GENOME WATCH

R–M systems go on the offensive

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This month's Genome Watch explores how genetic variation in restriction–modification systems from *Streptococcus pneumoniae* contributes to the defence against phages and to bacterial virulence.

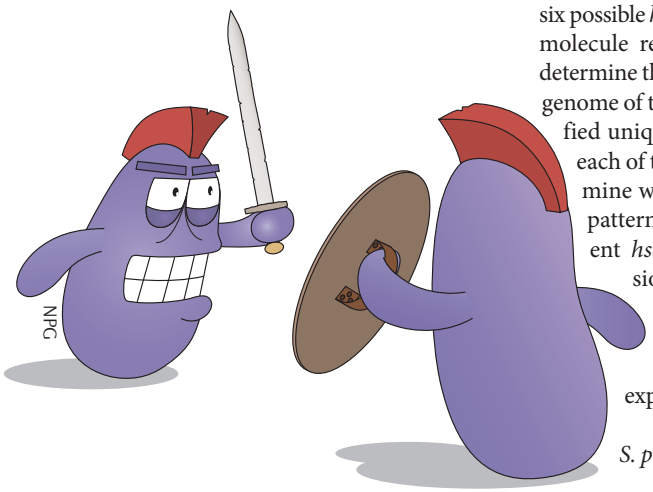
Restriction–modification (R–M) systems protect bacteria against the integration of foreign DNA from, for example, phages. These systems rely on restriction enzymes that cleave foreign unmethylated DNA at specific short sequence motifs; methylation of 'self' DNA protects the bacterial genome from its own restriction enzymes. Importantly, high frequency inversions in genes coding for R–M systems in *Bacteroides fragilis* have been shown to affect the cleavage specificity of these systems[1]. Now, Croucher *et al.*[2] and Manso *et al.*[3] describe a similar strategy in *Streptococcus pneumoniae*, and show that these R–M systems are not limited to the defence against phages but can also regulate the expression of genes involved in bacterial virulence.

Croucher *et al.* used a collection of 616 *S. pneumoniae* draft genomes to study the variation of mobile genetic elements across different lineages. They found that although integrative and conjugative elements (ICEs) were stably associated with specific *S. pneumoniae* lineages, prophage elements were highly variable across the collection, even within a single lineage. These data indicate a high rate of phage transmission among the strains and led the authors to hypothesize that *S. pneumoniae* has evolved a mechanism that prevents the spread of prophage elements through the population, which could otherwise result in overwhelming disruptive integration into the genome. In agreement with this hypothesis, Croucher *et al.* detected inversions in the *hsdS* locus — which encodes the specificity domain of a type I R–M system — that created six different alleles (named alleles A–F), each with a different target specificity. This locus rearranged rapidly enough that even a single colony contained a mixture of possible sequence arrangements, suggesting that this R–M system is effective at limiting the integration of diverse phages.

In addition to its role in preventing DNA integration, it has been proposed that the changes in methylation that result from the variation in R–M systems can also alter bacterial gene expression and influence virulence[4]. To investigate this possibility, Manso *et al.* constructed *S. pneumoniae* mutants that exclusively express each of the six possible *hsdS* alleles (A–F). By using single-molecule real-time (SMRT) sequencing to determine the methylation patterns across the genome of the different mutants, they identified unique motifs that are recognized by each of the specificity domains. To determine whether the different methylation patterns of the mutants carrying different *hsdS* alleles affected gene expression, Manso *et al.* then carried out RNA sequencing and observed a substantial downregulation of the capsule operon in mutants expressing the B allele of *hsdS*.

Increased capsule production by *S. pneumoniae* leads to the formation of opaque colonies, which are associated with invasive disease, as opposed to transparent colonies, which are associated with asymptomatic carriage. Indeed, bacterial mutants expressing the B allele of *hsdS* yielded only 7% of opaque colonies, whereas all of the colonies of bacterial mutants expressing the A allele of *hsdS* were opaque. The functional relevance of the variation in the *hsdS* locus was further confirmed *in vivo*, following the infection of mice with different *S. pneumoniae* mutants; capsule-producing mutants expressing the A allele caused invasive infection, but were unable to stably colonize the nasopharynx, whereas the opposite was true for the mutants expressing the B allele. These observations suggest that this R–M system could be the first genetic factor to be associated with virulence of *S. pneumoniae*.

Taken together, these two studies highlight the importance of R–M systems both in the defence against phages and in regulating the expression of virulence genes, and suggest that this method of gene regulation could also have similarly important roles in additional bacterial species — a key focus for future studies.



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Competing interests statement
The authors declare competing interests: see Web version for details.