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Spotlight

Beyond the Four Bases: A Home Run for Synthetic Epigenetic Control?

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Park et al. (2019) create a synthetic self-propagating adenine methylation system for epigenetic control in human cells. Targeting adenine allows their modular system to act orthogonally to most epigenetic processes, thereby opening the door for novel methods of controlling gene expression.

Reprogramming cells via gene editing is a central tenet of synthetic biology. However, in eukaryotes, extensive gene control is possible beyond altering nucleotide sequences. Additional information regarding if and when genes are expressed is added to the genome and its associated proteins through modifications such as DNA methylation, histone modifications, and interacting RNAs to create additional "epigenetic" regulation. What can synthetic biology do to take advantage of this epigenetic space? In a recent work, Park et al. (2019) present an adenine methylation system as a novel mechanism of epigenetic control.

The significance of methylation-based DNA modifications has only been uncovered recently, with many mechanistic details of this form of epigenetic control still to be elucidated. Millions of methylations in the human genome help control expression profiles that determine cell types (Lister et al., 2009; Yin et al., 2017), as different methylation patterns can be heritable from cell generation to cell generation. The potential extent of regulation by these methylations is expansive, as even a single-base methylation can inactivate a regulatory transcription factor gene that controls hundreds of downstream genes.

Synthetic control of genes typically relies on protein transcription factors because we think we best understand their mechanism of action. Scientists have developed complex systems based on TetR analogs or CRISPR dCas9 to create highly programmable digital logic gates (Gander et al., 2017; Nielsen et al., 2016). However, chromatin methylation is ubiquitous in nature and should be amenable to logical programming of gene circuits in many types of cells. As synthetic biologists engineer increasingly complex cellular functions, epigenetic control will be a vital tool for coordinating multiple cellular processes on a scale previously unachievable. Like protein transcription factor systems, a synthetic methylation method that is largely orthogonal to native epigenetic processes could create a powerful new system of control.

Park et al. have created such a synthetic epigenetic system that can read, write, and propagate DNA methylations over multiple cell generations in a modular fashion. The system is uniquely based on methylation of specific adenine bases to form N6-methyladenine (m6A), which is common in bacterial cells but relatively rare in mammalian cells, where cytosine methylation is more prevalent. Bacteria use these methylations at "GATC" sites to differentiate the native bacterial host DNA from invading DNA, which is subject to restriction enzyme cleavage, as well as to regulate genes and repair DNA (Luo et al., 2015).

To create the "write" function, the authors picked a bacterial DNA adenine methyltransferase (Dam) domain from a variant library screen to write specifically at GATC sites (Figure 1A). By fusing this Dam domain with a DNA-binding domain (a zinc finger [ZF] domain or endonuclease-dead dCas9), they showed site-specific targeted methylation of DNA.

To "read" these written methylations, the authors cleverly repurpose the recognition domain of DpnI, a bacterial restriction enzyme that cuts N6-methyladenine "GATC" sites, to allow specific binding to methylated GATC sites. They then fused this piece to a transcription factor (well-characterized VP64 or KRAB domains) that controls a promoter for GFP production, thus demonstrating methylated site-dependent transcription.

With the two-module system that can write and read methylation signals, the authors show the true power of their system by mixing and matching protein domains. They create another "read" piece that fuses the DpnI reader to a Dam "writer" domain instead of a transcription factor. This increases local methylation, allowing propagation spatially along neighboring DNA. A further modification created a chemically inducible temporal system by splitting domains with abscisic acid (ABA) recognition sites so that activity became ABA dependent. With just a 2 h pulse of ABA, a fraction of cells could propagate the signal for over 10 generations over a 20-day period.

Taken together, Park et al. have added a significant tool to synthetic epigenetic control methods. The orthogonality of the m6A methylation system is one of the impressive aspects of the work. An approach targeting adenine is complementary to many natural epigenetic marks and synthetic efforts to engineer them (Figure 1A). Those methods designed to edit and/or correct the natural epigenome are fundamentally and therapeutically important but are

