



Demystifying DNA Demethylation

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istic single-photon sources (4–7), quantum memories (8), or quantum switches with photons (9). In the present approach, an ensemble rather than a single atom was used, which led to a substantial reduction of the characteristic time scales by a factor $N^{1/2}$ for an N -atom cloud. Even more important, and in contrast to previous proposals, the input and output of the probe field was not mediated by the high-quality cavity but rather by the atomic ensemble. This approach allowed for longer operational times without sacrificing operational speed and also reduced the effects of losses. Finally, this experimental achievement is also quite different from recent experiments that

demonstrated EIT in strongly coupling cavities with individual atoms (10, 11). In those experiments, the control field was rather strong (essentially a classical external laser and closer to an EIT experiment) and not the field of a single photon scattered from the probe field into the cavity that would be needed for quantum switching.

The work by Tanji-Suzuki *et al.* has shown that transparency of an opaque medium and a substantial time delay can be induced by a few photons. Their demonstration suggests that the switching of light by light on the single-quantum level—an all-optical quantum gate—may soon be in reach.

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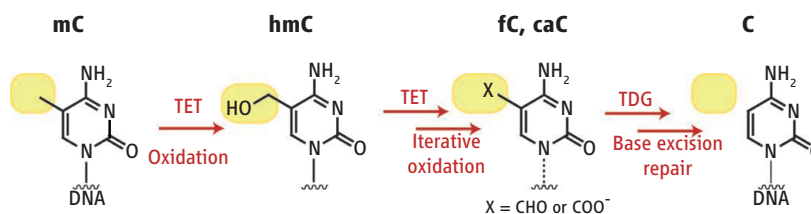
MOLECULAR BIOLOGY

Demystifying DNA Demethylation

Christopher S. Nabel and Rahul M. Kohli

Variability and adaptability are necessary for overcoming the challenges of multicellular life. To address this need, nature has evolved a substantial enzymatic toolbox for altering cytosine within the genome. Methylation of the nucleotide cytosine (C) at the 5-position of the base has profound impacts on gene expression and cellular identity. The reverse of this process, DNA demethylation, is equally important for cleaning the genomic slate during embryogenesis or achieving rapid reactivation of previously silenced genes. Although the mechanism of DNA methylation has been rigorously established, active DNA demethylation in mammals has remained enigmatic, as disparate observations have failed to coalesce into a consistent model. Cytosine deamination, oxidation, and base excision repair enzymes have been proposed in a dizzying variety of combinations (1). Against this backdrop, two reports in this issue, by Ito *et al.* (2) on page 1300 and He *et al.* on page 1303 (3), help bring new clarity to the mechanistic model for DNA demethylation.

The studies by Ito *et al.* and He *et al.* expand on the recent discovery that 5-methylcytosine (mC) can be oxidized to 5-hydroxy-



DNA demethylation. TET enzymes are proposed to oxidize 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC) and subsequently to generate the higher oxidation substituents 5-formylcytosine (fC) and 5-carboxylcytosine (caC) (shown as the structure with the 5-X substituent). Unmodified cytosine (C) is on the far right. Base excision repair, initiated by thymine-DNA glycosylase (TDG), releases and replaces the entire modified oxidized base with unmodified C.

methylcytosine (hmC) by TET enzymes, members of the α -ketoglutarate-dependent oxygenase family (4). Although hmC exists in low quantities—less than 1% of all cytosines (5)—the base has become a commodity in the epigenetics field, particularly given studies implicating TET in global and locus-specific DNA demethylation (6–8). One notable proposal posits that iterative oxidation by TET could yield 5-formylcytosine (fC) and 5-carboxylcytosine (caC) (1). Given the precedent of a decarboxylase in pyrimidine salvage, a similar enzyme could ultimately regenerate cytosine.

In evaluating this proposal, Ito *et al.* and He *et al.* both demonstrate that TET enzymes are capable of iterative oxidation of mC. Purified TETs converted hmC to fC and caC in oligonucleotides. In mouse embryonic stem (ES) cells, both fC and caC were detected in the genome by mass spectrometry, albeit at low amounts, confirming the recent detection of fC in ES cells (9). Accumulation of higher oxidation products depended on TET, as the

DNA modifying and repair enzymes make a new connection in the mechanism of DNA demethylation.

absence of a TET isotype decreased amounts of the modified bases in ES cells. The discovery of these seventh and eighth bases, after the sixth base hmC, provides clear evidence that we have underestimated the dynamic nature of the genome (10).

