Relative levels of methylation in human growth hormone and chorionic somatomammotropin genes in expressing and non-expressing tissues

Brian L.Hjelle*, John A.Phillips, III* and Peter H.Seeburg†

*Department of Pediatrics, John Hopkins University School of Medicine, Baltimore, MD 21205, and †Department of Molecular Biology, Genentech Inc., South San Francisco, CA 94080, USA

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ABSTRACT

It has been shown that the extent of methylation of cytosine in vertebrate DNA is inversely correlated with gene expression. We studied cytosine methylation in and around the homologous human growth hormone (GH) and chorionic somatomammotropin (CS) genes to determine if these genes are undermethylated in DNA from tissues in which they are expressed (pituitary and placenta, respectively) compared to other tissues. Hpa II and Hha I (which cleave only unmethylated 5' CCGG 3' and 5' GCGC 3' respectively) and Msp I (which cleaves CCGG and C^{me}CGG) were used to digest DNA samples followed by gel electrophoresis, Southern transfer and hybridization with a GH cDNA probe. The extent of methylation of Hpa II and Hha I sites in the GH and CS genes was leukocyte >> pituitary > placenta = hydatidiform mole. Taken as a whole, our data support the hypothesis that undermethylation is a necessary but not sufficient condition for gene expression since placental and pituitary DNAs are less methylated than leukocyte DNA in this region. However, the correlation between gene expression and undermethylation is imperfect since (1) hydatidiform mole DNA has a very similar methylation pattern compared to placental DNA even though moles make little or no CS and (2) the level of methylation of the GH gene compared to the CS gene does not vary in a tissue-specific manner.

INTRODUCTION

DNA Methylation

Approximately two to seven percent of the total cytosine residues in mammalian DNA is modified by the post-replicative addition of methyl groups to produce 5-methyl cytosine (^{me}C)(1). The great majority (90% or more) of such modifications occur on cytosine residues immediately 5' to guanine residues, i.e. in the sequence 5' CG 3'(2-5). The distribution of ^{me}C residues has been examined by digestion of DNA with restriction endonucleases with recognition sequences containing the dinucleotide CG. Many of these "CpG enzymes" will not cleave at their recognition sites if the cytosine residue is modified (6,7). Hha I, for example, will cleave GCGC, but not G^{me}CGC (4). Methyl-sensitive CpG enzymes have been used to study the extent

of methylation at specific cytosine residues and the regional distribution of ^{14}C in DNA (7-16). However, only a fraction of the total ^{14}C content in a given region of DNA can be detected by this approach.

One particularly useful system for studying methylation of DNA utilizes the isoschizomers Hpa II and Msp I. Msp I will cleave DNA at the sequence CCGG regardless of whether or not the internal cytosine is methylated, whereas Hpa II will cleave only unmethylated CCGG sequences (6,7). Digestion of genomic DNA separately with Hpa II and Msp I followed by transfer to nitrocellulose membranes and hybridization with a sequence-specific ^{32}P -labeled probe allows the detection, and to some extent, the quantitation of methylation at CCGG sites. When differences are observed between Hpa II digestions of DNA from different individuals, Msp I digestion can be used to determine if the differences are due to methylation or to restriction site polymorphisms.

Much of the DNA methylation literature has been reviewed by Razin and Riggs (4) and later by Ehrlich and Wang (5). While various functions have been proposed for methylation in eukaryotes, the greatest amount of attention has been focused on an association between methylation and gene expression. It has been demonstrated that local undermethylation at specific restriction sites correlates with gene activity in the chicken (8), human (9) and rabbit (7,10). β -globin-like genes, the ovalbumin and other chicken genes (11), and in viral genes in virus-transformed cell lines (12,13). Furthermore, five-azacytidine, which induces a decrease in DNA methylation, also appears to induce the expression of genes which had previously been inactive (14,15).

