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Review

γ-Glutamyl Transpeptidase

What Does the Organization and Expression of a Multipromoter Gene Tell Us about its Functions?

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γ-Glutamyl transpeptidase is a key enzyme in glutathione (GSH) salvage, metabolism of endogenous mediators such as leukotrienes and prostaglandins, detoxification of xenobiotics including environmentally important compounds and carcinogens, and cellular processes dependent on the oxidation/reduction of glutathione. The enzyme is widely distributed, and these functions often occur in separate tissues and in response to different stimuli. Evidence indicates that γ-glutamyl transpeptidase plays a direct role in some hepatic and renal responses to injury. In the mouse γ-glutamyl transpeptidase is a single copy gene expressed from at least seven promoters, and many of the transcribed γ-glutamyl transpeptidase RNAs are restricted in their expression. Studies that combine analyses of cellular processes with a knowledge of gene structure and expression hold promise for unravelling how these two different levels of function are integrated. (Am J Pathol 1995, 147:1175–1185)

γ-Glutamyl Transpeptidase

γ-Glutamyl transpeptidase (GGT) is the only enzyme known to cleave glutathione (GSH) and GSH S-conjugates in appreciable quantities (for reviews see refs. 1 and 2). The protein is a heterodimer processed from a single polypeptide chain and located on the plasma membrane of many cells. The active site is directed extracellularly. It is most prominent on the luminal surfaces (often in microvilli) of cells that have secretory or absorptive functions, but is also known to occur on the basolateral surfaces of renal epithelial cells and perhaps other cells as well (see refs. 1 and 2). The enzyme catalyzes the cleavage of GSH (γ-glutamylcysteinylglycine) to cysteinylglycine and a γ-glutamyl moiety that may be transferred to another amino acid, water, or even GSH itself (Figure 1). The enzyme will also catalyze the cleavage of S-conjugates of GSH (see below) and other γ-glutamyl compounds such as γ-glutamyl-*S*-methylcysteine.[2] GGT may not be unique in catalyzing these reactions. Recently another γ-glutamyl-cleaving enzyme, known as GGT-rel, with a 40% amino acid similarity to GGT, has been identified in humans.[3] It does not cleave substrates commonly used in GGT analysis; however, it will cleave leukotriene C_4 (LTC_4), a glutathione conjugate of leukotriene A_4, and possibly GSH itself. This gene has not been identified in mice.[3] GGT enzyme activity can be inhibited irreversibly by several compounds including

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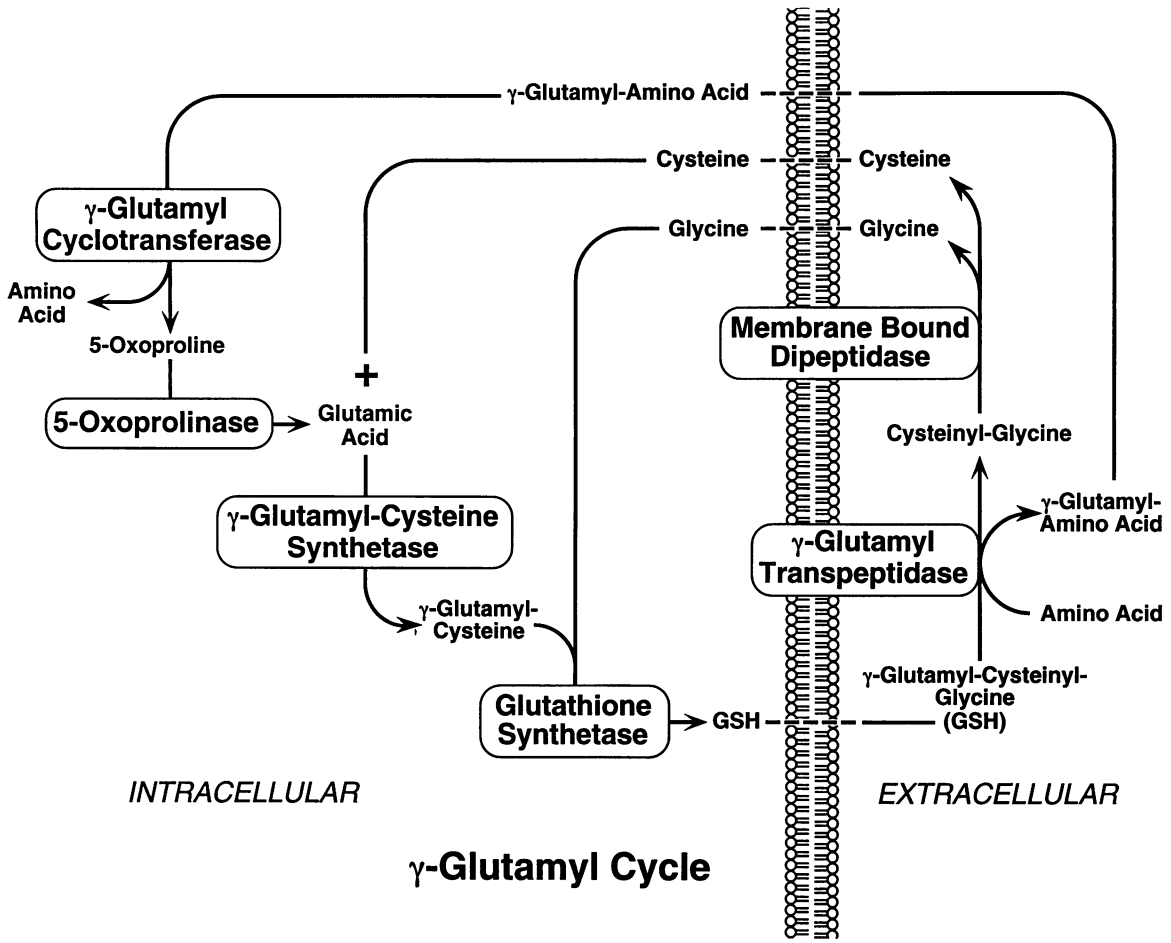

