cell proliferation. It seems reasonable to consider a possibility that some of the non-mutagenic carcinogens exert their action by epigenetic mechanisms. In this context, epigenomic analysis seems essential in toxicology, which has just started. Unfortunately, few reliable and sensitive methods specifically designed for toxicological analysis have been reported yet, and ordinary procedures for epigenetic and epigenomic analysis are used also for toxicological analysis. Their brief principles and efforts in development of convenient assay systems are described.

5.1 Principles of DNA Methylation Analysis

Methods can be divided into those for analysis of specific genomic regions and those for genome-wide analyses. DNA methylation at specific genomic regions is analyzed mainly based upon two principles of methylation detection; methylation-sensitive restriction enzymes, and bisulfite modification of DNA (Figure 8). Some restriction enzymes, such as *Hpa*II and *Sma*I, have recognition sequences with CpG sites, and cannot cleave if the CpG site is methylated. Bisulfite

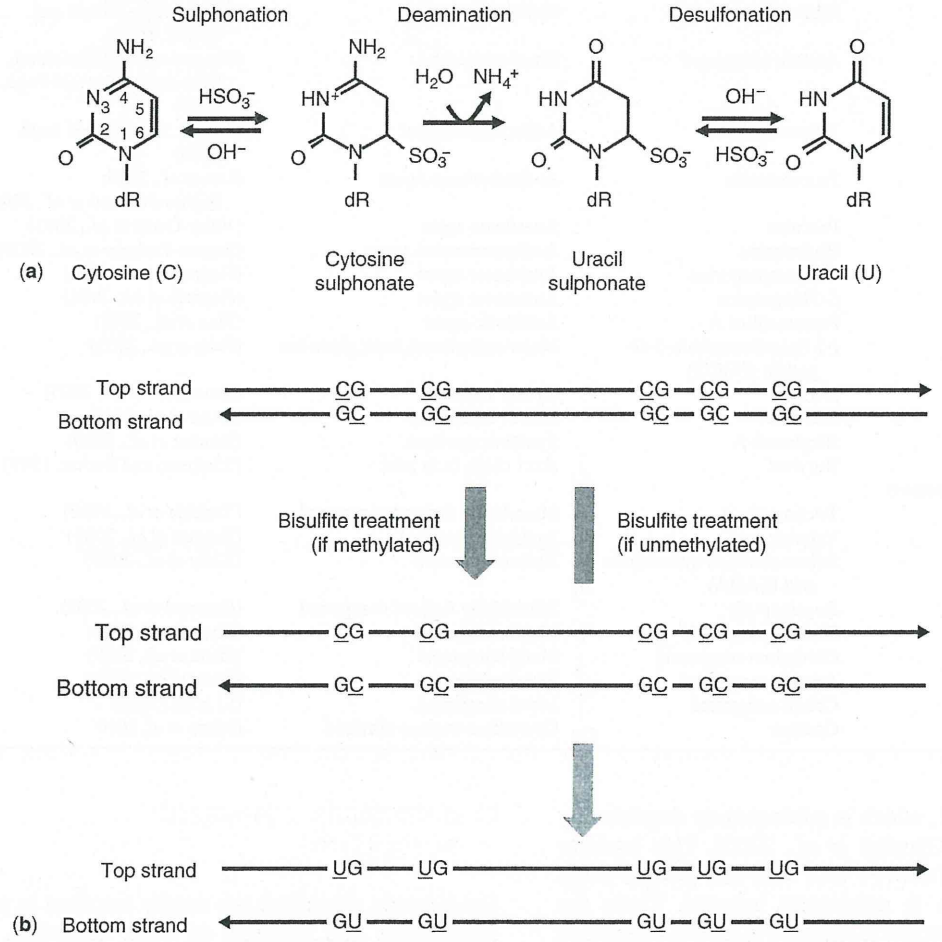

Figure 8. Principle of bisulfite modification: (a) chemical reactions for unmethylated cytosine; (b) sequence changes produced by bisulfite modification of methylated and unmethylated DNA. Different sequences are produced from methylated and unmethylated DNA, and the difference can be detected by various modalities.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

modification takes advantage of different efficiency in converting cytosine to uracil, which is very efficient for unmethylated cytosines but very slow for methylated cytosines. After bisulfite conversion, the top and bottom strands are no longer complementary. Methylated and unmethylated DNA will produce different sequences after the conversion, and the difference can be detected by various techniques, such as sequencing, allele-specific PCR, restriction digestion, and pyrosequencing. Depending upon the purpose of experiments, appropriate techniques should be selected, considering the required amount of DNA, flexibility in selection of CpG sites to analyze, how quantitative the method is, technical complexity, and the cost.

Genome-wide analyses are generally composed of a step of detection of DNA methylation and another step of genome-wide analysis (Ushijima, 2005; Laird, 2010). The methylation detection can be performed using affinity-based methods, such as use of anti 5-methylcytidine antibody and affinity column with methylated DNA binding domains, but also using methylation-sensitive restriction enzymes and bisulfite conversion. The detection step can be performed using microarray or next-generation sequencers.

