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Dynamics, stability and inheritance of somatic DNA methylation imprints

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Abstract

Recent research highlights the role of CpG methylation in genomic imprinting, histone and chromatin modification, transcriptional regulation, and 'gene silencing' in cancer development. An unresolved issue, however, is the role of stable inheritance of factors that manage epigenetic imprints in renewing or expanding cell populations in soma. Here we propose a mathematical model of CpG methylation that is consistent with the cooperative roles of de novo and maintenance methylation. This model describes (1) the evolution of methylation imprints toward stable, yet noisy equilibria, (2) bifurcations in methylation levels, thus the dual stability of both hypo- and hypermethylated genomic regions, and (3) sporadic transitions from hypo- to hypermethylated equilibria as a result of methylation noise in a finite system of CpG sites. Our model not only affords an explanation of the persistent coexistence of these two equilibria, but also of sporadic changes of site-specific methylation levels that may alter preset epigenetic imprints in a renewing cell population.

Keywords: DNA methyltransferase (Dnmt); CpG dinucleotide; Hypomethylation; Bistability; Markov chain model

1. Introduction

The existence of CpG islands (i.e. cytosine–guanine dinucleotide rich regions) in mammalian genomes that otherwise have a roughly 5-fold lower density of CpG sites is prescient of their developmental and regulatory significance (Bird, 1980; Jones et al., 1992). Indeed, over 70% of human genes now appear to be flanked 5′ by CpG islands that encompass their promoters (Saxonov et al., 2006). While dispersed CpGs and intragenic CpG islands are predominantly 5-methylcytosine methylated in soma, the majority of promoter-associated CpG islands are hypomethylated and associated with transcriptional activity. In contrast, the formation and maintenance of repressive heterochromatin is positively correlated with hypermethylation. A case in point is the stable clonal propagation of DNA methylation patterns on the inactive X chromosome in somatic cells of female mammals (Gartler et al., 1985; Lyon, 1988; Riggs and Pfeifer, 1992). Although the induction of a repressive chromatin structure may not require DNA methylation per se, there is evidence that methylation is required for the preservation of such imprints in dividing cells (Mohandas et al., 1981). Similarly, genomic regions belonging to endogenous retroviruses and transposable elements such as short/long interspersed nuclear elements (SINEs/LINEs) are also heavily methylated and transcriptionally silenced (Smit, 1999). Thus, one of the critical functions of methylation-based epigenetic imprinting is to encode the transcriptional state of the cell and to provide a mechanism for the controlled (de)activation of regulatory genes during development and differentiation. Although the term 'imprinting' is typically used in reference to heritable epigenetic methylation marks in the germline, here we extend this term to include somatic epigenetic patterns

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whose maintenance is critical for the proper development of the organism.

How do dividing cells manage to preserve their epigenetic imprints? Loss of methylation by the conversion of fully methylated CpG/GpC dyads to hemimethylated dyads is a simple consequence of DNA replication (when unmethylated cytosines are incorporated into the daughter DNA strand opposite parental CpG sites, see Fig. 1). Thus the question arises, how do dividing cells maintain simultaneously hypo- and hypermethylated CpG regions? Once they have been established, how is it possible that these two states can coexist with sufficient stability in distinct regions of the genome?

Recent experiments show that there are at least three DNA methyltransferases (Dnmt's) that are involved in post-replicative methylation of CpG sites (e.g. see Okano et al., 1999). While de novo methylation is attributed to the action of the isoforms Dnmt3a and Dnmt3b, Dnmt1 appears mainly responsible for maintaining the parental methylation pattern by methylating the correct daughter CpGs, as shown in Fig. 1 (Kim et al., 2002; Chen et al., 2003; Vilkaitis et al., 2005).