Figure 1. *The γ-glutamyl cycle.*

AT-125 (Acivicin) and γ-glutamyl-(*o*-carboxy)phenylhydrazides.[1,4,5] Administration of the hydrazides to mice and rats results in glutathionuria (from GGT inhibition in the kidneys) and an increase in serum GSH, which probably results from failure of GSH to be cleared.[4,6] Hepatic GSH levels did not change, but renal levels fell 30 to 40%.[4,6]

GGT is widely distributed in tissues and organs. It is abundant in the proximal convoluted tubules of the kidney, the ciliary body of the eye, seminal vesicles, the villi of the small intestine, fetal liver/liver, pancreas, and mammary glands. There are large differences among species in the relative levels of GGT expression in different organs; eg, in the rat the ratio of kidney to liver GGT specific activity is 875, whereas in the guinea pig it is 15.[7] These differences may dictate species differences in how both endogenous and exogenous substrates are metabolized (see below). In addition to well-studied sites associated with secretion where the enzyme is abundant, the enzyme appears to be present in cerebral vascular endothelial cells,[8–10] but its subcellular localization in endothelial cells has not been studied in detail. In addition, rare columnar epithelial cells in bronchi and terminal bronchioles are GGT-positive in the mouse (R. Barrios and M. W. Lieberman, unpublished observations). GGT RNA levels in the lung are many hundreds of times lower than those seen in kidney and are at the limits of detection when analyzed by extracting total organ RNA and performing Northern blot hybridization (G. M. Habib, R. Barrios, and M. W. Lieberman, unpublished observations). We do not have a clear understanding of the function of GGT at sites such as the cerebral vascular endothelium or occasional epithelial cells in the lungs, and even our knowledge of the role of GGT in metabolism in kidney, liver, and intestine remains incomplete.

GGT and the γ-Glutamyl Cycle

GSH is the principal non-protein intracellular thiol. In one study of mice on a standard protein diet GSH

concentrations were reported as 8.6 mmol/L in liver, 4 mmol/L in kidney, and 1.9 mmol/L in brain; a low protein diet reduced hepatic GSH by 30% and had no effect on the other tissues[11] (see also ref. 12). Plasma levels of GSH are three orders of magnitude lower (mouse and rat, ~15 to 35 μmol/L; human, ~1 to 3 μmol/L).[1,2,4,6] However, these "steady state" levels do not give an accurate picture of GSH turnover. This same group, for example, found that the $t_{1/2}$ of GSH was 215 minutes in liver and 15 minutes in kidney. Extraordinarily high rates of GSH synthesis are necessary to maintain these large intracellular GSH pools in the face of GSH export and its cleavage by GGT and dipeptidase (see below).

