COMMENTARY

A postreplicative C5-cytosine hypermodification triggered by bacteriophage methyltransferase and hydroxylase

Tzu-Yu Chen[a] and Wei-chen Chang[a,1]

Viruses of bacteria, also known as bacteriophages, harbor the greatest diversity of DNA modifications identified to date. To fight against restriction endonucleases of their hosts, bacteriophages modify their genomic DNA through introduction of various moieties including amino acids, polyamines, and sugars (1, 2). A series of transformations are involved in DNA base modification. Followed by formation of hydroxymethyl pyrimidine nucleotides, which are utilized by DNA polymerase, replication and postreplicative modifications furnish installation of these moieties onto the DNA polymer (3–7). Burke et al. (8) show that bacteriophages resort to C5-cytosine methyltransferase (C5-MT) and 5-methylcytosine dioxygenase ten-eleven translocation enzyme (TET) as an alternative mechanism to postreplicatively form hydroxymethylcytosine on DNA. The bacteriophage TET enables site-specific hydroxylation of 5-methylcytosine (5mC), installed by C5-MT, to produce 5-hydroxymethylcytosine (5hmC). Through bioinformatic screening, the authors identify and characterize tailoring enzymes, such as glycosyltransferases, that collaborate with phage C5-MT and TET to further elaborate DNA at 5hmC site.

TET/base J-binding proteins (TET/JBPs) are present in all domains of life. They belong to iron(II) and 2-oxoglutarate (Fe/2OG)-dependent dioxygenases (9). Fe/2OG enzymes are known to catalyze diverse, but well-controlled oxidative modifications, such as hydroxylation, halogenation, epoxidation, among many others, at the expense of 2OG and molecular oxygen (10–13). In eukaryotes, TETs have been demonstrated to catalyze consecutive reactions to covert 5mC to 5-carboxylcytosine (5caC) though 5hmC and 5-formylcytosine (5fC), en route to demethylation of 5mC (Fig. 1) (14–16). In addition to 5mC, its oxidized forms (5hmC, 5fC, and 5caC) have also been demonstrated to be stable epigenetic marks that have regulatory functions in chromatin remodeling and gene expression (17, 18).

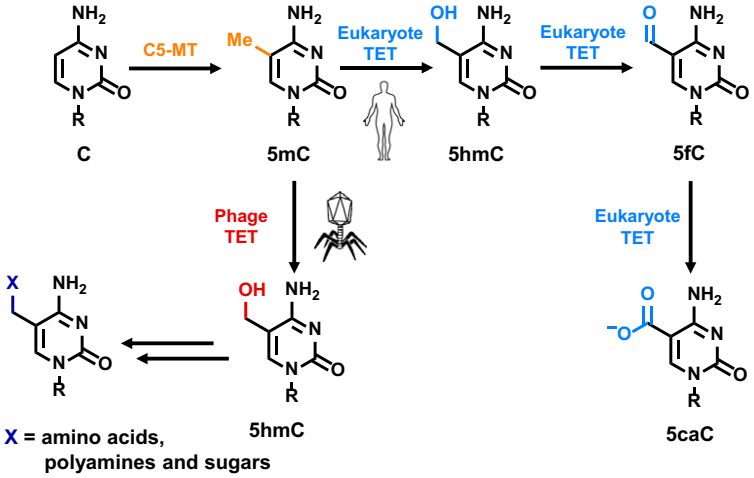

Fig. 1. TETs (ten-eleven translocation enzymes) in bacteriophage and eukaryote have distinctive substrate specificity and reactivity. In eukaryote, methylated cytosine (5mC) is converted to 5-carboxylcytosine (5caC) though 5-hydroxymethylcytosine (5hmC) and 5-formylcytosine (5fC), while 5hmC is the only product (5mC → 5hmC) catalyzed by phage TETs.

Notably, the work carried out by Burke et al. (8) demonstrates that bacteriophage TETs along with C5-MT and tailoring enzymes serve a role in phage DNA hypermodification. Using an *Escherichia coli* expression system and liquid chromatography-mass spectrometry/mass spectrometry, Burke et al. provide evidence that phage TETs perform a single oxidation event to convert 5mC to 5hmC, but no further oxidation as observed in eukaryotic TETs (Fig. 1). Additionally, the authors divulge a GpC-centered specificity for these enzymes using next-generation sequencing approaches. The findings are supported in vitro by testing the hydroxylation activity of a purified TET. The choice of a palindromic dinucleotide sequence could be significant in maintaining information during DNA replication, similar to what is shown for CpG (hydroxy)methylation in eukaryotes.

[a]Department of Chemistry, North Carolina State University, Raleigh, NC 27695

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The authors declare no competing interest.

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[1]To whom correspondence may be addressed. Email: wchang6@ncsu.edu.

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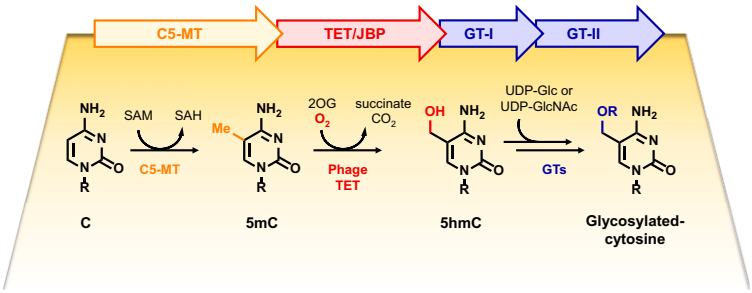

Fig. 2. C5-glycosylated cytosines are assembled through a collaborative work of C5-MT, TET, and glycosyltransferases (GTs) in bacteriophage.

On the other hand, the distinct difference in oxidation cycling between the phage (5mC → 5hmC) and eukaryotic TETs (5mC → 5hmC → 5fC → 5caC) hints a need of C5-MT/TET in phage to increase the chemical diversity of their genomic DNA, which can be used to employ new functionalities in the arms race against bacteria. The active-site features of phage TETs that enforce the single-oxidation event and, at the same time, avoid further oxidation are yet to be determined. The insight will undoubtedly contribute to a deeper understanding of these important enzymes.

Computational analyses of the gene neighborhoods of phage C5-MT/TET suggest that 5hmC, generated via C5-MT and TET, can be utilized by other adjacent enzymes. The authors examined two biosynthetic gene clusters composed of two glycosyltransferases clustered with C5-MT and TET. Through both in vivo and in vitro studies, the authors discovered formation of glycosylated cytosines on DNA (Fig. 2). These observations confirm the interdependency of these clustered genes in context of glycosylated cytosine formation and suggest that phage cytosine hypermodification is furnished through a collaborative enzymatic transformation.

Chemical modification of canonical nucleobases is expected to expand the DNA functions, similar to what has been shown for posttranscriptional modifications in RNA and posttranslational modifications in proteins. The rational exploitation of "genome neighborhood" information in phage using computation-guided strategies for functional discovery, followed by biochemical analysis of the predicted biosynthetic pathways by Burke et al. reveals a strategy to expand the structural diversity of 5mC and uncovers a biological function of Fe(II)/2OG-dependent 5mC TET other than epigenetic regulation demonstrated in eukaryotic systems.

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