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Dual role of phage terminase in *Salmonella enterica* oxidative stress response

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ABSTRACT

The adaptive survival mechanisms of bacterial pathogens under host-induced stress are crucial for understanding pathogenesis. Recently, Uppalapati et al. revealed a unique dual function of the Gifsy-1 prophage terminase in *Salmonella enterica*: it acts as a transfer ribonuclease (tRNase) under oxidative stress. The Gifsy-1 prophage terminase targets and fragments $tRNA^{Leu}$ to halt translation and temporarily impairs bacterial growth when exposed to high levels of ROS generated by the host immune cells. This response not only preserves genomic integrity by facilitating DNA repair but also inhibits prophage mobilization, thereby aiding in bacterial survival within vertebrate hosts. This study highlights a novel intersection between phage biology and bacterial adaptive strategies.

Recent discoveries in microbial pathogenesis have unveiled complex interactions between bacterial pathogens and their viral inhabitants, known as bacteriophages [1]. *Salmonella enterica*, a notorious pathogen capable of surviving inside immune cells of mammals, faces a respiratory burst of the phagocyte NADPH (nicotinamide adenine dinucleotide phosphate) oxidase [2,3]. To counteract this oxidative assault, bacteria typically employ a variety of defense mechanisms [4]. A groundbreaking study by Uppalapati et al., published in *Science*, revealed a novel phage protein enhancing the survival of *Salmonella enterica* during oxidative stress [5]. This unique discovery reshapes our understanding of bacterial adaptation to hostile environments [5].

In this study, *Salmonella enterica* demonstrated a significant suppression of protein synthesis in response to oxidative stress induced by hydrogen peroxide (H_2O_2). When exposed to 400 μM H_2O_2, the bacteria showed substantial inhibition of de novo protein synthesis, yet their viability remained unaffected, indicating resilience to moderate levels of oxidative stress through translation repression. Remarkably, *S. enterica* resumed normal translation activities 90 min after exposure (Fig. 1a). Further RNA-seq analysis revealed significant transcriptional changes, including the upregulation of genes associated with antioxidant defense and metabolic adjustments, and the downregulation of genes involved in oxidative phosphorylation and protein synthesis pathways. This genomic response, coupled with the activation of the *rtc* operon [6], which repairs fragmented RNA, underscores a strategic adaptation that enables bacteria to recover and sustain cellular function under oxidative stress (Fig. 1a).

The RecA protein, stimulated by single-stranded DNA, plays a central role in the SOS response by inactivating the repressor (LexA) of SOS response genes and thereby inducing the response, making it a key activator in this system. To understand the mechanisms behind transcriptional halt in *Salmonella enterica* following H_2O_2 exposure, Uppalapati et al. developed an *S. enterica* strain lacking the SOS recombinase RecA, which is essential for DNA repair post-oxidative damage. This ΔrecA mutant did not show accumulation of 5′-$tRNA^{Leu}$ fragments after H_2O_2 treatment, confirming that $tRNA^{Leu}$ cleavage under oxidative stress is SOS response-dependent (Fig. 1b and c). Moreover, at the absence of tRNA fragments, the transcription of RNA repair *rtc* genes was not triggered in the ΔrecA mutant. Together, these results suggest that the SOS response, which induces a DNA damage response coordinated by RecA, is essential for the RNA repair system.

Then the investigations revealed an unrecognized nuclease within the Gifsy-1 prophage that targets tRNA in *Salmonella enterica* under oxidative stress (Fig. 1d and e). This terminase shares structural similarities with colicin endoribonucleases known to target tRNA's anticodon loop (Fig. 1d). Systematic genetic and biochemical studies pinpointed the terminase encoded by the Gifsy-1 *gpA* gene in the genome of *Salmonella enterica* (Fig. 1e). Traditionally, terminase enzymes are known for their role in cleaving and packaging viral DNA during the assembly of new virus particles [7]. Intriguingly, this study found that under oxidative stress, the terminase exhibited a dual function by morphing into a transfer ribonuclease (tRNase) that targets the tRNA molecules essential for protein synthesis in the bacteria.