The transport of GSH into the extracellular space, its cleavage into its constituent amino acids, and their reabsorption and subsequent GSH resynthesis is known as the γ-glutamyl cycle (Figure 1). Because GSH appears not to be reabsorbed as the tripeptide in any appreciable quantity (however, see ref. 13), GGT is a key enzyme in this cycle.[1,2,14] That is, the reabsorption of cysteine or its S-conjugates is dependent on the action of GGT. There appear to be three sources of extracellular GSH: 1) GSH ingested as part of the diet; 2) intracellular GSH transported out of the cell and into the extracellular environment or blood; and 3) GSH secreted into lumens and extracellular spaces by secretory epithelia (proximal tubules of the kidney, seminal vesicle, ciliary body epithelium, hepatocytes, and choroid plexus epithelium).

The role of the γ-glutamyl cycle in recycling GSH and the metabolism of GSH conjugates is clear for some processes, but remains a major puzzle for others. For example, it is reasonable to attribute high levels of GGT in the villous epithelium to the need to absorb GSH released during digestion of food (although the real explanation may be that it is needed to cleave GSH not degraded in the hepatobiliary system after secretion). Likewise, the proposal of Meister and Larsson[1] that cells release GSH into the local environment to maintain the integrity of cell membranes (to protect them from oxidative damage) seems reasonable. Further, the release of relatively large amounts of GSH into the aqueous humor of the eye probably serves to prevent or reduce light damage to the lens.[15] It is less certain why GGT is present at such high levels in ciliary body epithelium. Why is it necessary to cleave and reabsorb the secreted GSH? One untested hypothesis is that GGT is present to cleave oxidized GSH, toxic GSH S-conjugates and other adducts, thus allowing their removal from the local environment by reabsorption and clearance in the urine or feces.

The reason that large amounts of GSH are secreted by hepatocytes into bile and plasma and by the proximal tubular cells into the urinary effluent remains uncertain. However, it is reasonable to presume that the high levels of GGT in the bilary system, intestine, and kidney are present to cleave GSH. It has been postulated that GSH serves as a reservoir for cysteine and facilitates the transport of this relatively toxic amino acid around the body,[1] but it seems likely that secretion of GSH into the blood and its maintenance there at μmol/L levels should be sufficient for this task. It has also been suggested that GSH release into the biliary epithelium serves as an osmotic pump to drive the formation of bile[16]; this mechanism also appears to serve as a way to rid the body of GSH conjugates formed in the liver. Yet these postulates do not seem to account for the enormous fluxes seen in these organs (see above). In the kidney, eg, only about 20% of the GSH in proximal tubular fluid is the result of glomerular filtration; the rest is secreted by tubular cells.[1,2,17] In seminal vesicles one can imagine that GSH secretion protects sperm from damage, but, once again, it is not clear why GGT should be present at high levels to cleave the secreted GSH or its conjugates. None of these puzzles is easily solved by conventional physiological or pharmacological experiments, but a better understanding of how the expression of the GGT gene is regulated may provide important clues to these questions. Ultimately, however, mouse models utilizing transgenic techniques and homologous recombination will provide the best tools for solving these problems.

GGT Deficiency and Overexpression in Humans

At present five individuals with GGT deficiency have been identified; those who have undergone metabolic testing are reported to show absent or barely detectable levels of GGT in serum, urine, and nucleated cells as well as very high GSH levels in urine and varying degrees of elevation of GSH levels in blood (summarized in refs. 1 and 14). One individual, eg, had urine GSH levels of 150 μg/ml (control value = 0.16 μg/ml) and plasma levels of 6.4 μg/ml (control value = 3.8 μg/ml). This individual and two others exhibited mental retardation and/or behavioral disorders; however, two individuals found by mass screening did not.[14] No other signs or symptoms were observed in any of the five individuals. It is not clear whether there is a direct relationship between

GGT deficiency and mental retardation and other central nervous system deficiencies.

