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Tissue Specificity of Methylation of Cytosines in Regulatory Regions of Four Genes Located in the Locus *FXYD5-COX7A1* of Human Chromosome 19: Correlation with Their Expression Level

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Abstract—In this study, we compared degree of methylation of selected CpG sites in CCGG sequences located in promoter regions of four human genes with expression level of these genes in several human cell lines and tissues. These genes were subdivided into two groups according to the dependence of their expression on CpG methylation in the 5′-regions. The first group, characterized by clear correlation of methylation with the transcription level, includes housekeeping gene *COX6B* (the absence of methylation unambiguously correlates with expression) and urothelium-specific uroplakin gene (the methylation coincides with absence of expression). The second group includes genes that are expressed in many, but not all tissues and cells. For these genes (*LEAP-1* and *ATP4A*), there was no correlation between methylation and expression. It is possible that methylation provides some basal level of gene repression, which is overcome by binding of tissue-specific transcription factors, whereas lack of methylation gives the opportunity for gene expression in various cells and tissues.

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The large content and relatively uniform distribution over the genome of methylated cytosine in CpG dinucleotide is typical for vertebrates. However, functions of genome methylation are not well understood. In mammals DNA, methylation is involved in inactivation of the X-chromosome [1], suppression of mobile element activity [2], imprinting [3], and tumor formation [4]. Adequate genome methylation is necessary for normal development of mammals [5].

Changes in distribution of 5-methylcytosines in genomes of various cell types and at various stages of development of an organism suggest that DNA methylation may be one of the epigenetic factors determining cell differentiation and tissue-specific gene expression.

Good experimental evidence has been accumulated indicating that degree of methylation of certain sites of a genome correlates with expression of corresponding genes in various vertebrate tissues [6-9]. For example, it is believed that methylation of certain sites, mainly in promoter region, may suppress activity of corresponding genes [10]. Methylation of some regulatory sequences may also correlate with activation of transcription and methylation of the ICR (imprinting control region) in human and mouse *H19/Igf2* locus is a good example illustrating this notion. ICR is an insulator of the *Igf2* gene; it uncouples *Igf2* from its enhancer positioned at a distance of several thousand base pairs. ICR methylation impairs its repressor function and activates *Igf2* transcription. Since ICR is methylated only on the father chromosome, *Igf2* expression is monoallelic: the father allele is active, whereas the mother allele is normally inactive, this correlating with lack of ICR methylation [11].

However, in some cases there is no correlation between methylation and gene expression level. Tissue-specific gene expression is suggested to be regulated by methylation, but some genes exhibit normal functioning in DNA methyltransferase-deficient mouse embryos [12]. So the problem of regulation of tissue-specific

Abbreviations: PCR) polymerase chain reaction; RT-PCR) reverse transcriptase polymerase chain reaction.

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expression by methylation requires further investigation, and the solution of this problem needs additional data of mammalian genome methylation profiling in various cell types.

A locus of the human genome of 1000 kb located on chromosome 19 between markers *FXYD5* and *COX7A1*, containing 40 identified and a number of hypothetical genes, is a convenient model for study of tissue-specific DNA methylation; many of these gene exhibit tissue-specific expression [13]. Determination of methylation status of potential regulator sequences of genes of this locus in various human tissues and cell lines in relation to activity of corresponding genes may promote better understanding of the role of methylation in regulation of gene expression.

MATERIALS AND METHODS

Human tissues and cell lines. The following cell lines were used in this study: Tera-1 (embryonal carcinoma, ATCC HTB-105), HL-60 (acute promyelocytic leukemia cells ATCC CRL-1964), NGP-127 (neuroblastoma kindly provided by Paul S. Meltzer [14]), and 293 line (embryonic kidney cells transformed by human adenovirus Ad5 DNA, ATCC CRL-1573), and also human cerebellum, liver, kidney, and stomach tissues.

