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# Tissue-specific levels of human glucose-6-phosphate dehydrogenase correlate with methylation of specific sites at the 3′ end of the gene

(DNA methylation/housekeeping genes/transcriptional regulation)

GIORGIO BATTISTUZZI*†, MICHELE D'URSO*‡, DANIELA TONIOLO‡, G. M. PERSICO‡, AND LUCIO LUZZATTO*

*Department of Haematology, Royal Postgraduate Medical School, Ducane Road, London W12 OHS, England; and ‡International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, Via Marconi 10, 80125 Naples, Italy

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ABSTRACT **Glucose-6-phosphate dehydrogenase (G6PD) is a ubiquitous enzyme that supplies the cell with NADPH required for a variety of reductive reactions and biosynthetic processes. Therefore, the gene *G6PD*, located in mammals on the X chromosome, that specifies G6PD can be regarded as a typical housekeeping gene. We have investigated the expression of human *G6PD* in eight different fetal and adult tissues by determining the level of enzyme activity, the level of G6PD mRNA, and the methylation pattern of the 3′ end of the gene, for which we have nucleic acid probes. By combining sequence information with results of Southern blot analysis of DNA samples digested with the methylation-sensitive restriction enzyme *Hpa* II, we have identified five specific sites that are unmethylated in all tissues examined, a number of sites that are uniformly methylated, and a number of sites that are sometimes methylated. A subset of *Hpa* II sites, designated on our restriction map as H37–H55, exhibit positive correlation between degree of methylation, level of mRNA, and level of G6PD activity. A comparison of these methylation patterns with those we previously have observed in the *G6PD* gene on the inactive X chromosome [Toniolo, D., D'Urso, M., Martini, G., Persico, M. G., Tufano, V., Battistuzzi, G. & Luzzatto, L. (1984) *EMBO J.* 3, 1987–1995] indicates that different sites are associated with X-inactivation and with the regulation of *G6PD* on the active X chromosome. We conclude that this housekeeping gene is subject to tissue-specific transcriptional regulation, which in turn correlates with methylation of specific sites located at and near the 3′ end of the gene.**

The mode of expression of eukaryotic "housekeeping" genes must differ markedly from that of genes specifying tissue-specific differentiation products (1). Methylation of certain residues in DNA may be involved in regulation of expression of differentiation genes (2–4), but we do not yet know whether methylation is relevant to the regulation of housekeeping genes, such as *G6PD*, the gene coding for glucose-6-phosphate dehydrogenase (G6PD) (see ref. 5). We have shown recently that in human leukocyte DNA the 3′ end of *G6PD* is extensively methylated, but we have identified a few individual sites that are specifically unmethylated (6). Since different levels of G6PD have been found in different mammalian tissues (5, 7), we can ask whether their *G6PD* DNA is associated with different methylation patterns. Here we show that the extent of methylation of certain cytidine residues near the 3′ end of *G6PD* correlates with the level of G6PD mRNA and with the level of G6PD activity.

## MATERIALS AND METHODS

**Human Tissues.** Samples of solid tissues were obtained postmortem (maximum 24 hr) from males aged 10 months to 42 yr (by courtesy of D. Hopkinson, Medical Research Council Human Biochemical Genetics Unit, Galton Laboratories, University College, London) and from male fetuses aborted at 19–24 weeks by prostaglandin induction (by courtesy of S. Lawler and T. Wong, Royal Marsden Hospital, London). Organs were washed, fragmented, washed again thoroughly with $P_i$/NaCl (0.15 M NaCl/5 mM phosphate, pH 7.4) to remove blood, and kept at −80°C for ≤30 days. No decay of G6PD activity was observed under these conditions. Blood cells were obtained from normal volunteers (22–50 yr old). Procurement of all samples was cleared through the Medical Ethics Committee of the Royal Postgraduate Medical School.

