cells exhibit prominent ribosome pausing at phenylalanine codons, leading to ribotoxic stress characterized by marked induction of ATF3. Conversely, the tRNA-mimicry mechanism tends to terminate translation by occupying the ribosome A-site. In the case of SidI, the N-terminal tRNA-mimicry domain does not appear to compete with A-site tRNAs, as indicated by the minimal effect on protein synthesis of the glycosyl transferase mutant R453P.

The discovery of the dual function of SidI opens up many questions about its mode of action and its impact on ribosome function during *Legionella* infection. A significant step forward would be the elucidation of the cryo-electron microscopy structure of elongating ribosomes in complex with SidI. Such studies could provide invaluable insights into the dynamics of ribosomes during translation elongation. Addressing these questions will deepen our understanding of the intricate interactions between bacterial pathogens and their host cells, potentially unveiling new targets for therapeutic interventions against infectious diseases.

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Competing interests

The authors declare no competing interests.