REVIEW

The Long (LINEs) and the Short (SINEs) of It: Altered Methylation as a Precursor to Toxicity

Ammie N. Carnell and Jay I. Goodman

Department of Pharmacology and Toxicology, Michigan State University, B-440 Life Science Building, East Lansing, Michigan 48824

Received March 28, 2003; accepted April 28, 2003

Although once thought of as “junk” DNA, the importance of interspersed elements in the genome has become increasingly appreciated in recent years. In a broad sense these are collectively referred to as transposable elements, which encompass both transposons and retrotransposons. The latter include long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). Expression of these elements leads to genetic instability. Therefore, it is important that they remain transcriptionally silenced, and DNA methylation plays a key role in this regard. A framework for understanding the possible interplay between altered DNA methylation, an epigenetic change, and mutational events is presented. A case is made as to how retrotransposable elements, specifically LINEs and SINEs, are likely to emerge as key players in furthering our understanding of mechanisms underlying a variety of toxicities, including carcinogenesis but not limited to this endpoint.

Key Words: DNA methylation; epigenetics; LINEs, retrotransposons; transposable elements; SINEs.

Overview of Retroelements

Conventional thinking has supported the notion that mutation/altered genome stability, produced in response to treatment with a chemical or physical agent, has as its basis one or a combination of the following: adduct formation, inhibition of apoptosis, strand breaks in DNA, altered DNA repair, and increased cell proliferation resulting in an increased chance for “errors” during DNA replication (Preston and Hoffmann, 2001). However, another key factor may be involved. This review focuses on (1) mutagenesis and/or genome stability as a consequence of transcriptional activation of mobile elements and (2) how altered DNA methylation, an indirect, secondary mechanism, may underlie the increased expression of these elements. It is appropriate to reflect upon the toxicological significance of these mobile elements.

DNA methylation plays a key role in maintaining stability of the genome by silencing the expression of mobile, retrotransposable elements. Altered DNA methylation, a secondary, epigenetic change (Goodman and Watson, 2001), may facilitate expression of these elements leading to a cascade of detrimental events resulting in mutation and decreased stability of the genome. This review extends the general notion that the study of altered DNA methylation has come of age in toxicological sciences (Watson and Goodman, 2002) by focusing upon a specialized area. We put forth a framework for understanding the interplay between epigenetic and mutational events and indicate how retrotransposable elements are likely to emerge as an important player in furthering our understanding of mechanisms underlying a variety of toxicities, including carcinogenesis but not limited to this endpoint.
Wolffe, 2000). Altering the methylation status of repetitive elements may foster a surge of events leading to toxicity, and a consideration of this is the focal point of this review.

Retrotransposable Elements: LINEs and SINEs

Retrotransposable elements are categorized as either autonomous or nonautonomous elements, where autonomous refers to the property of self-sufficiency for mobility. There are two classes of autonomous elements: long terminal repeat (LTR) and non-LTR subgroups. LTR-containing elements are structurally similar to retroviruses although they lack a functional env gene. Non-LTR elements contain an internal promoter for RNA polymerase II, a 5′ untranslated region (UTR) and a 3′ deoxyadenosine (A)-rich tract. Nonautonomous elements (SINEs) contain an internal promoter for RNA polymerase III and a 3′ A-rich tract (Ostertag and Kazazian, 2001). Unlike LINEs, SINEs have an internal promoter for RNA polymerase III and a 3′ A-rich tract (Weiner, 2002). The most prominent SINEs are the human Alu elements and the rodent B1 elements.