**Fractionation of Peripheral Blood Leukocytes.** Blood, collected by venipuncture and treated with anticoagulant citrate/dextrose solution (National Institutes of Health formula A), was diluted with 2 volumes of $P_i$/NaCl. To a 50-ml conical plastic centrifuge tube, 10 ml of a mixture containing 12% (wt/vol) Ficoll and 13.6% (wt/vol) Hypaque (density = 1.109 g/cm$^3$) was added. On top of this mixture was layered 10 ml of a mixture containing 6.72% Ficoll and 13.6% Hypaque (density = 1.090). The diluted blood was layered on top and the tube was centrifuged for 30 min at 20°C at 900 × *g*. The leukocytes at the two interfaces (mononuclear cells and granulocytes, respectively) were collected separately and washed twice with $P_i$/NaCl. Residual erythrocytes were removed by selective lysis (8). Final cross-contamination between mononuclear cells and granulocytes was <2%.

**Extraction of G6PD.** A fragment of frozen tissue was weighed. While still frozen it was minced with a scalpel and then ground in a mortar in liquid nitrogen until a fine powder was obtained. The powder was transferred to a Dounce homogenizer, and 2 ml of swelling solution (10 mM Tris Cl, pH 7.5/3 mM $MgCl_2$/10 mM NaCl/1 mM EDTA/1 mM 6-aminohexanoic acid/10 μM NADP) was added for each 100 mg of frozen tissue. Suspensions were kept on ice with occasional mixing for 20 min and then homogenized for 5 min at 2000 rpm at 4°C in a Sorvall tissue grinder with a tight-fitting Teflon pestle. Triton X-100 was then added to 0.2% final concentration and, after 30 sec, homogenization was carried out again for 10 sec at 2000 rpm. (In preliminary experiments we found that all G6PD was released from tissues by this method.) The suspension then was centrifuged at 2000 × *g* at 4°C for 10 min. G6PD activity and total protein were determined according to Battistuzzi *et al.* (9) and Bradford (10), respectively. Pellets were used for DNA content determinations (11). G6PD from leukocytes and from cultured fibroblasts were extracted by a similar procedure, starting with a cell suspension of about 5 × 10$^6$ cells per ml in swelling solution.

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Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; kb, kilobase(s); H, *Hpa* II site.

†On leave of absence from Istituto di Biologia Generale e Genetica, University of Napoli, Napoli, Italy.

**DNA Extraction.** Frozen fragments of tissue were finely ground in a mortar as for G6PD extraction. The powder then was transferred to a plastic tube containing 10 mM Tris Cl, pH 7.5/20 mM EDTA, pH 7.0 (approximately 10 ml/g of tissue). $NaDodSO_4$ then was added to a final concentration of 0.5%. After lysis had occurred, proteinase K was added (50 μg/ml) and the solution was incubated at 50°C for 3 hr. DNA then was extracted with 2 volumes of phenol, 2 volumes of phenol/chloroform, and 2 volumes of chloroform. The aqueous phase was dialyzed for 24 hr against several changes of 10 mM Tris Cl, pH 8.0/1 M NaCl at 20°C and then for 48 hr against 10 mM Tris Cl (pH 8.0) at 4°C. The dialyzed DNA solution was treated with RNase A (50 μg/ml) for 2 hr at 37°C and with proteinase K (50 μg/ml) for 8 hr at 37°C, extracted with 1 volume each of phenol, phenol/chloroform, and chloroform, and extensively dialyzed against 10 mM Tris Cl (pH 7.5) at 4°C.

**RNA Extraction.** Frozen tissue fragments (as above) were processed by the guanidinium isothiocyanate–hot phenol method (12). This was followed by CsCl centrifugation (13, 14). The RNA pellet was dissolved in water, ethanol-precipitated, and redissolved in water. Poly(A)$^+$ RNA separation on oligo(dT)-cellulose was done according to Aviv and Leder (15).

**Blot-Hybridization Analyses.** Electrophoresis of DNA was carried out in TAE buffer (14) and electrophoresis of RNA in Mops/formaldehyde buffer (14). Blotting of gels and hybridization were carried out as previously described (6), except that the pH of standard saline citrate solutions (NaCl/Cit) was 6.15.

