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CRISPR/Cas systems in archaea

What array spacers can teach us about parasitism and gene exchange in the 3rd domain of life

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CRISPR (Clustered, Regularly, Interspaced, Short, Palindromic Repeats) loci have been shown to provide prokaryotes with an adaptive immunity against viruses and plasmids. CRISPR arrays are transcribed and processed into small CRISPR RNA molecules, which base-pair with invading DNA or RNA and lead to its degradation by CRISPR-associated (Cas) protein complexes. New spacers can be acquired by active CRISPR/Cas systems, and thus the sequences of these spacers provide a record of the past "infection history" of the organism. Recently we used spacer sequences from archaeal genomes to infer gene exchange events among archaeal species and genera and to demonstrate that at least in this domain of life CRISPR indeed has an anti-viral role.

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In most known ecosystems, bacteria and archaea are under constant attack from viruses and the impact of this parasitism can often shape microbial assemblages.[1] Prokaryotes have evolved several defense systems to protect themselves from viruses, allowing for some long-term co-existence of viruses and their hosts. Perhaps the most elaborate defense system identified to date, CRISPR (Clustered, Regularly, Interspaced, Short, Palindromic Repeats)/Cas (CRISPR-associated), provides acquired and heritable immunity to prokaryotes against parasitic selfish elements, such as viruses.[2-4] These systems contain arrays of repeated sequences interspersed by short non-repetitive DNA segments (20–50 bp long), termed spacers.[5] The spacer array can be transcribed and processed into small CRISPR RNA (crRNA) molecules. These crRNA molecules base-pair with invading nucleic acids and mark them for degradation.[6,7] The fact that the CRISPR/Cas systems are able to continuously acquire new spacers allows the study of the history of past infections, as has been shown in acidic biofilms.[8,9]

Lately, the primary role of CRISPR as an anti-viral defense system, at least in Enterobacteriaceae, has been questioned by a study showing lack of acquisition of new spacers in *Escherichia coli* strains that diverged in the last 250,000 years and certainly exposed to numerous bacteriophages.[10] Furthermore, it was suggested that some CRISPR systems are actually specialized to have an exclusively anti-plasmid role.[10] We recently surveyed all archaeal spacers, using a BLASTN-based pipeline, in order to gain insights into which foreign DNA they come into contact with and whether anti-viral activity is their major function.[11]

About 50% of spacers that had high confidence database matches in our study appear to target mobile genetic elements. Over 40% of the matches were to viruses (including proviruses), implying that for archaea, anti-viral defense is probably the major known function of CRISPR/CAS systems. Further support for this notion was obtained when we examined the location of anti-viral spacers within the CRISPR array. Freshly-acquired spacers are incorporated next to the leader sequence of the CRISPR/Cas system, while older spacers are found toward the other end of the array. Newly acquired spacers will not have had time to undergo purifying selection, since there has not been sufficient time for mutations to accumulate. In contrast, older spacers can

accumulate mutations and be lost unless they are under selective pressure for their retention. This implies that over longer periods of time only spacers that are advantageous to the host organism would persist. If anti-viral spacers are indeed the most beneficial to the host one would expect enrichment for virus-matching spacers compared with other spacers toward the end of the array. Indeed, anti-viral spacers were significantly more distal on average, further supporting the importance of the anti-viral role of CRISPR/Cas in archaea. There were many instances where spacers from one genus matched a virus known to infect another, including 30 CRISPR spacers of *Metallosphaera sedula* (family Sulfolobaceae) that matched the genome of the Acidianus two-tailed virus.[12,13] Thus, some archaeal viruses may infect a relatively broad host range.

Viruses were by no means the only mobile element matching archaeal spacers —8.3% and 8.9% of hits matched plasmids and transposable elements, respectively. Strikingly, nearly 18% of the database hits were to CRISPR/CAS genes. This observation is somewhat expected since CRISPR loci themselves are often encoded on plasmids. We speculated that having one CRISPR/Cas system decreases the chances of acquiring additional such systems. Indeed some archaeal species have several distinct CRISPR loci, but only a single set of *cas* genes.[14] Touchon and Rocha have pointed out that plasmids carrying a CRISPR/Cas system are especially hazardous to the host as the newly introduced system can target host sequences and thus degrade its DNA, and that anti-*cas* spacers can provide defense from such dangerous plasmids.[10] Our findings reinforce that "Anti-CRISPR" view—the fact that we identified more than twice as many CRISPR/Cas-targeting spacers than we did for other plasmid-targeting spacers emphasizes just how important Anti-CRISPR activity probably is.

Additionally, 8.3% of the matches were to house-keeping chromosomal genes, such as the genes encoding the replication proteins Orc1 and PCNA, or their nearby non-coding regions, while an additional 11.1% matched hypothetical open reading frames. This implies that the CRISPR/cas systems of archaea are exposed to foreign DNA that is probably chromosomal in origin. In most cases the hits matched genes outside the specific CRISPR containing species. This DNA could theoretically have entered archaeal cells by transduction, conjugation or transformation. Sections of the chromosome can sometimes translocate to a plasmid by recombination and then be transferred by conjugation to a new host. Under such circumstances, the CRISPR/Cas system could become activated ending up in the acquisition of a spacer of chromosomal origin. Alternatively, there could be generalized transduction in some archaeal viruses that could package some host DNA into their capsid by mistake and then discharge it in a new cell, while activating its CRISPR/Cas. Natural competence may also be present at least in some archaea[15,16] and could serve nutritional purposes.[17] This could help explain spacers matching bacteria such as *Pseudomonas putida* or eukaryotes such as *Nasonia vitripennis.* One may speculate that similar to the animal immune system that can sometimes accidentally target food antigens, the archaeal CRISPR/Cas can occasionally become active during nutritional competence, resulting in DNA sequences acquired from the environment.

While primary role of CRISPR/Cas systems appears to provide immunity against viruses and harmful plasmids, many spacers that were acquired by archaea can target random genes from diverse sources. Thus, these spacers provide a glimpse of the DNA landscape that an archeon has been exposed to, both malevolent and benign.

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