DNA METHYLATION

Tying It All Together: Epigenetics, Genetics, Cell Cycle, and Cancer

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In eukaryotic cells, the methylation state of the base cytosine can be inherited without altering genetic material per se (*1*). This unusual—or "epigenetic"—form of inheritance generates patterns of DNA methylation that modulate overall genomic patterns of chromatin organization and gene expression. On page 1996 of this issue (*2*), Chuang *et al.* provide a potentially important entrée for understanding how, in humans, these patterns of DNA methylation are established and maintained. Further, their results show how epigenetic and genetic aspects of cancer might be married through events that control the cell cycle.

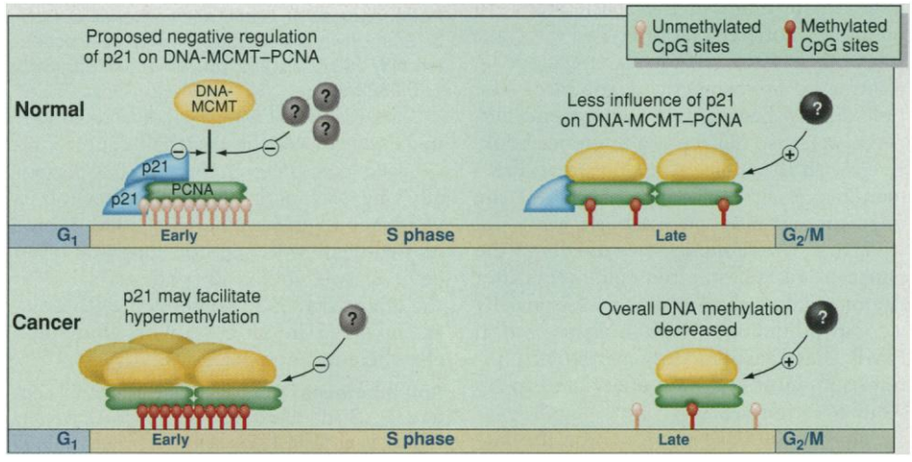
In higher order eukaryotes, DNA methylation and DNA-protein interactions together organize the genome into transcriptionally active and inactive zones (*3*). This organizational role is facilitated by an asymmetric pattern of DNA methylation. DNA methylation is absent in *Drosophila*, *Caenorhabditis elegans*, and yeast, but appeared as the vertebrate genome became more complex. Concurrently, during evolution, the CpG dinucleotide, the principal site of DNA methylation, has been selectively depleted through conversion of methylated cytosines to thymidines via a deamination process (*3*). The human genome has only 10% of the expected frequency of CpG's, and 70 to 80% of these are methylated (*3*). However, small regions of DNA remain (1 to 2%), termed "CpG islands," that are not CpG-depleted. These are rigorously protected from methylation and are associated with the transcription start sites in almost half, or some 40,000, human genes (*4*).

What is the purpose of this division of the genome? DNA methylation patterns closely correlate with patterns of gene expression. Heavily methylated DNA is generally associated with chromatin organization that is inhibitory to transcription (*3*). In humans, such repressed DNA often contains highly repeated sequences; methylation may help guard against transcriptional expression of these "parasitic" regions, which were introduced into the genome over evolution by transposable elements and DNA viruses (*5*). In contrast, the unmethylated CpG islands of most genes are associated with chromatin typical of highly transcribed DNA (*3*). But selected CpG islands are densely methylated. These regions have chromatin conformation typical of nontranscribed DNA and represent silenced alleles for mono-allelically expressed or "imprinted genes" (*6*) and for many genes on the transcriptionally inactivated X chromosome of the female (*3*). These normally silenced alleles can be expressed, and their CpG islands unmethylated, in mouse embryos with homozygous deletions of the DNA-methyltransferase (DNA-MCMT) gene, which encodes the major DNA-methylating enzyme (*7*). The methylation patterns generated by this enzyme are essential, because these mice die in early embryogenesis (*8*).

Cancer cells show altered patterns of DNA methylation (*9*). Overall DNA methylation is often decreased (*10*). This change may contribute to genomic instability (*11*). In these same tumors, the normally unmethylated CpG islands in the promoter region of critical genes can become densely methylated, and the associated transcriptional silencing is an epigenetic alternative to coding region mutations for causing loss of tumor suppressor gene function (*9*). Indeed, almost half of the suppressor genes known to underlie genetic forms of neoplasia—including VHL and p16 (*12*)—when mutated in the germ line exhibit CpG island hypermethylation in noninherited cancers.

