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Changes in translational yield regulate tissue-specific expression of β-glucuronidase

[protein synthesis/protein degradation/mRNA/poly(A)$^+$ RNA/"housekeeping" gene]

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ABSTRACT **The number of β-glucuronidase (GUS; β-D-glucuronoside glucuronosohydrolase, EC 3.2.1.31) molecules per cell varies as much as 12-fold among mouse tissues. To identify the regulatory mechanisms responsible, estimates of the rates of GUS protein synthesis (k_s) and degradation (k_d) were obtained for six tissues in the B6.PAC-*Gus*^{n} mouse strain, which carries the *N* haplotype of the GUS gene. Differences in enzyme levels among tissues were predominantly due to differences in rates of enzyme synthesis; only brain differed significantly in the rate of protein degradation. Typically, tissues contain about 2 molecules of GUS mRNA per cell. Differences in GUS mRNA levels were found among tissues, but these were not sufficient to account for observed differences in k_s. This suggests that tissues differ in translational yield, which is defined as the product of the efficiency with which the GUS message is translated and the fraction of newly made polypeptides that are successfully matured into GUS tetramers. Experimental estimates of translational yield confirmed that this is indeed a source of tissue differences in GUS gene regulation. This finding also proved to be true of the *B* haplotype of the GUS gene. The differential regulation of special-function genes is, in general, effected transcriptionally. In contrast, the differential regulation of several "housekeeping" genes has been reported to arise from changes in mRNA maturation and/or stability. It is now apparent that translational yield, which is an aspect of protein synthesis, can also serve as a differential regulatory mechanism.**

Current information regarding the regulation of gene expression largely derives from the study of special-function genes. Such genes are typically expressed in only one or a few tissues (such as globin and albumin) or exhibit a strong, tissue-specific inductive response (such as ovalbumin and metallothionein). In general, these genes are transcriptionally regulated (1, 2). Less is known about the regulation of "housekeeping" genes that are expressed in all cell types, even though these genes are thought to comprise about 95% of the active genes in most cells (3–5). There has been a tendency to assume, by extension, that such genes are also regulated transcriptionally. However, the information that is beginning to accumulate suggests that, in contrast to special-function genes, housekeeping genes are regulated primarily at the level of mRNA maturation and/or stability. Examples include rat glyceraldehyde-3-phosphate dehydrogenase (6), mouse dihydrofolate reductase (7), chicken thymidine kinase (8), chicken and human tubulin (9), and mouse histone H4 (10).

We have measured the parameters responsible for the differential expression between tissues of another housekeeping gene, encoding murine β-glucuronidase (GUS; β-D-glucuronoside glucuronosohydrolase, EC 3.2.1.31) and found a third mode of regulation, in which tissue differences result largely from changes in translational yield. Translational yield is defined as the number of mature enzyme molecules formed per minute per mRNA molecule. It is a function both of the efficiency with which the GUS message is translated and of the fraction of newly made polypeptides that are successfully matured into GUS tetramers. Six tissues of the B6.PAC-*Gus*^{n}, or B6.N, mouse strain were compared for their GUS activity, rates of enzyme synthesis and degradation, GUS mRNA levels, and translational yield. Estimates of translational yield for each tissue were obtained by determining the ratio of the relative rate of GUS synthesis to the relative concentration of GUS mRNA for each tissue. The findings indicate that the differential expression of the GUS gene between cell types results from changes in several aspects of GUS gene regulation, but the predominant effects result from changes in translational yield. These results were confirmed in the C57BL/6J mouse strain, which carries a different haplotype of the GUS gene.

MATERIALS AND METHODS

Mice. The GUS structural gene together with its associated regulatory sequences is referred to as the GUS gene complex, or [*Gus*]. Genetic variants of the complex are haplotypes. Chapman and coworkers (11) constructed the congenic strain B6.PAC-*Gus*^{n}, or B6.N, by transferring the [*Gus*]^N haplotype from the PAC/Cr strain to the C57BL/6 strain through a process of repeated backcrossing. Experimental animals were 2- to 3-month-old females raised in our own colony. C57BL/6J mice, which carry the [*Gus*]^B haplotype, were obtained from The Jackson Laboratory.