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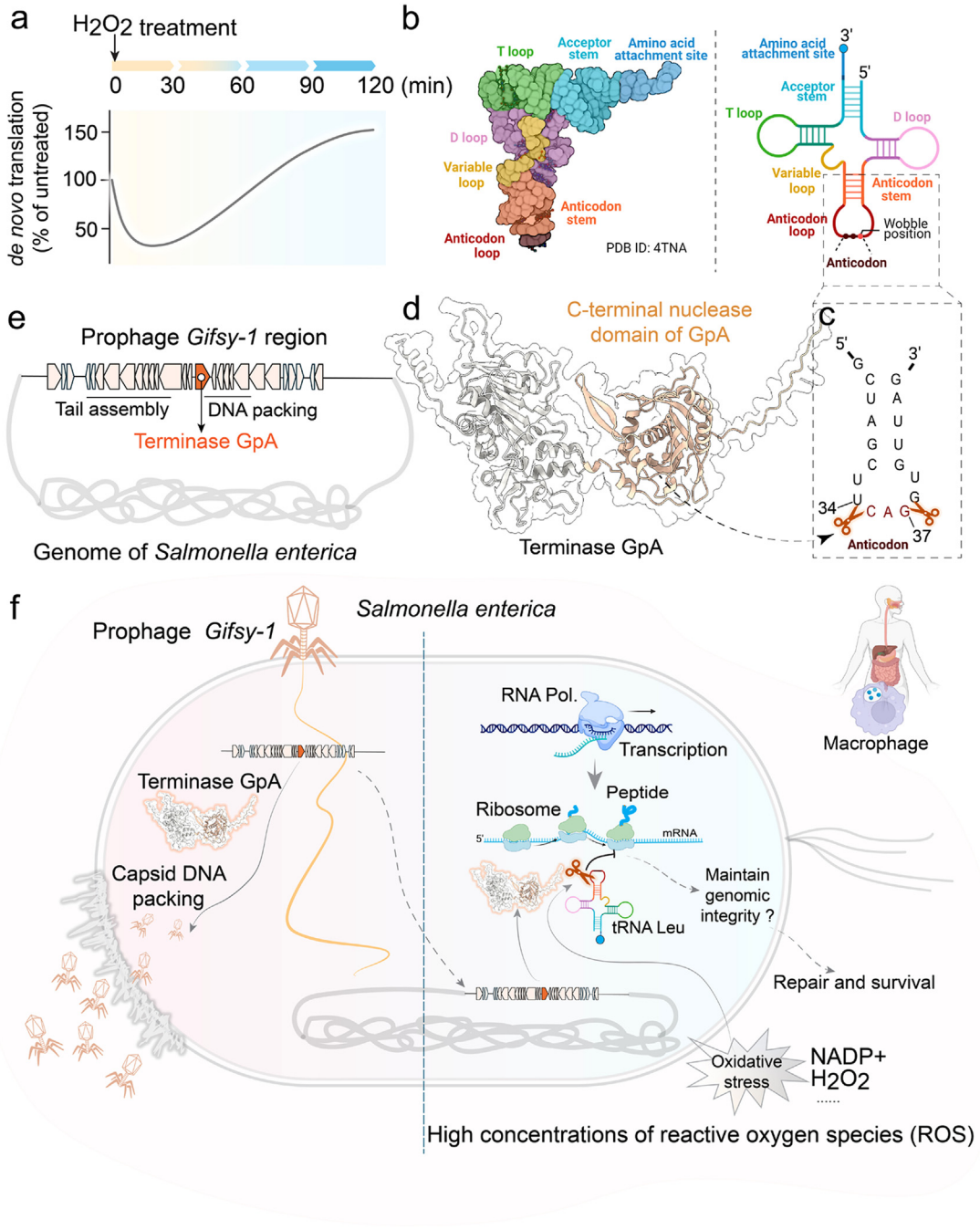

Fig. 1. Activation of the gifsy-1 prophage terminase and its role in tRNA cleavage and translation inhibition in *Salmonella enterica* under oxidative stress

a. Schematic representation of transcriptional adaptations in *S. enterica* during oxidative stress, illustrating how translation is initially repressed and allowing tolerance to moderate levels of peroxide stress. Translation resumes 90 min after exposure to H_2O_2. This figure presents a schematic representation of Fig. 1E from the original paper.

