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that by itself warrants future studies. After $IP_3$-triggered $Ca^{2+}$ release from the ER, the uptake of $Ca^{2+}$ into MFN2-deficient mitochondria was reduced markedly. These findings reveal a striking correlation between the number of ER-mitochondria contact zones and $Ca^{2+}$ uptake into mitochondria and therefore provide direct evidence that a close apposition of both organelles is crucial during $Ca^{2+}$ signaling. This may have important implications for mitochondrial movement, which varies in response to energy demand and other regulatory cues. $Ca^{2+}$-binding proteins at the mitochondrial surface that mediate the attachment of motor proteins to mitochondria have been proposed to sense local $Ca^{2+}$ oscillations (Pizzo and Pozzan, 2007). In this way, impaired ER tethering due to mutations in MFN2 may result in mitochondrial transport deficiencies.

The dual activity of MFN2 unraveled by de Brito and Scorrano could therefore be of direct relevance to our understanding of the pathogenesis of CMT2A, which is characterized by altered axonal transport of mitochondria (Baloh et al., 2007). A number of pathogenic MFN2 mutations, especially those affecting residues not conserved between MFN1 and MFN2, did not abolish the fusion activity of MFN2 but cause mitochondrial aggregation when overexpressed (Detmer and Chan, 2007). The corresponding region in MFN2 including a Ras-binding domain is now demonstrated to be required to maintain a normal ER morphology in mouse embryonic fibroblasts (de Brito and Scorrano, 2008). However, the presence of ER membranes in axons is controversial, and it appears likely that they are restricted to dendrites (Ye et al., 2007). Juxtaposition between ER and mitochondria may therefore be of critical importance for the transport of mitochondria into dendrites, explaining the accumulation of fragmented mitochondria at zones of dendritic outgrowth in MFN2-deficient Purkinje cells (Chen et al., 2007). How then can deficiencies in axonal transport be explained? Perhaps some MFN2 mutations are gain-of-function mutations sequestering mitochondria to ER membranes. Clearly, further studies are required to resolve the pathogenesis of CMT2A caused by MFN2 mutations. The dual activity of MFN2 in mitochondrial fusion and ER tethering may point the way.

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# DNA Cytosine Demethylation: Are We Getting Close?

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**Whether 5-methylcytosine (meC) can be enzymatically removed from vertebrate DNA has been the subject of extensive study and also some controversy. Rai et al. (2008) now report that cytosine demethylation can be accomplished in a one-cell zebrafish embryo by the combined action of a cytidine deaminase and a thymine DNA glycosylase.**

In eukaryotes, transcriptional activity of genes is often controlled by methylation of cytosines at CpG dinucleotides in their promoter regions. In general, densely methylated promoters are silenced, most likely through the binding of repressor proteins, whereas unmethylated promoters are largely active. Methylation patterns can change during fertilization, development, differentiation (Suzuki and Bird, 2008), and transformation (Baylin and Bestor, 2002). Although this phenomenon has attracted

