ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 5 & 6, 2005

Forum Review

The Role of Cystathionine β-Synthase in Homocysteine Metabolism

KWANG-HWAN JHEE[1] and WARREN D. KRUGER[2]

ABSTRACT

Cystathionine β-synthase (CBS) is the first enzyme in the transsulfuration pathway, catalyzing the conversion of serine and homocysteine to cystathionine and water. The enzyme contains three functional domains. The middle domain contains the catalytic core, which is responsible for the pyridoxal phosphate-catalyzed reaction. The C-terminal domain contains a negative regulatory region that is responsible for allosteric activation of the enzyme by *S*-adenosylmethionine. The N-terminal domain contains heme, and this domain regulates the enzyme in response to redox conditions. Besides its canonical reaction, CBS can catalyze alternative reactions that produce hydrogen sulfide, a novel neuromodulator in the brain. Mutations in human CBS result in homocystinuria, an autosomal recessive disorder characterized by defects in a variety of different organ systems. The most common *CBS* allele is 833T>C (I278T), which is associated with pyridoxine-responsive homocystinuria. A complementation system in *S. cerevisiae* has been developed for analysis of human CBS mutations. Using this system, it has been discovered that deletion of the C-terminal domain of CBS can suppress the functional defects of many patient-derived mutations. This finding suggests it may be possible to develop drugs that interact with the C-terminal domain of CBS to treat elevated homocysteine in humans. *Antioxid. Redox Signal.* 7, 813–822.

INTRODUCTION

Homocysteine is a thiol-containing amino acid derived from the metabolism of methionine (Fig. 1). Initial interest in homocysteine stemmed from the identification of children with cystathionine β-synthase (CBS) deficiency who had 20–30-fold increases in the level of total homocysteine present in blood (56). These children had a number of distinct pathologies, including defects in the visual system, circulatory system, nervous system, and skeletal system, suggesting that elevated homocysteine could affect a number of different biological processes (57). Treatments that lower plasma homocysteine reduce the severity of many of these pathologies, suggesting that homocysteine is the actual pathogenic agent (58). More recently, there has emerged large amounts of data suggesting that even moderately elevated homocysteine levels may cause increased risk for various human diseases, such as stroke, heart attack, peripheral vascular disease, dementia, and birth defects (63).

How could elevated homocysteine be involved in such a wide number of apparently unrelated diseases? One idea is that homocysteine may affect a very basic biological process central to a variety of diseases. A possible hypothesis has centered on the relationship between homocysteine and oxidative stress. Homocysteine itself has been shown to cause increased oxidative stress on cells, both through direct effects (*e.g.,* the production of hydrogen peroxide by oxidation of homocysteine to homocystine) and indirect effects (*e.g.,* reduction of glutathione peroxidase) (77). In addition, it is estimated that as much as 50% of the cellular antioxidant glutathione is produced from homocysteine by conversion through the transsulfuration pathway (55). A second popular hypothesis suggests

[1]Department of Applied Chemistry, Kumoh National Institute of Technology, Kyungbuk, Korea.
[2]Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA.

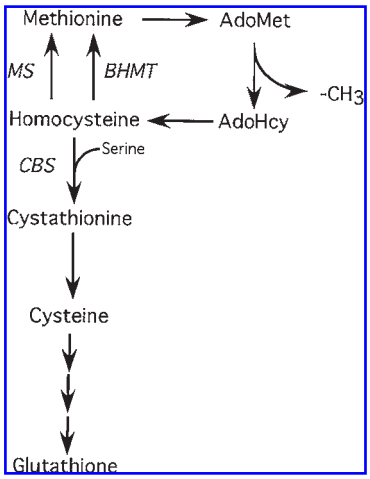

FIG. 1. Methionine metabolic pathway. Arrows indicate enzymatic reactions. Key enzymes are indicated in italics.

that elevated homocysteine affects the control of biologically important methylation reactions by causing a build-up of *S*-adenosylhomocysteine (AdoHcy). AdoHcy is a competitive inhibitor of *S*-adenosylmethionine (AdoMet) binding for methyltransferase enzymes. As methyltransferases are involved in a variety of important biological processes, inhibition of this class of enzymes could have extremely diverse effects on the organism (9).

The subject of this review is the metabolism of homocysteine through the transsulfuration pathway with a focus on the initial enzyme in this pathway, CBS. We will first discuss the enzymology of CBS and then present information on the consequences of defective CBS.

HOMOCYSTEINE METABOLISM

Homocysteine is formed from the hydrolysis of AdoHcy, which in turn is formed from AdoMet (Fig. 1). AdoMet is a methyl group donor in a large number of biochemical reactions. Therefore, homocysteine production increases as a result of increased methyl usage in the body. Once homocysteine is produced, it can have two metabolic fates: either remethylation to form methionine or transsulfuration to form cysteine. The remethylation reaction is carried out by two different enzymes, methionine synthase (MS) and betaine-dependent homocysteine methyltransferase (BHMT). MS transfers a methyl group from methylenetetrahydrofolate to homocysteine and uses vitamin B_{12} as an essential cofactor (48). MS appears to be expressed in most tissues, with very high levels found in the human heart, skeletal muscle and pancreas. In contrast, BHMT expression is restricted to the liver and kidney (13), where it uses betaine as the methyl-group donor for the conversion of homocysteine to methionine (24). The remethylation pathway is favored under conditions where methionine and AdoMet levels are low, resulting in conservation of these metabolites. When methionine and AdoMet levels are high, transsulfuration dominates (20).

The transsulfuration pathway catalyzes the conversion of homocysteine to cysteine, and is the only *de novo* pathway for cysteine production in mammals. The pathway consists of two steps. In the first step, homocysteine is condensed with serine to form cystathionine, whereas in the second step, cystathionine is cleaved to form cysteine and α-ketobutyrate. CBS is the first committed step in the transsulfuration pathway, and plays a key role in regulating the flux between transsulfuration and remethylation.

STRUCTURE–FUNCTION OVERVIEW OF CBS: THREE DOMAINS

The human CBS enzyme is a member of the pyridoxal 5′-phosphate (PLP) family of enzymes, which utilize PLP to catalyze their reactions. The enzyme exists as a homotetramer of four 63-kDa monomers (68). The monomer is 551 amino acids in length and is organized in a three-domain structure (72) (Fig. 2). The middle part of the enzyme contains the catalytic core, which binds the substrates and PLP. This part of the enzyme is the most conserved evolutionarily. The N-terminal portion of the human enzyme contains the heme-binding domain, which appears to be an evolutionary add-on. It is not found in any other PLP family members, nor is it observed in CBS enzymes outside of vertebrates (34). Based on studies described later, it appears that the heme plays a role in enzyme regulation as opposed to catalysis. The C-terminal domain is responsible for allosteric regulation of the enzyme by AdoMet. As will be discussed later, this domain acts as an inhibitor of the catalytic domain, and AdoMet appears to act by relieving this inhibition.

CBS CATALYTIC CORE

The most conserved portion of the CBS enzyme is the so-called "catalytic" core, which is located roughly between amino acids 70 and 400. The sequences of the catalytic core domains from rat, human, and yeast exhibit significant homology with the sequences of PLP-dependent enzymes that belong to the β-family (2) or Fold II (25). Related enzymes in Fold II include the β subunit of bacterial tryptophan synthase, bacterial and plant *O*-acetylserine sulfhydrylase (8), and the catalytic domain of bacterial threonine deaminase (34).