Isolation of genomic DNA. Genomic DNA was isolated from tissues by means of the Wizard Genomic DNA Purification Kit (Promega, USA) following supplier's recommendations.

The protocol for isolation of genomic DNA from cell lines is given below. A cell suspension ($3 \cdot 10^6$ cells) was pelleted by 5-min centrifugation at 150*g*; and the pellet was washed three times with cold PBS (phosphate buffered saline) solution and resuspended in 20 μl of TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). Then 200 μl of lysing buffer (10 mM Tris-HCl, pH 8.0, 100 mM EDTA, 0.5% SDS) and RNase A solution (final concentration 20 μg/ml) were added. This mixture was incubated at 37°C for 1-1.5 h, and after addition of proteinase K (final concentration 100 μg/ml), it was incubated overnight at 50-56°C. The cell lysate was deproteinized by a mixture of phenol (pH 8.0), chloroform, and isoamyl alcohol (25 : 24 : 1 v/v). The extraction was repeated until the whole interphase disappeared. Genomic DNA was pelleted by 96% ethanol in 0.3 M sodium acetate at −70°C for 1 h. The pellet was washed with 75% ethanol and dissolved at 65°C with stirring in TE buffer. DNA concentration was determined spectrophotometrically using GeneQuant spectrophotometer (Amersham Pharmacia Biotech, Sweden).

Isolation of total RNA. Total RNA was isolated from cell lines by means of the RNeasy kit following the supplier's recommendations (Qiagen, Germany). A cell suspension (about $1.5 \cdot 10^7$ cells) was centrifuged for 5 min at 150*g*; the pellet was washed with cold PBS and then subjected to isolation steps described in the supplier's protocols. The concentration of resultant RNA was determined using the GeneQuant spectrophotometer and quality of RNA was monitored by electrophoresis in 1% agarose gel.

For removal of contaminants of genomic DNA, 3 units of DNase RQ1 (Promega), 60 U RNasine, and dithiothreitol (DTT; final concentration 10 mM) were added to 6-10 μg total RNA and the reaction was carried out at 37°C for 10 min in the buffer recommended by the supplier. Then sodium acetate was added to final concentration of 0.3 M, and the RNA was deproteinized twice by a mixture of phenol (pH 5.5), chloroform, and isoamyl alcohol (25 : 24 : 1 v/v). RNA was pelleted by 96% ethanol overnight at −70°C. The pellet was washed twice with 70% ethanol and dissolved in water and stored at −70°C.

Hydrolysis of genomic DNA by restriction endonucleases. Before hydrolysis, genomic DNA was heated for 30 min at 65°C with stirring. Hydrolysis was carried out in 50 or 100 μl of the buffer containing 0.1% Triton X-100, 1 μg DNA, and 20 U of the enzyme (*Msp*I or *Hpa*II). The reaction mixture was incubated at 37°C for 4 h, and after subsequent addition of 20 U of the corresponding enzyme, this mixture was incubated overnight; then the endonuclease was inactivated by heating at 70°C for 10 min.

The degree of hydrolysis was evaluated by electrophoresis in an agarose gel and by PCR-amplification of the hydrolyzed DNA with the following primers to normally unmethylated site −166 in promoter region of the *BRCA1* gene [15]: 5′-CAGGCAAATTCGGCGCTCAC, 5′-GAGGGACAGAAAGAGCCAAGCG. Corresponding genomic DNA subjected to the same incubations in the restriction buffer but without enzymes (equal volume of deionized water was added) was used as control template.

Synthesis of the first chain of cDNA on the template of total RNA. Two mixtures with total volume of 20 μl each were prepared. Reaction mixture 1 contained 2 μg of total RNA and 0.2 μg of random hexanucleotide primer; reaction mixture 2 contained 20 U of RNasine and equimolar quantities of dNTP (each in final concentration of 125 μM), DTT (final concentration 0.1 M), and 10 U of AMV reverse transcriptase (Promega). Both mixtures were prepared in one-fold concentrated AMV reverse transcriptase buffer recommended by the supplier.