A complicating aspect of these studies is that it is now known that in humans there are at least 5 to 7 GGT genes and/or pseudogenes located on different chromosomes (see below). Although it is conceivable that some or all of these patients have a mutation at some site responsible for the regulation of all GGT genes, it is more likely that none of these individuals has complete absence of GGT activity. Further, in humans, GGT-rel (or other related but as yet undiscovered genes) may carry out some of the functions of GGT.[3] Because we do not as yet know very much about levels of expression of GGT genes in humans or the tissue distribution of their expression, it its difficult to know what to expect in complete GGT deficiency. Many of the roles of GGT relate to the function of GSH in protection from oxidative damage and toxic xenobiotics; thus the full effects of GGT deficiency may only become evident in the presence of environmental stress over a sustained period of time (eg, excessive exposure to sun light, cigarette smoke or air pollution). A family has recently been described in which individuals have serum enzyme GGT levels that are >100 times normal values.[18] The proband was found during routine laboratory screening; the trait appears to be inherited as an autosomal dominant. None of the individuals has other abnormal laboratory values and all are described as healthy.

The Role of GGT in Metabolism

GGT plays a key role in the metabolism of many physiologically important endogenous compounds as well as toxic xenobiotics and carcinogens. The first step in the biotransformation of these compounds is the intracellular formation of a GSH conjugate by the action of a GSH transferase (eg, refs. 19 to 21). After transport across the plasma membrane, conjugates are subject to an initial cleavage by GGT and subsequently to cleavage by a dipeptidase to yield the S-cysteinyl derivative of the compound. With many endogenous and exogenous compounds formation of a GSH conjugate is the first step in detoxification and excretion; however, conjugation with GSH sometimes yields active, physiologically important compounds, or results in the conversion of some xenobiotics to active alkylating agents.[22–25]

The endogenous compounds metabolized by the GSH transferase/GGT pathway include powerful mediators of physiological function (eg, leukotrienes, hepoxilins, and prostaglandins). LTC_4, eg, is formed by GSH conjugation with LTA_4 and is a powerful proinflammatory and a vasoconstrictive agent[26–31] (see also ref. 32 for a brief review).

LTC_4 is synthesized by neutrophils, monocytes, macrophages, and eosinophils and is degraded extracellularly or cleared by the liver, where it is secreted and metabolized in the biliary system. Bioconversion occurs by the sequential action of GGT, which results in the production of LTD_4, the cysteinylglycine conjugate of LTA_4 and its degradation to LTE_4 (the cysteinyl conjugate of LTA_4), and finally, after reabsorption, acetylation to *N*-acetyl-LTE_4 (the mercapturic acid). When [^{3}H] LTC_4 is administered to rats, most of the radioactivity is recovered as one of these metabolites, suggesting that modifications of the fatty acid portion of the molecule plays only a small role in metabolism.[29,30]

LTC_4 and LTD_4 share many physiological properties,[31] but differ markedly in others. The importance of GGT is illustrated by its the role in leukotriene-mediated hepatic injury. Administration of galactosamine and endotoxin to mice normally results in massive liver injury; however, if AT-125 (an inhibitor of GGT) is preadministered to animals liver damage is avoided. Further, infusion of LTD_4, but not LTE_4, into galactosamine-treated mice mimics the effect[33] and administration of an LTD_4 antagonist prevents damage. These results make it clear that GGT is involved in the generation of an important mediator of cell injury. It is likely that other such examples will come to light as more experiments of this sort are undertaken.

Hepoxilins are closely related to the leukotrienes. Hepoxilin A_3 is conjugated to GSH to form the biologically active compound hepoxilin A_3-C, which is exported and apparently binds to central nervous system receptors.[26,34] It is degraded by GGT. GSH transferases also catalyze the metabolism of prostaglandins PGA_1, PGA_2, and Δ^{12}-PGJ_2 to their glutathione conjugates, which are thought to be inactive.[26,31,32] It is likely that these compounds are metabolized by the GGT/dipeptidase pathway. Although the role of GGT in the metabolism and physiology of endogenous mediators has not been investigated in detail, it is clearly involved in both the activation and inactivation of these biomediators.

Hundreds of exogenous compounds are known to be conjugated by GSH transferases, secreted, and metabolized by GGT. The list includes anticancer drugs such as malphalan and widely recognized toxins and carcinogens such as polycyclic aromatic hydrocarbons, halogenated alkanes and alkenes, and aflatoxins. Although we cannot review this pro-

cess in detail here, it is worth reiterating that the overall effect of conjugation is to promote detoxification by rendering these compounds more water soluble and excretable. On occasion this conjugation to GSH results in intermediates that are more toxic than the original compound.[22–25] Most of the interest in this area has centered around the metabolism of carcinogens and toxic halogenated compounds. However, agents that are not metabolized and that form coordination complexes with GSH without the action of GSH transferases are also metabolized by GGT. One of the most important of these is CH_3Hg.