For "dot blot" analysis (16), RNA (40 μg) dissolved in 20 μl of 10 mM Tris Cl, pH 7.4/1 mM EDTA was added to 12 μl of 20 × NaCl/Cit and 8 μl of 37% formaldehyde. Samples (5–20 μl) were diluted to 150 μl with 15× NaCl/Cit and spotted, with the aid of a Perspex manifold (Schleicher & Schuell), onto nitrocellulose filters previously equilibrated with 3× NaCl/Cit. Filters were rinsed with 3× NaCl/Cit and then baked and hybridized as described for DNA (6).

**Probes.** Plasmids pGD3 and pGD1.4 (Fig. 2) have been described (6).

## RESULTS

**G6PD Activity Levels are Tissue-Specific.** Data on G6PD levels in rat organs have been known for 30 years (7), but data on human organs have been few. We have found that in solid tissues the levels of G6PD activity vary over an ≈10-fold range, being lowest in skeletal muscle and highest in adrenal and brain (Table 1). In free cells, such as circulating leukocytes, the activity is higher by another order of magnitude; the same is true in cultured fibroblasts (17). Considerable variability in G6PD activity was observed among samples of the same tissue both in adults and in fetuses (Fig. 1); this variability is probably due to a variety of causes, including age and possible pathological changes. However, it is clear that, for each organ, G6PD activity per unit protein is higher in the fetus than in the adult (Fig. 1). The relative ratios between various organs are roughly similar in fetal life and after birth.

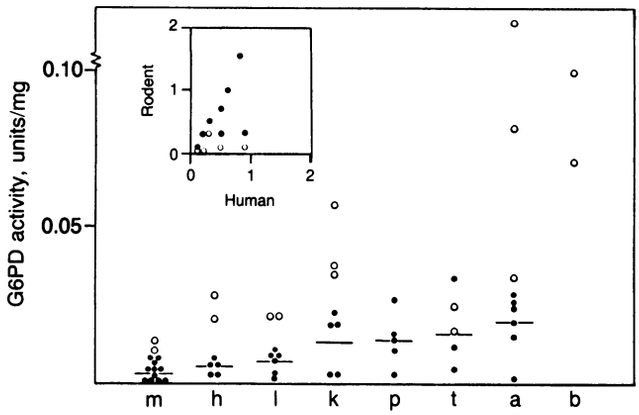


FIG. 1. G6PD activity in human adult (●) and fetal organs (○). Activity is given in units/mg of protein for muscle (m), heart (h), liver (l), kidney (k), pancreas (p), thymus (t), adrenal (a), and brain (b). Horizontal bars indicate arithmetic means for adult samples. (*Inset*) Correlation between data for humans (abscissa) and previous data for rodents (ordinate). ●, Human vs. rat (7); ○, human vs. mouse (7, 18). Each circle represents one tissue. Data in *Inset* are given in units/g of wet weight.

**Methylation Patterns of the *G6PD* Gene Are Tissue-Specific.** We previously have shown that the 3′ end of *G6PD* is rich in CpG sequences susceptible to methylation and that, in peripheral blood leukocytes, at least one-half of these sites are methylated, but some specific sites are unmethylated (6). We now have used the probes pGD3 and pGD1.4 (Fig. 2) to test what occurs in other tissues. In Southern blots of *Hpa*

