

# **Nuclear lncRNA stabilization in the host response to bacterial infection**

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## SUMMARY

Long non-coding RNAs (lncRNAs) play important roles in many cellular pathways, but their contribution to the defense of eukaryotic cells against pathogens remains poorly understood. A new study from Imamura *et al.* in *The EMBO Journal* reports that infection by *Salmonella* impacts nuclear RNA decay, which in turn results in the accumulation of otherwise unstable nuclear lncRNAs, some of which may have protective effects against this common bacterial pathogen. These unexpected findings demand more efforts to fully decrypt the molecular functions of lncRNAs in innate and adaptive immunity.

## MAIN TEXT

Infections are exquisitely complex organismic interactions ensuing from the dynamic interplay between a pathogen and its host. Infected host cells execute carefully orchestrated gene expression programs that involve pathways of both innate and adaptive immunity and integrate multiple different cues to mount and sustain an appropriate response. Traditionally, efforts to characterize these host gene expression changes have centered on the mRNA output, but more recently non-coding RNAs (ncRNAs) have garnered considerable attention, too (Duval *et al.*, 2017). This is well illustrated by studies of the non-coding RNA arm of the host response to *Salmonella enterica* serovar Typhimurium (henceforth *Salmonella*), a well-characterized invasive bacterium that causes a range of diseases in humans and livestock. For example, we now know of several members of the large family of ~22 nucleotides-long microRNAs that respond to infection by *Salmonella* and contribute [to its clearance](#) (Rodriguez *et al.*, 2007; Schulte *et al.*, 2011; Maudet *et al.*, 2014; Westermann *et al.*, 2016).

In addition to microRNAs, mammalian cells express many long non-coding RNAs (lncRNAs), an increasing number of which are associated with dysregulation of immunity and host defense (Chen *et al.*, 2017; Carpenter & Fitzgerald, 2017). This includes the [discovery of lncRNA NeST, which promotes clearance of \*Salmonella\*, a lncRNA that](#) through enhancing local IFN- $\gamma$  production [promotes clearance of \*Salmonella\*](#) in mice (Gomez *et al.*, 2013). Beyond individual examples, RNA-seq profiling of various host cell types infected with *Salmonella*

has provided a treasure trove of lncRNAs that are regulated upon immune cell stimulation or pathogen invasion (Westermann *et al*, 2016; Avraham *et al*, 2015; Haber *et al*, 2017; Saliba *et al*, 2016), but their molecular functions remain uncharacterized. In this issue of *The EMBO Journal*, Imamura *et al.* (2018) report the exciting observation that nuclear RNA degradation is dysregulated as *Salmonella* replicates inside host cells, prompting the accumulation of a distinct group of otherwise unstable lncRNAs, some of which are suggested to be important for antibacterial defense.

Unstable nuclear RNAs constitute a particularly enigmatic group of transcripts which are often viewed as the mere result of pervasive transcription of the eukaryotic genome. Since the mammalian nuclear RNA exosome quickly degrades most of ~~them~~ these RNAs (Schmid & Jensen, 2018), few accumulate to detectable levels. Therefore, it has been largely unclear how many of these transcripts are functional, and whether cells actively regulate the stability of specific nuclear RNAs in response to external or internal stimuli.

Profiling the transcriptome of HeLa cells at several time points after *Salmonella* invasion, the authors here observed 145 unstable nuclear ncRNAs that were significantly upregulated (Imamura *et al*, 2018; Imamura 18). Of these, 26 were classified as enhancer RNAs (eRNAs) based on histone modifications of their genomic loci. The authors then selected two eRNAs and two other lncRNAs to understand what causes their infection-induced accumulation. While all four of them required intracellular replication of *Salmonella* to accumulate, surprisingly, their transcription was found to be unchanged. Instead, they accumulated because their half-lives increased upon *Salmonella* infection.

To explain this specific lncRNA stabilization, the authors broadly monitored infection-induced changes in protein complexes that degrade unstable nuclear RNAs. Remarkably, protein levels of the nuclear exosome component RRP6 as well as of MTR4 (a component of the nuclear exosome targeting (NEXT) complex and the poly(A) exosome targeting (PAXT) complex; (Schmid & Jensen, 2018)) were diminished 18 hours post infection, just as the lncRNA-s NEAT1\_2 and eRNA07573 accumulated. Knocking down various RNA degradation factors in uninfected cells, the authors concluded that these

candidate RNAs were predominantly degraded by the NEXT complex (Imamura *et al*, 2018)(Imamura\_18).