A brief summary of the findings with CH_3Hg is helpful in illustrating the role of GGT in cell injury. In humans, CH_3Hg remains an important renal toxin and neurotoxin; exposure results primarily from the ingestion of fish and shell fish[35–39] (see also ref. 40). It is clear from animal studies that GGT plays a significant role in the clearance of CH_3Hg and the modulation of its toxicity. In fact, an interesting paradox exists; female mice retain CH_3Hg longer than males, but this chemical is more toxic to males. In "outbred" mice the $t_{1/2}$ of CH_3Hg clearance is 7 to 9 days for males and 10 to 16 days for females.[41] Intraperitoneal and oral administration gave similar values, but significant strain differences were noted. Although "outbred" males and females initially had similar liver levels, males had higher levels in kidney. This difference is thought to reflect the higher levels of renal GGT seen in males. It is believed that in males a greater fraction of CH_3Hg-GSH is cleaved by GGT and a dipeptidase to yield more of the *S*-cysteinyl conjugate, which is then reabsorbed by the kidney.[41] Another group reported a $t_{1/2}$ for Hg retention after administration of CH_3Hg of ~3.5 days for male mice and 6.75 days for females; they also noted that males died more frequently after high doses[42] and that males showed more proximal tubular damage than females. These studies suggest that GGT, which is so essential for the recycling of GSH, may promote the toxicity of some xenobiotics by facilitating their reabsorption. It is possible that the intracellular acetylation of cysteinyl-xenobiotic conjugates (ie, mercapturic acid formation) after reabsorption and the subsequent reexcretion of these acetyl derivatives may have developed in response to this toxicity.[43–45]

The details of CH_3Hg metabolism are species-specific and appear to depend in part on the relative distribution of GGT in different organs: mice and rats have high levels of GGT in kidney and relatively low levels in liver and gall bladder (in the mouse); the reverse is true for guinea pigs and rabbits.[7,45] In keeping with this observation Ballatori and his coworkers[45] have observed that a much higher fraction of CH_3Hg is reabsorbed in the liver and biliary tree of guinea pigs than of hamsters, which like rats and mice have relatively low levels of hepatic/biliary GGT. Although it has been realized that GGT is present in the small intestine,[46,47] it has not generally been appreciated that it is very abundant.[48] Thus it is likely that in species such as rats and mice with low levels of hepatic and biliary GGT, there is an enterohepatic recycling of compounds.

GGT and Neoplasia

For almost 20 years studies of rat and mouse liver carcinogenesis have relied on GGT as a positive marker for neoplastic and preneoplastic states.[49,50] In the rat, many but not all chemical carcinogens induce GGT-positive cells in liver as part of the neoplastic process, whereas in the mouse the usefulness of GGT as a marker is more variable.[49,50] Although in the past there has been considerable controversy about the cellular origin of chemically induced liver cancer in rodents, it seems clear that, as part of the carcinogenic process, the cell populations that emerge have many characteristics of fetal liver[51–53] and express high levels of GGT. It is known that transformation of cultured liver epithelial cells with the *ras* oncogene[54–56] results in cell populations that are GGT-positive. In this regard it is of interest that when carcinogen-induced rat liver tumors and *ras*-transformed rat liver epithelial cells were examined for GGT expression, both expressed primarily type III RNA, the most abundant GGT RNA in fetal liver[57] (see also below). GGT is also expressed in human cancers including those of the liver, skin, mammary gland, lungs, and colon.[58–60] GGT has not been used clinically as a marker for human liver cancer because serum α-fetoprotein is much more specific and sensitive.