Table 1. G6PD activity in normal human organs and leukocytes

| Tissue | *n* | G6PD activity: Units × $10^3$/mg of protein, Mean | | Range | Units × $10^3$ per $10^6$ nuclei*, Mean | | Range |
|---|---|---|---|---|---|---|---|
| Muscle | 11 | 3.28 | (1.0) | 0.57–8.34 | 0.70 | (1.0) | 0.25–1.38 |
| Heart | 5 | 5.54 | (1.7) | 2.75–8.88 | 0.54 | (0.8) | 0.14–0.98 |
| Liver | 6 | 7.24 | (2.2) | 2.02–11.3 | 0.89 | (1.3) | 0.22–2.04 |
| Kidney | 5 | 13.4 | (4.1) | 2.98–22.7 | 1.88 | (2.7) | 0.12–3.08 |
| Prostate | 1 | 14.1 | (4.3) | | 2.12 | (3.0) | |
| Pancreas | 5 | 14.3 | (4.4) | 2.93–26.7 | 0.82 | (1.2) | 0.13–1.51 |
| Thymus | 3 | 16.7 | (5.1) | 4.77–33.8 | 0.93 | (1.3) | 0.76–1.18 |
| Adrenal | 6 | 19.6 | (6.0) | 2.33–29.0 | 2.05 | (2.9) | 0.65–3.36 |
| Brain† | 2 | 84.8 | (6.9) | 70.8–98.8 | 3.56 | (4.7) | 2.85–4.26 |
| Mononuclear leukocytes | 21 | 207 | (63) | 48.9–787 | 5.94‡ | (8.5) | 4.31–8.04 |
| Granulocytes | 21 | 851 | (259) | 154–1666 | 28.6 | (40.8) | 21.2–39.7 |

Solid tissues (postmortem) and blood cells were obtained from male subjects as described in *Materials and Methods*, unless otherwise indicated. Each value in parentheses gives the ratio of the mean activity to that obtained for skeletal muscle.

*Based on DNA determination, assuming a DNA content of 5 pg per diploid nucleus.

†From two fetuses at 19 and 22 weeks of gestation. Ratios were obtained with mean activity value for fetal muscle (see Fig. 1).

‡Determined for only 4 subjects out of 21.