To determine if methylation differs in tissues with different patterns of gene expression, we compared the methylation of Hpa II and Hha I recognition sites within and around the highly homologous genes for human growth hormone (GH) and chorionic somatomammotropin (CS) in several tissues. The tissues studied included pituitary and placenta (which express GH and CS, respectively) and leukocyte and hydatidiform mole (which express little or no GH or CS).

GH and CS Genes

GH and CS are 191-amino acid polypeptide hormones with approximately 85 percent amino acid homology (17). GH-secreting cells comprise a subpopulation of the acidophilic cells in the anterior pituitary (adenohypophysis). These acidophilic cells, some of which secrete prolactin, represent about 35 percent of the total cell population in the adenohypophysis (18). CS is synthesized in the syncytiotrophoblastic cells of the normal placenta (19).

Synthesis of CS reaches a maximum at term gestation, at which time at least 20 percent of poly A-containing placental RNA is CS messenger RNA (mRNA) (20). In contrast to normal placenta, CS synthesis appears to be deficient or absent in the hydatidiform mole, a form of trophoblastic neoplasm (21).

The genes encoding GH and CS are 92 percent homologous in their coding regions and both sequences hybridize efficiently to an 800 base pair (bp) cDNA probe prepared from GH mRNA (22). Digestion of genomic DNA with EcoRI produces fragments of 2.6 and 2.9 kilobase-pairs (kb) which contain the entire GH and CS genes, respectively (22). At least two non-allelic GH genes, the normal (GH-N) and variant (GH-V) are found in the 2.6 kb fraction, only one of which (GH-N) is known to be expressed (22-24). The 2.9 kb fraction contains two or more non-allelic CS genes (23,25), but it is not known which of these is (are) expressed.

A 9.5 kb fragment containing an uncharacterized growth hormone-like (GH-L) sequence is also produced in EcoRI digestions. Because this sequence hybridizes less strongly to the chGH 800 probe than do the GH or CS gene sequences, it has been suggested that the GH-L sequence differs significantly in sequence and may be non-functional (22-23). Deletion of the GH-N locus or both CS loci results in the complete deficiency of GH or CS polypeptides, respectively, despite the presence of grossly intact GH-L sequences (24,26). These data tend to support the idea that the GH-L locus does not produce measurable amounts of GH or CS.

Although all of the GH-related genes are on the long arm of chromosome 17 (27,28), the restriction map of the GH/CS complex as a whole is not known.

MATERIALS AND METHODS

Genomic DNAs and Genomic DNA Clones

Pituitaries from cadavers were provided by Dr. S. Raiti of the National Pituitary Agency. Anterior lobes were separated from posterior lobes before DNA was extracted. Nuclear DNA was prepared from blood and from anterior pituitary tissue by the method of Kunkel et al (29). DNAs similarly prepared from term placentas and an hydatidiform mole as well as *E. coli* DNA were the generous gift of Dr. S. Wolf.

The clones used, which contain either the GH-N (λ GH-N) or the CS (λ CS) sequences had been characterized after isolation from a library of human genomic DNA (23). Aliquots of approximately 400 picograms of EcoRI-digested λ recombinants were digested in the presence of 1 μ g of *E. coli* DNA with 1 unit of Hpa II for 10 to 60 minutes to produce partial and complete

digestions.

Restriction Endonucleases

Restriction enzymes Hpa II, Msp I, Hha I, EcoRI and Hind III were obtained from Bethesda Research Laboratories and New England Biolabs. Digestions of genomic DNA were performed as recommended by the supplier except 2.5 to 12 units of enzyme were added per ug of DNA and reactions were allowed to proceed from 5 to 20 hours.

GH Probe Preparation

The recombinant plasmid chGH 800/pBR 322 contains nearly full-length complementary DNA (cDNA) to GH mRNA (30). The 800-bp GH cDNA insert was separated from pBR322 by Hind III digestion followed by electrophoresis and recovery from agarose gels (31). The purified 800-bp DNA fragment was labeled to a specific activity of approximately 10^8 cpm/ug using [α - ^{32}P] dATP and [α - ^{32}P] dCTP (400 Ci/mMol, Amersham) by "nick translation" with E. coli DNA polymerase I (32).