5.2 Principles of Histone Modification Analysis

Methods for histone modification analysis can be divided into: (i) those for analysis of global contents of histone modifications within a cell; (ii) those for analysis of histone modifications for a defined genomic region; (iii) those for histone modifications of defined genomic regions in a genome-wide manner. Global contents of histone modifications within a cell are mainly analyzed by immunohistochemistry and Western blotting. In contrast, histone modifications in defined genomic regions are analyzed by chromatin immunoprecipitation (ChIP). All of these methods are based upon the recognition of histone modifications by antibodies, and their specificity is critical for successful analysis.

The ChIP method can detect physical interactions between histones containing a specific modification and genomic DNA within a cell (Figure 9). The ChIP method is composed of four steps including: (i) preparation of fragmented chromatin from cells; (ii) immunoprecipitation by using a specific antibody; (iii) purification of immunoprecipitated (IP) DNA; (iv) analysis of IP DNA (Lee *et al.*, 2006). Fragmented chromatin is usually prepared by cross-linking DNA and histones by formaldehyde, followed by a fragmentation step by sonication or micrococcal nuclease. Immunoprecipitation is performed using a specific antibody, and then the immuno-complex of chromatin and antibody is collected and purified. IP DNA is analyzed by PCR of a specific genomic region, or by microarray or next-generation sequencers for a genome-wide analysis (Barski *et al.*, 2007; Lee *et al.*, 2006; Wang *et al.*, 2008).

5.3 Screening Methods for Epimutagens

A major reason why only a limited number of chemicals are reported to have epigenetic actions (see Section 4.6) is the lack of easy-to-use assay systems for chemicals' capacity to induce epigenetic alterations. For mutagens, there are various *in vitro* assays, using bacterial cultures or mammalian cells, and also *in vivo* assays using genetically-engineered animals (MacGregor, Casciano and Muller, 2000) (Table 3). In contrast, very limited assay systems are available for epimutagens. To construct an assay system for epimutagens, considerations should be given to what target genomic region is used as a marker for epigenetic effects, such as DNA demethylation and methylation, and what reporter

Table 3. Characteristics of assay systems for mutations and epigenetic alterations.

	Mutation assays	Assays for epigenetic alterations
Bacterial system	*Reversion in S. typhimurium* (Ames test)	Essentially impossible
Mammalian cell	*HPRT* or *TK* mutations Chromosome aberration test Mouse lymphoma assay Measurement of UDS	Under development (see text)
In vivo Assay	Micronucleus test Mouse specific locus test Tg mice for a marker gene (Big Blue, gpt-Δ, Muta-mouse etc.)	Not available yet

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

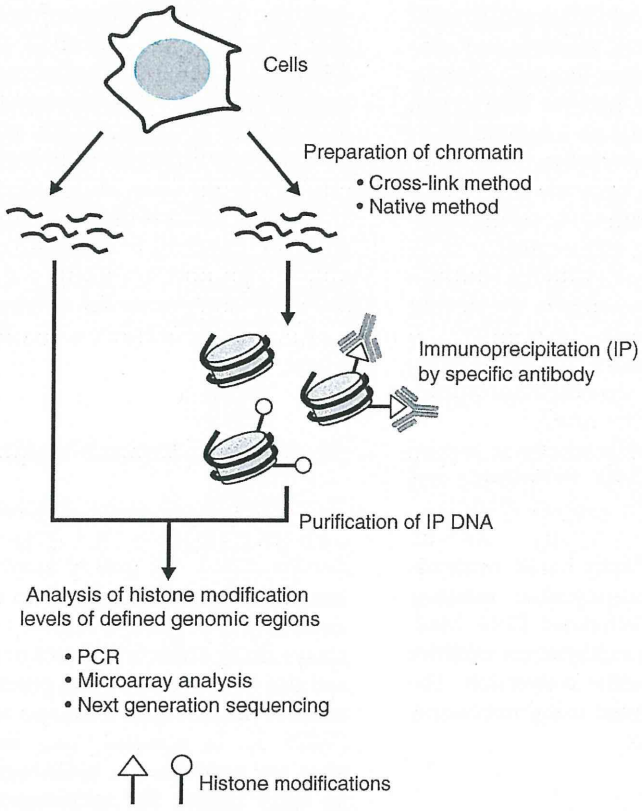

Figure 9. Principle of chromatin immunoprecipitation (ChIP). Fragmented chromatin is prepared, and then immunoprecipitated (IP) by using a specific antibody. DNA purified from the IP chromatin is used for analysis of histone modification levels for defined genomic regions by several technologies such as PCR, microarray, and next generation sequencing.

system is used. For screening purposes, a convenient and reliable assay system is essential.

So far, assay systems only for DNA demethylating agents have been reported. Three systems have been reported using a promoter of an exogenous gene and a reporter gene (Biard *et al.*, 1992; Cervoni and Szyf, 2001; Fan *et al.*, 2005). Among these, Fan *et al.*, 2005 successfully identified 5-bromo-2′-deoxyuridine (BrdU) as an anti-silencing agent without changing DNA methylation status. These exogenous promoters have a concern that they have epigenetic modifications different from endogenous genes. From this aspect, two assay systems are reported using a promoter of an endogenous gene (Okochi-Takada *et al.*, 2004; Oyer *et al.*, 2009). In addition to these efforts to use specific exogenous and endogenous promoters, hypomethylation of repeat sequences is also proposed as a precursor of toxicity (Carnell and Goodman, 2003).

