GENOME WATCH

It's diversity all the way down

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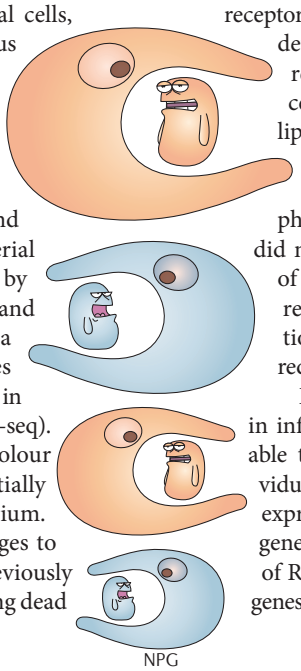
This month's Genome Watch highlights how single-cell transcriptomic analysis of infected macrophages has provided insight into the diversity in host–pathogen interactions.

Genomic studies of bacterial infections have traditionally relied on analysing a single representative isolate from each patient. However, accumulating evidence suggests that infections are often not composed of a homogeneous population, but rather consist of multiple strains or subtypes of the invading organism. This phenomenon, often referred to as 'within-host diversity', has important implications for inferring transmission networks, antibiotic resistance and, ultimately, disease outcome. Intriguingly, previous observations using a clonal population of the enteric pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium indicated that there is substantial variation in phenotypic traits, such as virulence factor expression and growth rate, between individual cells, even in genetically homogeneous populations[1]. This variation is paralleled by host macrophages in their response to infection; for example, some macrophages remain uninfected, some eliminate the bacterial pathogens and others allow intracellular bacterial replication. Now, a new study by Avraham *et al.*[2] seeks to understand how *S.* Typhimurium elicits a diverse response in macrophages by making use of recent advances in single-cell RNA sequencing (RNA-seq).

The authors used a two-colour fluorescence system to differentially label live and dead *S.* Typhimurium. This enabled mouse macrophages to be classified as uninfected, previously but no longer infected (containing dead bacteria) or currently infected (containing live bacteria). They observed large variations in the ability of host cells to phagocytose the bacteria, as well as to restrict bacterial growth after internalization. Single-cell RNA-seq of exposed macrophages, using the Illumina HiSeq platform, showed that transcriptomic profiles could distinguish between exposed and unexposed cells, and between infected and uninfected cells. The authors defined sets of genes that exhibit variable expression in response to infection. One set responded to extracellular exposure to bacteria, whereas another set was induced by intracellular bacterial signals, and showed greater diversity of expression between cells.

Genes involved in the type I interferon (IFN) response were upregulated in around one-third of infected macrophages, which would not have been observed using pooled sequencing and implies that there is extensive cell-to-cell variation after bacterial invasion. Using macrophages deficient in Toll-like receptor (TLR) signalling, the authors determined that the type I IFN response is triggered by intracellular detection of bacterial lipopolysaccharide (LPS) molecules by TLR4. However, pseudoinfections of macrophages with LPS-coated beads did not elicit the same high level of variation in the type I IFN response, suggesting that additional bacterial factors are required.



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By using a fluorescent reporter in infected cells, the authors were able to distinguish between individual macrophages differentially expressing type I IFN response genes. Simultaneous application of RNA-seq to bacterial and host genes in these macrophages showed that *phoP* and *phoQ* were upregulated in those bacteria that elicited a strong type I IFN response. These genes encode, respectively, the response regulator and sensor kinase of a two-component response regulator system that is activated upon macrophage invasion. The authors hypothesized that variation in the activity of PhoP may drive variation in the type I IFN response through the modification of LPS, which they confirmed using bacteria either deficient in or constitutively expressing PhoP.

In summary, this study demonstrates a causative link between bacterial phenotypic diversity and a variable host immune response. It is hypothesized that bacterial diversity is evolutionarily advantageous in the arms race between host and pathogen, as it increases survival chances in changing host environments. Building on previous observations of within-host genomic diversity, such as genetic diversity of the cystic fibrosis pathogens *Pseudomonas aeruginosa*[3] and *Burkholderia dolosa*[4], these latest results highlight another important layer of diversity within bacterial populations, which could only be revealed by studying single cells, rather than populations.

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Competing interests statement
The authors declare no competing interests.