Recently, the *x*-ray crystal structure of the catalytic core, along with the N-terminal domain (amino acids 1–413), has been determined (51, 73) (Fig. 3). This truncated form of the

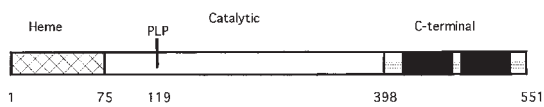

FIG. 2. Domain structure of human CBS. The different domains within CBS are indicated by shading. The approximate amino acid demarcating the differing regions is shown below the figure. The black shaded areas show the location of the CBS domains within the C-terminal region.

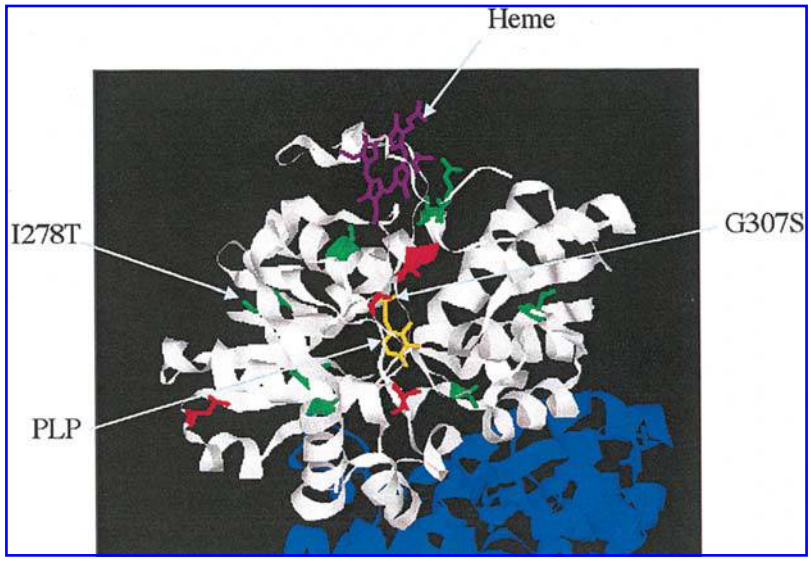

FIG. 3. Structure of amino acids 1–413 of human CBS. The white ribbon shows one monomer, whereas the blue ribbon shows the second monomer. The PLP molecule is indicated by the yellow stick representation, and the heme is represented by the purple stick representation. Shown as green and yellow sticks are mutations found in human patients with CBS deficiency. The green mutations are functionally suppressed in yeast by deletion of the C-terminal 143 amino acids, whereas the red mutations are not. In general, the nonsuppressible alleles tend to be closer to the active-site PLP than the suppressible alleles.

enzyme functions as a dimer as opposed to tetramer. It binds heme and PLP and is enzymatically active, but its activity is no longer regulated by AdoMet. The PLP moiety is deeply buried in a cleft within the catalytic core, such that the active site is accessible only via a narrow channel. Lysine-119 is the residue that forms a Schiff base with the PLP cofactor (19, 51). The hydroxyl group of serine-349 forms a hydrogen bond with the N1 of PLP and is also thought to play a key role in catalysis. The next step in the study of CBS will be the determination of the entire CBS crystal structure including the C-terminal regulatory domain. This will allow determination of the exact AdoMet-binding site and a detailed mechanism for the activation by AdoMet.

CATALYTIC MECHANISM OF CBS

PLP acts as the coenzyme in a number of significant reactions in amino acid metabolism. From the genomic study, we know that almost 1.5% of all genes code for PLP-dependent enzymes in many free-living prokaryotes, but in higher eukaryotes the percentage is substantially lower, consistent with these catalysts being involved mainly in basic metabolism (61). Assigning the function of PLP-dependent enzymes simply on the basis of sequence criteria is not straightforward because, as a consequence of their common mechanistic features, these enzymes have intricate evolutionary relationships (2, 17, 25, 50). The chemical basis explaining the catalytic versatility of PLP enzymes was proposed initially by Metzler in 1954 (53) and has been expanded since then (15, 16, 29, 32). Mechanistic investigation of a variety of PLP enzymes has generally confirmed these early proposals (12, 14, 26, 27, 33, 38, 54). Today the detailed and precise mechanisms of several types of PLP enzymes are known based on crystallographic, spectroscopic, and site-directed mutagenesis studies. As mentioned previously, human CBS is a unique enzyme that binds with PLP and heme as a cofactor. Until recently, the reaction mechanism of human CBS could not be extensively investigated because the heme largely masks the spectroscopic properties of the PLP coenzyme intermediates. However, yeast CBS lacks heme, and thus yeast CBS has proven useful for spectroscopic studies of reaction mechanism (35). The specific catalytic mechanism for CBS was first proposed by Borcsok and Abeles (5) (Fig. 4). Experimental support for this mechanism has been observed for both full-length and truncated yeast CBS through the use of steady-state kinetic analysis (34, 35) and rapid scanning stopped-flow spectroscopic studies (36, 37, 70). First, the substrate L-serine binds to PLP and undergoes hydrolysis to form an enzyme-aminoacrylate intermediate (E-AA) in the absence of L-homocysteine (Fig. 4, Stage I). This E-AA intermediate does not undergo hydrolysis or further reaction in the absence of an added nucleophile. Thus, the yeast CBS reaction does not proceed by direct displacement of the OH of L-serine, as first proposed by Braunstein and Goryachenkova (6). Addition of L-homocysteine to the preformed E-AA intermediate forms an external aldimine with cystathionine (Fig. 4, Stage II). The reaction of CBS with the product, L-cystathionine, also leads to formation of the E-AA intermediate. This means that the reaction is reversible (1). Rapid-scanning spectroscopy studies of the full-length yeast enzyme suggest the rate-limiting step is the release of cystathionine (70), whereas studies of the truncated enzyme indicate that the formation of cystathionine from the aminoacrylate is rate-limiting (36). Recent studies on a hemeless version of human CBS