6 EPILOGUE

Epigenomic alterations are important for cancer and possibly for other disorders. Nevertheless, epigenomic toxicology has just started, and scientists are not armed well yet. Application of findings in epigenetics and epigenomics to toxicology is now an exciting task.

ACKNOWLEDGMENTS

The work was supported by a Grant-in-Aid for the Third-term Cancer Control Strategy Program from the Ministry of Health, Labour and Welfare, Japan.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

RELATED ARTICLES

Molecular and Cellular Concepts in Toxicology
Carcinogenesis and Carcinogens that are also Genotoxic
Epigenetic Mechanisms and their Toxicological Significance
Oncology Biomarkers
Biomarkers of Exposure to Carcinogens

REFERENCES

Abe M, Okochi E, Kuramoto T, Kaneda A, Takato T, Sugimura T, Ushijima T. 2002. Cloning of the 5′ upstream region of the rat p16 gene and its role in silencing. *Jpn. J. Cancer Res.* **93**: 1100–1106.

Ahuja N, Li Q, Mohan AL, Baylin SB, Issa JP. 1998. Aging and DNA methylation in colorectal mucosa and cancer. *Cancer Res.* **58**: 5489–5494.

Anway MD, Cupp AS, Uzumcu M, Skinner MK. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**: 1466–1469.

Bachman AN, Phillips JM, Goodman JI. 2006. Phenobarbital induces progressive patterns of GC-rich and gene-specific altered DNA methylation in the liver of tumor-prone B6C3F1 mice. *Toxicol Sci.* **91**: 393–405.

Baranzini SE, Mudge J, van Velkinburgh JC, Khankhanian P, Khrebtukova I, Miller NA, Zhang L, Farmer AD, Bell CJ, Kim RW, May GD, Woodward JE, Caillier SJ, McElroy JP, Gomez R, Pando MJ, Clendenen LE, Ganusova EE, Schilkey FD, Ramaraj T, Khan OA, Huntley JJ, Luo S, Kwok PY, Wu TD, Schroth GP, Oksenberg JR, Hauser SL, Kingsmore SF. 2010. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature* **464**: 1351–1356.

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* **129**: 823–837.

Baylin SB, Ohm JE. 2006. Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction? *Nat. Rev. Cancer* **6**: 107–116.

Biard DS, Maratrat M, Thybaud V, Melcion C. Sarasin A. 1992. Flow cytometric detection of drugs altering the DNA methylation pattern. *Cancer Res.* **52**: 5213–5218.

Bird A. 2007. Perceptions of epigenetics. *Nature* **447**: 396–398.

Boffa LC, Mariani MR, Parker MI. 1994. Selective hypermethylation of transcribed nucleosomal DNA by sodium butyrate. *Exp. Cell Res.* **211**: 420–423.

Bromer JG, Wu J, Zhou Y, Taylor HS. 2009. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. *Endocrinology* **150**: 3376–3382.

Bromer JG, Zhou Y, Taylor MB, Doherty L, Taylor HS. 2010. Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming of uterine estrogen response. FASEB J

Brueckner B, Boy RG, Siedlecki P, Musch T, Kliem HC, Zielenkiewicz P, Suhai S, Wiessler M, Lyko F. 2005. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res.* **65**: 6305–6311.

Carnell AN, Goodman JI. 2003. The long (LINEs) and the short (SINEs) of it: altered methylation as a precursor to toxicity. *Toxicol Sci.* **75**: 229–235.

Cedar H, Bergman Y. 2009. Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet* **10**: 295–304.

Cervoni N, Szyf M. 2001. Demethylase activity is directed by histone acetylation. *J. Biol. Chem.* **276**: 40778–40787.

Chang MS, Uozaki H, Chong JM, Ushiku T, Sakuma K, Ishikawa S, Hino R, Barua RR, Iwasaki Y, Arai K, Fujii H, Nagai H, Fukayama M. 2006. CpG island methylation status in gastric carcinoma with and without infection of Epstein-Barr virus. *Clin. Cancer Res.* **12**: 2995–3002.

Chen H, Ke Q, Kluz T, Yan Y, Costa M. 2006. Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. *Mol. Cell. Biol.* **26**: 3728–3737.

Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. 1998. DNA hypomethylation leads to elevated mutation rates. *Nature* **395**: 89–93.

Cheng JC, Matsen CB, Gonzales FA, Ye W, Greer S, Marquez VE, Jones PA, Selker EU. 2003. Inhibition of DNA methylation and reactivation of silenced genes by zebularine. *J. Natl. Cancer Inst.* **95**: 399–409.

Costello JF, Fruhwald MC, Smiraglia DJ, Rush LJ, Robertson GP, Gao X, Wright FA, Feramisco JD, Peltomaki P, Lang JC, Schuller DE, Yu L, Bloomfield CD, Caligiuri MA, Yates A, Nishikawa R, Su Huang H, Petrelli NJ, Zhang X, O'Dorisio MS, Held WA, Cavenee WK, Plass C. 2000. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat. Genet* **24**: 132–138.

Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. 2002. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. *Cancer Res.* **62**: 6442–6446.

Curt GA, Kelley JA, Fine RL, Huguenin PN, Roth JS, Batist G, Jenkins J, Collins JM. 1985. A phase I and pharmacokinetic study of dihydro-5-azacytidine (NSC 264880). *Cancer Res.* **45**: 3359–3363.

Datta J, Ghoshal K, Denny WA, Gamage SA, Brooke DG, Phiasivongsa P, Redkar S, Jacob ST. 2009. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res.* **69**: 4277–4285.

de Smet C, Lurquin C, Lethe B, Martelange V, Boon T. 1999. DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. *Mol. Cell Biol.* **19**: 7327–7335.

Detich N, Bovenzi V, Szyf M. 2003. Valproate induces replication-independent active DNA demethylation. *J. Biol. Chem.* **278**: 27586–27592.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

Doerfler W, Orend G, Schubbert R, Fechteler K, Heller H, Wilgenbus P, Schroer J. 1995. On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. *Gene*. **157**: 241–245.

Eden A, Gaudet F, Waghmare A, Jaenisch R. 2003. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* **300**: 455.

Egger G, Liang G, Aparicio A, Jones PA. 2004. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* **429**: 457–463.

El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, Cooper ME, Brownlee M. 2008. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J. Exp. Med.* **205**: 2409–2417.

Ellinger J, Kahl P, von der Gathen J, Rogenhofer S, Heukamp LC, Gutgemann I, Walter B, Hofstadter F, Buttner R, Muller SC, Bastian PJ, von Ruecker A. 2010. Global levels of histone modifications predict prostate cancer recurrence. *Prostate* **70**: 61–69.

Fan J, Kodama E, Koh Y, Nakao M, Matsuoka M. 2005. Halogenated thymidine analogues restore the expression of silenced genes without demethylation. *Cancer Res.* **65**: 6927–6933.

Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS. 2003. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* **63**: 7563–7570.

Feinberg AP, Tycko B. 2004. The history of cancer epigenetics. *Nat. Rev. Cancer* **4**: 143–153.

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. 2005a. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. USA* **102**: 10604–10609.

Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Perez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M. 2005b. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat. Genet.* **37**: 391–400.

Fujita N, Watanabe S, Ichimura T, Tsuruzoe S, Shinkai Y, Tachibana M, Chiba T, Nakao M. 2003. Methyl-CpG binding domain 1 (MBD1) interacts with the Suv39h1-HP1 heterochromatic complex for DNA methylation-based transcriptional repression. *J. Biol. Chem.* **278**: 24132–24138.

Fuks F. 2005. DNA methylation and histone modifications: teaming up to silence genes. *Curr. Opin. Genet. Dev.* **15**: 490–495.

Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, Nakajima H, Tanaka A, Komatsu Y, Nishino N, Yoshida M, Horinouchi S. 2002. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* **62**: 4916–4921.

Gan Q, Yoshida T, McDonald OG, Owens GK. 2007. Concise review: epigenetic mechanisms contribute to pluripotency and cell lineage determination of embryonic stem cells. *Stem. Cells* **25**: 2–9.

Gao W, Kondo Y, Shen L, Shimizu Y, Sano T, Yamao K, Natsume A, Goto Y, Ito M, Murakami H, Osada H, Zhang J, Issa JP, Sekido Y. 2008. Variable DNA methylation patterns associated with progression of disease in hepatocellular carcinomas. *Carcinogenesis* **29**: 1901–1910.

Ghoshal K, Datta J, Majumder S, Bai S, Kutay H, Motiwala T, Jacob ST. 2005. 5-aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. *Mol. Cell Biol.* **25**: 4727–4741.

Graff JR, Herman JG, Myohanen S, Baylin SB, Vertino PM. 1997. Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in de novo methylation. *J. Biol. Chem.* **272**: 22322–22329.

Hayashi H, Nagae G, Tsutsumi S, Kaneshiro K, Kozaki T, Kaneda A, Sugisaki H, Aburatani H. 2007. High-resolution mapping of DNA methylation in human genome using oligonucleotide tiling array. *Hum. Genet.* **120**: 701–711.

Hellman A, Chess A. 2007. Gene body-specific methylation on the active X chromosome. *Science* **315**: 1141–1143.

Hermann A, Goyal R, Jeltsch A. 2004a. The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J. Biol. Chem.* **279**: 48350–48359.

Hermann A, Gowher H, Jeltsch A. 2004b. Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci.* **61**: 2571–2587.

Hino R, Uozaki H, Murakami N, Ushiku T, Shinozaki A, Ishikawa S, Morikawa T, Nakaya T, Sakatani T, Takada K, Fukayama M. 2009. Activation of DNA methyltransferase 1 by EBV latent membrane protein 2A leads to promoter hypermethylation of PTEN gene in gastric carcinoma. *Cancer Res.* **69**: 2766–2774.

Hogarth LA, Redfern CP, Teodoridis JM, Hall AG, Anderson H, Case MC, Coulthard SA. 2008. The effect of thiopurine drugs on DNA methylation in relation to TPMT expression. *Biochem Pharmacol* **76**: 1024–1035.