What do the new findings of Chuang *et al.* reflect about DNA methylation? One mystery has been how DNA-MCMT activity is coordinated with DNA replication to maintain both normal and abnormal DNA methylation patterns. This enzyme, conserved from sea urchin to human (*13*), preferentially methylates DNA that is already methylated on one strand. Thus, during DNA replication, DNA-MCMT recognizes methylated CpG sites on the parent strand and methylates correlating cytosines on the daughter strand (*13*). Chuang *et al.* now suggest that binding of the enzyme to a protein, proliferating cell nuclear antigen (PCNA), coordinates DNA-MCMT activity and DNA replication and that this step is negatively regulated by the protein p21.

PCNA facilitates DNA replication by loading delta and epsilon DNA polymerases onto DNA in cycling cells and during DNA repair (*14*). In intact cells, DNA-MCMT and PCNA were found by Chuang *et al.* to colocalize to DNA replication foci in early S phase, the cycle period for DNA synthesis. Such complexes are absent in G_1, which precedes the onset of DNA synthesis. p21 could



Proposed control of DNA methylation. In normal cells, the p21 protein negatively regulates targeting of DNA-MCMT to PCNA, primarily in early S phase, and protects CpG islands from methylation. Diminished effects of p21 in late S phase may target DNA-MCMT to methylated DNA. Other local regulators of methylation help block methylation of CpG islands in early S (symbol with ?) and facilitate interaction of DNA-MCMT with heavily methylated late-replicating DNA (symbol with ?). In cancer cells, loss of p21 function allows increased DNA-MCMT more access, via PCNA, to DNA replication foci, possibly facilitating aberrant methylation of CpG islands. Decreased relative targeting to late S phase foci results in lost sites of normal methylation. Also, decreased activity of early S phase negative modulators of methylation and of late S phase positive regulators (symbol with ?) may facilitate the aberrant methylation patterns.

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regulate these interactions in several ways. First, p21 binds PCNA (*15*) through a region found by Chuang *et al.* to be similar to, and to compete with, the site in DNA-MCMT that mediates PCNA binding. Second, when complexed with PCNA, p21 inhibits DNA synthesis (*16*). Finally, p21 also forms complexes with the cyclins, proteins that activate a series of cyclin-dependent kinases (CDKs) that allow cells to cycle and synthesize DNA (*17*). p21, by inhibiting this activity, especially at the G_1/S border, participates in the cell's decision whether to synthesize DNA (*17*).

How might these dynamics mediate normal and abnormal DNA methylation? Perhaps, in normal cells, when PCNA targets DNA-MCMT to early DNA replication foci, strategic placement of, or amounts of, p21 negatively modulate the complex such that early replicating DNA regions like CpG islands (*18*) are protected from methylation (see figure, top panel). Similarly, a diminished effect of p21 later in S phase, at the peak of PCNA localization to DNA (*19*), might positively regulate DNA-MCMT at normal sites of methylation (see figure, top panel).

Further, as stressed by Chuang *et al.*, loss of p21 function is a very common event in human cancers. Normally, the transcription of p21 is directly stimulated by p53, in a pathway for sensing DNA damage and signaling cells to die or cease DNA synthesis until DNA damage is repaired (*20*). p53 mutations are common in human cancer (*21*) leading to loss of p21 function. Other pathways signal increases in p21 and may also be lost in tumors (*17*). Chuang *et al.* then propose that, when p21 losses are juxtaposed with increased DNA-MCMT activity, as in SV40 transformation, this imbalance contributes to altered DNA methylation.