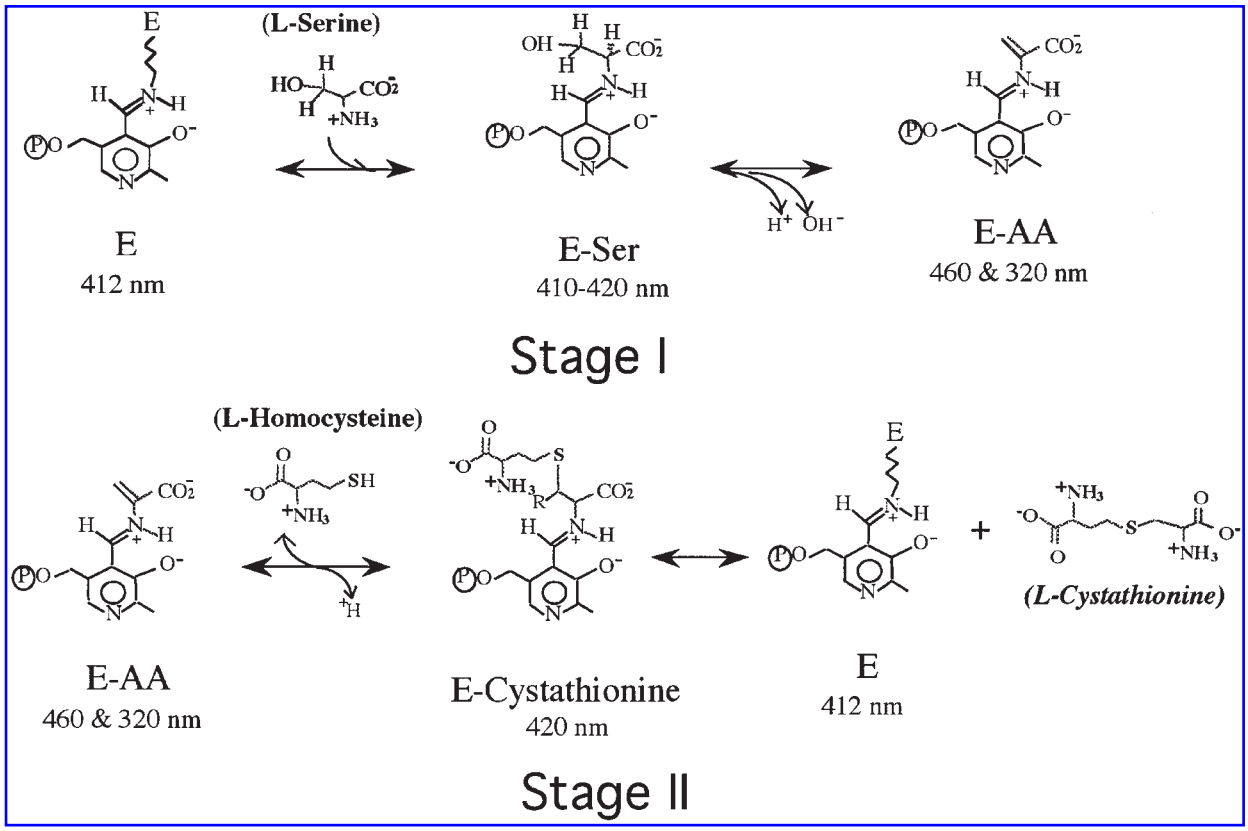

FIG. 4. Catalytic mechanism of CBS. Stage I shows the first half-reaction with L-serine in the absence of a cosubstrate. Note the loss of water to form enzyme-aminoacrylate (E-AA) intermediate. Stage II is the second half-reaction obtained upon addition of L-homocysteine to E-AA. Possible quinonoid intermediates between E-Ser and E-AA or between E-Cystathionine and E-AA are not shown.

also suggest the existence of an aminoacrylate intermediate (19). The type of reaction mechanisms used by the CBS is known as a double displacement or ping-pong mechanism (34).

INTERMEDIATES, SUBSTRATE/ PRODUCTION INHIBITION

In many PLP enzymes, high concentrations of substrates can cause enzyme inhibition. In the CBS reaction, L-serine does not show inhibition, but L-homocysteine exhibits the substrate inhibition (1, 34). This substrate inhibition is usually ascribed to the binding of one or both of the substrates to the wrong form of the enzyme, thereby diverting the enzyme from the productive pathway and altering the distribution of enzyme–substrate intermediates (65). For example, the binding of the cosubstrate indole to tryptophanase in the presence of an amino acid alters the equilibrium distribution of enzyme–substrate intermediates (40).

As mentioned above, steady-state and rapid-scanning spectroscopy studies indicate that the CBS product L-cystathionine can also form an E-AA intermediate (35, 36). This means that the entire reaction of CBS is reversible (1, 36). Thus, in theory, the presence of a large amount of L-cystathionine can inhibit the overall reaction of CBS. However, in most tissues, cystathionine levels are quite low so it is unlikely that this reverse reaction plays a significant role *in vivo.*

ROLE OF HEME

Human CBS binds heme as a cofactor in the N-terminal regulatory domain (Fig. 2). Based on the crystal structure, the heme iron is coordinated by interactions with His^{65} and Cys^{52} (52). CBS heme does not appear to be essential for catalysis because the deletion of the 70 N-terminal residues resulted in a heme-free protein retaining 10–20% of wild-type activity (60, 73). In addition, the CBS enzymes from both *S. cerevisiae* and *T. cruzi* lack heme but catalyze the overall reaction with similar steady-state kinetic parameters (34, 59).

One hypothesis to explain the presence of heme in mammalian CBS is that heme may act as a redox sensor to regulate CBS activity. Incubation of purified human CBS with the reductant titanium citrate resulted in a 1.7-fold reduction of CBS activity, which could be restored by oxidation with potassium ferricyanide (71). Although the distance between heme and PLP is ~20 Å, ^{31}P NMR reveals that the oxidation state of heme does affect the spectrum of PLP (39). It has also been observed

that treatment of a hepatoma-derived cell line with peroxide causes increased flux of homocysteine to glutathione without an increase in CBS enzyme level (55). However, it has not been proved that this increased flux is due to elevated CBS activity, and it is unclear how the cellular redox state affects the heme iron *in vivo*.

ALLOSTERIC REGULATION BY ADOMET AND THE CBS DOMAIN

AdoMet is a critical molecule in metabolism, being both the major methyl group donor and a key substrate in polyamine metabolism. It has been known for 30 years that AdoMet controls CBS enzyme activity (21). From a metabolic point of view, it is logical that CBS activity would be sensitive to AdoMet levels. When AdoMet levels are high, activation of the transsulfuration pathway would represent a way to get rid of excess AdoMet, whereas when levels are low, remethylation of homocysteine would allow its conservation.

AdoMet stimulates CBS activity by increasing the turnover rate rather than the binding of substrates to the enzyme (41, 71). This effect seems to occur via interactions of AdoMet with the C-terminal domain of the enzyme. Deletion of the C-terminal domain results in an enzyme that is in a superactivated state and no longer responds to AdoMet (41, 66). Certain point mutations in the C-terminal domain abolish AdoMet stimulation or increase the K_d for AdoMet (18, 67). AdoMet has been shown to bind to recombinant CBS with a stoichiometry of one molecule of AdoMet per subunit of CBS (72). This binding is abolished in an enzyme lacking the C-terminal 143 amino acids. These studies have led to a model of CBS activation in which the C-terminal domain of CBS acts as an inhibitory domain and AdoMet binding to this domain reduces this inhibition (18, 31, 67).

Several of the missense mutations that disrupt AdoMet activation of CBS lie within an evolutionarily conserved 53-amino acid motif known as the "CBS" domain. This motif is found in proteins in several diverse species ranging from humans to archaebacteria (4). In human CBS, it is located between amino acids 415 and 468 within the C-terminal regulatory region. Other proteins containing the CBS domain include inosine 5′-monophosphate dehydrogenase (IMPDH), 5′-AMP-activated protein kinase (AMPK), voltage-gated chloride channels (CLC2), ATP-binding cassette transporters, and several other proteins of undefined function. In all of these other cases, the CBS domain is found in multiple copies. Homology modeling of the C-terminal 143 amino acids of CBS with the CBS domain-containing region of IMPDH suggests that there is a possible second CBS domain between residues 486 and 543 (67).

The structure of the CBS domain has been determined for IMPDH (79). This enzyme is a triosephosphate isomerase (TIM) barrel, with two CBS domains inserted in a loop of the TIM barrel. Recently, it has been proposed that pairs of CBS domains act as binding domains for adenosine derivatives (64). Purified proteins containing tandem CBS domains from AMPK, IMPDH-2, and CLC2 bound adenosine monophosphate and adenosine triphosphate, whereas the CBS domains of CBS bound AdoMet. It was also shown that missense mutations in CBS domains of AMPK, IMPDH-2, CLC2, and CBS caused dramatic shifts in ligand-binding ability. This finding suggests that CBS domains act as sensors of cellular energy status and couple this information with enzyme function. Interestingly, yeast CBS is not affected by AdoMet even though it contains a CBS domain in the region of 371–424 (34).