Reaction mixture 1 was heated at 70°C for 3 min, cooled in ice, and then both mixtures were placed into a PCR-amplifier. Temperature was adjusted to 20°C and reaction mixture 2 was added to reaction mixture 1. cDNA was synthesized using the following mode: 20°C, 10 min; 37°C, 30 min; 42°C, 45 min. The reaction was stopped by adding EDTA (final concentration 20 mM).

Polymerase chain reaction (PCR). For detection of methylation of promoter regions the following primers

located adjacent to sites of interest were used: 5′-AAGAAATACCTGACACAGAAAAAGC, 5′-CCAGCAAGAGAACTACGACTTTC (*COX6B*); 5′-CTGCTCAGGGCTATCTAGTGTTCC, 5′-CACTGGGCTCTCACCTGTTGT (*LEAP-1*); 5′-CTTTACAGGGCTGCTGTTAGATG, 5′-AGCTGATTACAGGCTCCTTGG (*ATP4A*); 5′-CCATAGAGAGTGTTGTAAGCATGG, 5′-TGGAGTGAGAACCAGATCAGC (alternative primers located adjacent to the site −590 in promoter region of *ATP4A*); 5′-GCCCTGATTTGGATCTTGCTC, 5′-CCCCTCCTCACCTTCTCTGTC (*UPK1A*). Primers were constructed using the Primer3 program (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and verified using BLAST (www.ncbi.nlm.nih.gov/BLAST) and the human genome database.

PCR was carried out in 25-50 μl of the following mixture: 20 ng of genomic DNA (template), 10 pmol of each primer, 0.125 mM each dNTP, 1.5-2.0 mM $MgCl_2$, 1-2.5 U Taq polymerase (BIOTAQ, Dialat) in a one-fold buffer for Taq polymerase (16.6 mM $(NH_4)_2SO_4$, 67 mM Tricine, pH 9.1, 0.01% Tween-20). The following temperature mode was used: denaturation at 95°C, 2 min, one cycle; denaturation at 95°C, 45 sec; annealing at 57-63°C (depending on primer), 40 sec; elongation at 72°C, 50 sec for 23-36 cycles.

For determination of mRNA expression, the reaction was carried out under the same conditions but using cDNA as template; the amount of cDNA corresponded to 80 ng of initial total mRNA. Purity of resultant preparation and uniformity of mRNA concentrations were monitored by mRNA of the β-actin gene, which is expressed in all cell types (primers 5′-GAGCGGGAAATCGTGCGTGACATT, 5′-GATGGAGTTGAAGGTAGTTTCGTG). Transcription level of the genes studied was determined using primers to their neighboring exons: 5′-CGTGTGTACCAGTCCCTCTG, 5′-CACTTTTCCAATGAGTTTTTATTAGC (*COX6B*), 5′-CTGGTCCCCCTGCCCTAC, 5′-ATATCTTGGTGGCTGTCCAGAGG (*ATP4A*), 5′-GCCTGCTAGTTGTGGGCAATATC, 5′-ACTGGCTACCATGAAGAAGGAGAAG (*UPK1A*), 5′-AGAGCTGCAACCCCAGGAC, 5′-ACAAAAGAACCAGCCATTTTATTCC (*LEAP-1*).

Analysis of DNA sequences. Genes and promoter regions were analyzed using the UCSC Human Genome Browser databases and software (http://www.genome.ucsc.edu).

