Non-catalytic functions of DNMT1

Jesús Espada

Instituto de Investigaciones Biomédicas “Alberto Sols” CSIC-UAM, Departamento de Bioquímica, Universidad Autónoma de Madrid, and Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain

Address for correspondence:
Instituto de Investigaciones Biomédicas “Alberto Sols” CSIC-UAM
Arturo Duperier 4
28029 Madrid
Spain
e-mail: jespada@iib.uam.es

Key words: DNA methylation, DNA methyltransferase 1, E-cadherin, β-catenin, SNAIL1
ABSTRACT

Mammalian DNA methyltransferase 1 (DNMT1) is essential during early embryo development. Consistent with its key role in embryogenesis, depletion of this protein in adult somatic cells promotes severe cellular dysfunctions and cell death. DNMT1 contains a highly evolutionary conserved C-terminal catalytic DNA methyltransferase domain that is thought to be the responsible for the maintenance of CpG methylation patterns in the genome. DNMT1 has also a large N-terminal region with different functional protein-protein and protein-DNA binding domains. The multi-domain N-terminal region and the abundant molecular binding patterns suggest potential non-catalytic functions for DNMT1. However, this hypothesis remains controversial and conflicting results can be found in the literature. Here, recent results presenting a functional role for DNMT1 independent of its catalytic domain are discussed.
Methylation of the C-5’ position of cytosine (5mC) is a major epigenetic landmark of the DNA molecule that can be found in all vertebrates and flowering plants, in many bacterial species and in some invertebrates, fungi and protists (Goll and Bestor, 2005). As a rule, genomes of higher eukaryotes present extensive methylation at CpG dinucleotides and a global GC content that is lower than expected as a result of the cytosine to thymine transition mutations induced by cytosine methylation. Exceptions to this general rule are short stretches of DNA showing a high content of non-methylated CpG nucleotides, known as CpG islands. CpG islands serve as essential genomic regulatory elements for the transcriptional activity of a large proportion of annotated genes and non-coding RNAs (Deaton and Bird, 2011).

In higher eukaryotes, it is thought that methylation of CpG dinucleotides is intimately related in two ways to the regulation of transcription and, hence, to the configuration of particular outputs of cellular activity. At the chromatin fibre level, methylation of cytosine at CpG islands in promoter regions has been associated to a direct inhibition of the transcriptional machinery binding at transcription start sites and to a recruitment of 5mC-recognising transcriptional repressor complexes at these sites (Deaton and Bird, 2011). Cytosine methylation has a well-known key role in the transcriptional silencing of imprinted genes (Deaton and Bird, 2011; Goll and Bestor, 2005). However, it is worth to note that transcriptional silencing during normal development associated to CpG methylation of CpG island-containing promoters has been demonstrated for only a scarce number of non-imprinted genes (Deaton and Bird, 2011; Goll and Bestor, 2005). On the other hand, at a large scale genomic organization level, three-dimensional positioning in the nuclear compartment is globally associated to transcriptional activity (Fraser and Bickmore, 2007; Misteli, 2007). In this context, it is becoming clear that the extensive CpG methylation patterns of large genomic regions, mostly repetitive elements such as SINE, LINE and satellite families, which embrace the most part of 5mC in the genome of higher eukaryotes, can have a significant role in the positioning process of chromosome territories and nuclear components in the nuclear compartment (Espada and Esteller, 2009).
Despite the reported implication of CpG methylation in the regulation of gene expression, the precise role of this biochemical process during embryo development and in the regulation of cell function in adult tissues is not clear. As mentioned above, with the exception of imprinted genes, only a few genes containing CpG islands in the promoter region are silenced by CpG methylation. For many of these genes, active transcriptional silencing precedes cytosine methylation, suggesting that methylation acts as a mechanism to maintain silenced genes in a stable and heritable repressed state rather than a mechanism for a dynamic regulation of gene expression. On the other hand, organisms showing extensive genomic DNA methylation and organisms having little or no 5mC share conserved molecular signalling circuits that have key roles during development (Goll and Bestor, 2005). This fact suggests that the biochemical methylation of cytosine is not an essential process during the development of pluricellular organisms. However, it has been reported that extensive loss of genomic CpG methylation results in cell death during mammalian embryo development and in mammalian adult somatic cells (Chen et al., 2007; Egger et al., 2006; Li et al., 1992; Spada et al., 2007). These observations indicate that, at least in some organism, a correct pattern of genomic CpG methylation is required for cell function.