Assays. GUS activity was assayed by a fluorometric procedure with 4-methylumbelliferyl β-D-glucuronide as substrate (12). One enzyme unit is the amount of enzyme forming 1 μmol of product per hr at 37°C. For protein, DNA, and total RNA determinations, tissue homogenates (5% wt/vol) were prepared in 138 mM NaCl/3 mM KCl/10 mM sodium phosphate/2 mM EDTA, pH 7.4. Protein was determined by the method of Lowry *et al.* (13), and DNA was assayed by the method of Labarca and Paigen (14). Homogenates were assayed for total RNA by the method of Schmidt and Thannhauser (15) with modifications (16).

RNA. Total RNA was isolated by the guanidine hydrochloride method of Cox (17) as described by Labarca and Paigen (18). Poly(A)$^+$ RNA was quantitated by hybridization of dot blots with a ^{32}P-end-labeled (19) $(dT)_{12-18}$ probe. Total RNA from each tissue was spotted in triplicate on nitrocellulose, incubated with probe overnight at 30°C, and washed three times with 2× SSC (1× SSC is 0.15 M NaCl/0.015 M trisodium citrate, pH 7.0) containing 0.2% $NaDodSO_4$ and 5 mM sodium phosphate/0.015% $Na_4P_2O_7$ (pH 7) at 34°C for 30

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Abbreviation: GUS, β-glucuronidase.

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min. The dots were cut out for measurement of radioactivity by scintillation counting, and the poly(A) content was determined by comparison with a concentration curve of total RNA from mouse kidney that had previously been calibrated as containing 0.251% poly(A) using synthetic poly(A) as a standard. The amount of poly(A)$^+$ RNA was then estimated by assuming that poly(A) constitutes 10% of poly(A)-containing mRNA sequences. The length of poly(A) tails and the percent poly(A) sequences probably do not vary significantly among tissues, because we failed to detect any size variation in GUS mRNA among tissues, as is the case for many other mRNAs. GUS mRNA was quantitated by hybridization of triplicate dot blots with ^{32}P-labeled cRNA generated by Sp6 transcription of the plasmid Sp6-G3, as described elsewhere (20). Each blot contained a standard curve of total RNA from androgen-induced kidney of strain A/J mice containing 120 μg of GUS mRNA per gram of total RNA.

Relative Rates of Protein Synthesis. Relative rates of GUS synthesis were determined by pulse-labeling for 4 hr with [^{3}H]leucine as described (21) with minor modifications (unpublished). This pulse-labeling time was short compared to the half-life of the enzyme, which has been estimated at 3–6 days in liver, kidney, and spleen (22). Tissues from five mice were pooled for each determination. It is unlikely that incorporation is biased by enzyme secretion and reabsorption among tissues, since serum levels of GUS are less than 0.001 times those found in liver (ref. 23 and K.P., unpublished observation) and direct transfer to adjacent tissue cells would not affect measurements of total tissue synthesis. The relative rate of GUS synthesis was calculated as the ratio of label incorporated into purified GUS (corrected for recovery of enzyme activity) to label incorporated into total protein that was precipitated by trichloroacetic acid (24).

Calculations. For other calculations we used the value of 3.29 pg of DNA per haploid mouse genome (25), 0.40 μg of GUS protein per activity unit (26), 70,000 for the molecular weight of the mature GUS protein subunit (26), and 8.6×10^5 as the molecular weight of GUS mRNA (20).

RESULTS

Tissue Levels of GUS Protein. There is a 10- to 20-fold range in the amount of GUS protein per gram of tissue, per gram of protein, and per genome among six tissues of B6.N mice (Table 1). The catalytic activity per GUS molecule was compared between liver and kidney by antibody titration and found to be the same (L.T.B., unpublished data).

