A Partial Methylation Profile for a CpG Site Is Stably Maintained in Mammalian Tissues and Cultured Cell Lines*

(Received for publication, October 4, 1988)

Mitchell S. Turker,‡§∥, Karen Swisshelm,‡ Annette C. Smith,‡ and George M. Martin$†

From the ‡Department of Pathology, SM-30, University of Washington, Seattle, Washington 98195, the §Departments of Pathology and Microbiology/Immunology, University of Kentucky College of Medicine, and the †Lucille P. Markey Cancer Center, Lexington, Kentucky 40536

We wished to determine if a partial methylation profile for a specific CpG site was stably maintained in both mammalian tissues and cultured cell lines. To accomplish this, we identified a CpG site with a partial methylation profile located upstream of the mouse adenine phosphoribosyltransferase promoter region. This site was found to be methylated at a level of approximately 25% in mouse brain, kidney, lung, and skeletal muscle tissues, at a level close to 50% in liver, and at level close to 0% in tests. These tissue-specific methylation profiles were not altered during aging. A methylation profile of approximately 25% at this CpG site was also observed in five mouse teratocarcinoma stem cell lines and one additional cultured cell line. This profile, however, was altered upon cellular differentiation, adenine phosphoribosyltransferase hemizygosity, and a loss of adenine phosphoribosyltransferase activity in some of the cultured cell lines. We conclude that partial methylation of a specific CpG site can be stably maintained both in vivo and in vitro and that a mechanism exists for its maintenance. The functional significance of a partial methylation profile remains to be determined.

Many studies have demonstrated correlations between gene expression and the degree of cytosine methylation in mammalian somatic cells. A general rule is that there is an inverse correlation between the amount of methylation, particularly at the 5' end of a gene, and the degree of gene expression (Cedar, 1988). Methylation is also believed to play a role in X chromosome inactivation (Mohandes et al., 1981; Yen et al., 1984). Methylation in mammalian cells usually occurs at CpG sites. Although these sites are generally under-represented in the mammalian genome, they are frequently clustered at the 5' end of genes (Bird, 1986; Gardiner-Garden and Fromme, 1987). These clusters, termed CpG islands, are not methylated in constitutively expressed housekeeping genes and in some inducible genes (Gardiner-Garden and Fromme, 1987). In vitro methylation of this 5' region has been demonstrated to abolish expression of the Chinese hamster adenine phosphoribosyltransferase (APRT)† gene (Stein et al., 1982; Keshet et al., 1986).

Investigators have reported CpG sites with partial methylation profiles (i.e., methylation values greater than 0% and less than 100%) at specific CpG sites in DNA from both tissues (Stein et al., 1983; Yen et al., 1984; Mullins et al., 1987; Umero et al., 1988) and cell lines (Stein et al., 1983; Yisraeli et al., 1986; Schulz et al., 1988). To the best of our knowledge, however, attempts to determine the functional significance of these partial methylation profiles have not been attempted. As a first step we have chosen to determine if a partial methylation profile for a specific CpG site is stably maintained within different tissues of a single animal, in these tissues from a number of animals, as a function of aging, and in clonally derived cell lines. We report here the observation of a CpG site located upstream of the mouse APRT gene that displays a stable partial methylation profile of approximately 25% in cultured stem cell lines and in mouse brain, kidney, lung, and skeletal muscle tissues. Methylation at this site is close to 50% in mouse liver and close to 0% in mouse tests. The tissue-specific methylation profiles are not altered as a function of age. They are altered, however, in some cell lines as functions of cellular differentiation, APRT hemizygosity, and the loss of APRT activity. The results suggest that a mechanism exists to maintain this partial methylation profile. The functional significance of this partial methylation profile remains to be determined.

MATERIALS AND METHODS

Cell Culture and Lines—The PSA-1 derived stem cell lines were grown in Dulbecco's minimal essential medium (GIBCO) supplemented with 10% fetal bovine serum and penicillin-streptomycin (GIBCO) on a feeder layer as described elsewhere (Turker et al., 1984). The differentiated cell lines and the P-19 stem cell line were grown under identical conditions with the omission of the feeder layer. The isolation of the differentiated cell lines from the stem cells has been described in detail elsewhere (Turker et al., 1986). The proportion of differentiated cells in a well maintained stem cell line is approximately 5%.