The establishment of the novo CpG methylation patterns in the DNA, as well as the maintenance of these patterns through successive DNA replication rounds, is catalyzed by the DNA methyltransferases (DNMT) protein family. In mammals, three types of DNMTs have been identified so far, namely DNMT1, DNMT2 and DNMT3 (including DNMT3A, DNMT3B and DNMT3L forms)(Bestor, 2000; Goll and Bestor, 2005; Jurkowska et al., 2011). All these types share 10 conserved sequence motifs that configure a defined catalytic domain which is highly homologous to equivalent domains in different species, ranging from higher eukaryotes to bacteria. Indeed, this catalytic domain points a basic canonical sequence that serves to define this protein family (Goll and Bestor, 2005). Interestingly, genetic evidence indicates that the canonical DNMT catalytic domain has evolved from a bacterial DNA methyltransferase belonging to the type II restriction-modifications systems. These bacterial
systems confer resistance to viral infections but are not implicated in the regulation of gene expression (Goll and Bestor, 2005).

Mammalian DNMT1 was the first identified and the most abundant DNMT type in mammalian somatic cells (Bestor, 2000; Goll and Bestor, 2005). This enzyme is present at functional replication foci, showing a strong preference for hemimethylated DNA, and is, therefore, considered as the protein responsible for the maintenance of global methylation patterns in the genome. Mammalian DNMT1 shows a conserved C-terminal catalytic domain and a large N-terminal domain that contains different regulatory motifs, including DMAP1 and PCNA interaction domains, a replication foci-targeting domain, a KEN box/Zn finger domain and two bromo-adjacent homology (BAH) domains (Fig. 1A) (Jurkowska et al., 2011). The abundance of protein-protein and protein-DNA binding domains in the N-terminal region of DNMT1 indicates that this protein is potentially involved in a complex network of regulatory circuits in the cell nucleus, a fact that has been confirmed by different reports in the last years (Jurkowska et al., 2011).

The functional relevance of DNMT1 during mammalian embryo development and in the homeostasis of adult tissues is highlighted by genetic experiments in mouse models and human somatic cells. Deletion of both Dnmt1 alleles in the mouse results in embryonic lethality by day 8.5 post coitum (Li et al., 1992). Embryos showed an almost complete (up 95%) demethylation of the DNA molecule. Notably, this rate of DNA demethylation has no apparent effect on cell viability from day 0 to day 8.5 post coitum, during which time two sequential waves of complete demethylation and “de novo” methylation take place in the genome of the developing embryo. In line with this, mouse ES cells maintained in an undifferentiated state can survive to an almost complete CpG demethylation of the DNA molecule, but induction of a differentiation program in these demethylated ES cells rapidly results in cell death (Li et al., 1992). In a similar way, reduction of DNMT1 expression below a critical 20% threshold in human somatic (differentiated) cells results in extensive CpG demethylation, loss of cell viability and cell death after mitotic catastrophe (Chen et al., 2007; Egger et al., 2006; Spada et al., 2007). Equally important, a causal link between
DNMT1 mutations and human hereditary disease, namely a sensory neuropathy, has been recently reported (Klein et al., 2011).

The existence of functional roles for DNMT1 independent of its catalytic activity is a suggestive and plausible hypothesis when considering its large N-terminal domain and the plethora of molecular binding patterns attributed to this protein. However, no clear evidence to sustain such hypothesis has been reported to date and the matter has remained controversial in the literature. For example, it has been shown that depletion of mammalian DNMT1 can result in activation of gene transcription by a DNA methylation-independent mechanism (Milutinovic et al., 2004). In a similar way, it has been reported an essential gene silencing activity for xDnmt1 during early Xenopus leavis embryo development that is independent of its catalytic activity (Dunican et al., 2008). By contrast, it has also been claimed that a catalytic DNA methyltransferase activity is required for all biological functions of DNMT1 (Damelin and Bestor, 2007). In this context, it is to note that the ideal experimental framework to analyze potential non-catalytic functions of DNMT1 (and, by extension, of any other enzyme) is to introduce a catalytically inactive form of the enzyme in a defined cellular background. The complete elimination of the endogenous full-length enzyme is formally required in this experimental context, since all the non-catalytic domains of the endogenous protein can unmask the effects of the non-catalytic domains of the exogenous mutant protein. As mentioned above, a complete deletion of DNMT1 in committed mammalian cells results in cell death and, therefore, this experimental approach is not suitable to analyze non-catalytic functions of this protein. Different experimental designs and model systems are then required to elucidate non-catalytic functions of DNMT1.