It has been argued that GGT may provide a metabolic advantage in some tumors, especially those of the liver, and is not simply a marker of the preneoplastic or neoplastic phenotype.[49,50]

GGT may facilitate growth of neoplastic and preneoplastic cells by helping to provide high levels of intracellular GSH necessary for cell growth; membrane-bound GGT might cleave secreted extracellular GSH and thus initiate the process of the reabsorption of its constituents and the resynthesis of GSH.[50] It is also conceivable that GGT might make neoplastic cells more sensitive to a given dose of carcinogen. By analogy with the observation that higher levels of GGT promote the toxicity of CH_3Hg

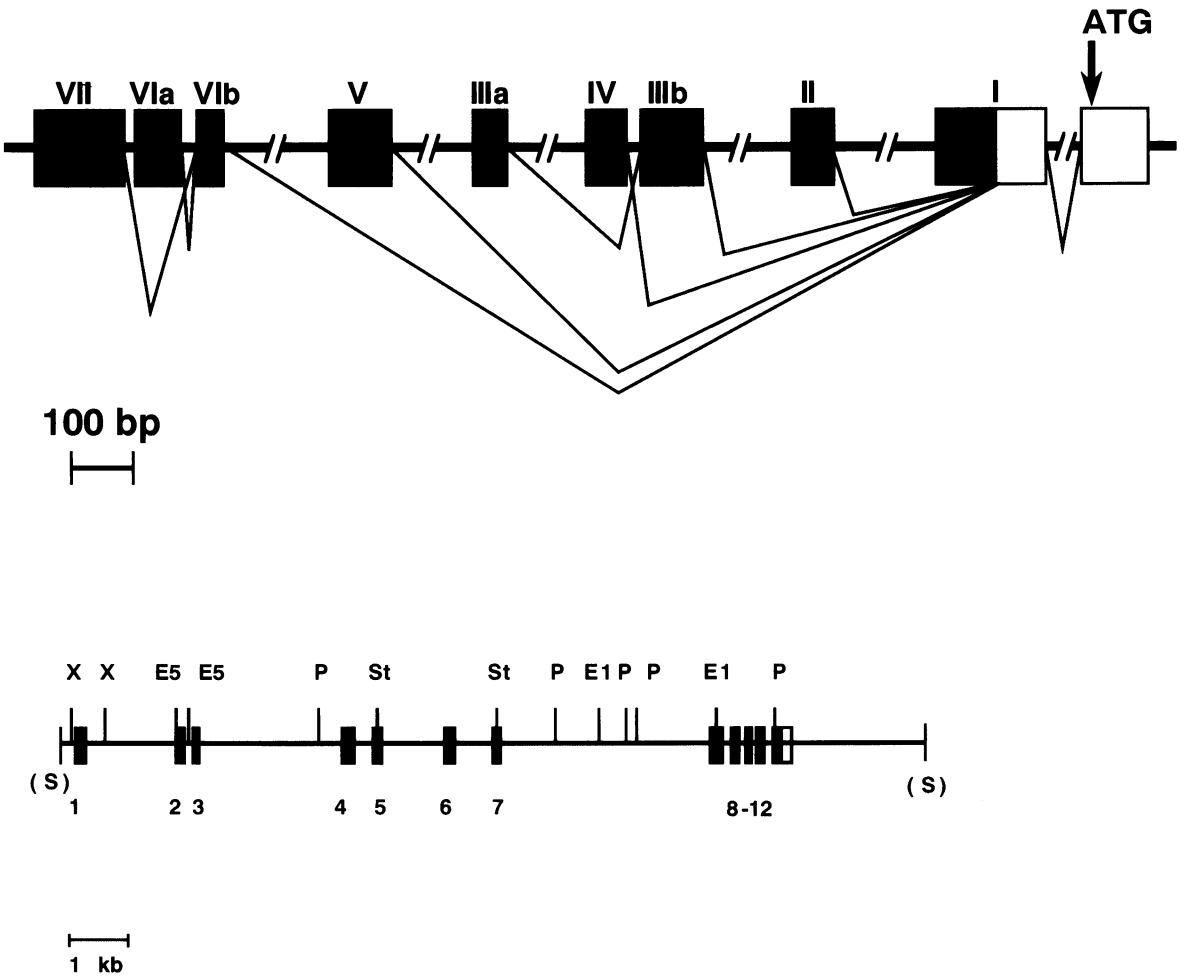

Figure 2. *Structure of the mouse GGT gene. The top row shows the 5' flanking region; the exon marked I is a common noncoding exon; ie, all seven known GGT transcripts contain it. The exon marked with the ATG is the first coding exon and corresponds to exon 1 in the bottom row*[71]. *The bottom row shows the coding exons that are identical for all GGT types examined.*[73]

by facilitating its reabsorption and retention in the kidney (see above), it is possible that a similar process occurs in GGT-positive hepatic cells exposed to carcinogens. Thus these cells would be exposed to a higher level of carcinogen damage and mutagenesis, which would facilitate neoplastic progression. In kidney and pancreas, which have the highest GGT levels in normal tissues, levels of the enzyme are reduced in carcinogen-induced tumors[61–63]; these data underscore the complexity of the process and demonstrate that the role of GGT in malignancy may be different in different tissues.