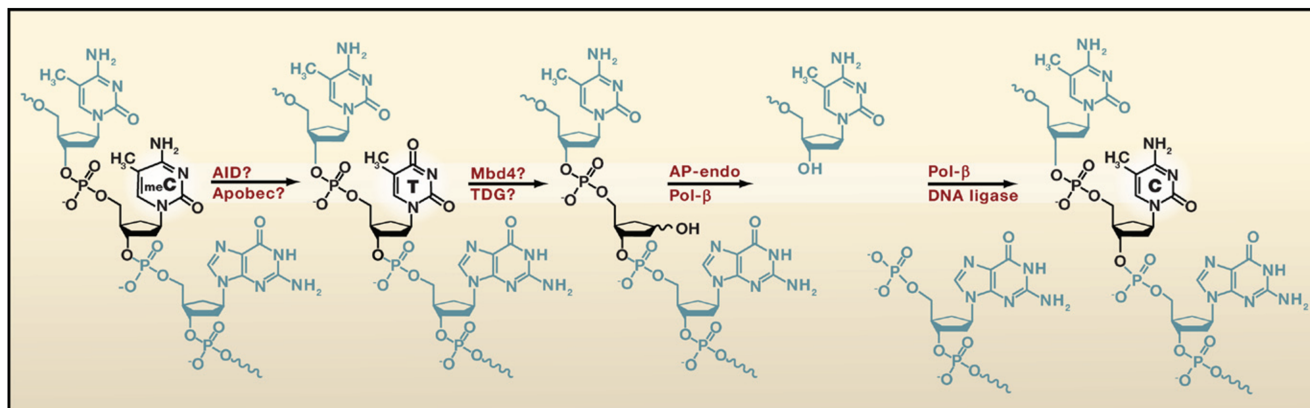


**Figure 1. A Potential Mechanism of Cytosine Demethylation**

In the first step, an enzyme of the Apobec family demethylates 5-methylcytosine (meC) to thymine. Because this thymine is mispaired with guanine in the opposite strand (not shown), it is recognized by a thymine DNA glycosylase and removed through cleavage of the glycosidic bond. The remaining apyrimidinic site (AP site) is cleaved by an AP endonuclease to generate a strand break. Polymerase-β then removes the abasic sugar-phosphate and inserts a deoxycytidine residue. The remaining nick is sealed by a DNA ligase. Note that cleavage of the AP sites in opposite strands, generated by the concurrent removal of both thymines, would give rise to a cytotoxic DNA double-strand break.

a great deal of attention, its control has been puzzling epigeneticists for more than four decades. In this issue, Rai et al. (2008) propose a two-step mechanism that mediates the enzymatic removal of methylated cytosine in zebrafish embryos.

5-methylcytosine (meC) is not incorporated into DNA by polymerases but is generated by the transfer of a methyl group by DNA methyltransferases to the 5-position of cytosine, using S-adenosylmethionine as the methyl donor (Goll and Bestor, 2005). DNA methyltransferases can either increase methylation density by modifying unmethylated CpG sites de novo or simply maintain the status quo by modifying CpG dinucleotides in the nascent, unmethylated strand after DNA replication, thus ensuring that a given methylation pattern is passed on to subsequent generations.

Methylation density can also be decreased. In a scenario known as “passive demethylation,” failure to methylate CpGs in the newly synthesized strand would result in 50% of progeny DNA lacking methylation on either strand after just two replication cycles. Several lines of evidence suggest that DNA can also be “actively” demethylated, that is in the absence of replication, but the mechanism of this reaction has remained enigmatic (Ooi and Bestor, 2008). The methyl-binding domain (Mbd) protein Mbd2 was initially reported to catalyze a direct removal of the methyl group from the 5-position of meC, but this energetically highly unfavorable process could not be reproduced in other laboratories. There is, however, another possibility: rather than converting meC to cytosine by removing the methyl group, meC could be converted to another base that does not belong in DNA and that can therefore be excised by a DNA repair enzyme. The most popular scenario involves the hydrolytic deamination of meC to thymine, where thymine could be removed from DNA by a thymine DNA glycosylase, such as TDG or Mbd4/Med1. It has been suggested that DNA methyltransferases could catalyze the meC to thymine conversion themselves—albeit in the absence of the methyl donor S-adenosylmethionine. This theme was recently revived (Metivier et al., 2008), but the reaction is extremely inefficient and therefore appears unlikely to be involved in global demethylation in vivo, especially given the omnipresence of S-adenosylmethionine.