In a recent report, an alternative experimental approach has been used to demonstrate that human DNMT1 can have biological functions that are independent of its DNA methyltransferase activity (Espada et al., 2011). In particular, it has been shown that the DMAP1/PCNA domains in the N-terminal region of DNMT1 are implicated in the transcriptional regulation of E-cadherin. Interestingly, the regulation of E-cadherin expression that occurs in this context is associated to a modulation of β-catenin signalling and can induce an
epithelial-to-mesenchymal transition. In this study, human cancer cells expressing low levels of a DNMT1 hypomorph that lacks exons 3-6 (DNMT1\(^{\Delta E3-6}\)) are used as experimental model system (Fig. 1A) (Egger et al., 2006; Rhee et al., 2000; Spada et al., 2007). This DNMT1 hypomorph can sustain a moderate catalytic activity that seems to be sufficient to maintain cell viability. However, DNMT1\(^{\Delta E3-6}\) expressing cells present an extensive and selective CpG demethylation at repetitive pericentromeric elements (Espada et al., 2004). Interestingly, this selective DNA demethylation pattern is associated to a shift in the histone H3 biochemical modification pattern in those regions and to an overall alteration of heterochromatin organization that results in an aberrant nuclear morphology (Espada et al., 2004). Demethylation of repetitive rRNA genes and a functional disorganization of the nucleolar compartment is also observed in DNMT1\(^{\Delta E3-6}\) expressing cells (Espada et al., 2007).

In the referred report it is shown that, while parental cells present well formed E-cadherin-dependent cell-cell contacts, DNMT1\(^{\Delta E3-6}\) expressing cells show a significant down regulation of the E-cadherin gene at the transcriptional level. This down regulation of E-cadherin results in a disorganization of Ca\(^{2+}\)-dependent cell-cell contacts, release of \(\beta\)-catenin from adherent junctions at the cell membrane, translocation of \(\beta\)-catenin to the cell nucleus and subsequent activation of Wnt transcriptional targets (Espada et al., 2011). This novel regulatory mechanism of E-cadherin expression strictly depends on the E-cadherin transcriptional repressor SNAIL1 and requires the interaction of this repressor with the DMAP1/PCNA domains of DNMT1. However, the mechanism is not associated to CpG methylation changes in either the E-cadherin or SNAIL1 promoters, which are otherwise completely demethylated in this cellular system (Espada et al., 2011). As the sole reported difference in the genetic background of DNMT1\(^{\Delta E3-6}\) expressing cells with respect to parental cell is the deletion of exons 3-6 in the DNMT1 gene, it is concluded that this region, encompassing the DMAP1 and PCNA domains, but not the catalytic domain, is the responsible for the regulation of E-cadherin expression in DNMT1\(^{\Delta E3-6}\) expressing cells.
Associated to the DMAP1/PCNA domains deletion in the N-terminal region of DNMT1, SNAIL1 binding to the *E-cadherin* promoter is strongly induced in DNMT1\(^{\Delta E3-6}\) expressing cells as compared to parental cells (Espada et al., 2011). It has also been previously reported that SNAIL1 can interact with and recruit the histone deacetylase modifier HDAC1 to the *E-cadherin* promoter to exert its repressor activity (Peinado et al., 2004). In agreement with this observation, DNMT1\(^{\Delta E3-6}\) expressing cells also shown a specific recruitment of HDAC1 to the *E-cadherin* promoter (Espada et al., 2011). On the other side, it was shown that a direct interaction can take place between the DMAP1/PCNA domain of DNMT1 and the SNAG domain of SNAIL1 (Espada et al., 2011). Taken into account these results, it can be hypothesized that the DMAP1/PCNA domain of DNMT1 can act as a binding domain to sequester the repressor SNAIL1/HDAC1 complex, thus preventing its interaction with the *E-cadherin* promoter (Fig 1B). An alternative splicing in the N-terminal region of DNMT1, deleting exons 2-6 but preserving the catalytic domain, can release the SNAIL1/HDAC1 repressor complex facilitating its interaction with the *E-cadherin* promoter (Fig. 1B). The presence of the C-terminal catalytic domain in the N-terminal spliced DNMT1 isoform assures the maintenance of essential CpG methylation patterns in DNMT1\(^{\Delta E3-6}\) expressing cells, preventing the induction of cell death (Fig. 1B). At the same time, the differential expression of this DNMT1\(^{\Delta E3-6}\) isoform can promote an epithelial-to-mesenchymal transition after an efficient *E-cadherin* gene transcriptional shut-down (Fig. 1B). Complete deletion of full-length DNMT1 can also result in the release of the SNAIL1/HDAC1 repressor complex, but the absence of a functional catalytic domain can compromise the maintenance of DNA methylation patterns promoting cell death (Fig. 1B).