RESULTS

Detection of methylation of promoter regions of *ATP4A*, *LEAP-1*, *UPK1A*, and *COX6B*. Methylation status of restriction sites was detected by using restriction endonucleases sensitive (*Hpa*II) and insensitive (*Msp*I) to inner methylated cytosine residue in CCGG followed by subsequent PCR-amplification of the fragment containing this restriction site. Oligonucleotides complementary to sequences positioned at both sides of the restriction sites were used as primers. Using such primers DNA amplification occurs only if the site between these primers is not cleaved (and is absent if this site has been cleaved). *Msp*I and *Hpa*II are isoschizomers; they cleave DNA at CCGG sites, but *Msp*I is insensitive to methylation of inner cytosine residue, whereas *Hpa*II cannot cleave this site when it is methylated. *Msp*I is used for control of cleavage and susceptibility of the restriction site. Non-hydrolyzed genomic DNA was used as a positive control for effectiveness of PCR. Effectiveness of cleavage by restrictases was additionally analyzed by using internal control, one of the sites at the 5′-region of *BRCA1* (breast cancer 1 gene). The latter is characterized by unmethylated state under normal conditions.

Regions of 1 kb in length flanking the 5′-side of the initiation transcription start point were selected as research objects. These regions contain the main regulatory elements influencing gene activity. Among all known and hypothetical genes of the *FXYD5–COX7A1* locus, we selected four genes. One of them is a housekeeping gene that is expressed in all cell types (subunit 6 of cytochrome oxidase, *COX6B*). Three other genes demonstrate tissue-specific expression. These are the hepsidin gene (*LEAP-1*), uroplakin 1a gene (*UPK1A*), and the gene encoding stomach parietal cell ATPase (*ATP4A*). *LEAP-1* is preferentially transcribed in liver and to a lesser extent in heart and brain [16]. Transcription of *UPK1A* is highly specific for urinary bladder urothelium [17], whereas *ATP4A* is expressed in stomach parietal cells [18]. (Some evidence also exists that *ATP4A* is transcribed in other organs and tissues including liver [19].)

5′-Regions of the selected genes contain specific binding sites of regulatory proteins and CpG island is located just before *COX6B* (Fig. 1). In each of these 5′-

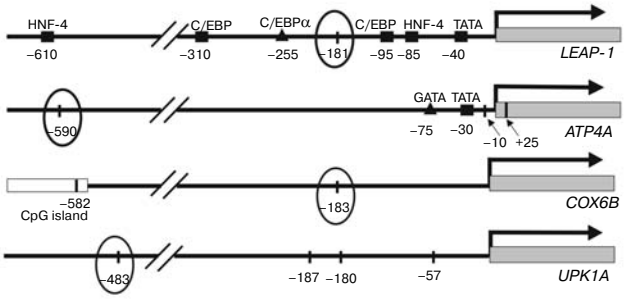

Fig. 1. Scheme of promoter regions of the studied genes. Grey rectangles mark the beginning of the first exons, arrows indicate transcription initiation start point, and vertical lines show CCGG sites. Ovals indicate sites investigated in this study. Quadrants mark putative regulatory sequences (TATA-sequence, protein binding sites HNF-4, C/EBP); triangles show regulatory sequences exhibiting experimentally validated binding of corresponding protein (*ATP4A* – GATA-6 [21], *LEAP-1* – C/EBP [22]).

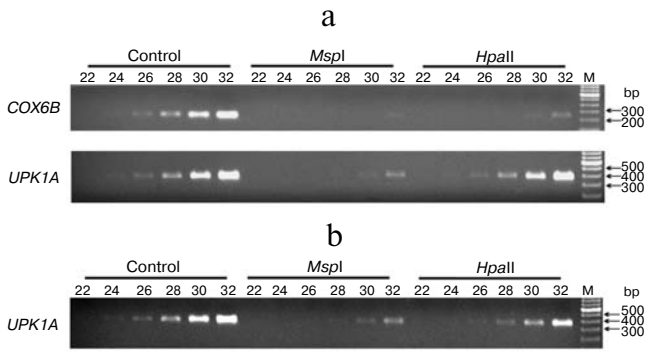

Fig. 2. Detection of methylation of –183 and –483 sites in promoter regions of *COX6B* and *UPK1A* genes, respectively. PCR-amplification on the template of non-hydrolyzed genomic DNA (control) and DNA cleaved by *Msp*I or *Hpa*II. Number of amplification cycles is indicated above lanes. Genomic DNA template from 293 cell line (a) and from kidney (b).

regions we selected one restriction site located within 600 bp of the transcription initiation start point. One of the criteria for such selection consisted in the possibility of construction of unique primers to regions adjacent to the site of interest.

