COMMENTARY

Reconstructing pathogen evolution from the ruins

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Advances in high-throughput sequencing have made genomic comparison an increasingly powerful tool for understanding pathogen evolution. From these analyses, elevated genome degradation has surfaced as a common trait among pathogens with a specialized lifestyle. Prime examples of this trend can be found within the species *Salmonella enterica*, wherein genomes of host-adapted serovars associated with extraintestinal disease contain more instances of degraded (i.e., hypothetically disrupted or deleted) genes than the genomes of broad host-range serovars associated with a localized gastrointestinal disease in humans (1, 2). Such degradative events can represent overt functional waypoints, demarcating relevant pathophysiological changes along the evolutionary path traversed by the respective pathogens.

A Genomic Signature Distinguishes Gastrointestinal from Extraintestinal Pathogens

Analysis of the degraded content from *S. enterica* genomes supports a division into a gastrointestinal pathovar, carrying a low number of hypothetically disrupted genes (HDGs) and an extraintestinal pathovar with a relatively higher number of HDGs (3). The extraintestinal pathovar includes *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B, Paratyphi C, and Sendai, host-restricted pathogens associated with extraintestinal disease in humans and collectively referred to as typhoidal *Salmonella* serovars (reviewed in ref. 4). In addition, the extraintestinal pathovar includes several other pathogens associated with extraintestinal disease, including *S. enterica* serovar Dublin (*S.* Dublin), a host-adapted cause of bacteremia in cattle, and *S. enterica* serovar Gallinarum (*S.* Gallinarum), the host-restricted causative agent of fowl typhoid (biovar Gallinarum) and pullorum disease (biovar Pullorum) in poultry.

A prominent genomic signature detected within genomes from the extraintestinal pathovar is the degradation of a large metabolic network involved in anaerobic growth in the intestinal lumen (3). Luminal growth in the gut is necessary for transmission of the gastrointestinal pathovar (5), but might be dispensable to the extraintestinal pathovar, where transmission is aided by persistence in the gallbladder in the case of *S.* Typhi (6), the ovaries in the case of *S.* Gallinarum (7), or the udder in the case of *S.* Dublin (8). Thus, a change in the mode of transmission associated with extraintestinal infection might be one of the main drivers of genome degradation (3).

Langridge et al. show that S. Gallinarum degraded the tetrathionate reductase gene cluster before its divergence into S. Gallinarum biovar Pullorum and S. Gallinarum biovar Gallinarum.

New Insights Garnered from Comparing Genomes of Closely Related Pathogens

In PNAS, Langridge et al. (9) provide further insight into the evolution of *S. enterica* by sequencing the known diversity of isolates within a single lineage comprising closely related members belonging to the extraintestinal (*S.* Gallinarum and *S.* Dublin) and gastrointestinal (*S.* Enteritidis) pathovars. Subsequent genome comparison enabled the authors to map the extent of gene degradation along the branches of a chromosomal phylogenetic tree, revealing that ancestral HDGs consisted mainly of phage, insertion sequences, and genes of unknown function. In contrast, the elevated HDG formation observed in members of the extraintestinal pathovar occurred only after serovar diversification and affected many genes involved in central anaerobic metabolism.

One metabolic function that enables the gastrointestinal pathovar to grow in the gut is the ability to perform tetrathionate respiration (10), which is required for the vitamin B12-dependent utilization of ethanolamine as a nutrient (11). Langridge et al. (9) show that *S.* Gallinarum degraded the tetrathionate reductase gene cluster before its divergence into *S.* Gallinarum biovar Pullorum and *S.* Gallinarum biovar Gallinarum. A subsequent loss of vitamin B12 biosynthesis occurred independently in each biovar, illustrating a knock-on effect of HDG formation in other related functions. Many additional functions involved in central anaerobic metabolism were affected in more than one host-adapted serovar within their dataset, and indeed in other members of the extraintestinal pathovar, such as typhoidal *Salmonella* serovars, suggesting that in most cases HDG formation does not correlate with the adaptation to a specific host species. Furthermore, degradation of these metabolic functions cannot account for host restriction, because HDG formation covered many of the same metabolic pathways in the human-restricted *S.* Typhi as it did in the domestic fowl-restricted *S.* Gallinarum. Instead, Langridge et al. (9) conclude that HDG accumulation in these pathways is likely a marker indicating a switch to invasive rather than enteric disease, a trend previously observed among extraintestinal pathovar members (3).

Interestingly, Langridge et al. (9) found *S.* Enteritidis to be heterogeneous, with a classic clade being formed by isolates commonly associated with human gastroenteritis, and a second clade clustering more closely to the *S.* Gallinarum lineage. Correlating with this finding, an isolate from the classic *S.* Enteritidis clade grew to higher numbers in the chicken gut than did an *S.* Gallinarum isolate or a second clade *S.* Enteritidis isolate, suggesting that a reduced

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ability to grow in the anaerobic environment of the gut may have preceded accelerated HDG formation in the *S.* Gallinarum lineage. Although the study does not provide insight into the mechanisms underlying these phenotypic differences, it is interesting to mention in this context that, unlike members of the gastrointestinal pathovar, *S.* Typhi and *S.* Gallinarum evade innate immunity (summarized in refs. 12 and 13). This evasiveness in turn enhances their dissemination from the intestinal mucosa into the bloodstream, thereby increasing the ability of *S.* Typhi and *S.* Gallinarum to reach organs important for transmission: the gall bladder or the ovaries, respectively (14, 15). An important consequence of innate immune evasion is an attenuation of intestinal inflammation (16–19), a host response that enhances luminal growth of members of the gastrointestinal pathovar (20). Thus, the common loss-of-functions required for anaerobic growth in the gut lumen observed in the *S.* Gallinarum and *S.* Typhi lineages might be a knock-on effect of their reduced ability to cause intestinal inflammation.

Overall, in addition to elucidating the timing and general nature of degradative events throughout a single lineage of closely related generalist and host-adapted *Salmonella* serovars, the study of Langridge et al. (9) provides a wealth of genomic data for the community to use. Although mechanisms of host adaptation remain elusive, future comparisons against the growing number of gastrointestinal and extraintestinal pathovar genomes for unique and more subtle patterns of alteration will likely prove fruitful in unraveling the mechanistic basis for differences in host range.

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