GUS Protein Synthesis and Degradation. At steady state, enzyme concentration = rate of enzyme synthesis (k_s)/rate of enzyme degradation (k_d) (27). Differences in steady-state levels of GUS protein among tissues, then, can arise from differences in k_s, or k_d, or both.

The pulse-labeling methods used to measure the rate of GUS synthesis in each tissue provide an estimate of the rate of GUS synthesis relative to total protein synthesis, k_s^{rel}, rather than an estimate of the absolute rate of GUS synthesis. For purposes of calculation, it is then necessary to convert the relationship to a form with equivalent units on both sides of the equation. As originally expressed in absolute units the steady-state relationship is

$$\text{GUS (g/liter)} = \frac{k_s^{GUS}}{k_d^{GUS}} = \frac{\text{GUS (g/liter) synthesized per hr}}{k_d^{GUS}}. \quad [1]$$

The conversion is accomplished using the equivalent equation for total protein:

$$\text{Total protein (g/liter)} = \frac{k_s^{prot}}{k_d^{prot}} = \frac{\text{protein (g/liter) synthesized per hr}}{k_d^{prot}}. \quad [2]$$

Dividing Eq. 1 by Eq. 2 gives the requisite relationship

$$\frac{\text{GUS (g)}}{\text{total protein (g)}} = \frac{k_s^{GUS}/k_s^{prot}}{k_d^{GUS}/k_d^{prot}} = \frac{k_s^{rel}}{k_d^{rel}}. \quad [3]$$

The use of relative parameters, as in Eq. 3, is essential if the rates of synthesis and degradation of a specific enzyme are to be compared among tissues. The reason is that average rates of protein synthesis (k_s^{prot}) and degradation (k_d^{prot}) differ markedly among tissues (ref. 28 and unpublished data). In effect, tissues achieve very similar protein contents and k_s^{prot}/k_d^{prot} ratios with markedly different absolute values of k_s^{prot} and k_d^{prot}. In order not to confuse effects specific to the enzyme in question with tissue differences in general, both k_s^{prot} and k_d^{prot} must be included in the steady-state relationship.

For the measurement of k_s^{rel}, the amount of label incorporated into GUS protein relative to the amount of label incorporated into total protein was determined after a 4-hr pulse of [^{3}H]leucine. k_d^{rel} was estimated using Eq. 3 and measurements of k_s^{rel} and the weight concentration of GUS. This is preferable to the technique of pulse-labeling protein and measuring the rate at which label is lost from GUS and total protein because it avoids the uncertainties that arise from reincorporation of labeled amino acids as proteins are degraded (29–31), particularly as reincorporation rates are likely to vary among tissues. The measured k_s^{rel} and the calculated values of k_d^{rel} are shown in Table 2. It is apparent that differences in enzyme content among tissues are primarily due to variations in the rate of enzyme synthesis. Tissue differences in the relative rate of GUS degradation were small except for brain, where an increased rate of enzyme degradation contributes significantly to low enzyme levels.

mRNA Concentration and Translational Yield. The relative rate of GUS synthesis is a function of the concentration of GUS mRNA relative to other mRNAs, the efficiency with

Table 1. Comparison of GUS protein levels in various tissues in B6.N mice

Tissue	Total protein,* mg per g of tissue	Genomes,† no. $\times 10^{-9}$ per g of tissue	GUS activity, units per g of tissue	GUS, μg per g of protein	GUS polypeptides, no. $\times 10^{-3}$ per gene
Liver	211 ± 7	2.48 ± 0.19	11.5 ± 0.4	22	16
Large intestine	72 ± 6	2.42 ± 0.13	7.54 ± 0.46	42	11
Lung	79 ± 5	2.74 ± 0.22	4.66 ± 0.17	24	5.8
Kidney	148 ± 16	3.16 ± 0.20	4.04 ± 0.10	11	4.4
Submaxillary gland	110 ± 13	3.70 ± 0.26	2.96 ± 0.10	11	2.8
Brain	93 ± 1	1.36 ± 0.14	0.51 ± 0.02	2.2	1.3

*Mean ± SEM, n = 4 individual mice.
†Mean ± SEM, n = 12–16 individual mice.