Taking into consideration tissue-specific expression of the studied genes, we selected four organs/tissues (liver, kidney, stomach, and cerebellum) for determination of methylation status. We also used genomic DNA isolated from four cell lines: Tera-1 (embryonal carcinoma), HL-60 (acute promyelocytic leukemia), NGP-127 (neuroblastoma), and 293 cell line (human embryonic kidney transformed with human adenovirus Ad5 DNA).

Figure 2a shows determination of methylation of sites –183 and –483 located in promoter regions of *COX6B* and *UPK1A* of cell line 293. It should be noted that even at optimal conditions restrictases do not cause 100% cleavage and after many amplification cycles at the cleaved DNA template it is possible to obtain electrophoretically detectable amount of product. So, site methylation was not evaluated by the presence or absence of PCR-product of expected length. This parameter was evaluated by difference in number of PCR cycles required for detection of corresponding product using as a template DNA preparations cleaved by *Msp*I and *Hpa*II, and also cleaved and not cleaved by *Hpa*II. In 293 cells the site –181 of *COX6B* was unmethylated, because difference in appearance of product on DNA template cleaved by *Hpa*II and non-hydrolyzed DNA was six cycles. On the contrary, site –483 in promoter region of *UPK1A* was methylated because there no difference was observed between amplification of non-hydrolyzed DNA and DNA cleaved by *Hpa*II, whereas during treatment with *Msp*I amplification was delayed by at least four cycles.

In similar manner, we analyzed methylation in all selected sites in four cell lines and four tissues/organs. These results are summarized in table.

It should be noted that some of the studied sites might be partially methylated (i.e., methylation involves some of the cells of the whole pool). Such conclusion would be based on difference in PCR cycles required for appearance of corresponding band on DNA templates cleaved by *Hpa*II and *Msp*I (Fig. 2b). However, such difference may also represent an artifact due to the following reasons: 1) we have used a semiquantitative method, allowing difference in accumulation of PCR-products to be detected; 2) *Hpa*II may be less effective than *Msp*I in cleaving these restriction sites. So, we consider such sites as preferentially methylated or preferentially unmethylated in dependence of difference of cycle number (see the table).

Link of methylation to tissue-specific expression. Results of numerous studies demonstrate correlation between methylation of promoter region and inactive state of certain genes [6, 9]. We have investigated putative link between CCGG methylation and mRNA transcription of the studied genes.

Tissues characterized by expression of *ATP4A*, *LEAP-1*, *UPK1A*, and *COX6B* are known in the literature and database available on the Internet (www.genome.ucsc.edu). However, expression of our genes of interest is less characterized in cell lines. So, we have detected level of mRNA transcription of these genes in cell lines using RT-PCR. The data suggest that *UPK1A* is not transcribed in any of cell lines studied; *COX6B* is transcribed in all cells; *ATP4A* is transcribed in all cell lines except NGP, whereas *LEAP-1* is transcribed only in NGP. We also confirmed transcription of *LEAP-1* in human brain tissue (cerebellum). The table summarizes the literature and

Methylation of promoter regions and transcription of *ATP4A*, *LEAP-1*, *UPK1A*, and *COX6B* genes in four cell lines and four human tissues/organs

Tissue/cell line	*ATP4A*	*LEAP-1*	*UPK1A*	*COX6B*
Brain (cerebellum)	+/–	+/+	+/–	–/+
Stomach	+/+	–/–	+/–	–/+
Liver	+/+	–/+	+/–	–/+
Kidney	+/–	–/–	+/–	–/+
NGP	+/–	+/+	+/–	–/+
Tera-1	+/+	+/–	+/–	–/+
HL-60	+/+	–/–	+/–	–/+
293	+/+	+/–	+/–	–/+

Note: +/+, the site is methylated and the gene is transcribed; +/–, the site is methylated but the gene is inactive; –/+, the site is unmethylated, but the gene is transcribed; –/–, the site is unmethylated and the gene is inactive.

experimental data on gene transcription and methylation of the studied sites in promoter regions of these genes in corresponding human tissues and cell lines.

