channel surface, as this effect has not been seen in conventional or non-2D materials. A moderate ion selectivity with a K^+/Li^+ ratio of about 9 was seen and was mechanistically based on a hydration sphere diameter or ion dehydration energies to insert ions between the graphene planes. Natural K^+ ion channels are still far superior, given the 1000:1 ratio for the even more difficult K^+-Na^+ separation.

The reported fast diffusion in many ways was unexpected, because normally, a large electrode bias creates a strong attractive interaction with interfacial ions. However, in this case, the slippery 2D surfaces counterintuitively enabled very fast coupled-ion transport through Coulombic forces. Voltage gating of the channel is also an important advancement and mimics the way that many protein channels regulate cellular chemistry by acting as chemical or charge-induced valves. Most approaches to date have used charged ligand chemistry at pore entrances or surfaces that modulate relatively large molecular species for applications such as drug delivery (*10*, *11*).

"The mechanism in this graphene channel can be visualized as a capacitative and ionic analog of 'Newton's cradle'..."

Gating small atomic ions is much more challenging, and generally only modest rectification in transmembrane currents are seen. In the work of Xue *et al.*, small ions were nearly completely blocked, and a sharp cutoff voltage was seen through a new coupled transport mechanism. Spatially addressable gates could find application in neural interfaces that require both voltage and chemical transport. ■

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MOLECULAR BIOLOGY

ZTCG: Viruses expand the genetic alphabet

Viruses build nucleotide Z, identified in meteorites, replacing adenine in DNA genomes

By **Michael W. Grome**[1,2,3] *and* **Farren J. Isaacs**[1,2,3]

Genomic DNA is composed of four standard nucleotides, each with a different nucleobase: adenine (A), thymine (T), cytosine (C), and guanine (G). These nucleobases form the genetic alphabet, ATCG, which is conserved across all domains of life. However, in 1977, the DNA virus cyanophage S-2L was discovered with all instances of A substituted with 2-aminoadenine (Z) throughout its genome (*1*, *2*), forming the genetic alphabet ZTCG. Studies revealed interesting properties of Z-substituted DNA (dZ-DNA) (*3*–*6*), but little of Z synthesis was understood. On pages 516 and 512 of this issue, Sleiman *et al.* (*7*) and Zhou *et al.* (*8*), respectively, characterize viral Z biosynthesis. On page 520, Pezo *et al.* (*9*) identify a Z-specific DNA polymerase that is responsible for assembling dZ-DNA from nucleotides. All three studies identify additional "Z-genomes" in diverse bacteriophages (viruses that infect bacteria), which may have offered evolutionary advantages alongside standard ATCG DNA since life began.

The nucleobases within standard DNA interact to form A:T and G:C base pairs. Two DNA single strands with complementary base sequences can recognize one another, form hydrogen bonds between matching base pairs, and zipper into a double helix. Notably, A:T forms two hydrogen bonds between bases, whereas G:C forms a stronger, more stable pair with three bonds. Within RNA, the nucleobase uracil (U) is substituted for T, forming A:U, but this does not alter the bond number. Within dZ-DNA, Z:T base pairs form with three hydrogen bonds, increasing bond number and thus stability compared with A:T (*2*, *5*). This makes Z the first instance of a nonstandard nucleobase that naturally alters canonical base pairing.

Although research on dZ-DNA has been scant, three studies in the late 1980s characterized synthetic and genomic dZ-DNA, confirming three major property enhancements over standard DNA: thermal stability: dZ-DNA is more stable at higher temperatures (*5*); sequence specificity: a single dZ-DNA strand is more accurate in binding complementary DNA sequences (*4*); and nuclease resistance: dZ-DNA is resistant to degradation by nucleases that recognize and cut specific DNA sequences containing A (*3*, *4*). Since those studies, the mechanical properties of helical dZ-DNA were examined (*6*), revealing increased rigidity, thermal and force stability, and a propensity to adopt a nonstandard helical form.