Table 2. Measured relative rates of GUS protein synthesis and calculated relative rates of degradation in B6.N mice

Tissue	GUS, μg per g of protein	$k_s^{rel} \times 10^6$*	k_d^{rel}
Liver	22	18.1 ± 1.1	0.83
Large intestine	42	25.7 ± 1.3	0.61
Lung	24	24.2 ± 2.1	1.0
Kidney	11	8.9 ± 1.3	0.82
Submaxillary gland	11	6.8 ± 0.8	0.63
Brain	2.2	5.0 ± 0.8	2.3

*Mean ± SEM, n = 3–6 independent experiments.

which the message is translated, and the fraction of newly made polypeptides that are successfully matured into catalytically active GUS tetramers recognizable by antibody to the enzyme. The concentration of GUS mRNA was measured by using hybridization probes. However, the last two components could only be estimated as a single combined parameter, which is the rate of mature enzyme synthesis per mRNA molecule. We refer to this combined parameter as the translational yield.

There are small differences in GUS mRNA concentrations among tissues (Table 3), but they are not sufficient to account for the differences in rates of enzyme synthesis among tissues. The implication is that tissues must differ in at least one of the two components of translational yield. Translational yield was calculated for each tissue from the following relationship: translational yield = k_s^{rel}/GUS $mRNA_{wt}^{rel}$. Since k_s is the relative rate, the equation uses the concentration of GUS mRNA relative to total mRNA in order to take into account possible differences in the total mRNA content of tissues. Measurements of poly(A)$^+$ RNA were used to estimate total mRNA. This estimate of translational yield allows a comparison among tissues of GUS synthesis per GUS mRNA molecule that is not complicated by any tissue differences in total mRNA content and/or overall rates of protein synthesis. The results (Table 4) confirm the existence of significant differences in GUS translational yield among tissues.

The *B* Haplotype. B6.N mice carry the *N* haplotype of the GUS gene complex (see *Materials and Methods*). The *N* haplotype differs from the commonly studied *B* haplotype present in B6 mice in several respects, including the production of a structurally variant form of enzyme and altered levels of gene expression. When tested, the *B* haplotype proved to be similar to the *N* haplotype in that the differences in GUS gene expression among tissues involved translational yield. In Table 5, the data for the two haplotypes are compared by reporting the parameters for each tissue normalized to the liver.

Table 3. GUS mRNA levels in B6.N mice

Tissue	Total RNA,* mg per g of tissue	GUS mRNA,† ng per g of RNA	Molecules of GUS mRNA, no. per gene
Liver	8.77 ± 0.2	648 ± 40	1.6
Large intestine	4.16 ± 0.3	843 ± 50	1.0
Lung	4.53 ± 0.3	966 ± 110	1.1
Kidney	5.57 ± 0.4	763 ± 90	0.94
Submaxillary gland	13.3 ± 0.7	473 ± 30	1.2
Brain	2.56 ± 0.12	410 ± 32	0.54

*Mean ± SEM, n = 5–9 individual mice.
†Mean ± SEM, n = 4 or 5 independent RNA preparations. Each preparation contained pooled tissues from 3 mice.

Table 4. Estimation of GUS translational yield in B6.N mice

Tissue	Poly(A)$^+$ RNA,* μg per mg of RNA	GUS $mRNA_{wt}^{rel}$,† μg per g of poly(A)$^+$ RNA	Translational yield‡
Liver	26.0 ± 1.8	25	0.72
Large intestine	15.0 ± 2.7	56	0.46
Lung	12.0 ± 1.2	81	0.30
Kidney	24.5 ± 0.6	31	0.29
Submaxillary gland	11.0 ± 0.9	43	0.16
Brain	31.5 ± 1.8	13	0.38

*Mean ± SEM, n = 10–20 RNA dots from 2 independent RNA preparations.
†Calculated from $\frac{\mu g \text{ of GUS mRNA}}{\mu g \text{ of RNA}} \div \frac{\mu g \text{ of poly(A)}^+ \text{ RNA}}{\mu g \text{ of RNA}}$.
‡Calculated as ($k_s^{rel} \times 10^6$) ÷ GUS $mRNA_{wt}^{rel}$.