Movement of Transposable Elements

Human L1 elements have been shown in vitro to ultimately move through a target-primed reverse transcription (TPRT) mechanism (Cost et al., 2002). Briefly, full-length L1 elements are transcribed from their internal promoter to produce a bicistronic mRNA (Ostertag and Kazazian, 2001). Association of a 7.5kb, sense strand mouse L1 RNA with protein from ribonucleoproteins (RNP) has been demonstrated (Eickbush and Malik, 2002). Up to several kb in length, the non-LTR retrotransposons are commonly referred to as long interspersed elements (LINEs). Structurally, they contain an internal promoter for RNA polymerase II, a 5′ untranslated region (UTR), two open reading frames (ORFs), and a 3′ terminal polyadenylation site (Loeb et al., 1986). The ORF1 protein is an RNA binding protein (Kolosha and Martin, 1997), while ORF2 encodes both a reverse transcriptase and a DNA endonuclease (Weiner, 2002). The most notable among the lines are the L1 elements, which are currently the object of a flurry of research concerning their mechanism of transposition, their role in the activation and movement of nonautonomous elements, and the health-related consequences of the movement of these elements within the genome.

An additional, important category of non-LTR interspersed elements is the nonautonomous retrotransposons. These are short interspersed elements (SINEs), which are approximately 80–400 bp in length and require activities encoded by the autonomous retrotransposons and/or the host for their mobility (Ostertag and Kazazian, 2001). Unlike LINEs, SINEs have an internal promoter for RNA polymerase III and a 3′ A-rich tract (Weiner, 2002). The most prominent SINEs are the human Alu elements and the rodent B1 elements.
scriptase or an endogenous enzyme (Evans and Palmiter, 1991) and integrated into the targeted site via TPRT (Ostertag and Kazazian, 2001). Truncations, producing inactive elements, frequently occur just prior to integration as a consequence of RNase H digestion or the inability of the reverse transcriptase to efficiently copy the entire L1 RNA before dissociating (Ostertag and Kazazian, 2001). These L1 element fragments are functionally inactive and hence “litter” the genome. Additionally, LINEs may facilitate the movement of SINEs. Recently, using a HeLa cell retrotransposition assay, an eel (Anguilla japonica) SINE with a similar 3’ end to a partner eel LINE was mobilized by the retrotransposition machinery of that partner LINE (Kajikawa and Okada, 2002). This, then, supports the notion that SINEs may exploit LINEs as a mechanism of retrotransposition.

Distribution and Abundance

Overall, approximately 33% of the human genome consists of transposable elements (Yoder et al., 1997), and they are distributed in a nonrandom fashion. According to the Human Genome Project Data, ~26% of the sequences on the X chromosome are L1 sequences, while the percentages in chromosomes 21 and 22 are approximately 13% and 10%, respectively (Hattori et al., 2000). In 2001, Ovchinnikov et al. hypothesized that distribution of L1 elements occurs as a result of selection either for the retention of L1s in regions of low GC content or for the loss of L1s from regions of high GC content. The same reasoning may not apply to SINEs, in particular Alu elements. Imprinted regions of the genome carry a very low density of SINE elements such as Alu elements (Greally, 2002). This raises questions concerning the accessibility of LINEs to imprinted regions versus a possible active selection process maintaining the intolerance to SINEs (Greally, 2002). The importance of these elements has been stressed in the context of mammalian genomes, but it should be noted that they, particularly SINEs, are present in both mammalian and nonmammalian species (Borodulina and Kramerov, 1999).

DNA Methylation Silences Transposable Elements

It is instructive to consider the role of altered methylation as an epigenetic mechanism for the activation of retrotransposable elements leading to their expression and possible retrotransposition (Fig. 2). Given the sheer number and distribution of these elements, both their movement and expression can lead to unstable conditions within the genome. The adverse consequences associated with the movement of these elements are diverse and will be discussed below. In order for the genome to maintain its integrity, retrotransposable elements need to be regulated. Methylation of cytosines to produce 5-methyl cytosine plays an important role as a transcriptional regulator (Jones and Baylin, 2002; Roberston and Wolffe, 2000). In general, increased methylation is associated with decreased gene expression. Indeed, in mammalian cells, methylation appears to be the prime regulator in terms of inhibition of transcription and formation of heterochromatin (Fahrner et al., 2002). The consequences of aberrant DNA methylation in terms of LINEs and SINEs is potentially far reaching when taking into account that methylation may regulate expression and movement of not only the parent LINEs but also SINEs (Kajikawa and Okada, 2002).