These features may offer evolutionary advantages in a world dominated by standard DNA. Bacteriophages (such as cyanophage S-2L) reproduce by injecting their genomic DNA into bacteria, hijacking host cellular machinery and manufacturing viral proteins to copy, build, and package new viral genomes inside the cell. To defend against infection, bacteria use a variety of mechanisms such as nucleases to destroy viral DNA. However, dZ-DNA could provide resistance to nucleases, evading host defenses. Additionally, the increased stability of dZ-DNA may permit viral persistence in extreme conditions to infect a broader range of hosts.

Despite research into dZ-DNA, little was known about the origin of Z or how it incorporates into the virus genome. Sleiman *et al.* and Zhou *et al.* used protein characterizations and metagenomic database analyses to identify key proteins of interest, determine mechanisms of action, and track sequence similarities within proteins across divergent evolutionary lineages (viruses, bacteria, and archaea). Although the entire mechanism of Z-genome replication remains unknown, two major proteins were found to be responsible for Z synthesis: PurZ (2-aminoadenylosuccinate synthetase), a virus-encoded protein that assembles a Z precursor dSMP (N6-succino-2-amino-2′-deoxyadenylate) from cellular dGMP (2′-deoxyguanosine-5′-monophosphate), and PurB (adenylosuccinate lyase), a bacterial protein that completes the synthesis of Z nucleotide dZMP (2-amino-2′-deoxyadenosine-5′-monophosphate) from dSMP (see the figure). Processing by host guanylate or nucleoside diphosphate kinases is needed to convert

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dZMP into dZTP (2-mino-2′-deoxyadenosine-5′-triphosphate) for DNA incorporation (*7*, *8*).

Searching genomic databases for similar PurZ sequences, a diverse set of 50 to 100 putative PurZ-containing genomes were identified, predominantly from bacteriophages, but also several archaea and bacteria. Putative bacteriophage hosts vary widely in environment, geography, and phylogeny (evolutionary relation), spanning actinobacteria, cyanobacteria, and proteobacteria (*7*–*9*). Further testing confirmed two additional Z-genome bacteriophages: Vibrio phage PhiVC8 (*7*) and Acinetobacter phage SH-Ab 15497 (*8*). When examined alongside cyanophage S-2L, other major Z-associated proteins were discovered, including a putative Z-specific DNA polymerase, DpoZ, which is considered responsible for Z-genome DNA assembly. Characterization of DpoZ by Pezo *et al.* confirmed not only its efficiency in assembling dZ-DNA but also its capacity to discriminate between A and Z during assembly: incorporating Z while excluding A. This ability contrasts the structurally analogous bacterial DNA polymerase-I Klenow fragment that has been shown to indiscriminately incorporate both nucleotides A and Z efficiently (*9*). The selective nature of DpoZ may ensure exclusion of A from Z-genomes. Whether Z is incorporated into bacterial genomes is an open question worthy of exploration.

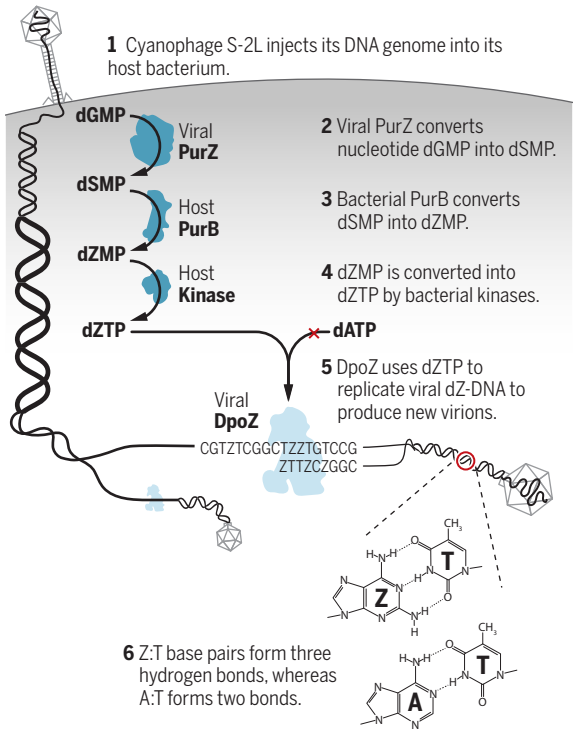
Diving deeper into phylogenetics, the viral PurZ was identified as an analog to archaeal PurA (adenylosuccinate synthetase), a protein responsible for A biosynthesis (*7*). This distant evolutionary connection raises questions of whether Z-specific proteins arose from bacterial host genes co-opted and adapted by viruses or are remnants of a preliminary biology, where Z-genomes existed in much greater frequency, perhaps even within cells. Phylogenetic analyses reveal that PurZ and DpoZ are often inherited together, suggesting that Z-genomes may have existed alongside canonical DNA since early evolution, before the divergence of actinobacteria, cyanobacteria, and proteobacteria ~3.5 billion years ago (*9*). Furthermore, a 2011 chemical analysis of a 1969 Antarctic carbonaceous meteorite revealed a composition of standard and noncanonical nucleobases of likely abiogenic extraterrestrial origin within the rock, including some deemed exceedingly rare on Earth: Z base (*10*). Origin-of-life researchers frequently explore meteorite compositions to gain insight into prebiotic chemical conditions on Earth, raising a potential role for Z in early forms of life.