Holleran JL, Parise RA, Joseph E, Eiseman JL, Covey JM, Glaze ER, Lyubimov AV, Chen YF, D'Argenio DZ, Egorin MJ. 2005. Plasma pharmacokinetics, oral bioavailability, and interspecies scaling of the DNA methyltransferase inhibitor, zebularine. *Clin. Cancer Res.* **11**: 3862–3868.

Holliday R. 1991. Mutations and epimutations in mammalian cells. *Mutat. Res.* **250**: 351–363.

Holliday R, Ho T. 2002. DNA methylation and epigenetic inheritance. *Methods* **27**: 179–183.

Hsieh CJ, Klump B, Holzmann K, Borchard F, Gregor M, Porschen R. 1998. Hypermethylation of the p16INK4a promoter in colectomy specimens of patients with long-standing and extensive ulcerative colitis. *Cancer Res.* **58**: 3942–3945.

Hublitz P, Albert M, Peters AH. 2009. Mechanisms of transcriptional repression by histone lysine methylation. *Int. J. Dev. Biol.* **53**: 335–354.

Issa JP, Kantarjian HM. 2009. Targeting DNA methylation. *Clin. Cancer Res.* **15**: 3938–3946.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.

DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. 2001. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res.* **61**: 3573–3577.

Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. 1994. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat. Genet.* **7**: 536–540.

Issa JP, Gharibyan V, Cortes J, Jelinek J, Morris G, Verstovsek S, Talpaz M, Garcia-Manero G, Kantarjian HM. 2005. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J. Clin. Oncol.* **23**: 3948–3956.

Jones PA 1985. Altering gene expression with 5-azacytidine. *Cell* **40**: 485–486.

Jones PA, Taylor SM. 1980. Cellular differentiation, cytidine analogs and DNA methylation. *Cell* **20**: 85–93.

Jones PA, Baylin SB. 2007. The epigenomics of cancer. *Cell* **128**: 683–692.

Jones PA, Baylin SB, Bernsten BE, Feinberg AP, Greally JM, Jenuwein T, Jirtle R, Ushijima T, Pierotta V, Allis CD, Elgin SC, Rine J, Wu C. 2008. Moving AHEAD with an international human epigenome project. *Nature* **454**: 711–715.

Kalhan SC 2009. Metabolism of methionine *in vivo*: impact of pregnancy, protein restriction, and fatty liver disease. *Nestle Nutr. Workshop Ser. Pediatr Program* **63**: 121-131; discussion 131–123, 259–168.

Kaneda A, Tsukamoto T, Takamura-Enya T, Watanabe N, Kaminishi M, Sugimura T, Tatematsu M, Ushijima T. 2004a. Frequent hypomethylation in multiple promoter CpG islands is associated with global hypomethylation, but not with frequent promoter hypermethylation. *Cancer Sci.* **95**: 58–64.

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H. 2004b. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* **429**: 900–903.

Kang GH, Lee S, Kim WH, Lee HW, Kim JC, Rhyu MG, Ro JY. 2002. Epstein-barr virus-positive gastric carcinoma demonstrates frequent aberrant methylation of multiple genes and constitutes CpG island methylator phenotype-positive gastric carcinoma. *Am. J. Pathol* **160**: 787–794.

Kelly WK, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, Tong W, Klang M, Schwartz L, Richardson S, Rosa E, Drobnjak M, Cordon-Cordo C, Chiao JH, Rifkind R, Marks PA, Scher H. 2003. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. *Clin. Cancer Res.* **9**: 3578–3588.

Keshet I, Schlesinger Y, Farkash S, Rand E, Hecht M, Segal E, Pikarski E, Young RA, Niveleau A, Cedar H, Simon I. 2006. Evidence for an instructive mechanism of de novo methylation in cancer cells. *Nat. Genet* **38**: 149–153.

Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA, Chinnaiyan AM. 2003. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci. USA* **100**: 11606–11611.

Klose RJ, Zhang Y. 2007. Regulation of histone methylation by demethylimination and demethylation. *Nat. Rev. Mol. Cell Biol.* **8**: 307–318.

Kondo Y, Kanai Y, Sakamoto M, Mizokami M, Ueda R, Hirohashi S. 2000. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis–A comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. *Hepatology* **32**: 970–979.

Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, Yamochi T, Urano T, Furukawa K, Kwabi-Addo B, Gold DL, Sekido Y, Huang TH, Issa JP. 2008. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat. Genet.* **40**: 741–750.

Kouzarides T. 2007. Chromatin modifications and their function. *Cell* **128**: 693–705.

Kramer OH, Zhu P, Ostendorff HP, Golebiewski M, Tiefenbach J, Peters MA, Brill B, Groner B, Bach I, Heinzel T, Gottlicher M. 2003. The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. *EMBO J.* **22**: 3411–3420.

Laird CD, Pleasant ND, Clark AD, Sneeden JL, Hassan KM, Manley NC, Vary JC, Jr., Morgan T, Hansen RS, Stoger R. 2004. Hairpin-bisulfite PCR: assessing epigenetic methylation patterns on complementary strands of individual DNA molecules. *Proc. Natl. Acad. Sci. USA* **101**: 204–209.

Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, Li E, Weinberg RA, Jaenisch R. 1995. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell.* **81**: 197–205.