ALTERNATIVE ENZYME REACTIONS

The reaction versatility of PLP enzyme originates from the structure of PLP and its capability to act as an "electron sink," which can withdraw electrons from the substrate (53). Given this general property, there are, in fact, many different reactions that can be catalyzed by PLP-containing enzymes. However, the unique environment provided by the protein part of each unique PLP enzyme directs the basic catalytic abilities of the coenzyme so as to provide the reaction specificity with its own substrate specificity. Because PLP reactions occur as a series of consecutive steps, the efficacy of each enzyme lies not only in its ability to accelerate the required reaction at each stage, but to prevent all the other side reactions. Sometimes a measurable and physiologically significant side reaction can occur as a result of "mistake." Like other PLP enzymes, CBS can catalyze alternative reactions such as β-elimination and β-replacement reaction with other substrates (6, 54).

The main CBS reaction catalyzes the β-replacement reaction of L-serine with L-homocysteine to form L-cystathionine (Fig. 5). One of the potential alternate reactions for CBS involves the condensation of cysteine with homocysteine to form cystathionine and hydrogen sulfide (H_2S) (Fig. 5, Alternate Reaction 1) (7). We have shown that this reaction can be catalyzed by CBS, with the K_m for cysteine being about half that of serine (Kruger, unpublished observations). This reaction may be physiologically important for two reasons. First, H_2S has been shown to be a neuromodulator in the brain (43). Second, it represents an alternative way to eliminate homocysteine. Another potential source of H_2S production would be from the breakdown of cysteine (Fig. 5, Alternate Reactions 3 and 4); however, we have not been able to show that CBS from any source has significant levels of cysteine hydrolase activity (Jhee, unpublished observations).

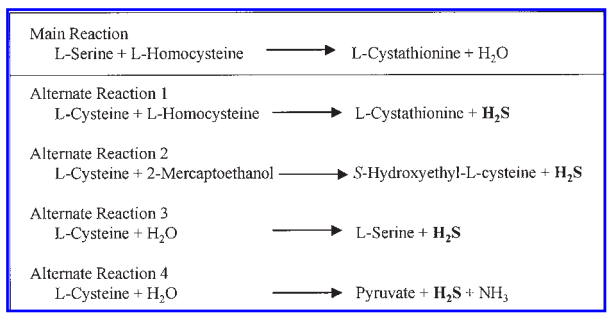

FIG. 5. Alternate enzyme reactions catalyzed by CBS.

CBS AND REDOX REGULATION

The transsulfuration pathway converts homocysteine to cysteine. It was reported that ~50% of the cysteine in the glutathione pool in cultured human liver cells is derived from homocysteine (55). Glutathione plays an important role in maintaining intracellular redox homeostasis and in cellular defense against oxidative stress. Therefore, the transsulfuration pathway is not only important to remove excess homocysteine in the body, but is also an essential pathway in terms of glutathione biosynthesis, to maintain redox homeostasis. Mice lacking CBS through homozygous deletion of the CBS gene have reduced levels of glutathione in the liver and brain (76). Analysis of CBS activity under a variety of growth conditions indicates that CBS is coordinately regulated with proliferation suggesting a redox-sensitive mechanism in both yeast and human cells (49).

In HepG2 cells, treatment with tumor necrosis factor-α, which is known to enhance production of reactive oxygen species, increased CBS activity and glutathione levels. Western blot data showed that the higher CBS activity originated from the cleavage of CBS to a truncated form. From those data, a novel redox regulation mechanism for CBS was proposed that oxidative stress targeted proteolysis of CBS to increase transsulfuration levels (80).

CBS EXPRESSION

CBS enzyme activity is not found in all tissues and cells. It is absent from heart, lung, testes, adrenal, and spleen in rats (20). In humans, it has been shown to be absent in heart muscle and primary cultures of human aortic endothelial cells (30). Examination of CBS protein by western blot suggests this lack of activity may result from posttranslational proteolysis of CBS. The lack of CBS in these tissues implies that these tissues are unable to synthesize cysteine and that cysteine must be supplied extracellularly (11). It also suggests that these tissues might have increased sensitivity to homocysteine toxicity because they cannot catabolize excess homocysteine via transsulfuration.

Four different CBS RNA isoforms have been described (3, 10). These differ only in their 5′-untranslated regions, and all of the isoforms would be expected to encode the same CBS protein. Adult tissues with the highest concentration of CBS mRNA are the liver, pancreas, and kidney. However, some CBS mRNA can be detected by northern blot analysis in all tissues tested. Certain RNA isoforms are expressed only in specific tissues. For example, isoform 5 is expressed exclusively in the pancreas, whereas the kidney expresses predominantly isoform 1. The significance of these differences is difficult to assess, as there are no data suggesting that the different mRNA isoforms produce differing levels of CBS protein.

CBS mRNA is also expressed during human fetal development. It is expressed at high levels in fetal human liver and brain (62). *In situ* hybridization has detected CBS mRNA in the central nervous system from embryos as young as 3 weeks old. The gene is expressed in all embryonic tissues that are affected by clinical homocystinuria. Thus, CBS RNA levels are high in the developing neural and vascular systems, and somewhat lower in the developing skeletal system.

MUTATIONS IN CBS

Mutations in *CBS* are the most common cause of homocystinuria, a genetic disorder characterized by extremely elevated levels of total homocysteine (tHcy) in plasma (58). Besides elevated tHcy, CBS-deficient patients also have severe hypermethionemia. Homocystinuric individuals have a variety of clinical phenotypes, including mental retardation, ectopia lentis, skeletal abnormalities, and thrombotic vascular disease. About half of CBS-deficient patients respond to pharmacological doses of pyridoxine (vitamin B_6) with significant lowering of tHcy and a reduction in the severity of disease phenotypes. Because CBS-deficient siblings are always concordant for pyridoxine responsiveness, it is thought that response to pyridoxine is associated with specific CBS alleles (57).

Over the past decade, more than 130 CBS mutations have been characterized from both responsive and nonresponsive patients (45). About 80% of the CBS alleles are missense mutations, with most of the others representing deletion alleles. Most alleles are rare alleles only observed in one or two specific individuals. In addition, there appear to be specific alleles favored in specific locations. For example, the 919G>A (G307S) allele is extremely frequent in Ireland, but rarely observed in Holland (23, 44). Similarly, the 797G>A (R266K) allele was the most frequent allele observed in a study of Norwegian families, whereas the T191M allele is common on the Iberian Peninsula (42, 75).

The most frequently observed allele, and an allele that is panethnic, is the 833T>C (I278T) allele (45). This allele tends to be associated with a pyridoxine responsiveness, but there appear to be many exceptions (44). This allele probably arose from a 68-bp repeat polymorphism within the CBS gene (69). This repeat is found in ~5% of all CBS alleles and creates a duplication border of intron 7 and exon 8 with the exception of a single base-pair change. Because this repeat is normally ignored by the splicing machinery, it has no effect on CBS production. However, it is hypothesized that occasionally a recombination event occurs such that the wild-type allele is spliced out and all that is left is the imperfect repeat, which causes the 833T>C allele (42).