With capacity to synthesize dZ-DNA, researchers can harness the properties of Z directly for applications in DNA technologies. Setting the stage, a 1988 study (*4*) revealed that dZ-DNA probes recognize and strongly bind specific genomic sequences in certain conditions better than standard DNA. Decades later, structural DNA nanotechnology uses hundreds of synthetic DNA strands that self-assemble into three-dimensional nano-scale structures in a test tube but typically suffer from low yields and instability (*11*). Incorporating Z into DNA strands may offer improved thermal stability, resistance to endonucleases, structural rigidity, and greater assembly yields from enhanced sequence specificity, advancing manufacturing of personalized, injectable drug-delivery vehicles or nanoscale scaffolding for nanomachines. For synthetic biology, the enhanced properties of Z could improve the targeting capabilities and longevity of designer DNA or RNA strands used in antisense DNA and gene-targeting therapies, along with genome engineering and synthesis technologies for improved safety and efficacy (*12*).

Researchers have long recognized the applications of expanding genetic alphabets in cells: incorporating nonstandard bases into DNA to enhance cell manufacturing capabilities, genetic isolation of strains, and biocontainment, among others (*13*, *14*). Although researchers have incorporated noncanonical bases into DNA, they have yet to extend this technology to whole genomes. Z is a natural case that may provide insight into this endeavor. However, before knowledge of Z-genomes can be fully applied, many questions remain. For example, with the selective nature of DpoZ, a cell could theoretically build a Z-genome, but it is not known whether dZ-DNA is compatible with most DNA-interacting cellular machinery. Many proteins interact with genomic DNA to maintain a healthy, functioning cell. As Z-substitution alters DNA structure, many proteins may require reengineering to accommodate dZ-DNA.

Another question is whether Z-genomes encode Z-specific RNA polymerases, making Z-substituted RNA. Cellular translation machineries recognize RNA nucleotides to assemble proteins. If Z replaces A, this changes the code, and re-engineering of cellular machinery might be required to recognize Z and assemble protein. However, if feasible, engineered cellular machinery that recognizes Z, alongside natural machinery recognizing A, could incorporate synthetic protein subunits into natural protein production to make unnatural designer proteins for therapeutics and biomaterials or to engineer dependencies on synthetic molecules for biocontainment (*15*). Renewed interest in Z should spark investigations into Z-genome biology and spur new innovations in materials and biotechnology. ■

Z nucleotides in viral genomes



dATP, 2′-deoxyadenosine-5′-triphosphate; dGMP, 2′-deoxyguanosine-5′-monophosphate; DpoZ, Z-specific DNA polymerase; dSMP, N6-succino-2-amino-2′-deoxyadenylate; dZMP, 2-amino-2′-deoxyadenosine-5′-monophosphate; dZTP, 2-amino-2′-deoxyadenosine-5′-triphosphate; PurB, adenylosuccinate lyase; PurZ, 2-aminoadenylosuccinate synthetase.

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