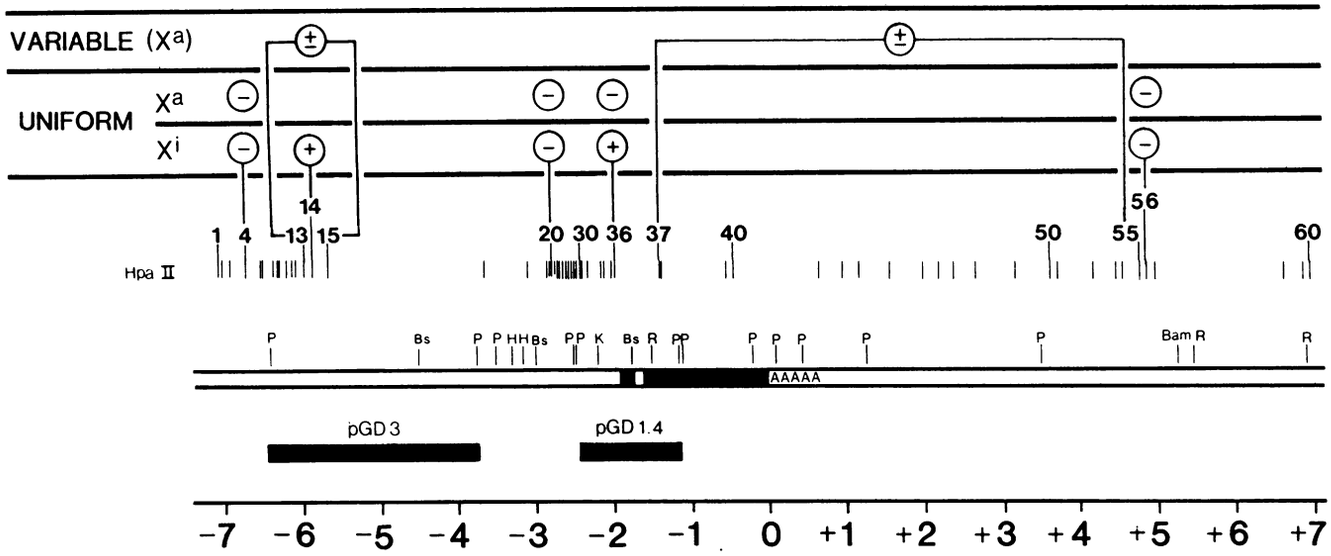


FIG. 2. Restriction map of the 3′ end of the *G6PD* gene. From bottom to top: abscissa in kb (we have chosen as 0 the end of the transcript); the two probes used in this analysis; diagram of gene (exons shaded) with restriction enzyme sites (P, *Pst* I; Bs, *Bst*EII; H, *Hin*dIII; K, *Kpn* I; R, *Eco*RI; Bam, *Bam*HI) and region corresponding to poly(A) of the transcript (AAAAA); *Hpa* II (*Msp* I) sites. The map is from Toniolo *et al.* (6). The symbols at top illustrate the methylation status of cytosine in a number of CpG sites. ⊖ and ⊕, Unmethylated and fully methylated, respectively. ⊖, "Partially" methylated. Horizontal lanes at top: uniform, methylation status of sites in the inactive X chromosome ($X^i$) of females (see ref. 6) and in the X chromosome of males and the active X chromosome of females ($X^a$); variable ($X^a$), sites for which the methylation status was found to be different in different tissues. This variability concerns only $X^a$.

II-digested DNA from various tissues, pGD3 hybridizes to either two or three bands [3.0, 3.2, and 3.4 kilobases (kb) (Fig. 3)]. The quantitative ratios among these bands vary considerably. Double digestions with *Hpa* II and *Bst*EII yield bands corresponding to the smaller fragments expected from our restriction map (Fig. 2). These data indicate that, as in leukocytes, also in other tissues the 3.0- and 3.2-kb *Hpa* II fragments extend from *Hpa* II site 20 (H20) to H15 and H14, respectively. The third band at 3.4 kb found in several tissues can be inferred, from the double digestion, to originate similarly from cleavage at H20 and H13. In addition, the quantitative relationships observed in single digests agree with those observed in double digests (Table 2). In various tissues, unmethylation of H15 ranges from 10% to 75%, and that of H14 from 25% to 90%.

Variation in the methylation pattern was even more striking when analyzed with the probe pGD1.4 (Fig. 4). We have shown previously that the major 6.8-kb *Hpa* II fragment revealed by this probe in leukocyte DNA arises from cleavage of H36 and H56 (6), indicating that all intervening sites are fully methylated. With other tissues, we observed additional fragments of 3.6, 3.3, 3.0, 1.65, and 1.5 kb (Fig. 4: some additional bands were so faint that they do not appear on the prints of the autoradiography films). Since in all of these tissues *Hpa* II/*Pst* I double digests always yield a single band of 0.9 kb (see lane 15, Fig. 4), we infer that the *Hpa* II fragments smaller than 6.8 kb must extend from H36 to other sites ranging from H39 (1.5 kb) to H44 (3.6 kb). Thus, one or more of these sites are unmethylated in tissues other than leukocytes. The amount of 6.9-kb fragment indicates what fraction of all of the sites H37–H55 are methylated in the DNA from various tissues, arranged in order of decreasing G6PD activity in Fig. 4, lanes 1–8.

**Correlation Between G6PD Activity, G6PD mRNA, and Methylation Pattern in Various Tissues.** To test whether tissue-specific methylation patterns are related to the expression of the *G6PD* gene, we needed first to have evidence that enzyme activity levels correlated with G6PD-specific mRNA levels. We chose to examine muscle (lowest G6PD activity), liver (intermediate), and brain (high). Using the dot blot hybridization method, we have found that the relative levels of G6PD mRNA in these three organs are in the same