DNA Isolation—Cell pellets were resuspended in STE buffer (150 mM NaCl, 10 mM Tris (pH 8.0), and 10 mM EDTA) and genomic DNA isolated as described in Davis et al. (1986) with minor modification. For isolation of DNA from tissues, the tissues were homogenized in a buffer containing 10 mM KCl, 10 mM Tris (pH 8.5), 1.5 mM MgCl₂, and 0.5% Nonidet P-40. The resultant preparation of nuclei was pelleted with centrifugation and resuspended into STE buffer prior to DNA isolation.

DNA Analysis and Probes—The published sequence of the mouse APRT gene (Dush et al., 1985), which includes approximately 800 bp of upstream sequences, was used to identify a potential methylation

* This work was supported by National Institutes of Health Grants AG00057, AG01751, and AG08199.
† To whom correspondence should be addressed: Dept. of Pathology, Markey Cancer Center, University of Kentucky College of Medicine, Lexington, KY 40536.
‡ The abbreviations used are: APRT, adenine phosphoribosyltransferase; kbp, kilobase pair.
∥ M. McBurney, personal communication.
Partial Methylation of a CpG Site

The critical sequences within this region necessary for promoter function have recently been determined (Dush et al., 1988). The H2 methylation site falls within the sequence CCGG which codes for the isoschizomer restriction enzymes HpaII and MspI. When the internal cytosine is methylated, MspI can digest the site whereas HpaII will not function. We note that the HpaII restriction enzyme cannot function on hemimethylated DNA (i.e. one strand is methylated and the other is not) (Gruenbaum et al., 1981). Therefore, the methylation values we reported could represent CpG sites methylated on either one or both DNA strands.

Four DNA probes were used for this study. The 966-base pair pN1 probe was obtained with a NaeI digest from a 3.1-kbp (kilobase pair) DNA fragment (pSam 3.1) containing the entire mouse APRT gene and upstream sequences (Dush et al., 1985). The pN1M1, pN1M2, and pN1M3 probes were obtained by digesting the pN1 probe with MspI. Fig. 1 shows the relative location of each of these probes.

**Use of Restriction and Methylase Enzymes**—The MspI, NaeI, and TaqI enzymes were obtained from either Boehringer Mannheim or Bethesda Research Laboratories (BRL) (Bethesda, MD). Due to the importance of the HpaII enzyme in this study, we obtained this enzyme from four sources (Boehringer Mannheim, BRL, U. S. Biochemical Corp., Cleveland, OH, and New England Biolabs). Similar results were obtained regardless of the manufacturer. For all experiments 10 μg of genomic DNA was digested with 15 units of the appropriate restriction enzyme and incubated at 37 °C overnight. This was followed by the addition of a second aliquot of 15 units of enzyme, with incubation for approximately 5-7 h. The HpaII methylase enzyme was obtained from New England Biolabs. In all cases the manufacturer’s recommended buffer conditions for each enzyme were followed.

**RESULTS**

**Identification of a CpG Site with a Partial Methylation Profile**—A preliminary study was undertaken to determine the methylation profile of the H2 site (Fig. 1) in mouse tissues. DNA was extracted from lung, kidney, liver, and testis of a 12-month 129SV mouse, digested individually with HpaII and MspI, separated by agarose gel electrophoresis, and blot-hybridized with a nonlabeled pN1 probe. Kidney DNA digested with MspI (msp) is also observed when the amount of DNA loaded on the gel was tripled (not shown). By referring to the APRT sequence (Dush et al., 1985), the relative map location of each hybridization band was determined (Fig. 1). In addition to the MspI/HpaII sites identified from the DNA sequence at −420 (H2), −33 (H3), and +94 (H4), a MspI/HpaII site was approximately localized to position −1120 (H1) (Fig. 1). When the HpaII digests for kidney, liver, testis, and lung were compared with the MspI digest, we observed that the 0.7-kbp band was missing. It was replaced by two hybridization bands of approximately 2.4 and 2.0 kbp (Fig. 2). We interpreted this result as indicating that the H1 site was completely methylated in all samples and that the H2 site was partially methylated. A methylated H2 site would yield the 2.4-kbp band whereas a nonmethylated H2 site would yield the 2.0-kbp band. From the size of these bands we determined that a HpaIIMspI site was located at approximately −2420 (H0, Fig. 1) and that it was not methylated. We have not determined if additional HpaIIMspI sites are located between the H0 and H1 sites, but note that if they exist they would also be methylated.