How can higher oxidation products of mC revert to cytosine? Although Ito *et al.* suggest an unknown decarboxylase, He *et al.* propose direct removal of the entire caC nucleobase by thymine-DNA glycosylase (TDG). Subsequent repair of the resulting abasic site would restore unmodified cytosine. TDG has been previously implicated in demethylation, as its absence is embryonic lethal and perturbs DNA methylation patterns (11, 12). A requirement for TDG has fostered the prevailing assumption that demethylation must involve deamination, as the canonical TDG substrates are mismatches between thymine and guanine nucleotides that result from deamination of genomic mC. However, TDG has some activity against cytosine analogs, particularly when substituents weaken the N -glycosidic bond between the nucleobase and the sugar (13), as would likely be the case for fC and caC. Indeed, He *et al.* show that ES cell lysates contain glycosylase activity against caC-containing oligonucleotides—activity that is lost when TDG is depleted. Furthermore, TDG overexpression decreases genomic

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caC, whereas caC accumulates when TDG is depleted. These observations overturn the assumption associating TDG solely with deamination-mediated demethylation; TDG activity on the higher oxidation products of mC links two proposed players in DNA demethylation—oxidation and base excision repair—in a new and plausible manner.

As an important point of discrepancy, Ito *et al.* find that fC accumulates relative to caC, whereas He *et al.* report that hmC is efficiently converted to caC without any accumulation of fC. This raises the question of the identity of the penultimate cytosine oxidation product prior to the action of base excision repair. Mechanistically, it is feasible that fC could be the better substrate for TDG (13), given impacts on *N*-glycosidic bond stability. Higher relative amounts of fC in ES cells would support this possibility, and caC accumulation may reflect altered steady-state amounts given perturbed TDG or TET expression. A kinetic appraisal of the relative rates of formation of hmC, fC, and caC by TETs should resolve these possibilities.

The factors regulating the extent of TET-mediated oxidation must also be explored. Why does TET sometimes oxidize to hmC and at other times iteratively to fC or caC? Do fC and caC have roles in shaping the genome other than as intermediates in demethylation? Further, Ito *et al.* and He *et al.* demonstrate a viable demethylation pathway by examining oligonucleotides or by assessing global amounts of nucleotides. Neither shows that caC is specifically present in promoters undergoing demethylation. Additional experiments will need to confirm the coupling of iterative oxidation and base excision repair at activated promoters for this model to gain acceptance as a bona fide mechanism of DNA demethylation.

Although iterative oxidation coupled to base excision repair provides a plausible demethylation pathway, what becomes of observations that favor other pathways? Rather than viewing these mechanisms as mutually exclusive, it is possible that they may assume accessory roles. Pathways such as those involving deamination by activa-

tion-induced cytidine deaminase (6, 14, 15) may serve as bypass routes to cytosine in specific physiological settings such as primordial germ cells. Given the multitude of ways to manipulate cytosine, it is fortunate that a framework for demystifying demethylation is now at hand.

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MATERIALS SCIENCE

Through Thick and Thin

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The ratio of shear stress to shear rate in a flowing fluid defines its viscosity, or resistance to shear. For a Newtonian fluid like water, the viscosity is constant. Such simple behavior can change drastically, however, when small particles are suspended in the liquid. In some instances, the viscosity decreases with increasing shear rate and the fluid is said to exhibit shear thinning. For applications such as paints, this is desirable because it keeps suspended pigments on the painted surface at rest but lets them flow easily when brushed. There is the opposite possibility of shear thickening whereby the viscosity increases with shear rate. For some suspensions, such as cornstarch in water, this effect can be so dramatic that a person can run across the surface of a pool filled with the suspension, but sinks when standing still. Such non-Newtonian flow behaviors are thought to be caused by changes in the particle arrangements under shear. To investigate

this, Cheng *et al.* (1), on page 1276 of this issue, report direct measurements of particle arrangements while moving between regimes of shear thinning and thickening.

A textbook example of the role of particle arrangement in driving the behavior of suspensions is shear thinning resulting from the organization of particles into layers oriented along the direction of flow in which they can slide over each other more easily than if they were randomly distributed (2). Similarly, it was predicted that shear thickening could result from the formation of particle clusters (3, 4). These “hydroclusters” form when the particles are pushed together by shear and can bunch together transiently as a result of large viscous drag forces in the thin lubrication layers between the particles, which slow their separation.

To test these ideas of suspension rheology, Cheng *et al.* developed a particularly fast and sensitive confocal rheometer that allows them to track the three-dimensional locations of individual, 1- μm -diameter particles suspended in a liquid while simultaneously shearing the sample and measuring the stresses. They can quantify subtle changes in

New experimental results probe the relationship between rheology and particle-scale structure of suspensions.

the local particle arrangements as the sample transitions between shear-thinning, Newtonian, and shear-thickening regimes. Their data lead to two important results. First, the viscosity decrease during shear thinning can be quantitatively characterized as the sum of two contributions: a constant, Newtonian portion resulting from viscous stresses, and an entropic contribution that comes from the pressure produced by random collisions of particles under thermal motion, which decreases with shear rate. Second is the first direct experimental verification of clusters of nearly touching particles that grow as the suspension thickens, consistent with earlier predictions (3, 4).

Not all suspensions show all the flow regimes seen in the model system investigated. For example, some suspensions do not shear thicken, some do not have any appreciable intervening Newtonian regime, and others do not exhibit entropic effects. However, the particle-scale detail revealed by the elegant Cornell experiments informs the broader problem in rheology of how to attribute suspension properties to particle interactions on the one hand and structural

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