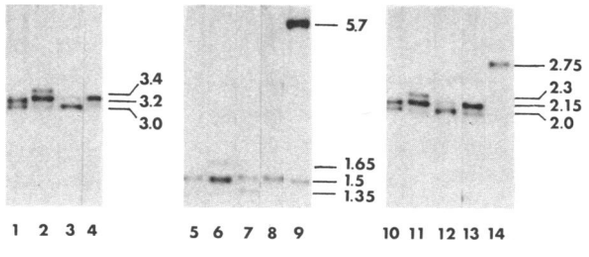


FIG. 3. Southern blot analysis with pGD3 probe of DNA digests from different tissues. Lanes 1, 5, and 10: fetal skeletal muscle (heart and adrenal yielded identical patterns). Lanes 2, 6, and 11: fetal liver (thymus and brain yielded identical patterns). Lanes 3 and 7: fetal kidney. Lane 12: adult kidney. Lanes 4, 8, 9, 13, and 14: granulocytes (mononuclear leukocytes yielded identical patterns). Restriction enzymes used in digestions were as follows: Lanes 1–4, *Hpa* II; lane 9, *Bst*EII; lane 14, *Pst* I; lanes 5–8, *Hpa* II + *Bst*EII; lanes 10–13, *Hpa* II + *Pst* I. Leukocytes were from adults; solid tissues from adults and fetuses gave similar results. Markers in kb.

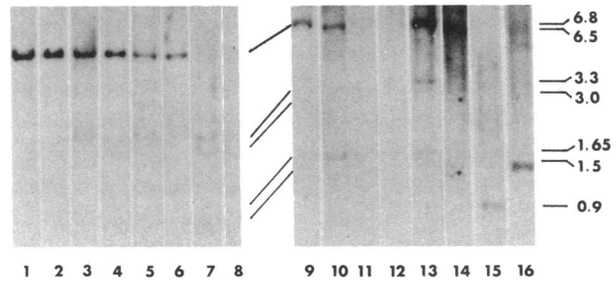


FIG. 4. Southern blot analysis with pGD1.4 probe of DNA digests from granulocytes (lanes 1, 9, 10, and 13–16), peripheral blood mononuclear cells (lane 2), brain (lane 3), thymus (lane 4), kidney (lane 5), liver (lane 6), heart (lane 7), and skeletal muscle (lanes 8, 11, and 12). The amount of DNA applied to each lane was 20 μg, except for lane 11, which had 4 μg. Restriction enzymes used: lanes 1–9, *Hpa* II; lanes 10–12 and 14, *Bst*EII; lane 16, *Pst* I; lane 13, *Hpa* II + *Bst*EII; lane 15, *Hpa* II + *Pst* I. Leukocytes were from adults; solid tissues were from fetuses. Markers in kb.

Table 2. Tissue-specific differences in DNA methylation revealed by probe pGD3 after digestion of genomic DNA with a methylation-sensitive enzyme

| | % total hybridization signal | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Hpa* II + *Bst*EII | | | | | | | | |
| | *Hpa* II | | | 1.65 | | 1.5 | | 1.35 | | % of sites methylated | | |
| Tissue | 3.0 | 3.2 | 3.4 | Exp | Obs | Exp | Obs | Exp | Obs | H15 | H14 | H13 |
| Muscle | 55 | 45 | | | | 68 | 70 | 32 | 30 | 55 | ≥45 | |
| Heart | 40 | 60 | | | | 80 | 70 | 20 | 30 | 40 | ≥60 | |
| Liver | 10 | 65 | 25 | 12 | 11 | 78 | 82 | 5 | 7 | 10 | 65–75 | ≥25 |
| Kidney | 75 | 25 | | | | 63 | 59 | 37 | 41 | 75 | ≥25 | |
| Thymus | 12 | 64 | 24 | 12 | 17 | 82 | 77 | 6 | 6 | 12 | 64–76 | ≥24 |
| Adrenal | 55 | 45 | | | | 77 | 79 | 23 | 21 | 55 | ≥45 | |
| Brain | 10 | 65 | 25 | 12 | 10 | 83 | 71 | 5 | 9 | 10 | 65–75 | ≥25 |
| Mononuclear cells | 9 | 91 | | | | 95 | 95 | 5 | 5 | 10 | ≥90 | |
| Granulocytes | 20 | 80 | | | | 90 | 90 | 10 | 10 | 20 | ≥80 | |