Rai et al. now describe efficient demethylation of an in vitro-methylated ~740 bp DNA fragment upon microinjection into a one-cell zebrafish embryo. The process depends on the combined action of a cytosine deaminase (AID [activation-induced deaminase] or Apobec2a) and a thymine DNA glycosylase (Mbd4). Moreover, the authors build on the findings from the Niehrs laboratory, which implicated Gadd45α (growth arrest and DNA-damage-inducible protein 45 alpha) in DNA demethylation in *Xenopus laevis* oocytes (Barreto et al., 2007): Rai et al. show that Gadd45α increases the efficiency of the demethylation reaction. Interestingly, microinjection of larger amounts of the methylated DNA fragment also simulated the demethylation of several genomic loci and decreased the global meC content of the genomic DNA by ~10%. The suggested mechanism posits that meC is first deaminated to thymine by one of the Apobec class enzymes and that the thymine is then efficiently removed by Mbd4 due to its mispairing with guanine (Figure 1). The potentiating effect of Gadd45α is well documented, but no mechanistic insight is offered.

Careful consideration of the mechanistic aspects of the above process raises a host of questions. The first concerns the deamination reactions. Apobecs are efficient in deaminating cytosines in certain sequence contexts, but always in single-stranded RNA (Apobec1) or single-stranded DNA (AID) because the amino group that has to undergo hydrolysis is hydrogen bonded to guanine in double-stranded substrates (Conticello et al., 2007). Of course, double-stranded DNA can be made transiently single stranded during replication or transcription, but the substrate used in the current experiments, a linear ~740 bp DNA duplex, is neither replicated nor transcribed.

The second caveat concerns the action of Mbd4. This glycosylase removes thymines from G/T mispairs to generate abasic sites that are rapidly cleaved by an AP endonuclease (so named because it cleaves on the 5′ side

of an apyrimidinic site). In a symmetrically methylated CpG, deamination of both strands would give rise to a TG/GT double mispair. Is this a substrate for Mbd4? If yes, it might be processed by the AP endonuclease to generate a double-strand break, and this would almost certainly result in a loss of the microinjected substrate. The apparent absence of degradation suggests that the deamination events must be consecutive rather than concurrent, but would the Apobecs deaminate meC in a hemimethylated CpG? Most importantly, Apobecs deaminate cytosine much more efficiently than meC, and Mbd4 removes uracil from G/U also more rapidly than thymine from G/T (Hendrich et al., 1999). So why wasn't the microinjected substrate degraded? And why wasn't the substrate substantially mutagenized when Mbd4 was downregulated by a short-interfering RNA?

Other questions concern the phenotype of Mbd4-deficient cells. Given the deleterious consequences of perturbations in DNA methylation during development, it might be anticipated that a fertilized egg unable to demethylate the paternal nucleus (Oswald et al., 2000) would not develop into a viable organism. Yet, mice lacking Mbd4 are born healthy. This suggests that, at least in higher organisms, there is a redundant activity that compensates for the lack of Mbd4, such as the other thymine DNA glycosylase, TDG. And what about the XPG endonuclease implicated in the process observed by Niehrs and colleagues (Barreto et al., 2007)? It is also surprising that none of the mechanisms implicating the deamination of meC to thymine followed by thymine excision and substitution with deoxycytidine actually showed that the latter nucleotide is indeed incorporated into the demethylated DNA.

In sum, the experiments described in the detailed and extensive study by Rai et al. are highly convincing yet leave a feeling that there is a great deal more to the observed demethylation process than suggested by the simple three-protein scheme. We look forward to what future experiments will reveal about the uncertainties concerning DNA cytosine demethylation in vertebrates.

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# A Charged Performance by gp17 in Viral Packaging


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**Packaging of viral genomes into virus capsids requires powerful motors to overcome the repulsive force that builds as the nucleic acids are compressed. Through structural analyses of the T4 bacteriophage packaging motor gp17, Sun et al. (2008) now propose a packaging mechanism in which electrostatic forces cause the motor to alternate between tensed and relaxed conformational states.**


In cells, motor-driven movement usually occurs by transformation of chemical energy stored as ATP into mechanical energy through the activity of an ATPase domain (Rayment et al., 1993). The trick to understanding these molecular motors is to envision how the free energy released from hydrolysis of the ATP bond between the $\beta$ and $\gamma$ phosphates is coupled to work performed by the attached functional modules. Although these modules vary between different motors, the underlying structural biochemistry involved in the coupling process may be more general. In