Agarose Gel Electrophoresis and Transfer to Nitrocellulose

Agarose gel electrophoresis of DNA, transfer to nitrocellulose membranes, and hybridization to the ^{32}P -labeled chGH800 probe were as previously described (28,33-34). In some cases EcoRI-digested genomic DNA was subjected to electrophoresis in agarose, certain size fractions were excised and the DNA was purified by electroelution and DEAE-cellulose chromatography (35). Fragments purified in this manner were then digested with a second restriction enzyme followed by electrophoresis and transfer to nitrocellulose.

RESULTS

The locations of the Hpa II sites (downward arrows) and Hha I sites (upward arrows) within the 2.6 kb GH-N fragment and 2.9 kb CS fragment produced by digestion with EcoRI are shown in Figure 1. The locations of these sites were determined by DNA sequencing (36). Within the GH-N gene fragment six out of the 65 CG dinucleotides are found in Hpa II recognition sites, while seven out of 70 CG sequences in the CS gene fragment are in Hpa II sites. The distribution of Hpa II and Hha I sites within the GH-V gene is similar to that of the GH-N gene. One additional Hpa II site (not shown) was present in one but not in another CS clone and may represent a polymorphic restriction site.

Hpa II Digestions

Figure 2 is an autoradiogram of 5 ug aliquots of Hpa II digested genomic

Figure 1 - Hpa II (downward arrows) and Hha I (upward arrows) restriction map of the 2.6 kb EcoRI-generated fragment containing the complete GH gene (left). The map of the 2.9 kb EcoRI fragment containing the intact CS gene is also shown (right). The organization of these genes on the chromosome is not known. Exons are shown as boxes.

DNA from leukocytes (L1 and L2), pituitary (PI 1), placenta (PL 1 and PL 2) and hydatidiform mole (HM). In each pair of lanes the DNA samples were identical but in each case the DNA in the right lane was digested with twice as much enzyme. The average size of the GH-hybridizing fragments in Hpa II digestions was greatest in leukocyte DNA and smallest in placental and hydatidiform molar DNA, indicating that leukocyte DNA is the most heavily

Figure 2 - Autoradiogram of Hpa II-digested genomic DNAs (5 μ g/lane) from leukocytes (L1 and L2), anterior pituitary (PI 1), placenta (PL 1 and PL 2), and hydatidiform mole (HM) subjected to electrophoresis in 1% agarose, transferred to nitrocellulose and hybridized with chGH800. The right lane DNA sample in each pair of lanes was digested with twice as much Hpa II as was the left lane sample. Sizes (in kb) are shown on the left.

methyated. Because of the cross-hybridization between the GH and CS genes, it is not possible to determine the gene from which each of the fragments was derived. It should be noted that a very large number of distinct bands are present, particularly in the pituitary, placental and hydatidiform molar DNAs. This indicates that a wide variety of methylation patterns are present in different cells in this region of DNA. It is surprising that hydatidiform molar DNA is virtually indistinguishable from placental DNA in its methylation pattern even though moles appear to synthesize little or no CS.