DISCUSSION

We find that differences in steady-state levels of GUS enzyme between tissues are based primarily on differences in relative rates of enzyme synthesis (k_s^{rel}), which are only due in part to differences in GUS mRNA abundance. This is in contrast to other gene systems in which differences in enzyme levels reflect the relative abundance of the specific mRNA (1, 2, 6–10). Instead, tissue differences in the relative rate of GUS synthesis largely result from tissue differences in translational yield.

The translational yield estimates reflect the outcome of a sequence of events beginning with the initiation of translation and ending with formation of the mature, catalytically active tetramer. Within this sequence are several steps that could readily differ among tissues and that might be the source of tissue differences in GUS expression.

Translational efficiency itself could differ among tissues. To our knowledge, tissue-specific differences in the efficiency of translation of a specific mRNA have not been reported. There are, however, many cases where a regulatory or developmental signal changes the translational efficiency of a specific mRNA in a single cell type (32–37).

A second source of tissue differences in translational yield could lie in one of the events that determine the proper maturation and cellular localization of the GUS polypeptide. GUS is synthesized on membrane-bound polysomes (38). In eukaryotic cells, it is generally assumed that the binding of secretory proteins to the endoplasmic reticulum membrane and their subsequent translocation across the membrane are mediated by two proteins, a soluble protein called signal-recognition particle and a membrane-bound receptor protein called docking protein (39–41). Upon entering the lumen of the endoplasmic reticulum, the GUS polypeptide undergoes several modifications, including the addition and modification of oligosaccharide side chains and the phosphorylation of mannose residues, that are important in localization of the polypeptide (38). This sequence of events suggests two possible sources of the tissue differences. One concerns the efficiency of binding of signal-recognition particle. If the GUS peptide signal sequence fails to bind a signal-recognition particle, the polypeptide chain will not be released into the lumen of the endoplasmic reticulum where it can be glycosylated and transported. Instead, translation will continue and the nascent polypeptide will be released into the cytosol, where it presumably will be degraded. The other possibility is that binding of signal-recognition particle is efficient, along with translocation of the growing polypeptide into the lumen, but that the efficiency of subsequent glycosylation and transport varies among tissues, and some GUS polypeptides are lost.

Finally, a third possibility for tissue differences in translational yield is suggested by the recent finding of Oshima *et*

Table 5. Normalized GUS protein levels, k_d^{rel}, k_s^{rel}, and translational yield

Tissue	GUS activity per g of protein	GUS polypeptides per gene	k_d^{rel}	k_s^{rel}	Molecules of GUS mRNA per gene	Translational yield
		B6.N mice				
Liver	1.00	1.00	1.00	1.00	1.00	1.00
Large intestine	1.94	0.68	0.74	1.42	0.63	0.63
Lung	1.09	0.36	1.24	1.34	0.70	0.41
Kidney	0.50	0.28	0.98	0.49	0.59	0.39
Submaxillary gland	0.50	0.17	0.76	0.37	0.74	0.22
Brain	0.10	0.08	2.76	0.28	0.33	0.52
		B6 mice				
Liver	1.00	1.00	1.00	1.00	1.00	1.00
Large intestine	1.31	0.54	0.86	1.13	—	—
Lung	1.05	0.31	1.07	1.13	0.50	0.48
Kidney	0.46	0.26	1.78	0.82	0.55	0.77
Submaxillary gland	0.33	0.13	1.17	0.43	0.60	0.28
Brain	0.12	0.10	1.81	0.22	0.32	0.49

al. (42) of two GUS mRNAs in human tissues. One of these mRNAs codes for complete functional enzyme molecules. The other lacks a short, central sequence of 51 codons and cannot synthesize functional enzyme molecules. It is possible that a similar situation exists in the mouse and the proportion of these two message types differs among tissues. When tested by blot hybridization analysis of electrophoretically fractionated RNA, each of the six tissues contained a predominant 2.6-kilobase band of GUS mRNA (data not shown), but differences as small as 150 bases might not have been detected. There is presently no evidence for the existence of two mRNA types among the variety of mouse GUS cDNA clones isolated in three laboratories (20, 43, 44).

