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GENE EXPRESSION

Host–pathogen duels revealed by dual RNA-seq *in vivo*

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Dual RNA sequencing (RNA-seq) provides a powerful means for obtaining detailed insights into host and pathogen gene expression responses simultaneously and from the same samples. Building on earlier applications of dual RNA-seq in cell culture, new studies apply dual RNA-seq *in vivo* to uncover complex multicellular interactions during infections with bacterial or viral pathogens.

Nuss *et al.* studied *Yersinia pseudotuberculosis* infections in the Peyer's patches of mice, as a model for enteropathogenic yersiniae infections in humans. The authors isolated total RNA from infected Peyer's patches and enriched these for informative mouse and bacterial RNA by depleting mouse ribosomal RNAs (ribo depletion) before RNA-seq. Mouse and bacterial RNA reads were separated bioinformatically based on mapping to mouse versus bacterial genomes. As control samples, they also carried out RNA-seq on uninfected mouse Peyer's patches and on *Y. pseudotuberculosis* grown *in vitro*.

One of the major host responses was an inflammation-based immune response, with a particular signature of neutrophil RNAs. This observation is consistent with the known infiltration of infected Peyer's patches by neutrophils, but additionally revealed molecular details of the chemokines and signalling molecules that are involved. Further host defence mechanisms activated were the acute-phase response and coagulation cascade, both of which have antibacterial and tissue-healing roles.

Next, focusing on infection-triggered transcriptional changes in *Y. pseudotuberculosis*, Nuss *et al.* observed mRNA changes that were indicative of altered stress responses (such as detoxification of reactive nitrogen species from the host immune attack) and host-adapted metabolism (such as enzymes to import and metabolize simple sugars from the microenvironment). Additionally, various metabolic regulatory non-coding RNAs (ncRNAs) had altered expression, and mutant strains confirmed that combinations of these ncRNAs were required for full virulence.

A major strength of dual RNA-seq is that it can be used to examine the strategies that hosts and pathogens use to compete for limited biochemical resources. Nuss *et al.* characterized a complex battle for metal ions as protein cofactors, with the host upregulating numerous metal ion sequestration systems, whereas the pathogen upregulated metal ion uptake transporters and ion-independent replacement proteins. These findings are consistent with a related recent study by Damron *et. al.*, which also used dual RNA-seq to characterize a battle for iron in mouse lungs infected with *Pseudomonas aeruginosa*.

In a distinct study, Wesolowska-Andersen *et al.* applied dual RNA-seq for host–virus interactions in human nasal airway brushing samples from 48 children. All were negative for acute respiratory illness, but the group comprised a mixture of subjects with or without respiratory viruses detected by quantitative PCR (qPCR) and with or without asthma. Focusing on RNA viruses, the study was conceptually distinct from bacterial dual RNA-seq: standard poly(A)-based RNA-seq was sufficient owing to the poly(A)-containing nature of host and viral mRNAs, and the viral RNA reads reflected copy numbers of the viral RNA genomes, as well as their transcriptional activity through the pile-up of reads across viral genes.

Relative to qPCR (which focused on six common respiratory viruses), dual RNA-seq had an 86% sensitivity for detecting respiratory viral infections, but crucially identified the exact strain in five cases that were either not detected or misidentified by qPCR. The most notable finding was that the presence of virus, even in the absence of overt illness, was associated with substantial remodelling of the host airway transcriptome, including immune-related signatures and modulation of the translation machinery. The virus-disrupted host gene modules include the type 2 inflammatory and ciliated cell development pathways, both of which are known to be altered in asthma disease. However, longitudinal studies will be required to definitively test molecular links between these silent childhood infections and any future asthma symptoms.

It will be interesting to see whether dual RNA-seq can be sufficiently rapid and cost-effective for future clinical adoption for identifying the specific pathogen strain in an infection, as well as a molecular window into the ongoing pathogenesis.

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ORIGINAL ARTICLES Nuss, A. M. *et al.* Tissue dual RNA-seq allows fast discovery of infection-specific functions and riboregulators shaping host-pathogen transcriptomes. *Proc. Natl Acad. Sci. USA* **114**, E791–E800 (2017) | Damron, F. H. *et al.* Dual-seq transcriptomics reveals the battle for iron during *Pseudomonas aeruginosa* acute murine pneumonia. *Sci. Rep.* **6**, 39172 (2016) | Wesolowska-Andersen, A. *et al.* Dual RNA-seq reveals viral infections in asthmatic children without respiratory illness which are associated with changes in the airway transcriptome. *Genome Biol.* **18**, 12 (2017)

FURTHER READING Westermann, A. J., Gorski, S. A. & Vogel, J. Dual RNA-seq of pathogen and host. *Nat. Rev. Microbiol.* **10**, 618–630 (2012)