Hha I Digestions

To determine if other CG sequences were methylated to the same extent as those in Hpa II recognition sites, aliquots of leukocyte, pituitary, placental and hydatidiform molar DNA were digested to completion with Hha I. The autoradiogram obtained following Hha I digestion is shown in Figure 3. Again, for each sample, the aliquot in the right lane was digested with twice as much endonuclease as the aliquot in the left lane. The four tissues can once again be ordered in decreasing levels of DNA methylation as follows: leukocyte (L1 and L2) >> pituitary (PI 1) > placenta (PL 1 and PL 2) =

Figure 3 - GH and GH-related Hha I generated restriction fragments from leukocytes (L1 and L2), anterior pituitary (PI 1), placental (PL 1 and PL 2) and hydatidiform molar (HM) genomic DNA. Arrows indicate fragments produced in complete Hha I digestions of genomic DNA clones containing the intact GH (λ GH) and CS (λ CS) genes. Twice as much restriction enzyme was used for digestion of DNA in the right lane of each pair as in the left.

hydatidiform mole (HM). Once again, a large variety of fragments were observed, including many that are larger than would be expected if no methylation were present (see Figure 1). The fragments observed following complete digestion of the unmethylated λ GH-N and λ CS recombinants with Hha I are shown by arrows. Not all of the fragments produced by Hha I digestion of genomic recombinants can be predicted from the maps shown in Figure 1 because additional Hha I sites flank the EcoRI sites.

Nearly every fragment seen in DNA from one tissue is seen in the other DNAs as well, except that the smaller fragments are not visible in leukocyte DNA. This may be because the average level of methylation in leukocyte DNA is too high for the smallest fragments to be present in appreciable quantities. A few bands do appear to be disproportionately represented in pituitary compared to placental DNA or in placental compared to pituitary DNA. These differences may be due to specific differences in methylation, or to restriction site polymorphisms. Other investigators have demonstrated a high level of polymorphism in the recognition sites of CpG endonucleases (11), presumably because of a high rate of transition of ^{me}C to thymidine (37).

Several pieces of evidence suggest that these differences observed in Hpa II and Hha I digestion patterns between DNAs of different tissues were not due to incomplete digestions. All digestions of DNA from a single tissue produced very similar patterns and no changes were observed when larger amounts of enzyme and longer digestion times were employed. All digestions were performed with a large excess of endonuclease, typically three to ten units of enzyme per μ g of DNA for 16 hours.

Msp I Digestions

The large variations in Hpa II digestion patterns between DNAs from different tissues (Figure 2) could be due primarily to differences in methylation levels or to differences in DNA sequence (restriction site polymorphisms). Arguing against the latter alternative is the observation that the digestion patterns are tissue-specific. To further demonstrate that polymorphisms do not represent a major determinant of the observed variation in Hpa II digestion patterns, Msp I digestions were performed (Figure 4) on DNAs from leukocyte (L 1), pituitary (PI 2), placenta (PL 2) and hydatidiform mole (HM). In contrast to the striking differences seen in patterns produced by Hpa II digestion of DNAs from the four tissues, relatively small differences (limited to the four largest fragments) were observed following Msp I digestion. These four large fragments (3.3 to 4.3 kb) are derived from

Figure 4 - Msp I digestions of DNA from leukocytes (LI), anterior pituitary (PI 2), placenta (PL 2) and hydatidiform mole (HM). Twice as much Msp I was used in the right lane of each pair of lanes.

the GH-L sequences and from the 3' portions of the two CS genes (25). The variations in Msp I generated fragments could be due either to methylation of the external cytosine of the CCGG sequence (^{me}CCGG) or to sequence polymorphisms (38). We believe these different patterns are caused by two independent restriction polymorphisms because the differences are not tissue-specific and are inherited in a Mendelian fashion in leukocyte DNA from nine families (25). In addition, several other closely linked polymorphic restriction sites (non-CG-containing) segregated with the Msp I markers in these families in a manner which was also compatible with simple Mendelian inheritance (25). Thus it is evident that polymorphisms in the Hpa II/Msp I recognition sites alone are not adequate to explain the tissue-specific differences seen in Hpa II digestion patterns (Figure 2) and that methylation must be considered to be by far the major contributor.