The authors have also obtained results to suggest that these nuclear lncRNAs are stabilized because they are required for full host defense (Imamura *et al*, 2018)(Imamura\_18). They show that NEAT1 knockout cells (lacking both its NEAT1\_1 (3.7 kb) and NEAT1\_2 (23 kb) transcripts—which partly overlap isoforms) are more permissive to *Salmonella* infection, and that there is a set of 126 genes, including those of several immune regulators, whose induction upon infection requires an intact NEAT1 locus. Thus, not only antiviral genes, as reported previously (Imamura *et al*, 2014), but also the antibacterial response may rely on a yet-to-be-defined molecular function of the NEAT1\_1 and NEAT1\_2 lncRNAs. Similarly, deletion of the eRNA07573 locus renders HeLa cells more susceptible to *Salmonella* (Imamura *et al*, 2018)(Imamura\_18). Whether this is because of a lost enhancer activity of this locus is currently unclear. That is notably, eRNA07573 lies upstream the genes of glucose transporters SLC2A3 and SLC2A14, and the authors show that both of them are transcriptionally induced upon *Salmonella* infection, in an eRNA07573 locus-dependent manner. In the cis-activation scenario, however, the putative cis enhancer function of the eRNA07573 locus would help to feed the enemy rather than to constrain it, for since cytoplasmic glucose is a preferred carbon source of intracellular *Salmonella* (Eisenreich *et al*, 2010). Therefore, the answer An alternative explanation for the observed phenotype may be found in those other ~200 genes whose induction by *Salmonella* requires the eRNA07573 locus (Imamura *et al*, 2018)(Imamura\_18).

Taken together, the authors present intriguing data suggesting that an infection-induced loss of specific nuclear RNA degradation factors causes the accumulation of a subset of otherwise unstable nuclear lncRNAs, some of which may be involved in helping to clear the replicating pathogen. The extent to which these RNA products themselves regulate specific immune response pathways remains an open question that also highlights a grand challenge in the field: connecting lncRNA expression to lncRNA function. A mechanistic dissection of lncRNAs will include strategies that deplete the respective transcripts without

perturbing genomic DNA elements, while monitoring infection phenotypes or the expression of distinct immune genes. One fast track to molecular functions and mechanisms of various infection-induced lncRNAs would be a systematic dissection of biochemical interaction partners of endogenous lncRNAs in intact cells that undergo infection, for example, by RNA antisense purification in combination with next-generation sequencing or quantitative mass-spectrometry (McHugh *et al*, 2015; Engreitz *et al*, 2013).

The observations by Imamura *et al*, 2018 prompt many additional questions as to host RNA metabolism being a target during infection. In the present case, it will be exciting to understand the chain of molecular events that leads to a depletion of the RRP6 and MTR4 proteins. Does *Salmonella* secrete a specific effector protein that targets these components for proteolysis? Further downstream, how are the 145 affected unstable nuclear ncRNAs selected for targeting by the exosome/NEXT RNA degradation machineries? Is their stabilization a general phenomenon *Salmonella* shares, at least to some extent, with other well-characterized intracellular model bacteria such as *Listeria* or *Shigella* species? Addressing these questions from a view point of lncRNA biology can be expected to provide more unexpected insights into both, the many clever tricks whereby bacteria manipulate eukaryotic cells to their own benefit, and in turn how host cells use their arsenal of thousands of non-coding transcripts to mount the right response to their prokaryotic enemies.

## FIGURE LEGEND

XXXXXXFigure 1: The mammalian non-coding RNA response to bacterial infections. Host cells utilize microRNAs (e.g. let-7, miR-15, miR-30, miR-128, miR-146, miR-155) and lncRNAs (e.g. NeST) to respond to *Salmonella* infection. Imamura *et al*, 2018 now report loss of nuclear RNA degradation factors and accumulation of unstable nuclear lncRNAs in response to intracellular *Salmonella* growth. These lncRNA candidates may contribute to host defense mechanisms that help clear the invading pathogen.



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