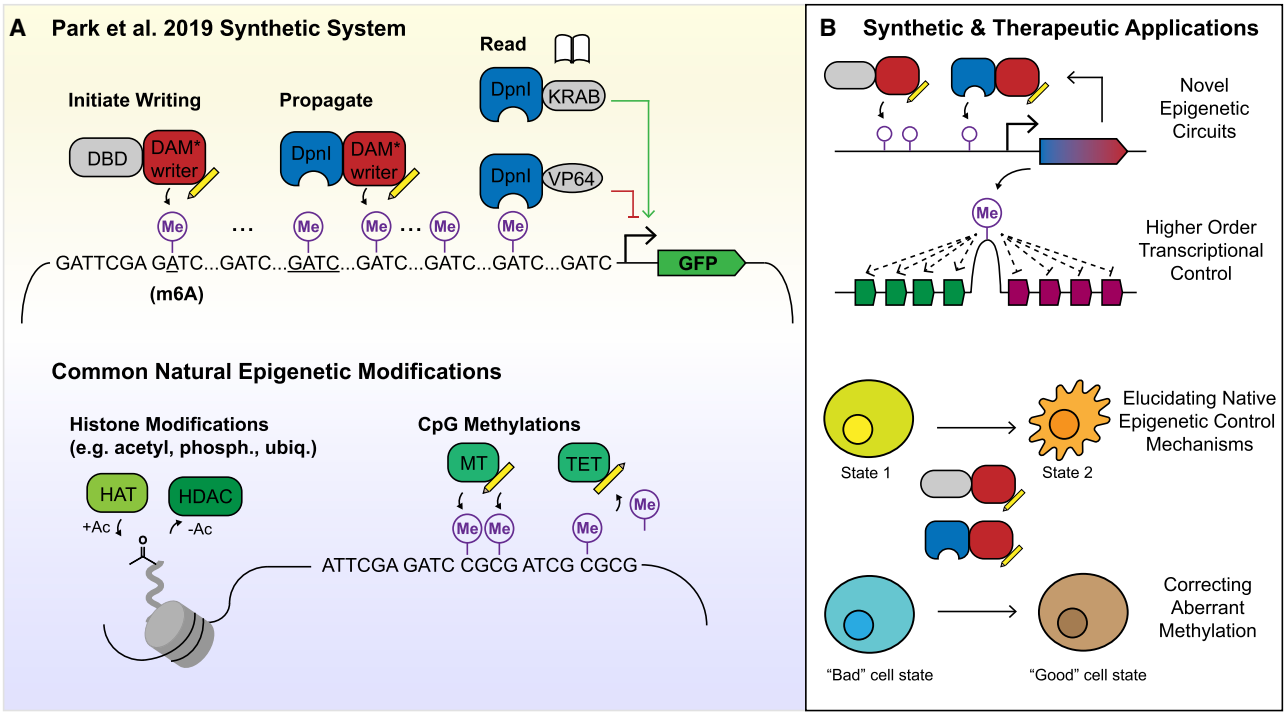

Figure 1. An Orthogonal Epigenetic Control System

(A) The synthetic epigenetic methylation system developed by Park et al. is largely orthogonal to known natural modifications. In their system, a fusion protein of a DNA-binding domain (DBD) with a DNA adenine methyltransferase (Dam) variant DAM* "writer" domain allows initial adenine methylation (m6A). A DpnI binding domain recognizes these methylated GATC sites, and a fused writer domain methylates further. A fused transcription factor domain allows direct expression control of reporter gene GFP. This system acts alongside common epigenetic processes of histone modifications (acetylation, phosphorylation, ubiquitinylation, etc.) and cytosine methylations by other methyltransferase (MT) and ten-eleven translocation (TET) family enzymes that eventually demethylate.
(B) The synthetic system may allow for new applications and studies using epigenetic control.

more susceptible to native regulatory processes that counteract the desired effects. Instead, Park et al. try to get around that challenge by creating a DNA module insert designed to act outside of those processes. With only the introduced proteins responsible for the ultimate phenotype, fundamental parameters of methylation nucleation, propagation, and preservation are easier to elucidate. These parameters can then be used for therapeutic design or to produce epigenetic circuits that control expression of multiple genes.

To that end, engineering targeted demethylation would allow for even more sophisticated circuit development. Negative feedback loops governed by alternatively expressed methylation and demethylation enzymes could form the basis of an epigenetic oscillator, for example. Memory circuits that can track *in situ* exposure of cells to dilute or transient stimuli can help elucidate fundamental knowledge and develop *in vivo* diagnostics. Indeed, one such epigenetic memory circuit has been demonstrated in *E. coli* to remember DNA damage, heat, and metabolite exposure (Maier et al., 2017). Transplanting these types of sensors into mammalian cells could involve interfacing with host biosensory processes or developing orthogonal sensors and actuators from non-human (e.g., bacterial) parts as Park et al. have done.

Another application is the promise of epigenetically targeted therapeutics, such as for cancer treatment (Nyer et al., 2017). Perhaps inserting the m6A methylation system in front of a gene natively regulated by epigenetic methylation could allow synthetic control of oncogenes and their targets or other therapeutically relevant loci. Abnormal methylation patterns have also been tied to aging (Booth and Brunet, 2016), so synthetic control over these patterns could delay or reverse cellular senescence.

Park et al. demonstrate that synthetic biologists can contribute fundamental knowledge of epigenetic processes with engineered, orthogonal systems. Moving forward, this knowledge will be indispensable in developing epigenetic circuits for diagnostic and therapeutic cellular engineering.

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