EcoRI plus Hpa II Digestions

To estimate the relative levels of methylation at CCGG sequences in the CS genes compared to the GH genes it is necessary to resolve GH-derived Hpa

II restriction fragments from CS-derived fragments and to identify the origin of each. Although resolution of analogous fragments from highly homologous genes may be difficult, it is possible to achieve some separation of GH- and CS-derived fragments using EcoRI plus Hpa II digestions (Figures 5 and 6).

In Figure 5 partial Hpa II digestions of EcoRI-digested λ GH and λ CS genomic clones (lanes λ GH and λ CS) were subjected to electrophoresis beside a limit EcoRI plus Hpa II digestion of a placental DNA sample (PL2). These partial digestions of genomic clones containing the GH or CS genes produced an array of fragments which can be aligned with the fragments resulting from complete digestion of a genomic DNA sample. It is not possible to prove that a band seen in the genomic DNA digestion is derived exclusively from only one of the two genes by this method. To determine if a "single" band in the genomic DNA digests might be composed of fragments derived from both the GH

Figure 5 - EcoRI-digested genomic DNA clones containing the intact GH-N (λ GH) and CS (λ CS) genes were digested partially with Hpa II and subjected to electrophoresis beside a complete EcoRI plus Hpa II digestion of placental DNA (lane PL 2). Lanes 2.6 GH and 2.9 CS contain 2.6 kb and 2.9 kb fractions of EcoRI-digested, gel-fractionated placental DNA which were purified from the gel and redigested with a large excess of Hpa II. Sized (in kb) are shown on the left.

Figure 6 - Complete EcoRI plus Hpa II digestions of the λ GH-N genomic clone (lane 1), two different preparations of leukocyte DNA (lanes 2 and 3), anterior pituitary DNA (lanes 4 and 5), placental DNA (lanes 6 and 7), an hydatidiform mole DNA sample (lane 8), and the λ CS genomic clone (lane 9). The λ clones are completely cleaved with Hpa II because they are not methylated at Hpa II sites.

and CS genes, EcoRI-digested placental DNA was fractionated in agarose gels and the 2.6 kb GH- and 2.9 kb CS-containing fractions were purified, digested with a large excess of Hpa II, and subjected to electrophoresis (lanes 2.6 GH and 2.9 CS, Figure 5). The isolated genomic fractions produced a pattern similar to those obtained from the Hpa II partial digestions of the EcoRI-digested λ GH and λ CS clones.

Two bands (migrating at 1.8 kb and 0.4 to 0.6 kb) in the total placental DNA EcoRI plus Hpa II digestion (Figure 5, lane PL2) appear to arise from both the GH and CS genes, by virtue of their appearance in the Hpa II digests of both the 2.6 kb GH and 2.9 kb CS fractions (lanes 2.6 GH and 2.9 CS). However, most of the other fragments appear to be derived exclusively from one gene or the other. Thus an estimate of the degree of methylation of the GH and CS genes can be obtained in each tissue type to determine, for example, if the CS gene is less methylated than the GH gene in placenta. It is apparent from Figure 5 (lanes 2.6 GH, 2.9 CS and PL 2) that the CS genes

in placental DNA do not appear to be any more susceptible to digestion with Hpa II than do the GH genes; thus, the CS genes do not appear to be less methylated than do the transcriptionally inactive GH genes.

The patterns seen after EcoRI plus Hpa II digestions of DNAs from leukocytes (Lanes 2 and 3), pituitaries (lanes 4 and 5), placenta (lanes 6 and 7) and an hydatidiform mole (lane 8) are shown in Figure 6. Limit Hpa II digestions of the λ GH (lane 1) and λ CS (lane 9) clones were done to identify the fragments expected from Hpa II cleavage at unmethylated CCGG sites. As one might expect from the digestions with Hpa II alone (Figure 2), most of the signal in the leukocyte DNA EcoRI plus Hpa II digestions is in the 2.9 kb and 2.6 kb bands. These fragments result from all of the Hpa II recognition sites in the region of the GH and CS genes being in the methylated state (see Figure 1). Numerous other bands are present in the DNAs of the four tissues; all of those below 2.6 kb correspond to GH and CS fragments with one or more Hpa II sites in the unmethylated state, whereas all fragments above 2.9 kb are derived from the weaker-hybridizing GH-L sequence (which migrates at 9.5 kb in digestions with EcoRI alone). This 9.5 kb GH-L fragment, when excised from a gel and digested with Hpa II, does not contribute any significant amount of signal in the region below 2.6 kb (data not shown).