Laird PW. 2010. Principles and challenges of genome-wide DNA methylation analysis. *Nat. Rev. Genet* **11**: 191–203.

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM,

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.

DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, Szustakowki J, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. 2001. Initial sequencing and analysis of the human genome. *Nature* **409**: 860–921.

Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet* **11**: 204–220.

Lee BH, Yegnasubramanian S, Lin X, Nelson WG. 2005. Procainamide is a specific inhibitor of DNA methyltransferase 1. *J. Biol. Chem.* **280**: 40749–40756.

Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* **36**: 900–905.

Lee TI, Johnstone SE, Young RA. 2006. Chromatin immunoprecipitation and microarray-based analysis of protein location. *Nat. Protoc.* **1**: 729–748.

Li B, Carey M, Workman JL. 2007. The role of chromatin during transcription. *Cell.* **128**: 707–719.

Li E, Bestor TH, Jaenisch R. 1992. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell.* **69**: 915–926.

Li Q, Ke Q, Costa M. 2009. Alterations of histone modifications by cobalt compounds. *Carcinogenesis* **30**: 1243–1251.

Lin JC, Jeong S, Liang G, Takai D, Fatemi M, Tsai YC, Egger G, Gal-Yam EN, Jones PA. 2007. Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. *Cancer Cell.* **12**: 432–444.

Loeb LA 2001. A mutator phenotype in cancer. *Cancer Res.* **61**: 3230–3239.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**: 251–260.

MacGregor JT, Casciano D, Muller L. 2000. Strategies and testing methods for identifying mutagenic risks. *Mutat. Res.* **455**: 3–20.

Maciejewska Rodrigues H, Jungel A, Gay RE, Gay S. 2009. Innate immunity, epigenetics and autoimmunity in rheumatoid arthritis. Mol Immunol

MacPhee DG. 1998. Epigenetics and epimutagens: some new perspectives on cancer, germ line effects and endocrine disrupters. *Mutat. Res.* **400**: 369–379.

Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, Sugimura T, Ichinose M, Ushijima T. 2006. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. *Clin. Cancer Res.* **12**: 989–995.

Manuyakorn A, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Guha C, Dawson DW. 2010. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. *J. Clin. Oncol.* **28**: 1358–1365.

Margueron R, Reinberg D. 2010. Chromatin structure and the inheritance of epigenetic information. *Nat. Rev. Genet.* **11**: 285–296.

Maze I, Covington HE, 3rd, Dietz DM, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovsky A, Schaefer A, Nestler EJ. 2010. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* **327**: 213–216.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci* **12**: 342–348.

Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES. 2008. Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**: 766–770.

Minucci S, Pelicci PG. 2006. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat. Rev. Cancer* **6**: 38–51.

Muller K, Heller H, Doerfler W. 2001. Foreign dna integration. genome-wide perturbations of methylation and transcription in the recipient genomes. *J. Biol. Chem.* **276**: 14271–14278.

Nagao M, Ochiai M, Okochi E, Ushijima T, Sugimura T. 2001. LacI transgenic animal study: relationships among DNA-adduct levels, mutant frequencies and cancer incidences. *Mutat. Res.* **477**: 119–124.

Nakajima T, Yamashita S, Maekita T, Niwa T, Nakazawa K, Ushijima T. 2009. The presence of a methylation fingerprint of Helicobacter pylori infection in human gastric mucosae. *Int. J. Cancer* **124**: 905–910.

Nakajima T, Maekita T, Oda I, Gotoda T, Yamamoto S, Umemura S, Ichinose M, Sugimura T, Ushijima T, Saito D. 2006. Higher methylation levels in gastric mucosae significantly correlate with higher risk of gastric cancers. *Cancer Epidemiol Biomarkers Prev.* **15**: 2317–2321.

Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. 2008. Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. *Hepatology* **47**: 908–918.

Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. 2010. Inflammatory processes triggered by Helicobacter pylori infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res.* **70**: 1430–1440.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

Oka D, Yamashita S, Tomioka T, Nakanishi Y, Kato H, Kaminishi M, Ushijima T. 2009. The presence of aberrant DNA methylation in noncancerous esophageal mucosae in association with smoking history: a target for risk diagnosis and prevention of esophageal cancers. *Cancer* **115**: 3412–3426.

Okano M, Xie S, Li E. 1998. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat. Genet.* **19**: 219–220.

Okano M, Bell DW, Haber DA, Li E. 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell.* **99**: 247–257.

Okochi-Takada E, Ichimura S, Kaneda A, Sugimura T, Ushijima T. 2004. Establishment of a detection system for demethylating agents using an endogenous promoter CpG island. *Mutat. Res.* **568**: 187–194.

Oyer JA, Chu A, Brar S, Turker MS. 2009. Aberrant epigenetic silencing is triggered by a transient reduction in gene expression. *PLoS One* **4**: e4832.

Ozsolak F, Song JS, Liu XS, Fisher DE. 2007. High-throughput mapping of the chromatin structure of human promoters. *Nat. Biotechnol* **25**: 244–248.