The relative intensities of the 2.0- and 2.4-kbp bands for each tissue shown in Fig. 2 were determined using scanning densitometry. Based on the assumption that these two bands represented partial methylation at the H2 site (verified below), we determined the degree of methylation at this site to be as follows: kidney, 27%; liver, 44%; lung, 20%; and testis, 8%. A Southern blot analysis with tissues from a 3-month C3H animal also demonstrated partial methylation at the H2 site and complete methylation at the H1 site. These results, however, were not quantitated (see Fig. 3 for C3H kidney).

To confirm that the 2.0- and 2.4-kbp hybridization bands seen with the HpaII digests represented alternate states of methylation at the H2 site, two experiments were performed. One was to use the pN1M1, pN1M2, and pN1M3 probes (see Fig. 1) to determine if each hybridized with either the 2.0- or 2.4-kbp bands or both. The pN1M1 probe hybridized effectively with both the 2.0- and 2.4-kbp bands, whereas the pN1M2 probe hybridized almost exclusively with the 2.4-kbp band (data not shown). Therefore, the 2.4-kbp hybridization band included the 383-bp N1M2 fragment, confirming methylation at the H2 site. The pN1M3 probe (Fig. 1) failed to hybridize with either the 2.0- or 2.4-kbp HpaII digestion

---

**Fig. 1.** HpaII methylation sites located upstream of the promoter region for the mouse APRT gene. Each HpaII site (H0-H4) is represented by a bubble. The percent of coloring within each bubble represents the average amount of methylation at that site (see text). The distances between each HpaII methylation site are indicated. The relative genomic locations of the probes used in this study (pN1, pN1M1, pN1M2, and pN1M3) are also shown. The start site and direction for APRT transcription is indicated with an arrow. Two TaqI restriction sites are also indicated (Tq).

**Fig. 2.** Partial methylation at the H2 site in tissues isolated from a 12-month 129SV animal. DNA samples from kidney (Ki), liver (Ll), testis (Te), and lung (Lu) were digested with HpaII, separated by agarose gel electrophoresis, and blot-hybridized with 32P-labeled pN1 probe. Kidney DNA digested with MspI (msp) is also shown.

---

**Note:** The HpaII enzyme was obtained from New England Biolabs. In all cases the manufacturer’s recommended buffer conditions for each enzyme were followed.
Partial Methylation of a CpG Site

bands, indicating that the H3 site (Fig. 1) was not methylated. Consistent with this, an expected 0.1-kbp hybridization band was observed in both the HpaII and MspI digestion lanes when pN1M3 was used as a probe (data not shown). This result also demonstrated that the H4 methylation site was not methylated (Fig. 1). Both the H3 and H4 methylation sites lie within the CpG island located at the 5′ end of the mouse APRT gene.

For a second experiment, the effect of in vitro methylation at the H2 site was examined. Fig. 3 demonstrates the expected 1:3 ratio of the 2.4- and 2.0-kbp hybridization bands for a HpaII digest of kidney DNA from the C3H animal. When this DNA was digested with both HpaII and TaqI (TaqI sites bracket the H2 methylation site, Fig. 1), hybridization bands of approximately 1.1 and 0.7 kbp were seen, again at a 1:3 ratio. In contrast, when the DNA was treated with HpaII methylase prior to a double digest with HpaII and TaqI, only the 1.1-kbp hybridization band was observed. The same 1.1-kbp hybridization band was observed for the methylase-treated DNA digested with TaqI alone (the TaqI sites were not affected by the HpaII methylation treatment) (Fig. 3). Therefore, the 0.7-kbp hybridization band seen with the double digest was due to the H2 site not being methylated, and the 1.1-kbp hybridization band was due to its being methylated.