The significance of tumor-associated increases in DNA-MCMT activity (*22*) has been debated. Normal cells increase DNA-MCMT activity during DNA synthesis and the increase in tumors could simply be a nonfunctional consequence of increased cell cycling (*23*). However, DNA-MCMT increases may actually be pivotal for driving tumorigenesis. Increases in DNA-MCMT activity and overall DNA methylation occur in specific cells after carcinogen exposure (*24*). Also, insertion of the gene for DNA-MCMT can cause cellular transformation (*25*) and hypermethylation of selected CpG islands (*26*). Genetically engineered lowering of DNA-MCMT activity can slow tumor progression (*27*). The loss of p21 might facilitate increased DNA-MCMT effects in tumors by simultaneously allowing unchecked cell cycling, increased DNA synthesis, and shifting of increased DNA-MCMT–PCNA complexes from late to early DNA replication (see figure, lower panel). Both abnormal gains and losses of DNA methylation sites might ensue. Further, Chuang *et al.* suggest that in tumors p21 loss from PCNA complexes could cause abnormal gains of methylation during repair of DNA damage. This is intriguing because both increased methylation of inserted gene sequences (*11*) and of endogenous gene CpG islands (*28*) have been found in a type of colon cancer associated with mismatch repair deficiency.

These new findings thus potentially bring together the fields of DNA methylation, cell cycle regulation, control of chromatin organization, and cancer genetics. However, the future challenge is to integrate them with other events that could modulate DNA methylation. First, p21 is only one of a number of related proteins that might modulate PCNA–DNA-MCMT (*29*). Second, other proteins likely influence DNA methylation such as transcription factors that may block access of DNA-MCMT to CpG islands (*30*). Some transcriptional coactivators alter chromatin organization by controlling the acetylation of histones and other proteins (*31*). Histone variant H1 inhibits the activity of DNA-MCMT for CpG-rich sequences (*32*). Further, proteins that bind preferentially to methylated cytosines could help guide DNA-MCMT to areas of normal methylation. One such protein MeCP1 (methyl-CPG binding protein) shares a domain with the enzyme itself and with a mammalian homolog to a chromatin modeling protein in *Drosophila* (*33*).

Finally, the initial unmethylated status of CpG islands lessens their affinity for DNA-MCMT, given its preference for hemimethylated DNA, and CpG-rich areas may be poor intrinsic substrates for DNA-MCMT (*34*). Also, specific demethylation events could help protect CpG islands (*35*). Exploring all of these interacting events, including the role of proposed additional DNA-MCMTs (*36*), will dominate DNA methylation research over the next years. The knowledge gained should prove invaluable for understanding how this DNA modification is essential for normal cell function and, when disrupted, can contribute to cancer and other disease states.