YEAST ASSAY FOR HUMAN CBS

Although many mutations in the *CBS* gene have been identified, in only a minority of cases have the functional effects of these mutations been analyzed. One system that has proved useful in the evaluation of CBS mutants is through functional expression and complementation in the yeast *S. cerevisiae* (46). The basis for this assay is that human CBS can functionally correct the cysteine auxotrophy associated with deletion of the endogenous yeast CBS gene *CYS4*. By expressing various mutant forms of CBS in yeast, it is possible to determine whether a particular missense mutation is disease-causing or whether it is a neutral polymorphism (Fig. 6). Our lab has

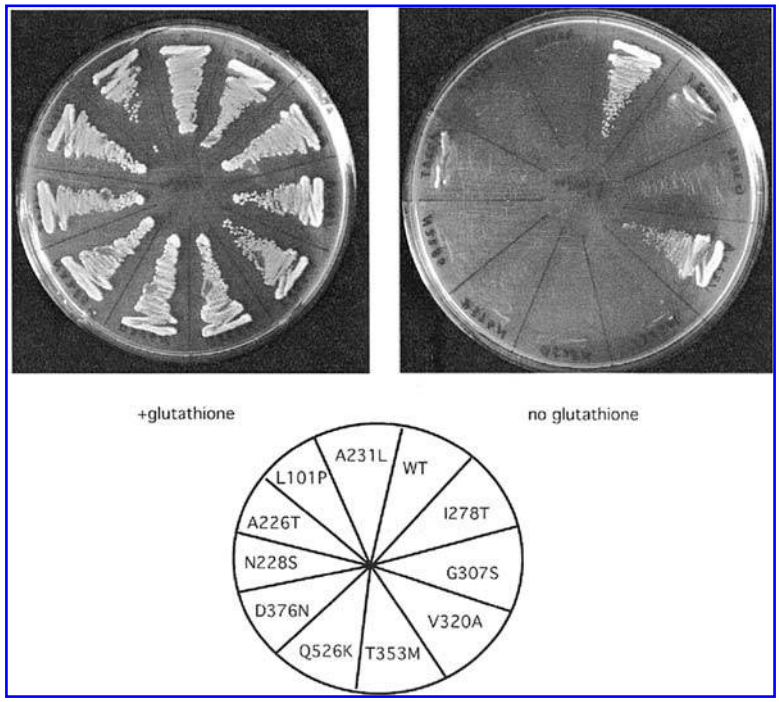

FIG. 6. Yeast functional assay for CBS. CBS-deficient yeast strain WY35 (46) was used as a host strain to express the indicated mutant alleles of human CBS. The left panel shows yeast growth on synthetic complete medium containing glutathione (a stable source of cysteine). The right panel shows the AdoMet yeast on medium lacking cysteine. The wheel in the center shows the key to the plate.

used this method to assess the functional effects of over 20 patient-derived alleles (42, 46, 47). The modeling of mutant forms of CBS in yeast allows the determination of the functional consequences of specific changes. For example, the R266K mutation found in several Norwegian patients with pyridoxine-responsive homocystinuria, was shown to give a pyridoxine-responsive phenotype in yeast (42).

The yeast system can also be used for mutational analysis of the enzyme. The observation that the C-terminus of CBS encoded a regulatory domain that is required for AdoMet regulation was inferred from a mutation that was originally discovered in the yeast system (66). An important finding from the yeast system is that deletion of the regulatory domain of CBS can suppress the functional effects of many missense mutations located within the catalytic core of CBS (67). This finding suggests that many missense mutations affect the interaction of the regulatory domain with the catalytic domain, such that the enzyme is an inactive form. Additional deletion or mutations within the regulatory domain can restore enzyme function. These findings suggest it might be possible to discover small-molecule drugs that bind to the C-terminal domain and cause activation of CBS activity. Such compounds could potentially be useful in the treatment of CBS deficiency or other homocysteine-related disease.

ACKNOWLEDGMENTS

This work was supported by grants HL57299 and core grant CA06927 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania for W.D.K. We also thank Rose Sonlin for secretarial support.

ABBREVIATIONS

AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; AMPK, 5′-AMP-activated protein kinase; BHMT, betaine-dependent homocysteine methyltransferase; CBS, cystathionine β-synthase; CLC2, voltage-gated chloride channels; E-AA, enzyme-aminoacrylate; H_2S, hydrogen sulfide; IMPDH, inosine 5′-monophosphate dehydrogenase; MS, methionine synthase; PLP, pyridoxal 5′-phosphate; tHcy, total plasma homocysteine; TIM, triosephosphate isomerase.

REFERENCES

1. Aitken SM and Kirsch JF. Kinetics of the yeast cystathionine beta-synthase forward and reverse reactions: continuous assays and the equilibrium constant for the reaction. *Biochemistry* 42: 571–578, 2003.
2. Alexander FW, Sandmeier E, Mehta PK, and Christen P. Evolutionary relationships among pyridoxal-5′-phosphate-dependent enzymes. Regio-specific α, β, and γ families. *Eur J Biochem* 219: 953–960, 1994.
3. Bao L, Vlcek C, Paces V, and Kraus JP. Identification and tissue distribution of human cystathionine beta-synthase mRNA isoforms. *Arch Biochem Biophys* 350: 95–103, 1998.