The concept of methylation as a genome defense system assumes that retrotransposable elements are inherently detrimental to the genome. Protection and conservation of the integrity and fidelity of an organism’s DNA serves as the overriding goal. Therefore, an important aspect of DNA methylation is its connection to the host-defense system, which acts to offset the threats from these largely parasitic sequences by maintaining them in a methylated, transcriptionally silent state (Yoder et al., 1997). Genome instability is a common feature of tumorigenesis (Loeb, 2001). Extensive hypomethylation results in genome instability reflected by an increase in mutation frequency (Chen et al., 1998). Hypomethylation-induced transcriptional activation of LINEs and SINEs contributes to this instability (Takai et al., 2000). Furthermore, hypomethylation of LINE-1 sequences has been observed in various cancers, such as colon cancer and chronic lymphocytic leukemia (Dante et al., 1992). More recently, when nine human hepatocellular carcinoma samples were tested, eight samples showed evidence of L1 hypomethylation. Even more interesting is the finding that hypomethylated L1 sequences in the surrounding noncancerous liver tissue were absent (Takai et al., 2000). The story is much the same for urothelial carcinomas, where hypomethylation of the L1 sequences is consistently seen as compared to the normal bladder mucosa (Jürgens, 1996). This led to the speculation that demethylation of LINE-1 sequences may promote genomic instability and facilitate tumor progression (Jürgens, 1996).

An early study which looked at the link between DNA methylation and gene expression found that infectious proviral DNA could be generated following removal of methyl groups from an inactive viral genome (Harbers et al., 1981). This initial look at the transcriptional regulation of an endogenous retroviral genome helped to spur a parallel interest in DNA methylation influence over retrotransposons. L1 sequences in the mouse, human, and rat have been characterized structurally as having a promoter region which controls the expression of the two open reading frames, ORF1 and ORF2 (Nur et al., 1988; Severynse et al., 1992; Swergold, 1990). Expression of these regions, encoding an RNA binding protein, a reverse transcriptase, and an endonuclease, is required for integration of a new copy of the original element into the genome. For this reason, a closer look at the methylation status of the promoter region is a focus of investigation. A foundation was laid by Thayer in 1993 with the finding that hypomethylation of the 5’ region of L1Hs correlated with increased amounts of ORF1 protein, and specific nucleotides were alluded to as critical regulatory sites for methylation. Subsequent to these findings,
the effect of methylation on transcription was examined in vivo by transfecting a plasmid containing a L1 promoter linked to chloramphenicol acetyl transferase reporter (CAT) gene into HeLa cells and in vitro by performing transcription studies with mutagenized templates (Hata and Sakaki, 1997). Here it was found that methylation of the L1 promoter completely suppressed the transcription of the CAT gene, and methylation of four particular CpG sites was necessary and sufficient to inhibit L1 transcription in vitro (Hata and Sakaki, 1997).

SINEs, being much a counterpart to LINEs, also fall under the influence of methylation status. Certain human alu elements associated with the genes for α1 globin, tissue plasminogen activator, adrenocorticotropic hormone, and angiogenin, were found to display a high degree of methylation supporting the role of methylation in silencing SINEs (Kochanek et al., 1993). In addition, cell-free transcription experiments suggested that alu element transcription in vitro can be inhibited by 5′CG 3′ methylation (Kochanek et al., 1993). Alu sequences are structurally set up as a right and left monomer linked by an oligo-d(A) tract. The left monomer, only, contains the functional two box RNA polymerase III (pol III) promoter, but curiously no terminator sequence is found within the alu element; downstream RNA polymerase III terminators are utilized (Fuhrman et al., 1981). Based on artificial methylation of CpG sites, methylation anywhere within or near the internal pol III promoter can result in transcriptional repression (Liu and Schmid, 1993). However, the context of the methylation patterning may also positively affect the transcriptional re-