Values given are percentages of total signal associated with each fragment on Southern blots, such as those in Figs. 3 and 4, as measured by densitometry. From repeat experiments, estimated accuracy is ±10%. Expected (Exp) values were calculated by assuming that the left-side boundaries (see Fig. 2) of the fragments obtained in double digests are sites H13–H15. The agreement between Exp and observed (Obs) values confirms that this assumption is correct. In the last two columns, ≥ signs are used because the methylation status of site H14 cannot be assessed for the extent that H15 is unmethylated; similarly for site H13.

order as the relative G6PD activities (Fig. 5). When the intensity of the spots was measured by densitometry, the average ratios of the signals are 5.5 for liver to muscle and 7.0 for brain to muscle. These values compare with 2.2 and 6.9 for the respective ratios of enzyme activity (see Table 1).

We next investigated whether the extent of methylation of those sites in which it is most variable in turn correlates with G6PD activity in various tissues. The answer was negative with respect to sites H13–H15 (compare Tables 1 and 2). On the other hand, there was a striking positive correlation between the proportion of DNA in the 6.8-kb *Hpa* II fragment seen with probe pGD1.4 and G6PD activity (Fig. 6). We conclude that, for this portion of this gene and among the tissues examined, the highest level of expression, found in granulocytes, is associated with full methylation of sites H37–H55.

## DISCUSSION

The possible role of DNA methylation in controlling gene expression has been reviewed comprehensively by Cooper (3), whose conclusions were based almost entirely on data pertaining to differentiation genes. With respect to housekeeping genes, Stein *et al.* (19) have shown that certain sites near the 5′ end of the hamster adenine phosphoribosyl transferase gene (*APRT*) are unmethylated in five tissues, including sperm cells in which the gene probably is not expressed. For the mouse dihydrofolate reductase gene (*DHFR*), no differences were found in the five tissues tested. Yen *et al.* (20) have observed differences in the methylation pattern of the human hypoxanthine phosphoribosyl transferase gene (*HPRT*) in placenta vs. leukocytes, but no correlation with enzyme activity was sought.

Considering the four housekeeping genes *APRT*, *DHFR*, *HPRT*, and *G6PD* as a whole, it is apparent that the relationship between methylation and expression is complex, as it is for differentiation genes (3). A common feature in housekeeping genes is the "partial" methylation of numerous sites. For X-linked genes in males, with only one copy per cell, this must mean that a particular site is methylated in some cells and not in others. This may be related to heterogeneity in cell populations (for instance, in the brain: see ref. 21); or to different functional states within the same cell population (for instance, in the liver: see ref. 22).

Regulation of genes in mammalian cells has a variety of functionally different features. (*i*) Tissue-specific genes associated with differentiation are expressed at a very high lev-

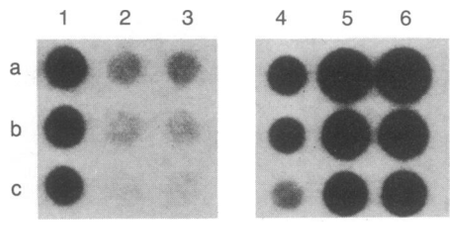


FIG. 5. Dot blot hybridization assay of G6PD-specific mRNA in different tissues. Amount of RNA applied to filters was 20, 10, and 5 μg in lines a, b, and c, respectively. Filter at left was prepared using total RNA. Column 1, liver; columns 2 and 3, muscle (two different preparations). Exposure to x-ray film was 15 days. Filter at right was prepared using poly(A)$^+$ RNA. Column 4, muscle; columns 5 and 6, brain (two different preparations). Exposure to x-ray film was 8 hr.