References and Notes

1. A. Bird, *Cell* **70**, 5 (1992).
2. L. S. H. Chuang *et al.*, *Science* **277**, 1996 (1997).
3. F. Antequera and A. Bird, in *DNA Methylation: Molecular Biology and Biological Significance*, J. P. Jost and H. P. Saluz, Eds. (Birkhauser Verlag, Basel, 1993), p. 169.
4. ———, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11995 (1993).
5. M. S. Turker and T. H. Bestor, *Mutat. Res.* **386**, 119 (1997); T. H. Bestor and B. Tycko, *Nat. Genet.* **12**, 363 (1996).
6. D. P. Barlow, *Science* **270**, 1610 (1995).
7. E. Li, C. Beard, R. Jaenisch, *Nature* **366**, 362 (1993).
8. E. Li, T. H. Bestor, R. Jaenisch, *Cell* **69**, 915 (1992).
9. P. W. Laird and R. Jaenisch, *Hum. Mol. Genet.* **3**, special issue no. 1487 (1994); P. A. Jones, *Cancer Res.* **56**, 2463 (1996); S. B. Baylin, J. G. Herman, J. R. Graff, P. M. Vertino, J. Issa, *Adv. Cancer Res.*, in press.
10. M. A. Gama-Sosa, *et al.*, *Nucleic Acids Res.* **11**, 6883 (1983); S. E. Goelz, B. Vogelstein, S. R. Hamilton, A. P. Feinberg, *Science* **228**, 187 (1985); A. P. Feinberg, C. W. Gehrke, K. C. Kuo, M. Ehrlich, *Cancer Res.* **48**, 1159 (1988).
11. C. Lengauer, K. W. Kinzler, B. Vogelstein, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 2545 (1997).
12. J. G. Herman *et al.*, *ibid.* **91**, 9700 (1994); A. Merlo *et al.*, *Nat. Med.* **1**, 686 (1995).
13. T. Bestor, A. Laudano, R. Mattaliano, V. Ingram, *J. Mol. Biol.* **203**, 971 (1988); J. A. Yoder, R. C. Yen, P. M. Vertino, T. H. Bestor, S. B. Baylin, *J. Biol. Chem.* **271**, 31092 (1996); K. L. Tucker, D. Talbot, M. A. Lee, H. Leonhardt, R. Jaenisch, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12920 (1996).
14. G. Prelich and B. Stillman, *Cell* **53**, 117 (1988); M. K. K. Shivji, M. K. Kenny, R. D. Wood, *ibid.* **69**, 367 (1992); B. Stillman, *ibid.* **78**, 725 (1994); A. Umar *et al.*, *ibid.* **87**, 65 (1996).
15. J. Chen, P. K. Jackson, M. W. Kirschner, A. Dutta, *Nature* **374**, 386 (1995); J. M. Gulbis, Z. Kelman, J. Hurwitz, M. O'Donnell, J. Kuriyan, *Cell* **87**, 297 (1996).
16. S. Waga, G. J. Hannon, D. Beach, B. Stillman, *Nature* **369**, 574 (1994); H. Flores-Rozas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8655 (1994).
17. S. J. Elledge, *Science* **274**, 1664 (1996); C. J. Sherr, *ibid.*, p. 1672.
18. S. Selig, K. Okumura, D. C. Ward, H. Cedar, *EMBO J.* **11**, 1217 (1992).
19. G. F. Morris and M. B. Mathews, *J. Biol. Chem.* **264**, 13856 (1989).
20. A. J. Levine, *Cell* **88**, 323 (1997); I. J. Ko and C. Prives, *Genes Dev.* **10**, 1054 (1996); W. S. El-Deiry *et al.*, *Cell* **75**, 817 (1993).
21. B. Vogelstein and K. W. Kinzler, *Cell* **70**, 523 (1992); M. Hollstein, *Science* **253**, 49 (1991).
22. J. P. Issa *et al.*, *J. Natl. Cancer Inst.* **85**, 1235 (1993); T. L. Kautiainen and P. A. Jones, *J. Biol. Chem.* **261**, 1594 (1986).
23. P. J. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10366 (1996).
24. S. A. Belinsky, K. J. Nikula, S. B. Baylin, J.-P. Issa, *ibid.*, p. 4045.
25. J. Wu *et al.*, *ibid.* **90**, 8891 (1993).
26. P. M. Vertino, R. C. Yen, J. Gao, S. B. Baylin, *Mol. Cell. Biol.* **16**, 4555 (1996); J. R. Graff, J. G. Herman, S. Myohanen, S. B. Baylin, P. M. Vertino, *J. Biol. Chem.* **272**, 22322 (1997).
27. P. W. Laird *et al.*, *Cell* **81**, 197 (1995).
28. N. Ahuja *et al.*, *Cancer Res.* **57**, 3370 (1997).
29. J. LaBaer *et al.*, *Genes Dev.* **11**, 847 (1997); C. J. Sherr and J. M. Roberts, *ibid.* **9**, 1149 (1995).
30. M. Brandeis *et al.*, *Nature* **371**, 435 (1994); D. Macleod, J. Charlton, J. Mullins, A. P. Bird, *Genes Dev.* **8**, 2282 (1994); P. Mummaneni, K. A. Walker, P. L. Bishop, M. S. Turker, *J. Biol. Chem.* **270**, 788 (1995).
31. P. A. Wade, D. Pruss, A. P. Wolffe, *Trends Biochem. Sci.* **22**, 128 (1997); M. J. Pazin and J. T. Kadonaga, *Cell* **89**, 325 (1997).
32. R. Santoro *et al.*, *J. Biochem.* **305**, 739 (1995).
33. P. M. Waring and M. L. Cleary, *Curr. Top Microbiol. Immunol.* **220**, 1 (1997); S. H. Cross, R. R. Meehan, X. Nan, A. Bird, *Nat. Genet.* **16**, 256 (1997).
34. T. H. Bestor, G. Gundersen, A. B. Kolsto, H. Prydz, *Genet. Anal. Tech. Appl.* **9**, 48 (1992).
35. M. Szyf, J. Theberge, V. Bozovic, *J. Biol. Chem.* **270**, 12690 (1995); A. Weiss, I. Keshet, A. Razin, H. Cedar, *Cell* **86**, 709 (1996).
36. H. Lei *et al.*, *Development* **122**, 3195 (1996).

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