4. Bateman A. The structure of a domain common to archaebacteria and the homocystinuria disease protein. *Trends Biochem Sci* 22: 12–13, 1997.
5. Borcsok E and Abeles RH. Mechanism of action of cystathionine synthase. *Arch Biochem Biophys* 213: 695–707, 1982.
6. Braunstein AE and Goryachenkova EV. The beta-replacement-specific pyridoxal-P-dependent lyases. *Adv Enzymol* 56: 1–89, 1984.
7. Braunstein AE, Goryachenkova EV, Tolosa EA, Willhardt IH, and Yefremova LL. Specificity and some other properties of liver serine sulphhydrase: evidence for its identity with cystathionine β-synthase. *Biochim Biophys Acta* 242: 247–260, 1971.
8. Burkhard P, Rao GSJ, Hohenester E, Schnackerz KD, Cook PF, and Jansonius JN. The three-dimensional structure of *O*-acetylserine sulfhydrylase from *Salmonella typhimurium*. *J Mol Biol* 283: 121–133, 1998.
9. Caudill MA, Wang JC, Melnyk S, Pogribny IP, Jernigan S, Collins MD, Santos-Guzman J, Swendseid ME, Cogger EA, and James SJ. Intracellular *S*-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. *J Nutr* 131: 2811–2818, 2001.
10. Chasse JF, Paul V, Escanez R, Kamoun P, and London J. Human cystathionine beta-synthase: gene organization and expression of different 5′ alternative splicing. *Mamm Genome* 8: 917–921, 1997.
11. Chen P, Poddar R, Tipa EV, Dibello PM, Moravec CD, Robinson K, Green R, Kruger WD, Garrow TA, and Jacobsen DW. Homocysteine metabolism in cardiovascular cells and tissues: implications for hyperhomocysteinemia and cardiovascular disease. *Adv Enzyme Regul* 39: 93–109, 1999.
12. Christen P and Metzler DE. Transaminases. In: *Biochemistry: A Series of Monographs, Vol. 2,* edited by Meister A. New York: John Wiley & Sons, 1985, p. 643.
13. Delgado-Reyes CV, Wallig MA, and Garrow TA. Immunohistochemical detection of betaine-homocysteine *S*-methyltransferase in human, pig, and rat liver and kidney. *Arch Biochem Biophys* 393: 184–186, 2001.
14. Denesyuk AI, Denessiouk KA, Korpela T, and Johnson MS. Functional attributes of the phosphate group binding cup of pyridoxal phosphate-dependent enzymes. *J Mol Biol* 316: 155–172, 2002.
15. Dunathan HC. Conformation and reaction specificity in pyridoxal phosphate enzymes. *Proc Natl Acad Sci U S A* 55: 712–716, 1966.
16. Dunathan HC. Mechanism and stereochemistry of transamination. *Vitam Horm* 28: 399–414, 1970.
17. Dunathan HC and Voet JG. Stereochemical evidence for the evolution of pyridoxal phosphate enzymes of various function from a common ancestor. *Proc Natl Acad Sci U S A* 71: 3888–3891, 1974.
18. Evande R, Blom H, Boers GH, and Banerjee R. Alleviation of intrasteric inhibition by the pathogenic activation domain mutation, D444N, in human cystathionine beta-synthase. *Biochemistry* 41: 11832–11837, 2002.
19. Evande R, Ojha S, and Banerjee R. Visualization of PLP-bound intermediates in hemeless variants of human cystathionine beta-synthase: evidence that lysine 119 is a general base. *Arch Biochem Biophys* 427: 188–196, 2004.
20. Finkelstein JD. The metabolism of homocysteine: pathways and regulation. *Eur J Pediatr* 157: S40–S44, 1998.
21. Finkelstein JD, Kyle WE, Martin JL, and Pick AM. Activation of cystathionine synthase by adenosylmethionine and adenosylethionine. *Biochem Biophys Res Commun* 66: 81–87, 1975.
22. Gallagher DT, Gilliland GL, Xiao G, Zondlo J, Fisher KE, Chinchilla D, and Eisenstein E. Structure and control of pyridoxal phosphate dependent allosteric threonine deaminase. *Structure* 6: 465–475, 1998.
23. Gallagher PM, Naughten E, Hanson NQ, Schwichtenberg K, Bignell M, Yuan M, Ward P, Yap S, Whitehead AS, and Tsai MY. Characterization of mutations in the cystathionine beta-synthase gene in Irish patients with homocystinuria. *Mol Genet Metab* 65: 298–302, 1998.
24. Garrow TA. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase. *J Biol Chem* 271: 22831–22838, 1996.
25. Grishin NV, Phillips MA, and Goldsmith EJ. Modeling of the spatial structure of eukaryotic ornithine decarboxylase. *Protein Sci* 4: 1291–1304, 1995.
26. Hayashi H. Pyridoxal enzymes: mechanistic diversity and uniformity. *J Biochem (Tokyo)* 118: 463–473, 1995.
27. Hayashi H, Wada H, Yoshimura T, Esaki N, and Soda K. Recent topics in pyridoxal 5′-phosphate enzyme studies. *Annu Rev Biochem* 59: 87–110, 1990.
28. Hyde CC, Ahmed SA, Padlan EA, Miles EW, and Davies DR. Three-dimensional structure of the tryptophan synthase $\alpha_2\beta_2$ multienzyme complex from *Salmonella typhimurium*. *J Biol Chem* 263: 17857–17871, 1988.
29. Ivanov VI and Karpeisky MY. Dynamic three-dimensional model for enzymic transamination. *Adv Enzymol* 32: 21–53, 1969.
30. Jacobsen DW and Poddar R. Vascular dysfunction at the cellular level. *Neth J Med* 52: S1, 1998.
31. Janosik M, Kery V, Gaustadnes M, Maclean KN, and Kraus JP. Regulation of human cystathionine beta-synthase by *S*-adenosyl-L-methionine: evidence for two catalytically active conformations involving an autoinhibitory domain in the C-terminal region. *Biochemistry* 40: 10625–10633, 2001.
32. Jenkins WT, Yphantis DA, and Sizer IW. Glutamic aspartic transaminase. I. Assay, purification and general properties. *J Biol Chem* 234: 51–57, 1959.
33. Jhee K-H, Yoshimura T, Kurokawa Y, Esaki N, and Soda K. A stereochemical aspect of pyridoxal 5′-phosphate dependent enzyme reactions and molecular evolution. *J Microbiol Biotechnol* 9: 695–703, 1999.
34. Jhee KH, McPhie P, and Miles EW. Domain architecture of the heme-independent yeast cystathionine beta-synthase provides insights into mechanisms of catalysis and regulation. *Biochemistry* 39: 10548–10556, 2000.
35. Jhee KH, McPhie P, and Miles EW. Yeast cystathionine beta-synthase is a pyridoxal phosphate enzyme but, unlike the human enzyme, is not a heme protein. *J Biol Chem* 275: 11541–11544, 2000.
36. Jhee KH, Niks D, McPhie P, Dunn MF, and Miles EW. The reaction of yeast cystathionine beta-synthase is rate-