FIG. 2. Schematic representation of an epigenetic change as a precursor to expression and movement of retrotransposable elements. (1) An epigenetic change, e.g., hypomethylation of the retrotransposable element allows for (2) enhanced transcriptional activity. (3) RNA processing and (4) mRNA export ensue. (5) Translation and (6) posttranslational modification precede the formation of a ribonucleoprotein particle in which ORF1 and ORF2 encoded proteins are associated with the original mRNA. (7) Once entry into the nucleus has occurred, (8 and 9) reverse transcription and integration are achieved via the encoded reverse transcriptase and endonuclease through a mechanism termed target primed reverse transcription (TPRT). Modified from Ostertag and Kazazian, Jr. (2001), and reprinted with permission from the Annual Review of Genetics, vol. 35 © 2001, by Annual Reviews, www.annualreviews.org.
sponse (Vorce et al., 1994). It was postulated that transcriptional inhibition could be additionally dependent on a methylated DNA binding protein. The loss of inhibition and, in fact, activation may then be explained by high template concentrations, which effectively titrate out the methylated DNA binding protein (Vorce et al., 1994). One more possibility for selective control over these elements lies with the context of the surrounding regions. The methylation and chromatin patterns of regions or genes surrounding alu elements could also mediate the transcriptional activity of alu elements rendering transcriptionally competent elements incompetent (Schmid, 1991) or vice versa.

The mouse genome has proved useful for SINE research in utilizing the B1 and B2 elements, counterparts to human alu elements. On par with topics discussed for alu elements is that of the function and genomic target sites of de novo and maintenance methylation enzymes. When asking whether B1 elements provide a target for de novo methylation, an 838-base pair methylation center located upstream of the mouse adenine phosphoribosyltransferase gene was targeted. This region was previously described as serving as a de novo methylation signal upon transfection into mouse embryonal carcinoma (EC) cells (Yates et al., 1999). Two tandem B1 elements located at the 3’ end of the methylation center are methylated at relatively high levels in embryonic cells with severe maintenance methylation deficiency but intact de novo activity (Yates et al., 1999). Upon transfection, the B1 elements became methylated de novo, indicating a significant role in the methylation center (Yates et al., 1999).

Genomic Consequences

At the forefront of genomic consequences due to retrotransposon expression and movement is insertional mutagenesis. Insertion of these elements, whether random or targeted, represents a mutation, and therefore, retrotransposition poses a clear risk to the stability of the genome. Not only is movement of these elements critical but also their capability to transduce surrounding DNA sequences. At times this may promote genomic diversification (Moran et al., 1999), but more apparent is the possible contribution to mutagenicity. On a larger scale, L1 integrants and/or their transduced sequences can result in chromosomal rearrangements (Symer et al., 2002). Muscular dystrophy, characterized by a progressive loss of muscle strength which develops around age 10 in humans, has been associated with a L1 insertion within exon 48 of the dystrophin gene (Holmes et al., 1994). These findings supported recent L1 retrotransposition activity (Holmes et al., 1994) and directly demonstrated the consequential toxicity associated with the aberrant regulation of these elements. L1 insertions account for 13 isolated cases of human disease, while alu insertions account for 19 cases (Ostertag and Kazazian, 2001). For a comprehensive and detailed list of human disease resulting from movement of transposable elements, refer to Table 1 of Ostertag and Kazazian (2001). Additionally, altered regulation of gene expression by insertion of L1 elements as a direct mutation has been documented numerous times. Britten (1997) cited twenty-one examples of sequence element inclusion from Drosophila, sea urchin, human, and mouse genomes that serve a function in terms of transcriptional competency.