Partial Methylation Profiles at the H2 Site Are Reproducible with Tissues from Different Animals—We next wished to determine if the tissue-specific partial methylation profiles observed for the 192SV and C3H animals were reproducible for a number of animals. We also wanted to determine if these methylation profiles were stable with age. Therefore, 6- and 30-month CBA/ca mice were obtained from the NIA colony maintained by Charles River Laboratories (Stone Ridge, NY). In addition to DNA preparations from kidney, liver, and lung tissues, we also isolated DNA from skeletal muscle and brain tissues. Testis DNA was isolated from only one CBA/ca animal. The methylation values at the H2 site for all samples analyzed by scanning densitometry during this study are presented in Table I. The average values obtained for the brain (26%), kidney (27%), lung (24%), and skeletal muscle (28%) tissues were essentially the same. However, the average value obtained by combining the data from these four tissues (26%) was significantly different from that obtained for the liver samples (44%) (p value < 0.0001). The average value for the two testis DNA samples examined was 6%. Table II demonstrates that the tissue-specific methylation values at the H2 site were not altered during aging. For this analysis we pooled the values from the brain, kidney, lung, and skeletal muscle DNA preparations obtained from CBA/ca mice.

Partial Methylation at the H2 Site in Clonally Derived Stem Cell Lines—The observation of a reproducible methylation value for the H2 site of approximately 25% in four separate tissues (brain, kidney, lung, and skeletal muscle) was intriguing. However, since each of the different tissues is composed of a variety of cell types, we felt it was important to also examine pure populations of cells. Therefore, DNA from clonally derived mouse teratocarcinoma stem cell lines (P-19 (McBurney and Rogers, 1982), NG2 (Dewey et al., 1977), E140, DAP1C, and DAP1B (Turker et al., 1984)) was examined. E140 is an APRT heterozygous deficient derivative (i.e. containing only one active APRT allele) of the wild type PSAl-1 stem cell line. DAP1B and DAP1C, which are derived from E140, are APRT homozygous deficient (i.e. they lack an active APRT allele) stem cell lines (Turker et al., 1984). NG2 is a hypoxanthine phosphoribosyltransferase deficient clone derived from PSAl-1 (Dewey et al., 1977); it is wild type for APRT expression. All of these stem cell lines have been karyotyped in our laboratory (Turker et al., 1984, 1989). They were found to have stable pseudodiploid karyotypes; most importantly, each contained two copies of chromosome 8 which contains the mouse APRT gene (Kozak et al., 1975).

Table III demonstrates methylation profiles at the H2 site for these stem cell lines near 25%. Therefore, a partial methylation profile for the H2 site was observed in clonal cell lines.

Alterations in Methylation Values at the H2 Site—Previ-
cell lines ranged from 3 to 8% (Table III). Therefore, there was a reduction in methylation at the H2 site when the APRT-deficient stem cell lines underwent cellular differentiation. In other work, we isolated APRT hemizygous deficient mutants (i.e., with a deletion of one of the two APRT alleles) from the P-19 stem cell line (Turker et al., 1989). To determine if deletion of one of the two APRT alleles had an effect on the methylation level for the H2 site, we compared two hemizygous mutants (P19H1 and P19H4) with the P-19 cell line. Table III demonstrates approximately a 50% reduction in methylation at the H2 site for both P19H1 and P19H4 at the H2 site (13 and 10%, respectively) as compared with P-19 (26%). We have also isolated P-19 mutants which had deletions of both APRT alleles (Turker et al., 1989). DNA from these deletion mutants lacked the 2.0- and 2.4-kbp methylation-related hybridization bands (not shown) indicating that these regions are not duplicated elsewhere in the mouse genome.