b. Diagram of tRNA, displaying both its 3D and secondary structures. Key structural features are highlighted in different colors to emphasize important functional regions.

c. Detailed view of the anticodon region of tRNA from panel (b), indicating the specific cleavage site targeted by the putative tRNase on $tRNA^{Leu}$ PQTV under H_2O_2-induced stress.

d. AlphaFold prediction model visualization of the terminase GpA with its C-terminal nuclease domain highlighted in orange, demonstrating the structural basis for its function under oxidative conditions.

e. Schematic of the Gifsy-1 prophage genomic region within the *Salmonella enterica* genome, highlighting elements relevant to phage and host interactions.

f. Conceptual model illustrating how the terminase GpA serves dual functions in phage propagation and as a tRNase activated by reactive oxygen species (ROS) generated by host immune cell, such as macrophage. This model underscores how the Gifsy-1 terminase-mediated translation inhibition in H_2O_2-treated *S. enterica* contributes to genomic integrity maintenance. In this scenario, *S. enterica* potentially co-opts host cell-derived ROS to activate a lysogenic phage element, thereby halting bacterial growth and translation during severe oxidative stress, which facilitates DNA repair and enhances survival prospects.

This study illustrates the strategic benefits of tRNA cleavage in Salmonella, where the immediate detrimental effects of translational halts are offset by long-term advantages (Fig. 1f). The temporary cessation of translation allows the bacteria to divert resources towards DNA repair and genome integrity, providing a survival edge during peak oxidative stress [8-10]. Additionally, the dual functionality of the Gifsy-1 terminase prevents the activation of the prophage's lytic cycle, aiding survival of *Salmonella* against the host immune response while preserving crucial viral genes for potential future adaptation. The findings of Uppalapati et al. challenge the conventional view of prophages merely as vehicles of virulence factors [11-13]. Instead, they presented a nuanced picture where phage proteins can have critical, context-dependent roles that influence the pathogenicity and survival of bacterial hosts under environmental stress. The study also shed light on the intricate dynamics of host-pathogen interactions, highlighting how pathogens exploit both their own genetic elements and those borrowed from phages to navigate and adapt to the hostile environments within host organisms.

At last, several intriguing questions remain unresolved in the context of the Gifsy-1 terminase GpA's response to oxidative stress in *Salmonella*. First, the molecular mechanisms by which terminase GpA senses and responds to oxidative stress are not clear. Understanding the specific signals or structural changes in GpA that trigger its shift from a DNA-processing enzyme to a tRNase would provide deeper insights into its regulatory mechanisms. Additionally, it is intriguing why GpA specifically targets $tRNA^{Leu}$ for cleavage instead of other tRNAs. Indeed, considering that approximately 20 % of all codons in the Salmonella enterica genome encode for leucine [5], the combined impact of oxidative stress on dehydroxyacid dehydratase and the $tRNA^{Leu}$ cleavage mediated by Gifsy-1 terminase could profoundly affect the synthesis of the nascent proteome. This specificity suggests a potential unrecognized codependency between leucine usage in proteins and stress responses. Moreover, the reasons behind GpA's activity being restricted to oxidative stress conditions need further exploration to determine if this is a common feature among other terminase enzymes in similar contexts or unique to GpA. Finally, the mechanisms through which cellular pathways repair DNA and maintain genomic integrity following tRNA cleavage induced by GpA terminase are yet to be fully elucidated. Future studies should focus on identifying the signaling pathways and repair mechanisms activated by tRNA cleavage, potentially involving the *rtc* gene cluster, and how these contribute to cellular recovery and survival. Addressing these questions will not only fill critical gaps in our understanding of bacterial stress responses but also might reveal novel targets for antimicrobial strategies.

Declaration of Competing Interest

Given his role as editorial board member, Dr. Chunyi Hu, had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Dr. Qunxin She.

CRediT authorship contribution statement

Senfeng Zhang: Writing – original draft. **Shengsheng Ma:** Writing – review & editing. **Feizuo Wang:** Writing – review & editing. **Chunyi Hu:** Writing – original draft.

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