Counterpart to insertions are deletions and duplications, which can arise from unequal crossing-over and mispairing of homologous L1 sequences (Kazazian and Goodier, 2002). As much as a 3% frequency of DNA deletions due to L1 retrotransposition has been proposed (Kazazian and Goodier, 2002). Gilbert et al. (2002) observed a large deletion of the genomic DNA following the retrotranspositional event. A common mechanism preceding this deletion, among other alterations, was shown to involve cleavage of the genomic top strand. Variations of this model also suggest that chimaeric L1s, large deletions, and long duplications are also possible (Gilbert et al., 2002). Clinically, inactivating mutations arising from L1-mediated recombination can lead to the accumulation of mutations in specific target genes during cancer and development (Viel et al., 2002). This highlights the fact that L1 elements are capable of reshaping the genome through direct mutation.

Mutation as a result of movement of L1 elements is not the only method for creating instability within the genome. Aberrant activity in terms of expression of both LINEs and SINEs can result in altered gene expression and/or production of mRNAs bearing some sequence combination of the transposable element, the original gene, or both. Antisense promoters provide a recent example of the impact of altered gene expression (Speek, 2001). The power and capability of antisense promoters is not limited by distance from protein coding sequences, and they are scattered over the chromosomes, which only increases their importance in terms of transcriptional control and command (Speek, 2001). In addition to antisense promoters, transcriptional interference and, hence, altered gene expression, can arise when a retrotransposon interrupts a gene. This creates an environment for internal initiation via the transposable element promoter or disrupted transcriptional initiation via the endogenous promoter. Resulting from this, chimaeric mRNA molecules may be generated (Robertson and Wolffe, 2000). In simpler terms, retrotransposons can increase the level of nonfunctional or error-prone mRNAs. B2 SINEs were implicated in providing a pol II promoter at sites throughout the genome, likely a method for creating ectopic start sites leading to erroneous mRNA production (Ferrigno et al., 2001). SINEs scattered throughout introns allow for pol III-derived transcription of genes. In addition to this, pol II transcription initiated from neighboring regions may also alter the nature of mRNA products. Decreased mRNA levels and subsequent protein levels, along with truncated proteins, may also be a result of aberrant activity of LINEs and SINEs. Antisense mechanisms initiating degradation of the message have been
suggested as a possible cause of this (Robertson and Wolfe, 2000).

Toxicological Implications

Deviant transposable element activity has been presented as a possible mechanism underlying toxicity. The mutagenicity and/or altered genome stability resulting from expression and movement of transposable elements, in particular, LINEs and SINEs, may occur subsequent to epigenetic changes, e.g., altered DNA methylation. Interestingly, there are multiple ways that DNA methylation may be modified (Goodman and Watson, 2002). This can occur, for example, as a consequence of inhibition of DNA methyltransferases (Trinh et al., 2002), altered levels of methyl donors, and disruption of one-carbon metabolism (Sibani et al., 2002). Furthermore, DNA adduct formation can also lead to altered methylation, which might persist after the adduct is repaired (Turk et al., 1995). In this manner, there exists a possible interplay between epigenetic changes, mutation, and altered stability of the genome, bearing in mind that the altered DNA methylation leading to transcriptional activation of LINEs and SINEs may occur by a secondary, threshold-exhibiting mechanism (Goodman and Watson, 2001). Within this context, it is important to note that DNA methylation is more stable in human cells as compared to rodent cells (reviewed in Goodman and Watson, 2002). Therefore, compounds that produce toxicity in rodents, through a mechanism involving hypomethylation and consequent increased transcriptional activity of transposable elements, may have a relatively low potential to cause toxicity in humans. However, even though humans may possess a “built in safety factor” when compared to rodents, exploration of epigenetic mechanisms initializing transposable element activity should not simply be discounted as a basis for potential toxicity.

ACKNOWLEDGMENT

A.N.C. is a predoctoral fellow, supported by NIH-NIEHS Training Grant ES-04911.

REFERENCES