From the sizes of fragments obtained following Hpa II plus EcoRI digestion (see Figures 5 and 6) and the known Hpa II restriction maps of the GH and CS genes (Figure 1), it is possible in many cases to determine the origin of specific bands. The presumed origin of each band was confirmed by isolation of the 2.9 and 2.6 kb EcoRI-generated fragments of DNA from all tissues except hydatidiform mole from gels and digestion with Hpa II (data not shown). The assignments of the various EcoRI plus Hpa II-generated bands observed in Figure 5 and 6 are shown in Figure 7.

Methylated Hpa II sites are represented in Figure 7 as closed circles whereas unmethylated Hpa II sites are represented as open circles. In cases where it could not be determined with certainty how many fragments might actually be present in a dark band or smear, all fragments in that size range are depicted. The fragments diagrammed in Figure 7 appeared to be present in EcoRI plus Hpa II digestions of pituitary, placental and hydatidiform molar DNA. Similarly digested leukocyte DNA yielded all fragments seen in DNA from the other tissues of more than 1.1 kb, and some of the smaller fragments became visible after prolonged exposure. Thus, a wide variety of methylation arrangements are present among the different cells in each tissue.

Figure 7 - *Hpa* II recognition sites (arrows) in the 2.6 kb *Eco*RI-generated GH gene fragment (left) and in the 2.9 kb CS gene fragment (right) are methylated (●) or unmethylated (○) in various combinations to produce the array of fragments shown beneath the maps. All or nearly all of the fragments depicted were observed in *Eco*RI plus *Hpa* II-digested DNA from the four tissues examined (Figures 5 and 6), although in some cases exposure times of up to 1 to 2 weeks were required to visualize small fragments. Two or three *Hpa* II recognition sites occurring close together were treated as one because the precise patterns of methylation at those sites could not be distinguished by our methods. When several fragments appeared to be present in a particular band (smear), all of the possible fragments in that size range are shown. Sizes of the fragments are indicated in bp.

DISCUSSION

A number of reports have shown that local undermethylation of cytosine residues appears to be correlated with gene expression (7-15). Our data are consistent with these observations in that undermethylation was shown to occur in the transcriptionally active CS genes in placenta. The GH gene is shown to be less methylated in pituitary DNA than in leukocyte DNA. In addition, leukocytes, which express neither GH nor CS, were found to be heavily methylated at all sites within both the GH and CS genes.

If methylation is an important determinant of gene expression, it should be possible to establish that undermethylation is specific, i.e., that the expressed gene is less methylated than unexpressed genes in the same tissue. For example, since placental DNA appears to have a low overall level of cytosine methylation (5,9), it is possible that the CS genes are not specifically undermethylated compared to loci which are not expressed. To address this question, we attempted to compare the degree of methylation at *Hpa* II sites in the CS genes in placental DNA to that of the homologous but non-expressed GH genes (Figure 6). A similar comparison was made between the two genes in pituitary, where GH is expressed and CS is not. Hydatidiform mole was used as an additional control tissue because it is closely related to the placenta but is deficient in CS synthesis (21).