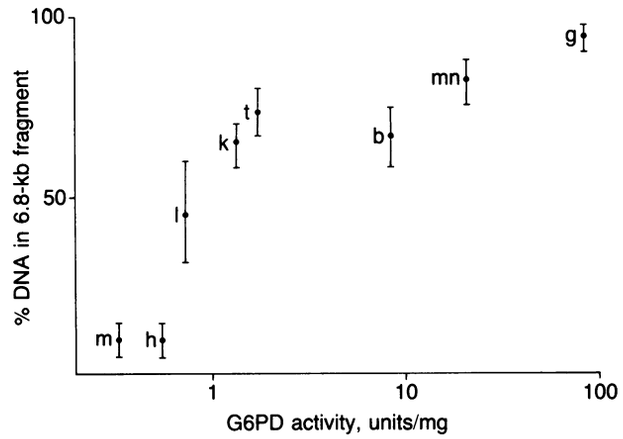


FIG. 6. Correlation between methylation within *G6PD* gene and level of G6PD activity. The proportion of *G6PD* DNA that hybridizes with probe pGD1.4 and that is 6.8 kb in size after *Hpa* II digestion was measured densitometrically on the autoradiographs shown in Fig. 4, by comparison with the bands of similar size obtained in *Bst*EII digests. Mean values obtained for eight tissues are plotted against the respective values (on a logarithmic scale on the abscissa) of G6PD activity (from Table 1). Vertical bars indicate the range for at least three samples for each tissue. Linear correlation coefficient, $r = 0.86$ ($P < 0.01$). m, Skeletal muscle; h, heart; l, liver; k, kidney; t, thymus; b, brain; mn, mononuclear cells; g, granulocytes.

el in some cells and not at all in others. This type of control is largely transcriptional (1). (*ii*) The majority of X-linked genes are subject to an all-or-none control, which we call inactivation and which is also transcriptional (see ref. 23). (*iii*) Housekeeping genes are expressed, by definition, in all cells at a low but not necessarily uniform level, and this variability of expression from cell to cell may result from transcriptional regulation.

*G6PD* is an X-linked housekeeping gene and therefore lends itself to an analysis of both *ii* and *iii*. With respect to item *ii*, we have shown previously (6) that in the inactive X chromosome of females, sites H14 and H36 are methylated (see Fig. 2). With respect to item *iii*, we have shown here that G6PD mRNA levels, measured in three tissues by hybridization to a specific probe, are roughly proportional to the enzyme activity levels (Fig. 5). Thus, although a housekeeping gene is not turned off in any tissue, it appears that it is still susceptible to a transcriptional regulation that can modulate its activity.

If tissue-specific transcriptional regulation does take place, it is possible that methylation of the *G6PD* gene is involved. We find, indeed, that *G6PD* DNA from various tissues has different but still specific methylation patterns. The most distinctive differences were observed in a DNA region extending over about 6 kb, straddling the putative transcription termination site (Fig. 2). In this region we have observed in eight different tissues a striking empirical positive correlation between methylation of CpG sites (H37–H55) and G6PD activity (Fig. 6). In contrast, for each tissue the patterns observed did not differ between fetus and adult. In a related study, by using partial digestions with *Hpa* II, Wolf *et al.* (24) have shown that within the two clusters illustrated in Fig. 2, hypomethylation is associated with spontaneous or 5-azacytidine-induced *G6PD* reactivation.

In summary, three main features of methylation have emerged thus far from our analysis of *G6PD* DNA. First, tissue-specific patterns involve, at different sites, both methylation and nonmethylation. In all tissues, the gene is expressed and five sites are unmethylated; but what correlates with G6PD activity is methylation rather than nonmethylation of sites H37–H55. [A positive correlation between methylation and expression of the *H-2K* gene has been reported recently in F9 embryonal carcinoma cells differentiating *in vitro* (25)]. Second, although the probes available have limited our study to the 3′ end of the gene, it is significant that tissue-specific DNA methylation changes are seen in this portion of the gene. This finding is in keeping with other recent data which indicate that sequences responsible for transcriptional regulation may exist at the 3′ end of eukaryotic genes (26). Third, because G6PD is X-linked, we had an opportunity to compare changes in methylation associated with X-inactivation with those associated with tissue-specific modulation. The methylation pattern of *G6PD* DNA on the inactive X chromosome (6) is different from and simply additive with what has been described here. Thus, the all-or-none control that is characteristic of X-inactivation is associated with changes in methylation that are distinct from those associated with fine control of tissue-specific expression of the same housekeeping gene.

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