Park SY, Yoo EJ, Cho NY, Kim N, Kang GH. 2009. Comparison of CpG island hypermethylation and repetitive DNA hypomethylation in premalignant stages of gastric cancer, stratified for Helicobacter pylori infection. *J. Pathol.* **219**: 410–416.

Pina IC, Gautschi JT, Wang GY, Sanders ML, Schmitz FJ, France D, Cornell-Kennon S, Sambucetti LC, Remiszewski SW, Perez LB, Bair KW, Crews P. 2003. Psammaplins from the sponge Pseudoceratina purpurea: inhibition of both histone deacetylase and DNA methyltransferase. *J. Org. Chem.* **68**: 3866–3873.

Poirier LA. 2002. The effects of diet, genetics and chemicals on toxicity and aberrant DNA methylation: an introduction. *J. Nutr.* **132**: 2336S-2339S.

Pulling LC, Vuillemenot BR, Hutt JA, Devereux TR, Belinsky SA. 2004. Aberrant promoter hypermethylation of the death-associated protein kinase gene is early and frequent in murine lung tumors induced by cigarette smoke and tobacco carcinogens. *Cancer Res.* **64**: 3844–3848.

Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP. 2009. A human B cell methylome at 100-base pair resolution. *Proc. Natl. Acad. Sci. USA* **106**: 671–678.

Rauch TA, Zhong X, Wu X, Wang M, Kernstine KH, Wang Z, Riggs AD, Pfeifer GP. 2008. High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. *Proc. Natl. Acad. Sci. USA* **105**: 252–257.

Reichard JF, Schnekenburger M, Puga A. 2007. Long term low-dose arsenic exposure induces loss of DNA methylation. *Biochem Biophys Res. Commun* **352**: 188–192.

Richards EJ, Elgin SC. 2002. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell.* **108**: 489–500.

Riggs AD, Xiong Z. 2004. Methylation and epigenetic fidelity. *Proc. Natl. Acad. Sci. USA* **101**: 4–5.

Robertson KD. 2005. DNA methylation and human disease. *Nat. Rev. Genet.* **6**: 597–610.

Ruthenburg AJ, Li H, Patel DJ, Allis CD. 2007. Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell. Biol.* **8**: 983–994.

Segura-Pacheco B, Trejo-Becerril C, Perez-Cardenas E, Taja-Chayeb L, Mariscal I, Chavez A, Acuna C, Salazar AM, Lizano M, Duenas-Gonzalez A. 2003. Reactivation of tumor suppressor genes by the cardiovascular drugs hydralazine and procainamide and their potential use in cancer therapy. *Clin. Cancer Res.* **9**: 1596–1603.

Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, Tze S, Wang Q, Chia D, Goodglick L, Kurdistani SK. 2009. Global levels of histone modifications predict prognosis in different cancers. *Am. J. Pathol* **174**: 1619–1628.

Shi Y. 2007. Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat. Rev. Genet* **8**: 829–833.

Shivapurkar N, Wilson MJ, Poirier LA. 1984. Hypomethylation of DNA in ethionine-fed rats. *Carcinogenesis* **5**: 989–992.

Snyder RD, Green JW. 2001. A review of the genotoxicity of marketed pharmaceuticals. *Mutat. Res.* **488**: 151–169.

Stadtman ER, Barker HA. 1949. Fatty acid synthesis by enzyme preparations of Clostridium kluyveri; a consideration of postulated 4-carbon intermediates in butyrate synthesis. *J. Biol. Chem.* **181**: 221–235.

Stewart MD, Li J, Wong J. 2005. Relationship between histone H3 lysine 9 methylation, transcription repression, and heterochromatin protein 1 recruitment. *Mol. Cell. Biol.* **25**: 2525–2538.

Takeshima H, Ushijima T. 2010. Methylation destiny: Moira takes account of histones and RNA polymerase II. *Epigenetics* **5**: 89–95.

Takeshima H, Yamashita S, Shimazu T, Niwa T, Ushijima T. 2009. The presence of RNA polymerase II, active or stalled, predicts epigenetic fate of promoter CpG islands. *Genome Res.* **19**: 1974–1982.

Takeshima H, Suetake I, Shimahara H, Ura K, Tate S, Tajima S. 2006. Distinct DNA methylation activity of Dnmt3a and Dnmt3b towards naked and nucleosomal DNA. *J. Biochem.* **139**: 503–515.

Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. 1999. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA* **96**: 8681–8686.

Toyota M, Itoh F, Kikuchi T, Satoh A, Obata T, Suzuki H, Ishii S, Endo T, Tokino T, Imai K. 2002. DNA methylation changes in gastrointestinal disease. *J. Gastroenterol* 37 Suppl **14**: 97–101.

Ushijima T. 2005. Detection and interpretation of altered methylation patterns in cancer cells. *Nat. Rev. Cancer* **5**: 223–231.

Ushijima T. 2007. Epigenetic field for cancerization. *J. Biochem Mol. Biol.* **40**: 142–150.

Ushijima T, Sasako M. 2004. Focus on gastric cancer. *Cancer Cell.* **5**: 121–125.

Ushijima T, Okochi-Takada E. 2005. Aberrant methylations in cancer cells: Where do they come from? *Cancer Sci.* **96**: 206–211.