Because the Hpa II sites occur close together in the GH and CS genes (Figure 1), a large number of small restriction fragments would have to be resolved and accurately quantitated to estimate the extent of methylation at each Hpa II site. However, a comparison of the overall level of methylation in these genes in different tissues can be made by examining Figure 6, since many of the bands in this autoradiogram can be assigned to either the GH or CS genes (cf Figures 5 and 7). Both genes appear to be present in fully methylated copies (2.9 and 2.6 kb for CS and GH respectively) and less methylated copies (1.8, 1.4-1.2, 1.0, 0.8 and 0.6-0.4 kb for GH and 2.1, 1.6, 1.1 and 0.6-0.4 kb for CS) in both pituitary and placental DNA (Figure 6). Both genes appear to be divided to a similar extent between fully methylated and less methylated copies in both pituitary (lanes 4 and 5) and placenta (lanes 6 and 7). Thus, we cannot conclude from these data that expressed genes are specifically undermethylated compared to closely linked genes which are not expressed. In addition, the methylation pattern of DNA from hydatidiform mole appears to be virtually indistinguishable from that of placental DNA even though the hydatidiform mole makes little or no CS. This result suggests that methylation patterns may more closely reflect the state of differentiation of a tissue than its pattern of gene expression.

There are several possible explanations for our inability to demonstrate relative undermethylation of active genes compared to related inactive genes. The GH and CS loci may lie sufficiently close to one another on chromosome 17 that all of the loci must be undermethylated for one to be expressed. Another explanation may be that specific undermethylation does occur, but cannot be detected by our methods. For example, the large population of anterior pituitary cells which does not synthesize GH may obscure the signal from the small population of GH-secreting cells and make it difficult to detect any specific undermethylation of GH genes which are expressed. A similar argument could be made for the case of the CS genes in the placenta, although in this tissue it is likely that a large fraction of cells synthesize CS since a high percentage of placental mRNA is CS mRNA (20).

The fact that the GH and CS genes both occur in at least two copies per haploid in humans is another factor which may make it difficult to demonstrate that undermethylation is specific to the active gene. It is possible that the two loci of each gene are not expressed equally. For example, the GH-V gene product appears to be deficient and/or poorly functional compared to the GH-N gene product (24). Since the Hpa II - generated restriction fragments from the GH-N and GH-V genes should be

indistinguishable by our methods, we cannot compare the relative methylation of the GH-N and GH-V loci in pituitary DNA. Nor can we exclude the possibility that one CS locus is more methylated than the other. The presence of a methylated copy of one GH (CS) gene may make it more difficult to show that the other GH (CS) gene is specifically undermethylated.

Taken as a whole, our data support the conclusion made by other investigators that undermethylation is a necessary but not sufficient condition for gene expression (9). This is illustrated by our finding that the GH and CS genes are undermethylated in tissues in which they are expressed compared to a control tissue (leukocytes). However, we were unable to show that such undermethylation is specific, i.e., occurs to a greater extent in active genes than in inactive genes in the same region of DNA. Finally, the close similarity between the methylation patterns of hydatidiform molar and placental DNAs suggests that tissues with similar histology and at similar stages of differentiation may have nearly identical methylation patterns in a given gene region even though certain genes within that region are expressed to very different degrees.

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REFERENCES

1. Vanyushin, B.F., Tkacheva, S.G., & Belozersky, A.N. (1970) *Nature* (London) 225, 948
2. Doskocil, J. & Sorm, F. (1962) *Biochim Biophys Acta* 55, 953
3. Grippo, P., Iaccarino, M., Parisi, E. & Scarano, E. (1968) *J Mol Biol* 36, 195
4. Razin, A. & Riggs, A.D. (1980) *Science* 210, 604
5. Ehrlich, M. & Wang, R.Y.H. (1981) *Science* 212, 1350
6. Bird, A.P. & Southern, E.M. (1978) *J Mol Biol* 118, 27