Finally, in two cases we have identified cell lines in which methylation at the H2 site exceeded 90%. One is the Su cell line, which is an APRT-deficient derivative of the ST0 fibroblast mouse cell line (Turker et al., 1984) (Fig. 4). The second case was an APRT-deficient cell line, TT-31D2, derived from the APRT hemizygous deficient differentiated cell line, TT-31. Fig. 4 demonstrates little methylation at the H2 site for the TT-31 cell line and nearly complete methylation at this site for the TT-31D2 cell line. Therefore, a loss of APRT activity in the TT-31D2 cell line was accompanied by a marked increase in methylation at the H2 site.

**DISCUSSION**

Partial methylation levels have been reported for numerous CpG sites in the mammalian genome (see the introduction), yet the relative stability and therefore potential significance of these profiles has not been rigorously tested. Theoretically, for a given CpG site of an autosomal gene in a specific cell type, there should be only three potential methylation values: 0, 50, or 100%. These values would represent methylation of neither allele, one allele, or both alleles, respectively. Methylation of only one allele could be indicative of parental imprinting (Swain et al., 1987). In tissues with complex mixtures of cell types, however, each cell type might be expected to have a specific methylation value for a specific CpG site. The relative proportion of the different cell types within each tissue would determine a consensus methylation profile. Therefore, when considering a single CpG site, it is reasonable to predict a wide range of methylation profiles when comparing a variety of tissue types.

In this work we have identified a CpG site (H2) with a partial methylation profile located upstream of the mouse APRT promoter and have demonstrated a consistent consensus value of 25% methylation for four of six tissues examined: brain, kidney, lung, and skeletal muscle. We consider it unlikely that these four tissues would achieve similar methylation profiles at the H2 site by random chance. Moreover, we note the possibility of discrete shifts in the methylation profile at the H2 site from the observations of methylation profiles near 50% (44%) for liver and near 0% (6%) for testis. In addition to comparing tissues from a number of animals of one strain (CBA/ca) and two additional strains (C3H and 129SV), we also demonstrated stability of the partial methylation profiles by examining animals of different ages. By pooling the data from the brain, kidney, lung, and skeletal muscle tissues, we demonstrated that aging did not alter the 25% methylation profile at the H2 site. The liver methylation profile near 50% (44–45%) was also not altered with aging.

---

**TABLE III**

**Methylation values at the H2 CpG site in clonal cell lines**

| Cell line* | Phenotype* | Assay no. | Methylation % |
|------------|------------|----------|---------------|
| NG2        | SC; WT     | 1        | 19            |
| NG2T3      | DC; WT     | 4        | 27            |
| E140       | SC; Het    | 2        | 22            |
| TT-3       | DC; Het    | 1        | 3             |
| TT-31      | DC; Het    | 1        | 3             |
| DAP1C      | SC; Hom    | 2        | 25            |
| TC-21      | DC; Hom    | 2        | 8             |
| DAP1B      | SC; Hom    | 1        | 22            |
| TC-B-6     | DC; Hom    | 1        | 6             |
| P-19       | SC; WT     | 2        | 27            |
| P19H1      | SC; Hem    | 1        | 13            |
| P19H4      | SC; Hem    | 1        | 10            |

---

* The origin of the cell lines listed above can be found in Turker et al. (1984, 1986, 1989). Pertinent information concerning these cell lines can be found in the text of this manuscript.

* SC, stem cell line; DC, differentiated cell line derived from a stem cell line (see Turker et al., 1986); WT*, APRT, wild type; Het-APRT, heterozygote-deficient (i.e., both alleles are present, but only one is expressed); Hom-APRT, homozygous deficient (i.e., both alleles are present and neither is expressed); Hem-APRT, hemizygous deficient (i.e., one of the alleles has been deleted).

* "no." represents the number of times a given DNA preparation was analyzed. Each analysis required a separate restriction enzyme digestion, blot hybridization, and scanning densitometry determination.