DISCUSSION

Promoter regions. We have tried to interpret results of this study from the viewpoint of the hypothesis on interrelationship between methylation state of promoter region and inactive state of a gene.

The housekeeping gene *COX6B* is expressed in all cell types. The promoter region of this gene, site −183, is unmethylated. This is typical for all housekeeping genes. Lack of methylation of promoter region of housekeeping genes is usually associated with overlapping of 5′-regions of most of these genes with CpG islands. Under normal conditions, these islands exist in unmethylated state. Site −183 studied by us is located outside of the CpG island, but its methylation status is the same as of CpG dinucleotides located within CpG islands.

UPK1A is strictly transcribed in urinary bladder urothelium and so the unmethylated state of site −483 may be associated with inactive state of this gene. Interestingly, at low transcription rate of uroplakin 1a in kidney [19] we found that this site is partially demethylated.

However, we did not find a clear interrelationship between transcription of *LEAP-1* and *ATP4A* and demethylation of corresponding promoter regions. The site CCGG −181 in the promoter region of *LEAP-1* is unmethylated in liver expressing this gene; however, unmethylated state is also observed in DNA of stomach, kidney, and HL-60 cell line where hepsidin mRNA has not been found. This site is methylated in the brain and neuroblastoma cells; some transcription level of *LEAP-1* has been found in human brain tissue and neuroblastoma. Site −590 in the promoter region of *ATP4A* is methylated in all studied tissues including stomach, expressing H^+/K^+-ATPase, and also in liver where *ATP4A* transcription has been reported [19]. This site is also methylated in HL-60, Tera-1, and 293 where ATPase mRNA is detected (table).

It should be noted that cell lines are more homogenous than tissues. Determining methylation state of some site in a tissue/organ containing a heterogeneous cell population, we just find some proportion of cells in which the site of interest is methylated. In such cases, we basically observe so-called partial methylation, i.e., methylation of the site in some proportion of cells of the studied population. It should be noted that we cannot always evaluate adequately how methylation state is linked to gene expression because the methylation sites may exist only in some (micro)population of cells, characterized by specific expression behavior compared to other cells of a given tissue. It is also possible that these sites may be uniformly distributed among all cells irrespectively to expression level of the corresponding gene. Use of cell lines overcomes this problem, but cell cultures do not adequately reflect the situation *in vivo*, because they are often characterized by abnormal methylation [20]. These features of research objects could influence our results and to some extent pervert interrelationship between methylation and transcription activity of *LEAP-1* and *ATP4A*.

We have analyzed data obtained from the viewpoint of consistence with the hypothesis that methylation of promoter region corresponds to inactive state of gene controlled by this promoter and *vice versa*. In the table, gray color designates 10 cases that contradict this hypothesis. In the other 22 cases, such correspondence has been observed.

Thus, genes analyzed in this study can be subdivided into two groups by dependence of their expression on methylation of CpG in their 5′-regions. The first group, characterized by clear interrelationship between methylation and transcription level, includes the housekeeping gene *COX6B* (lack of methylation unequivocally correlates with expression) and the uroplakin gene. The latter is expressed in only one tissue (urothelium) and its methylation strictly coincides with lack of expression. Genes expressed in some but not all tissues and cells constitute the second group. These genes (*LEAP-1* and *ATP4A*) are characterized by lack of correlation between methylation and expression.