Ushijima T, Asada K. 2010. Aberrant DNA methylation in contrast with mutations. *Cancer Sci.* **101**: 300–305.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.

DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

Ushijima T, Watanabe N, Okochi E, Kaneda A, Sugimura T, Miyamoto K. 2003. Fidelity of the methylation pattern and its variation in the genome. *Genome. Res.* **13**: 868–874.

van Panhuys N, Le Gros G, McConnell MJ. 2008. Epigenetic regulation of Th2 cytokine expression in atopic diseases. *Tissue Antigens* **72**: 91–97.

Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, Chinnaiyan AM. 2002. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**: 624–629.

Villar-Garea A, Fraga MF, Espada J, Esteller M. 2003. Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res.* **63**: 4984–4989.

Waki T, Tamura G, Sato M, Motoyama T. 2003. Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. *Oncogene* **22**: 4128–4133.

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. 2008. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* **40**: 897–903.

Waterland RA, Jirtle RL. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell. Biol.* **23**: 5293–5300.

Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D. 2007. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* **39**: 457–466.

Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. 2007. The genomic landscapes of human breast and colorectal cancers. *Science* **318**: 1108–1113.

Yamada Y, Jackson-Grusby L, Linhart H, Meissner A, Eden A, Lin H, Jaenisch R. 2005. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. *Proc. Natl. Acad. Sci. USA* **102**: 13580–13585.

Yamashita S, Hosoya K, Gyobu K, Takeshima H, Ushijima T. 2009. Development of a novel output value for quantitative assessment in methylated DNA immunoprecipitation-CpG island microarray analysis. *DNA Res.* **16**: 275–286.

Yasunaga J, Taniguchi Y, Nosaka K, Yoshida M, Satou Y, Sakai T, Mitsuya H, Matsuoka M. 2004. Identification of aberrantly methylated genes in association with adult T-cell leukemia. *Cancer Res.* **64**: 6002–6009.

Yoshida M, Kijima M, Akita M, Beppu T. 1990. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* **265**: 17174–17179.

Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes MP. 1997. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proc. Natl. Acad. Sci. USA* **94**: 10907–10912.

Zheng C, Hayes JJ. 2003. Structures and interactions of the core histone tail domains. *Biopolymers* **68**: 539–546.

Zhou X, Li Q, Arita A, Sun H, Costa M. 2009. Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. *Toxicol. Appl. Pharmacol* **236**: 78–84.

General, Applied and Systems Toxicology, Online © 2011 John Wiley & Sons, Ltd.
This article is © 2011 John Wiley & Sons, Ltd.
DOI: 10.1002/9780470744307.gat230
This article was first published in print in the *Handbook of Systems Toxicology* ISBN: 978-0-470-68401-6 © 2011 John Wiley & Sons, Ltd.

CHAPTER 8

Analysis of Gene-specific DNA Methylation

Naoko Hattori and Toshikazu Ushijima
National Cancer Center Research Institute, Tokyo 104-0045, Japan

INTRODUCTION

Gene- or region-specific DNA methylation analysis is necessary in various situations, and a variety of methods are available. It is important to become familiar with the characteristics of each technique, including the required amount of DNA, flexibility in selection of CpG sites to analyze, how quantitative the technique is, technical complexity, and the cost (Table 8.1). For example, if one wants to analyze DNA methylation as a cause of gene silencing, a specific region that controls gene expression should be analyzed [1], and a method with flexibility in selecting a region to analyze should be used. If one aims for diagnostic applications, a method that is highly accurate should be adopted.

In this chapter, we first introduce principles of DNA methylation analysis, and then summarize characteristics of individual methods. Finally, we will provide tips necessary to perform bisulfite sequencing, methylation-specific PCR (MSP), and quantitative MSP.

PRINCIPLES OF DNA METHYLATION ANALYSIS

DNA methylation can be analyzed based on several principles that differentially recognize 5-methylcytosine (C^m) from cytosine (C). The first principle depends upon methylation-sensitive restriction enzymes whose activity is affected by the presence of a methyl group on a cytosine at a CpG site(s) within restriction sites (Fig. 8.1A). The vast majority of methylation-sensitive restriction enzymes, such as *Hpa*II and *Sma*I, are inactive on methylated CpG sites, but a unique methylation-sensitive restriction enzyme, *Mcr*BC, is inactive on unmethylated CpG sites. Differential cleavage can be detected by Southern-blot hybridization.

The second principle depends on bisulfite-mediated DNA conversion. This treatment converts unmethylated C into uracil (U) very rapidly, whereas it converts methylated C extremely slowly [2]. Under optimized conditions, a difference in methylation status of a CpG site can be converted into a difference of sequence, UpG or CpG. Once a difference of methylation status is converted into a difference of DNA sequence, it can be detected by various techniques, such as bisulfite sequencing, methylation-specific PCR (MSP), real-time MSP, combined bisulfite restriction analysis (COBRA), pyrosequencing, and MassARRAY® analysis (Table 8.1).

Third, methylated cytosines can be specifically recognized by an anti-methylcytidine antibody or a methylated DNA binding (MBD) protein. After appropriate shearing of DNA,

Handbook of Epigenetics: The New Molecular and Medical Genetics. DOI: 10.1016/B978-0-12-375709-8.00008-3