GGT is also present in most carcinomas of mouse skin induced by initiation/promotion protocols and focally in some large papillomas.[64–67] Recently Slaga and his associates[68] have shown that subcutaneous transplantation of cultured mouse papilloma cell lines carrying a GGT cDNA driven by a cytomegalovirus promoter resulted in four times as many subcutaneous tumors as did transplantation of cells carrying a vector control (T. J. Slaga, personal communication). In addition, when the cells carrying GGT were grafted into the skin the resulting tumors were 2.5 to 3.0 times larger than when cells carrying the vector were grafted. These findings clearly demonstrate that GGT provides a selective growth advantage to cultured papilloma cells. They suggest that in skin at least the expression of GGT is not simply a marker for "primitive" or neoplastic cells and that critical evaluations of other organ systems should be undertaken as well.

Organization of the Mouse and Rat GGT Genes

In the past few years our group characterized the structure and organization of the mouse *GGT* gene

Table 1. *Expression of GGT RNAs in Mouse Organs*

Type	I	II	III	IV	V	VI	VII
Adult kidney	3	13–46	6–10	26–33	7	5	–
Fetal kidney	+	+	+	+	+	+	ND
Small intestine	+	–	–	–	+	90	+
Fetal liver	–	–	80	–	–	–	–
Adult liver	–	–	–	–	–	–	ND
Eye (ciliary body)	+	–	–	–	–	–	ND
Lung	–	–	–	–	–	+	ND
Pancreas	–	–	+	–	–	+	ND
Seminal vesicle	–	–	–	–	+	–	–
Epididymis	ND	+	ND	80	ND	ND	–
Mammary gland	+	+	+	+	–	+	–
Skin	–	–	–	–	–	+	–

Numerical values are the percentage of steady state GGT RNA represented by a given type in individual organs. Data are from nuclease protection experiments[48,70,71] and unpublished observations. +/–, present or absent by reverse transcript polymerase chain reaction[48,69–71] and A. Sepulveda and M. W. Lieberman, unpublished observations. ND, not determined.

(Figure 2). *GGT* is a single copy gene transcribed from six promoters that have been mapped and characterized (I to VI) and at least one additional promoter (VII) upstream of them.[48,69–71] They reside in a ~10 kb region immediately 5′ of the first coding exon. One of the promoters contains a TATA box (V), and transcription is initiated from a single site.[70] Promoters III and IV have pyrimidine-rich sequences at +1 similar to an "initiation" sequence first identified in the *TdT* gene and show one predominant transcription start site.[72] Promoters I, II, and VI have no obvious TATA or initiator sequences and have multiple transcription start sites spread over 40 to 60 nucleotides.[70] The transcripts from all these promoters splice or are transcribed through a common noncoding exon (exon I), which in turn splices to 12 coding exons.[73] The result is the generation of GGT RNAs that have different 5′ ends but appear to code for the same protein. cDNA clones and genomic clones predict a polypeptide with 541 amino acids, which is then cleaved into a heavy chain (amino terminal) and a light chain (carboxyl terminal). Within the coding region we have not identified any splice variants or alternative poly(A) addition sites.

The organization of the rat *GGT* gene is similar to that of the mouse.[47,74,75] It too is a single copy gene with multiple promoters in the 5′ flanking region. Three rat promoters have been identified (I, II, III); the 5′ flanking regions and noncoding exons of mouse and rat types I and II show great sequence identity, although the transcription start sites for type II RNA are different. Type III is more complicated in that in the mouse, transcription of type III is initiated upstream of type IV[71] (Figure 2), whereas in the rat, "type III" contains sequences homologous to the mouse type IIIb region but is initiated in a region corresponding to the mouse type IV region.[75] In both species RNAs transcribed from all three types are expressed in kidney, and mouse and rat type III RNA is the predominant GGT RNA in fetal liver.[57,69–71,74–78] A comparison of mouse and rat cDNA clones for GGT RNA transcribed from promoter VI reveals long stretches with near identity in the two species.[48,79] The protein coded for by the respective cDNAs must be very similar since the nucleotide sequences predict a 95% amino acid identity.[68,69,73]