limited by the conversion of aminoacrylate to cystathionine. *Biochemistry* 40: 10873–10880, 2001.
37. Jhee KH, Niks D, McPhie P, Dunn MF, and Miles EW. Yeast cystathionine beta-synthase reacts with L-allothreonine, a non-natural substrate, and L-homocysteine to form a new amino acid, 3-methyl-L-cystathionine. *Biochemistry* 41: 1828–1835, 2002.
38. John RA. Pyridoxal phosphate-dependent enzymes. *Biochim Biophys Acta* 1248: 81–96, 1995.
39. Kabil O, Toaka S, LoBrutto R, Shoemaker R, and Banerjee R. Pyridoxal phosphate binding sites are similar in human heme-dependent and yeast heme-independent cystathionine beta-synthases. Evidence from ^{31}P NMR and pulsed EPR spectroscopy that heme and PLP cofactors are not proximal in the human enzyme. *J Biol Chem* 276: 19350–19355, 2001.
40. Kazarinoff MN and Snell EE. Some effects of indole on the interaction of amino acids with tryptophanase. *J Biol Chem* 255: 6228–6233, 1980.
41. Kery V, Poneleit L, and Kraus JP. Trypsin cleavage of human cystathionine beta-synthase into an evolutionarily conserved active core: structural and functional consequences. *Arch Biochem Biophys* 355: 222–232, 1998.
42. Kim CE, Gallagher PM, Guttormsen AB, Refsum H, Ueland PM, Ose L, Folling I, Whitehead AS, Tsai MY, and Kruger WD. Functional modeling of vitamin responsiveness in yeast: a common pyridoxine-responsive cystathionine beta-synthase mutation in homocystinuria. *Hum Mol Genet* 6: 2213–2221, 1997.
43. Kimura H. Hydrogen sulfide as a neuromodulator. *Mol Neurobiol* 26: 13–19, 2002.
44. Kluijtmans LA, Boers GH, Kraus JP, van den Heuvel LP, Cruysberg JR, Trijbels FJ, and Blom HJ. The molecular basis of cystathionine beta-synthase deficiency in Dutch patients with homocystinuria: effect of CBS genotype on biochemical and clinical phenotype and on response to treatment. *Am J Hum Genet* 65: 59–67, 1999.
45. Kraus JP, Janosik M, Kozich V, Mandell R, Shih V, Sperandeo MP, Sebastio G, de Franchis R, Andria G, Kluijtmans LA, Blom H, Boers GH, Gordon RB, Kamoun P, Tsai MY, Kruger WD, Koch HG, Ohura T, and Gaustadnes M. Cystathionine beta-synthase mutations in homocystinuria. *Hum Mutat* 13: 362–375, 1999.
46. Kruger WD and Cox DR. A yeast assay for functional detection of mutations in the human cystathionine beta-synthase gene. *Hum Mol Genet* 4: 1155–1161, 1995.
47. Kruger WD, Wang L, Jhee KH, Singh RH, and Elsas LJ 2nd. Cystathionine beta-synthase deficiency in Georgia (USA): correlation of clinical and biochemical phenotype with genotype. *Hum Mutat* 22: 434–441, 2003.
48. Li YN, Gulati S, Baker PJ, Brody LC, Banerjee R, and Kruger WD. Cloning, mapping, and RNA analysis of the human methionine synthase gene. *Hum Mol Genet* 5: 1851–1858, 1995.
49. Maclean KN, Janosik M, Kraus E, Kozich V, Allen RH, Raab BK, and Kraus JP. Cystathionine beta-synthase is coordinately regulated with proliferation through a redox-sensitive mechanism in cultured human cells and *Saccharomyces cerevisiae*. *J Cell Physiol* 192: 81–92, 2002.
50. Mehta PK and Christen P. Homology of pyridoxal-5′-phosphate-dependent aminotransferases with the cobC (cobalamin synthesis), nifS (nitrogen fixation), pabC (p-aminobenzoate synthesis) and malY (abolishing endogenous induction of the maltose system) gene products. *Eur J Biochem* 211: 373–376, 1993.
51. Meier M, Janosik M, Kery V, Kraus JP, and Burkhard P. Structure of human cystathionine beta-synthase: a unique pyridoxal 5′-phosphate-dependent heme protein. *EMBO J* 20: 3910–3916, 2001.
52. Meier M, Oliveriusova J, Kraus JP, and Burkhard P. Structural insights into mutations of cystathionine beta-synthase. *Biochim Biophys Acta* 1647: 206–213, 2003.
53. Metzler DE, Ikawa M, and Snell EE. A general mechanism for vitamin-B6 catalyzed reactions. *J Am Chem Soc* 76: 648–652, 1954.
54. Miles EW. Pyridoxal phosphate enzymes catalyzing β-elimination and β-replacement reactions. In: *Pyridoxal Phosphate: Chemical, Biochemical and Medical Aspects, Part B, Vol. 1B,* edited by Avramovic O. New York: John Wiley & Sons, 1986, pp. 253–310.
55. Mosharov E, Cranford MR, and Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. *Biochemistry* 39: 13005–13011, 2000.
56. Mudd SH, Finkelstein JD, Irreverre F, and Laster L. Homocystinuria: an enzyme defect. *Science* 143: 1443–1444, 1964.
57. Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GH, Bromberg IL, and Cerone R. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 37: 1–31, 1985.
58. Mudd SH, Levy HL, and Skovby F. Disorders of transsulfuration. In: *The Metabolic Basis of Inherited Disease,* 6th edit., edited by Valle D. New York: McGraw–Hill, 1995, pp. 1272–1327.
59. Nozaki T, Shigeta Y, Saito-Nakano Y, Imada M, and Kruger WD. Characterization of transsulfuration and cysteine biosynthetic pathways in the protozoan hemoflagellate, *Trypanosoma cruzi*. Isolation and molecular characterization of cystathionine beta-synthase and serine acetyltransferase from *Trypanosoma*. *J Biol Chem* 276: 6516–6523, 2001.
60. Oliveriusova J, Kery V, Maclean KN, and Kraus JP. Deletion mutagenesis of human cystathionine beta-synthase. Impact on activity, oligomeric status, and *S*-adenosylmethionine regulation. *J Biol Chem* 277: 48386–48394, 2002.
61. Percudani R and Peracchi A. A genomic overview of pyridoxal-phosphate-dependent enzymes. *EMBO Rep* 4: 850–854, 2003.
62. Quere I, Paul V, Rouillac C, Janbon C, London J, Demaille J, Kamoun P, Dufier JL, Abitbol M, and Chasse JF. Spatial and temporal expression of the cystathionine beta-synthase gene during early human development. *Biochem Biophys Res Commun* 254: 127–137, 1999.
63. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, and Scott JM. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 50: 3–32, 2004.
64. Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, Norman DG, and Hardie DG. CBS domains form energy-sensing modules whose binding of adenosine

ligands is disrupted by disease mutations. *J Clin Invest* 113: 274–284, 2004.
65. Segel IH. *Enzyme Kinetics. Behavior and analysis of rapid equilibrium and steady-state enzyme systems.* New York: John Wiley & Sons, 1975, p. 957.
66. Shan X and Kruger WD. Correction of disease-causing CBS mutations in yeast. *Nat Genet* 19: 91–93, 1998.
67. Shan X, Dunbrack RL Jr, Christopher SA, and Kruger WD. Mutations in the regulatory domain of cystathionine beta synthase can functionally suppress patient-derived mutations *in cis*. *Hum Mol Genet* 10: 635–643, 2001.
68. Skovby F, Kraus JP, and Rosenberg LE. Homocystinuria: biogenesis of cystathionine beta-synthase subunits in cultured fibroblasts and in an in vitro translation system programmed with fibroblast messenger RNA. *Am J Hum Genet* 36: 452–459, 1984.
69. Sperandeo MP, de Franchis R, Andria G, and Sabastio G. A 68bp insertion found in a homocystinuric pateint is a common varient and is skipped by alternative splicing of the cystathionine beta-synthase mRNA. *Am J Hum Genet* 59: 1391–1392, 1996.
70. Taoka S and Banerjee R. Stopped-flow kinetic analysis of the reaction catalyzed by the full-length yeast cystathionine beta-synthase. *J Biol Chem* 277: 22421–22425, 2002.
71. Taoka S, Ohja S, Shan X, Kruger WD, and Banerjee R. Evidence for heme-mediated redox regulation of human cystathionine beta-synthase activity. *J Biol Chem* 273: 25179–25184, 1998.
72. Taoka S, Widjaja L, and Banerjee R. Assignment of enzymatic functions to specific regions of the PLP-dependent heme protein cystathionine β-synthase. *Biochemistry* 38: 13155–13161, 1999.
73. Taoka S, Lepore BW, Kabil O, Ojha S, Ringe D, and Banerjee R. Human cystathionine beta-synthase is a heme sensor protein. Evidence that the redox sensor is heme and not the vicinal cysteines in the CXXC motif seen in the crystal structure of the truncated enzyme. *Biochemistry* 41: 10454–10461, 2002.
74. Thomazeau K, Curien G, Dumas R, and Biou V. Crystal structure of threonine synthase from *Arabidopsis thaliana. Protein Sci* 10: 638–648, 2001.
75. Urreizti R, Balcells S, Rodes M, Vilarinho L, Baldellou A, Couce ML, Munoz C, Campistol J, Pinto X, Vilaseca MA, and Grinberg D. Spectrum of CBS mutations in 16 homocystinuric patients from the Iberian Peninsula: high prevalence of T191M and absence of I278T or G307S. *Hum Mutat* 22: 103, 2003.
76. Vitvitsky V, Dayal S, Stabler S, Zhou Y, Wang H, Lentz SR, and Banerjee R. Perturbations in homocysteine-linked redox homeostasis in a murine model for hyperhomocysteinemia. *Am J Physiol Regul Integr Comp Physiol* 287: R39–R46, 2004.
77. Weiss N, Heydrick S, Zhang YY, Bierl C, Cap A, and Loscalzo J. Cellular redox state and endothelial dysfunction in mildly hyperhomocysteinemic cystathionine beta-synthase-deficient mice. *Arterioscler Thromb Vasc Biol* 22: 34–41, 2002.
78. Yao M, Ose T, Sugimoto H, Horiuchi A, Nakagawa A, Wakatsuki S, Yokoi D, Murakami T, Honma M, and Tanaka I. Crystal structure of 1-aminocyclopropane-1-carboxylate deaminase from *Hansenula saturnus. J Biol Chem* 275: 34557–34565, 2000.
79. Zhang R, Evans G, Rotella FJ, Westbrook EM, Beno D, Huberman E, Joachimiak A, and Collart FR. Characteristics and crystal structure of bacterial inosine-5′-monophosphate dehydrogenase. *Biochemistry* 38: 4691–4700, 1999.
80. Zou CG and Banerjee R. Tumor necrosis factor-alpha-induced targeted proteolysis of cystathionine beta-synthase modulates redox homeostasis. *J Biol Chem* 278: 16802–16808, 2003.