We suggest that methylation provides some basal level of gene repression, which may be overcome due to tissue-specific transcription factors, whereas lack of methylation "gives" genes the possibility to be expressed in many cells and tissues. However, this hypothesis requires methylation analysis with simultaneous determination of expression level in many genes.

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REFERENCES

1. Panning, B., and Jaenisch, R. (1998) *Cell*, **93**, 305-308.
2. Yoder, L. A., Walsh, C. P., and Bestor, T. H. (1997) *Trends Genet.*, **13**, 335-340.
3. Feil, R., and Khosla, S. (1999) *Trends Genet.*, **15**, 431-435.
4. Jones, P. A., and Baylin, S. B. (2002) *Nat. Rev. Genet.*, **3**, 415-428.
5. Li, E., Bestor, T. H., and Jaenisch, R. (1992) *Cell*, **69**, 915-926.

6. Peek, R., Niessen, R. W., Schoenmakers, J. G., and Lubsen, N. H. (1991) *Nucleic Acids Res.*, **19**, 77-83.
7. Kudo, S., and Fukuda, M. (1995) *J. Biol. Chem.*, **270**, 13298-13302.
8. Thomassin, H., Flavin, M., Espinas, M. L., and Grange, T. (2001) *EMBO J.*, **20**, 1974-1983.
9. Futscher, B. W., Oshiro, M. M., Wozniak, R. J., Holtan, N., Hanigan, C. L., Duan, H., and Domann, F. E. (2002) *Nat. Genet.*, **31**, 175-179.
10. Cedar, H. (1988) *Cell*, **53**, 3-4.
11. Pfeifer, K. (2000) *Am. J. Hum. Genet.*, **67**, 777-787.
12. Walsh, C. P., and Bestor, T. H. (1999) *Genes Dev.*, **13**, 26-34.
13. Chernov, I. P., Akopov, S. B., Nikolaev, L. G., and Sverdlov, E. D. (2002) *J. Cell Biochem.*, **84**, 590-600.
14. Elkahloun, A. G., Bittner, M., Hoskins, K., Gemmill, R., and Meltzer, P. S. (1996) *Genes Chromosomes Cancer*, **17**, 205-214.
15. Magdinier, F., Ribieras, S., Lenoir, G. M., Frappart, L., and Dante, R. (1998) *Oncogene*, **17**, 3169-3176.
16. Krause, A., Neitz, S., Magert, H. J., Schulz, A., Forssmann, W. G., Schulz-Knappe, P., and Adermann, K. (2000) *FEBS Lett.*, **480**, 147-150.
17. Olsburgh, J., Harnden, P., Weeks, R., Smith, B., Joyce, A., Hall, G., Poulsom, R., Selby, P., and Southgate, J. (2003) *J. Pathol.*, **199**, 41-49.
18. Saccomani, G., Helander, H. F., Crago, S., Chang, H. H., Dailey, D. W., and Sachs, G. (1979) *J. Cell. Biol.*, **83**, 271-283.
19. Su, A. I., Cooke, M. P., Ching, K. A., Hakak, Y., Walker, J. R., Wiltshire, T., Orth, A. P., Vega, R. G., Sapinoso, L. M., Moqrich, A., Patapoutian, A., Hampton, G. M., Schultz, P. G., and Hogenesch, J. B. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 4465-4470.
20. Antequera, F., Boyes, J., and Bird, A. (1990) *Cell*, **62**, 503-514.
21. Yoshida, T., Sato, R., Mahmood, S., Kawasaki, S., Futai, M., and Maeda, M. (1997) *FEBS Lett.*, **414**, 333-337.
22. Courselaud, B., Pigeon, C., Inoue, Y., Inoue, J., Gonzalez, F. J., Leroyer, P., Gilot, D., Boudjema, K., Guguen-Guillouzo, C., Brissot, P., Loreal, O., and Ilyin, G. (2002) *J. Biol. Chem.*, **277**, 41163-41170.