---

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Cell lines with nearly complete methylation at the H2 site. DNA samples from the Su (A), TT-31 (B), and TT-31D2 (C) cell lines were digested with HpaII, separated by agarose gel electrophoresis, and blot-hybridized with 32P-labeled pN1 probe. An MspI digest of the Su cell line DNA is indicated (map).
Therefore, despite presumed cell turnover in these tissues over the lifespan of the animals, age-related changes in the methylation profiles did not occur. Biochemical analyses have demonstrated hypomethylation with aging in the mouse (Singhal et al., 1987; Wilson et al., 1987) and a molecular analysis has demonstrated hypomethylation specifically for the mouse intracisternal A particle genes (Mays-Hoopes et al., 1983). Hypomethylation has also been demonstrated to occur during in vitro aging for most human diploid fibroblast lines, although at least one line exhibited hypermethylation (Shmookler Reis and Goldstein, 1982a). This may explain why clonal variation for methylation patterns has been observed for specific genes in such cell lines (Shmookler Reis and Goldstein, 1982b; Goldstein and Shmookler Reis, 1985).

A methylation profile of approximately 25% at the H2 site was also observed in six cultured cell lines, five of which were stem cell lines. Since each cell line represented a pure cell population, this result was particularly informative. Each of these cell lines was examined cytogenetically and determined to have two copies of chromosome 8 and therefore presumably two copies of the APRT gene. Moreover, these lines were clonally isolated. The observed methylation value of 25%, however, implied that only one in four APRT alleles was methylated at the H2 site. Therefore, either every other cell was methylated at one allele, every fourth cell was methylated at both alleles or a combination of both of these possibilities existed. Regardless, this observation raises the distinct possibility that the cells possess a mechanism for maintenance of a partial methylation profile at the H2 site. Such a mechanism could also explain the 25% profiles for the brain, kidney, lung, and skeletal muscle tissues. At this time, however, we do not understand how such a mechanism would work and in particular if it would be an active or passive mechanism. The possibility of an active mechanism, while intriguing, implies intercellular communication of intracellular methylation levels.

A premise which led us to undertake this study was that the stable maintenance of a partial methylation profile may have functional significance. Due to our interest in APRT gene expression (Turker et al., 1984, 1986, 1989; Turker and Martin, 1985), we chose to use this gene as a model system. Since the CpG island at the 5′ end of the mouse APRT gene is not methylated, we decided to examine a CpG site (H2) located slightly upstream of APRT promoter region. We note that the H2 site is also located several hundred bases downstream of an apparent consensus polymerase II promoter (Dush et al., 1985). At the present time, however, there is no evidence that this promoter region is functional. Three results presented in this work suggested that changes in the methylation profile at the H2 site could perhaps be correlated with changes in APRT activity. The first was that the partial methylation level of 25% in the APRT-deficient stem cell lines was reduced when these lines underwent cellular differentiation. This change in methylation was not observed when an APRT wild type stem cell line underwent differentiation. The second was that the deletion of one of the two APRT alleles, inclusive of the H2 site, in the P-19 stem cell line was correlated with a 50% reduction in relative methylation at the H2 site for the remaining APRT allele. Finally, a potential relationship between methylation of the H2 site and APRT expression was seen with two APRT-deficient cell lines, Su and TT-31D2. DNA from both cell lines was almost completely methylated at the H2 site (Fig. 4). In particular, we were able to correlate the acquisition of methylation at the H2 site with a loss in APRT activity for the TT-31D2 cell line. A tentative conclusion that can be drawn from these results is that a degree of methylation at the H2 site may be involved in the regulation of APRT expression in some cell types. More work, however, will be required to validate this possibility.

In conclusion, we have demonstrated a high level of tissue specificity and stability for partial methylation at a specific CpG site located upstream of the promoter region for the mouse APRT gene. This stability was determined by examining DNA from different tissues, from a number of animals of a single strain (CBA/ca) and two additional strains (C5H and 129SV), animals of different ages, and a number of clonally derived stem cell lines representing pure cell populations. In four of six tissues examined and the stem cell lines a partial methylation level of approximately 25% was consistently observed. These results suggest a mechanism for the maintenance of partial methylation profiles in mammalian cells. The functional significance of such profiles remains to be determined.