Address reprint requests to:
Warren D. Kruger, Ph.D.
Division of Population Science
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111

E-mail: wd_kruger@fccc.edu

Received for publication December 3, 2004; accepted December 10, 2004.

This article has been cited by:

1. K. G. Flores, C. A. Stidley, A. J. Mackey, M. A. Picchi, S. P. Stabler, J. M. Siegfried, T. Byers, M. Berwick, S. A. Belinsky, S. Leng. 2012. Sex-specific association of sequence variants in CBS and MTRR with risk for promoter hypermethylation in the lung epithelium of smokers. *Carcinogenesis* **33**:8, 1542-1547. [CrossRef]
2. Maria E. R. C. Santos, Francisco C. L. e Silva, Karina B. Gomes, Ana Paula M. Fernandes, Fernanda R. Freitas, Mayara C. Faria, Ana Paula L. Mota, Maria G. Carvalho. 2011. Mutations in methylenetetrahydrofolate reductase and in cysthationine beta synthase: is there a link to homocysteine levels in peripheral arterial disease?. *Molecular Biology Reports* **38**:5, 3361-3366. [CrossRef]
3. Amany K. Elshorbagy, A. David Smith, Viktor Kozich, Helga Refsum. 2011. Cysteine and Obesity. *Obesity* . [CrossRef]
4. S. Harvey Mudd. 2011. Hypermethioninemias of genetic and non-genetic origin: A review. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics* **157**:1, 3-32. [CrossRef]
5. Elena Bravo, Simonetta Palleschi, Patricia Aspichueta, Xabier Buqué, Barbara Rossi, Ainara Cano, Mariarosaria Napolitano, Begoña Ochoa, Kathleen M Botham. 2011. High fat diet-induced non alcoholic fatty liver disease in rats is associated with hyperhomocysteinemia caused by down regulation of the transsulphuration pathway. *Lipids in Health and Disease* **10**:1, 60. [CrossRef]
6. Xianfeng Gu, Yi Zhun Zhu. 2011. Therapeutic applications of organosulfur compounds as novel hydrogen sulfide donors and/or mediators. *Expert Review of Clinical Pharmacology* **4**:1, 123-133. [CrossRef]
7. Baiqing Tang, Aladdin Mustafa, Sapna Gupta, Stepan Melnyk, S. Jill James, Warren D. Kruger. 2010. Methionine-deficient diet induces post-transcriptional downregulation of cystathionine #-synthase. *Nutrition* **26**:11-12, 1170-1175. [CrossRef]
8. Amany K. Elshorbagy, Maria Valdivia-Garcia, Helga Refsum, A. David Smith, Dwight A.L. Mattocks, Carmen E. Perrone. 2010. Sulfur amino acids in methionine-restricted rats: Hyperhomocysteinemia. *Nutrition* **26**:11-12, 1201-1204. [CrossRef]
9. Qiong Wei, Liqun Wang, Qiang Wang, Warren D. Kruger, Roland L. Dunbrack. 2010. Testing computational prediction of missense mutation phenotypes: Functional characterization of 204 mutations of human cystathionine beta synthase. *Proteins: Structure, Function, and Bioinformatics* NA-NA. [CrossRef]
10. Soumi Kundu, Neetu Tyagi, Utpal Sen, Suresh C. Tyagi. 2009. Matrix imbalance by inducing expression of metalloproteinase and oxidative stress in cochlea of hyperhomocysteinemic mice. *Molecular and Cellular Biochemistry* **332**:1-2, 215-224. [CrossRef]
11. M.O. Krebs, Alfredo Bellon, Gaell Mainguy, T.M. Jay, Helge Frieling. 2009. One-carbon metabolism and schizophrenia: current challenges and future directions. *Trends in Molecular Medicine* **15**:12, 562-570. [CrossRef]
12. Jung H. Suh, Robert Kim, Burcu Yavuz, Daniel Lee, Ashutosh Lal, Bruce N. Ames, Mark K. Shigenaga. 2009. Clinical assay of four thiol amino acid redox couples by LC–MS/MS: Utility in thalassemia#. *Journal of Chromatography B* **877**:28, 3418-3427. [CrossRef]
13. Tom G. Holt , Bernard K. Choi , Neil S. Geoghagen , Kristian K. Jensen , Qi Luo , William A. LaMarr , Gergely M. Makara , Lorraine Malkowitz , Can C. Ozbal , Yusheng Xiong , Claude Dufresne , Ming-Juan Luo . 2009. Label-Free High-Throughput Screening via Mass Spectrometry: A Single Cystathionine Quantitative Method for Multiple Applications. *ASSAY and Drug Development Technologies* **7**:5, 495-506. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
14. S. Ozaki, A. Nakahara, C. Sakaguchi. 2009. Mutagenesis of Gln-142 and Phe-143 of O-Acetylserine Sulfhydrylase. *Asian Journal of Biochemistry* **4**:4, 117-124. [CrossRef]
15. Anna E. Beaudin, Patrick J. Stover. 2009. Insights into metabolic mechanisms underlying folate-responsive neural tube defects: A minireview. *Birth Defects Research Part A: Clinical and Molecular Teratology* NA-NA. [CrossRef]
16. Sapna Gupta, Liqun Wang, Xiang Hua, Jakub Krijt, Viktor Koz#ich, Warren D. Kruger. 2008. Cystathionine #-synthase p.S466L mutation causes hyperhomocysteinemia in mice. *Human Mutation* **29**:8, 1048-1054. [CrossRef]
17. Maxim Pimkin, George D. Markham. 2008. The CBS subdomain of inosine 5#-monophosphate dehydrogenase regulates purine nucleotide turnover. *Molecular Microbiology* **68**:2, 342-359. [CrossRef]
18. Andrew McCaddon, Peter R Hudson. 2007. Alzheimer's disease, oxidative stress and B-vitamin depletion. *Future Neurology* **2**:5, 537-547. [CrossRef]
19. E MARTIGNONI, C TASSORELLI, G NAPPI, R ZANGAGLIA, C PACCHETTI, F BLANDINI. 2007. Homocysteine and Parkinson's disease: A dangerous liaison?. *Journal of the Neurological Sciences* **257**:1-2, 31-37. [CrossRef]
20. Eitan Okun, Yahav Dikshtein, Alon Carmely, Hagar Saida, Gabi Frei, Ben-Ami Sela, Lydia Varshavsky, Asher Ofir, Esthy Levy, Michael Albeck, Benjamin Sredni. 2007. The organotellurium compound ammonium trichloro(dioxoethylene-

o,o')tellurate reacts with homocysteine to form homocystine and decreases homocysteine levels in hyperhomocysteinemic mice. *FEBS Journal* **274**:12, 3159-3170. [CrossRef]

21. X. Chen, L. Wang, R. Fazlieva, W.D. Kruger. 2006. Contrasting behaviors of mutant cystathionine beta-synthase enzymes associated with pyridoxine response. *Human Mutation* **27**:5, 474-482. [CrossRef]
22. Hideo Kimura . 2005. Hydrogen Sulfide as a Biological Mediator. *Antioxidants & Redox Signaling* **7**:5-6, 778-780. [Citation] [Full Text PDF] [Full Text PDF with Links]