Human GGT Genes

GGT expression in humans appears more complex than in the mouse and the rat.[80–86] To date, human GGT genomic sequences have not been completely characterized, but chromosomal mapping studies as well as genomic and cDNA cloning suggest that the human genome has a number (at least 5 to 7) of GGT genes and pseudogenes.[85,86] In general, cDNA analysis indicates that these have different, but related 5′ untranslated ends and, in one case at least, an RNA that appears to be derived from an alternative splice.[80–85] It is not known whether multiple RNAs with different 5′ ends are transcribed from individual human GGT genes as they are in the mouse and the rat. As mentioned above, a cDNA representing a GGT-related gene has also been identified in humans.[3] It is of interest, however, that in humans, multiple GGT genes show restricted tissue expression of different GGT RNAs;[86] a similar restriction occurs in the mouse, which has a single gene with multiple promoters (see below). These findings serve to underscore the problem (and the opportunity) of using humans with GGT deficiency ("loss of function" mutations) to examine GGT function.

Regulation of GGT Expression

Perhaps because GGT is so widely distributed, it is not surprising that GGT expression is not highly inducible. Agents such as steroids and ethanol induce two- to threefold increases in GGT levels, and examination of many other agents has failed to uncover any that stimulate large inductions.[87,88] The large increases in GGT seen after treatment with chemical carcinogens are the result of emerging new cell populations that are GGT-positive rather than activation or up-regulation of GGT expression. It is well known that GGT levels in kidney rise about 10-fold after birth and that a similar fall in GGT levels occurs in liver.[89,90] It is likely that virtual disappearance of GGT from mouse liver after birth represents a negative regulation of expression similar to that seen for α-fetoprotein. However, interpretation of increases seen in kidney is more complicated; part of this rise may be the result of more GGT-positive tubules per unit weight rather than an up-regulation of gene expression (R. Barrios and M. W. Lieberman, unpublished observations).

Given this set of circumstances, it is somewhat puzzling that the regulation of GGT expression is so complex. In the mouse, transcription is initiated from at least seven promoters (Figure 1), many more than appear to be needed to produce the observed constant steady state levels of GGT. Although levels of GGT expression in individual organs seem largely unperturbable, there is surprising specificity of GGT RNA type in many organs (Table 1). Although six types of GGT RNA are detectable in kidney, the great bulk of intestine and fetal liver GGT RNA expression is restricted to one type: VI in the intestine and III in the liver. It is unknown whether these findings reflect promoter usage, differences in the stability or processing of different GGT RNAs, or both. It is also interesting that in less well studied tissues such as the ciliary body (eye) and seminal vesicle epithelium only one type of GGT RNA (types I and V, respectively) has been detected. Why should the GGT gene need so many promoters to express the same protein? Does GGT RNA require different 5′ ends for stability or processing in different tissues? Is it possible that translation is dependent on different 5′ untranslated regions in different tissues? GGT is highly glycosylated, and patterns of glycosylation are different in different tissues (eg, 91). Is there some relationship between the untranslated region of GGT RNAs and glycosylation patterns?

Another issue is the relationship between function and expression. Is it possible that differential expression reflects different functions of GGT in the same or different tissues? We know that GGT is involved in GSH salvage via the γ-glutamyl cycle in the kidney and liver; protection against photochemical damage in the eye; an unknown, but probably protective function in the male genitourinary system; lactation; metabolism of GSH conjugates of leukotrienes, hepoxilins, and prostaglandins in liver and other organs; and metabolism of toxic xenobiotics and carcinogens in the hepatobiliary-intestinal system and kidneys. It is possible that the reason we have failed to find regulation of expression is that we have looked in the wrong place with the wrong agents. Perhaps some of less well understood functions of GGT that occur in tissues where the enzyme is less abundant and in which unusual substrates are used may be the key to understanding the puzzle of complex regulation of a gene whose functions appear largely uninducible, standard and even prosaic. Conversely, one can view the molecular biology findings as clues to the different physiological functions of GGT. They serve to highlight that in fact GGT function is complex, and they provide potential tools for understanding this complexity.

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