In an early paper, Otto and Walbot (1990) provided a first description of methylation dynamics in terms of both de novo and maintenance methylation. Although the specific enzymes responsible for these processes had not yet been identified, their model predicted globally stable methylation equilibria in dividing cell populations as a consequence of the recurrent actions of DNA replication and joint de novo and maintenance methylation. A similar model (in continuous time, rather than discrete time) was put forward by Pfeifer et al. (1990). The former was recently improved by Genereux et al. (2005) to allow for differential de novo methylation between parental and daughter DNA strands, including the case when de novo methylation occurs on one strand but not the other. Their model was also formulated to allow parameter estimation via maximum likelihood based on counts of fully methylated, hemimethylated, and unmethylated CpG dyads (see also Laird et al., 2004). Their analysis of CpG methylation within the promoter of the human gene *FMR1* also confirmed previous estimates of methylation efficiencies that lacked multi-site information. However, no model of methylation dynamics has this far addressed the stability question raised above, in particular, the possibility of metastable equilibria in dividing cell populations.

Here we recast the model by Otto and Walbot (1990) in terms of a Markov chain process for which the steady-state equilibrium solutions can be readily computed. In doing so, we allow for a more general methylation dynamics, including asymmetry of DNA strand segregation. Site-specific transitions between the methylation states of a CpG dyad are associated with the rates of both de novo and maintenance methylation. The basic premise of the model is that after DNA replication Dnmt1 methylates any hemimethylated CpG dyad with probability ρ, while the combined role of Dnmt3a/b is that of methylating both hemi- and unmethylated CpG dyads with probability μ. Although there is evidence that members of these two Dnmt families cooperate, the details of their interaction are not well understood (Kim et al., 2002). However, weak de novo CpG methylation by Dnmt1 has been demonstrated in the absence of Dnmt3a/b in mouse embryonic stem cells (Lorincz et al., 2002), further complicating our model.

Analysis of the steady-state solutions of the linear methylation model, in which the actions of maintenance and de novo methylation are assumed independent, suggests that stable maintenance of hypomethylated states requires an exquisite repression of de novo methylation (i.e. $\mu < 0.01$). Although it has been hypothesized that the methyltransferases may require specific histone modifications possibly involving Lys9 methylation on H3, and presence of HDAC and/or HP1 for their activation, it is not clear how such complex modifications (or persistent lack of such modifications) would be faithfully inherited from one cell generation to the next (e.g. see the discussions in Wolffe et al., 1999; Jones and Baylin, 2002).

Here we propose an alternative solution to this question based on a modification of the basic Markov chain model that assumes cooperativity of the Dnmt's. Specifically, we propose that the efficiency of de novo methylation, μ, is dependent upon the region-specific density of hemimethylated sites immediately after DNA replication. Because the activity of Dnmt1, which has a preference

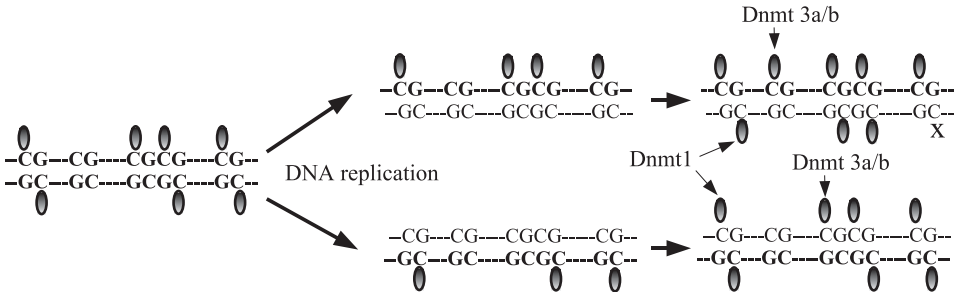

Fig. 1. Illustration of the CpG methylation model. DNA replication (left) leads to hemimethylated DNA double strands. After DNA replication, maintenance methylation (via Dnmt1) methylates hemimethylated CpG dyads, but occasionally fails to do so (marked 'X'). De novo methylation (primarily via Dnmt3a/b) is assumed to act either concomitantly or in tandem, and with some probability methylates any unmethylated CpG site (indicated by lightly shaded ovals).