Acknowledgments—We thank Drs. M. Fry and J. Davidson for helpful discussions and A. Nelson for technical assistance.

REFERENCES

Bird, A. P. (1986) Nature 321, 209-213

Cedar, H. (1986) Cell 53, 2-4

Davis, L. G., Dibner, M. D., and Battey, J. F. (1986) Basic Methods in Molecular Biology, Elsevier Science Publishing Co., New York

Dewey, M. J., Martin, D. W., Jr., Martin, G. R., and Mintz, B. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5564-5568

Dush, M. K., Sikela, J. M., Khan, S. A., Tischfield, J. A., and Stamhrook, P. J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2731-2735

Dush, M. K., Briggs, M. R., Royce, M. E., Schaff, D. A., Khan, S. A., Tischfield, J. A., and Stamhrook, P. J. (1988) Nucleic Acids Res. 16, 8393-8414

Goldstein, S., and Shmookler Reis, R. J. (1985) Nucleic Acids Res. 13, 7055-7065

Gruenbaum, V., Cedar, H., and Razin, A. (1981) Nucleic Acids Res. 9, 2509-2515

Gardiner-Garden, M., and Frommer, M. (1987) J. Mol. Biol. 196, 261-262

Keshet, I., Yisraeli, J., and Cedar, H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2560-2564

Kozak, C. E., Nichols, E. A., and Ruddie, F. H. (1975) Somat. Cell Genet. 1, 371-382

Mays-Hoopes, L. L., Brown, A., and Huang, R. C. (1983) Mol. Cell. Biol. 3, 1371-1378

Mckinney, M. W., and Rogers, B. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 82, 4962-4966

McBurney, M. W., and Rogers, B. J. (1988) Dev. Biol. 131, 503-508

Mohandes, T., Sparker, R. S., and Shapiro, L. J. (1981) Science 211, 394-396

Mullins, L. J., Veres, G., Caskey, C. T., and Chapman, V. (1987) Mol. Cell. Biol. 7, 3916-3922

Schnitzler, W. A., Crawford, N., and Locker, J. (1989) Exp. Cell Res. 174, 433-447

Singhal, R. P., Mays-Hoopes, L. L., and Elchhorn, G. L. (1987) Mech. Ageing Dev. 38, 189-210

Shmookler Reis, R. J., and Goldstein, S. (1982a) Proc. Natl. Acad. Sci. U. S. A. 79, 3949-3953

Shmookler Reis, R. J., and Goldstein, S. (1982b) Nucleic Acids Res. 10, 4293-4304

Stein, R., Razin, A., and Cedar, H. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 5418-5422

Stein, R., Scialy-Gallili, N., Razin, A., and Cedar, H. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2427-2430

Swan, J. W., Stewart, T. A., and Leder, P. (1987) Cell 50, 719-727

Turker, M. S., and Martin, G. M. (1985) Mol. Cell. Biol. 5, 2662-2668

Turker, M. S., Smith, A. C., and Martin, G. M. (1984) Somat. Cell Mol. Genet. 10, 189-199

Turker, M. S., Tischfield, J. A., Rahmohinov, P., Stambrook, P. J., Trill, J. J., Smith, C., Ogburn, C. E., and Ogburn, C. R. (1986) J. Exp. Pathol. 2, 299-311

Turker, M. S., Stambrook, P. J., Tischfield, J. A., Smith, A. C., and Martin, G. M. (1980) Somat. Cell Mol. Genet. 6, 55-60

Umero, M., Song, B. J., Gozak, C., Gelboin, H. V., and Gonzales, F. J. (1988) J. Biol. Chem. 263, 4906-4912

Wilson, V. L., Smith, R. A., Ma, S., and Cutler, R. G. (1987) J. Biol. Chem. 262, 8591-8595

Yaffe, D., and Cedar, H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7631-7635

Yeh, P. H., Paki, P., Chiinault, H. A. C., Mohandes, T., and Shapiro, L. J. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 1759-1763

Yisraeli, J., Adelstein, R. S., Melilad, N., Nudel, U., Yaffe, D., and Cedar, H. (1986) Cell 46, 409-410