7. Waalwijk, C. & Flavell, R.A. (1978) *Nucl Acids Res* 5, 4631
8. McGhee, J.D. & Ginder, G.D. (1979) *Nature* 280, 419
9. Van der Ploeg, L.H.T. & Flavell, R.A. (1980) *Cell* 19, 947
10. Shen, C.K.J. & Maniatis, T. (1980) *Proc Natl Acad Sci USA* 77, 6634
11. Mandel, J.L. & Chambon, P. (1979) *Nucl Acids Res* 7, 2081
12. Desrosiers, R.C., Mulder, C. & Fleckenstein, B. (1979) *Proc Natl Acad Sci USA* 76, 3839
13. Sutter, D. & Doerfler, W. (1980) *Proc Natl Acad Sci USA* 77, 253
14. Groudine, M., Eisenman, R. & Weintraub, H. (1981) *Nature (London)* 292, 311
15. Compere, S.J. & Palmiter, R.D. (1981) *Cell* 25, 233
16. Stearman, R.S., Lowell, C.A., Pearson, W.R. and Morrow, J.F. (in press) *Annals NY Acad Sci*
17. Niall, H.D., Hogan, M.L., Sauer, R., Rosenblum, I.Y. & Greenwood, F.C. (1971) *Proc Natl Acad Sci USA* 68, 866
18. Rasmussen, H. (1974) *Textbook of Endocrinology* (Robert H. Williams, Editor), W.B. Saunders Co, Philadelphia
19. McWilliams, D. & Boime, I. (1980) *Endocrinology* 107, 761
20. McWilliams, D., Callahan, R.C. & Boime, I. (1977) *Proc Natl Acad Sci USA* 74, 1024
21. Mochizuki, M., Morikawa, H., Kawaguchi, K. & Tojo, S. (1976) *J Clin Endocrinol Metab* 43, 614
22. Fiddes, J.C., Seeburg, P.H., DeNoto, F.M., Hallewell, R.A., Baxter, J.D. & Goodman, H.M. (1979) *Proc Natl Acad Sci USA* 76, 4294
23. Goodman, H.M., DeNoto, F., Fiddes, J.C., Hallewell, R.A., Page, G.S., Smith, S. & Tischer, E. (1980) *Miami Winter Symposium* 155
24. Phillips, J.A.III, Hjelle, B.L., Seeburg, P.H. & Zachmann, M. (1981) *Proc Natl Acad Sci USA* 78, 6372
25. Phillips, J.A.III, Parks, J.S., Hjelle, B.L., Herd, J.E., Plotnick, L., Migeon, C.J. & Seeburg, P.H. manuscript submitted
26. Wurzel, J.E., Parks, J.S., Herd, J.E., & Nielsen, P.V. manuscript submitted
27. Owerbach, D., Rutter, W.J., Martial, J.A., Baxter, J.D. & Shows, T.B. (1980) *Science* 209, 289
28. George, D.L., Phillips, J.A.III, Francke, U. & Seeburg, P.H. (1981) *Hum Genet* 57, 138
29. Kunkel, L.M., Smith, K.D., Boyer, S.H., Borgaonkar, D.S., Wachtel, S.S., Miller, O.J., Breg, W.R., Jones, H.W. & Rary, J.M. (1977) *Proc Natl Acad Sci USA* 74, 1245

30. Martial,J.A., Hallewell,R.A., Baxter,J.D., & Goodman,H.M. (1979) Science 205, 602
31. Vogelstein,B. & Gillespie,D. (1979) Proc Natl Acad Sci USA 76, 615
32. Maniatis,T., Kee,S.G., Efstratiadis,A. & Kafatos,F.C. (1976) Cell 8, 163
33. Southern,E.M. (1975) J Mol Biol 98, 503
34. Jeffreys,A.J. & Flavell,R.A. (1977) Cell 12, 429
35. McDonell,M.W., Simon,M.N. & Studier,F.W. (1977) J Mol Biol 110, 119
36. Seeburg,P.H., unpublished data
37. Salser,W. (1977) Cold Spring Harbor Symp Quant Biol 42, 985
38. Van der Ploeg,L.H.T., Groffen,J. & Flavell,R.A. (1980) Nucl Acids Res 8, 4563