Genetic changes within the GUS gene complex are also known to affect the translational yield of GUS mRNA within a single tissue. Studies of these genetic variants provide additional data regarding possible mechanisms for the tissue-specific changes in translational yield reported here.

The *CS*, *CL*, and *H* haplotypes of the GUS gene all have reduced activity compared to the standard *B* haplotype as a consequence of changes in translational yield (20, 45). Measurements of polysome profiles of GUS mRNA in kidneys of $[Gus]^H$ mice failed to reveal any differences from those of $[Gus]^B$ mice (K. Denich, G. Watson, and K.P., unpublished data). This implies that the reduced translational yield resulting from genetic change in the *H* haplotype is not a consequence of defective initiation or termination of translation and probably lies in the fraction of nascent GUS polypeptides that are successfully converted to mature GUS tetramers. It is possible that the differences in GUS translational yield among tissues reflect the same mechanistic changes as the differences between haplotypes within a single tissue.

In all of the tissues we have tested, the steady-state levels of GUS are maintained by low levels of mRNA, with an average of 1–3 molecules per cell. Precedents exist for mRNA levels in this range (3–5, 46, 47).

For GUS, the measured levels of message coupled with the estimated half-life of the message allows the calculation of replacement rates for GUS mRNA. In C57BL/6J kidney the message turns over with a half-life of approximately 0.65 day (k_d = 1.10 day^{-1}; ref. 16), which approximates the average half-life for kidney mRNA (48). Similar estimates have been made for the $[Gus]^N$ message (L.T.B., unpublished data). Since there is approximately 0.94 mRNA molecule per gene in kidney (Table 3), the replacement rate for GUS mRNA is (1.10/day) × (0.94 mRNA per gene), or 1.0 transcript per gene per day, suggesting that each gene is transcribed about once a day.

In the case of GUS protein in kidney, Smith and Ganschow (22) have estimated the enzyme half-life, allowing for amino acid reincorporation, to be approximately 3 days (k_d = 0.23 day^{-1}). The replacement rate for GUS protein is then (0.23/day) × (4.4×10^3 polypeptides per gene), or 1.0×10^3 polypeptides per gene per day. With 0.94 transcript per gene, the replacement rate for GUS protein is 1.1×10^3 polypeptides per mRNA per day, or 0.7 polypeptide per mRNA per min. With an average GUS polysome size of 11 in kidney (K. Denich, G. Watson, and K.P., unpublished data), the transit time per ribosome is about 15 min, or about 44 amino acids per min. These values fit reasonably well with estimates for other proteins (49).

The differential expression of special-function genes is, in general, regulated transcriptionally. In contrast, the differential expression of several housekeeping genes has been shown to result from changes in mRNA maturation and/or stability. The findings with GUS introduce a third mode of differential regulation of gene expression. In addition to transcription and mRNA stability, both of which control mRNA levels, it is now apparent that translational yield, which involves protein synthesis, can also be utilized. At first, the differential use of translational yield to achieve tissue-specific gene expression appears to be energetically inefficient. From the relative numbers of GUS mRNA and protein molecules produced, it is apparent that more than 99% of the energy expenditure for GUS production comes in synthesizing the protein. It would seem energetically more efficient to regulate mRNA production than to carry out inefficient protein synthesis. A possible explanation lies in the small numbers of GUS molecules present in cells. Regulatory systems probably require on the order of 10^4 molecules per cell to operate effectively; this is the case for steroid receptors (50). Since GUS is only present at 3–40 × 10^3 molecules per cell, the cost of coding, synthesizing and operating a tissue-specific regulatory system for this enzyme could be as great or greater than the cost of inefficient GUS protein synthesis.

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