The N-terminal region of mammalian DNMT1 encompassing DMAP1 and PCNA domains has been implicated in specific recognition of AT-rich regions and localization on Line-1 and satellite repeats (Araujo et al., 2001; Suetake et al., 2006). In the mouse, alternative splicing affecting the first N-terminal exons of the *Dnmt1* gene is a common phenomenon during oogenesis (Mertineit et al., 1998), muscle differentiation (Aguirre-Arteta et al., 2000) and in different germinal and somatic tissues (Gaudet et al., 1998; Lin et al., 2000; Sakai et al.,
Differential splicing affecting the first N-terminal region of *DNMT1* has been also reported in human cells and tissues (Bonfils et al., 2000; Hsu et al., 1999). These observations indicate that this region is especially prone to genetic regulation by alternative splicing. In this context, it is tempting to speculate that mammalian cells can use the differential splicing of the N-terminal region of DNMT1 as a common mechanism to regulate DNMT1 localization and function. This is a novel concept that has been reinforced by the experimental use of human cells genetically manipulated to force the expression of a DNMT1 isoform lacking a competent DMAP1/PCNA region but having a functional catalytic domain. As a whole, the observations discussed here suggest that the existence of biologically significant functions of DNMT1 that are independent of its catalytic activity is a plausible hypothesis. Further experiments are required to probe this hypothesis in “in vivo” systems.
ACKNOWLEDGEMENTS

The author is a Ramón y Cajal Program researcher supported by the Spanish Ministry for Science and Innovation (MICINN; SAF2008-00609).
REFERENCES


FIGURE LEGEND

Figure 1. Non-catalytic functions of DNMT1. A) Schematic representation of the DNMT1 full-length protein and of a DNMT1 isoform resulting from a differential splicing of exons 3 to 6, encompassing the DMAP1 and PCNA domains. The extension and localization of the different structural domains of DNMT1 are indicated. Representations are not drawn to scale. B) Hypothetical model depicting a potential non-catalytic function of DNMT1 that is implicated in the regulation of E-cadherin expression. The DMAP1 and PCNA domains in the N-terminal region of DNMT1 can interact with SNAIL1/HDAC1 repressor complex, impeding the interaction of this repressor complex with the E-cadherin promoter (upper panels). An alternative splicing deleting the DMAP1 and PCNA domains of DNMT1 promotes the release of the SNAIL1/HDAC1 complex that can then interact with and repress the activity of the E-cadherin promoter; down regulation of E-cadherin expression can promote an epithelial-to-mesenchymal transition (EMT) (middle panels). The presence of a fully active catalytic domain in this DNMT1 isoform assures the maintenance of DNA methylation patterns through successive DNA replication rounds, preventing cell death. However, full length deletion of DNMT1 can release SNAIL1/HDAC1 repressor complexes but also promotes an extensive demethylation of the DNA that ultimately results in cell death (lower panels).
Espada, 2011