HOW ARE MAMMALIAN METHIONINE ADENOSYLTRANSFERASES REGULATED IN THE LIVER? A FOCUS ON REDOX STRESS.

María A. Pajares[1,2*], Luis Álvarez[2,3], Dolores Pérez-Sala[4]

[1]Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Arturo Duperier 4, 28029 Madrid, Spain. [2]Molecular Hepatology group, IdiPAZ, Pº de la Castellana 261, 28046 Madrid, Spain. [3]Hospital Universitario La Paz, Pº de la Castellana 261, 28046 Madrid, Spain. [4]Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain.

*To whom correspondence should be addressed: Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM), Arturo Duperier 4, 28029 Madrid, Spain (Phone: 34-915854414; FAX: 34-915854401; email: mapajares@iib.uam.es)

ABSTRACT

S-adenosylmethionine synthesis is a key process for cell function, and needs to be regulated at multiple levels. In recent years, advances in the knowledge of methionine adenosyltransferases have been significant. The discovery of nuclear localization of these enzymes suggests their transport to provide the methyl donor, S-adenosylmethionine, for DNA and histone methyltransferases in epigenetic modifications, opening new regulatory possibilities. Previous hypotheses considered only the cytoplasmic regulation of these enzymes, hence the need of an update to integrate recent findings. Here, we focus mainly on the liver and redox mechanisms, and their putative effects on localization and interactions of methionine adenosyltransferases.

Keywords: methionine adenosyltransferase, S-adenosylmethionine, methylation, redox regulation, association state, epigenetic modifications.

Highlights:

- Redox stress differentially regulates methionine adenosyltransferases
- Redox stress may regulate subcellular localization of methionine adenosyltransferases
- S-adenosylmethionine homeostasis is regulated by redox stress

1. INTRODUCTION

Cells use methylation reactions to synthesize a large variety of compounds, including neurotransmitters and phospholipids, but also to regulate important functions such as gene expression [1-3]. For this purpose, a methyl donor is needed, a role performed mainly by S-adenosylmethionine (AdoMet). This metabolite is synthesized from methionine and ATP by methionine adenosyltransferases (MATs), which also hydrolyze the triphosphate chain of the nucleotide to yield pyrophosphate and inorganic phosphate (Fig. 1)[4]. Mammalian genomes contain three MAT genes that code for two catalytic subunits, α1 (*MAT1A*) and α2 (*MAT2A*), and a regulatory β-subunit (*MAT2B*). These subunits associate into three isoenzymes that are homo- (MAT I and III) and hetero-oligomers (MAT II)[2,5]. MAT I and MAT III are a tetramer and a dimer of α1 subunits, respectively, whereas MAT II was recently postulated to be a hetero-trimer of α2 and β subunits in a 2:1 ratio (Fig. 1) [6]. These isoenzymes show different affinities for methionine ($S_{0.5}^{Met}$), which are low for MAT III (1 mM), intermediate for MAT I (100 μM) and high for MAT II (3.3-16 μM). Their V_{max} follow the opposite trend compared to the $S_{0.5}^{Met}$, hence low AdoMet levels correlate with MAT II expression and high levels of the methyl donor with MAT I/III expression [2,3,5] (Fig. 1). Isoenzyme expression is tissue-dependent, that of MAT I/III being maximal in the liver [7,8] whereas MAT II is the prevalent isoenzyme in extrahepatic tissues [2]. Based on these kinetic and expression data, the capacity to synthesize AdoMet is higher in the liver than in other tissues and its levels are the highest followed closely by those found in kidney [8]. In contrast, the hepatic methylation index (AdoMet/S-adenosylhomocysteine ratio) is lower than in other tissues, a fact related to the number and importance of hepatic transmethylation reactions, and the resulting S-adenosylhomocysteine production [1,8].

Classically, MATs were considered cytosolic proteins and MAT I/III the hepatic isoenzymes; therefore, the previous hypothesis on their regulation was focused on cytosolic MAT I/III activity and association state [9], suggesting the need to explore post-translational modifications affecting these isoenzymes. This led to detailed studies on the redox and phosphorylation mechanisms acting on MAT I/III, which assumed that AdoMet synthesized in the cytosol was transported to compartments such as the nucleus for its use in methylation reactions (*e.g.* histone and DNA methylation). However, in 2009 our group demonstrated the nuclear localization of MAT I [7], a report that was shortly followed by the description of MAT II and splicing variants of the β-subunit in the nucleus [10,11]. These articles related nuclear localization of MATs to enhanced histone methylation, therefore suggesting that MATs are recruited to these locations where AdoMet synthesis is needed, as pointed out in an editorial article accompanying Katoh's paper [12]. These data, together with the accumulated knowledge on structure, post-translational modifications, and nuclear association states, prompted us to update and expand the previous regulatory model, incorporating also data concerning MAT II.

2. STRUCTURAL DATA ON METHIONINE ADENOSYLTRANSFERASES

Crystal structures of several MAT homo-oligomers in free active site conformations or complexed with substrates, products, or their analogues are now available [5,13,14]. These structures also included Mg^{2+} and K^{+} ions and $NADP^{+}$. The three-domain organization of α1-monomers is preserved in α2-subunits and, given the high level of sequence conservation among catalytic subunits [15], this organization is expected to be a common feature throughout the MAT family. A large hydrophobic surface constitutes the monomer-monomer interface, where the two dimer active sites are opposed to one another. Both monomers contribute residues to the active sites, and

hence monomers are unable to synthesize AdoMet. Access to the active site is controlled by a flexible loop that contains a cysteine residue in α1 [5,13]. The small interface between dimers in the tetramer involves the central domain of the α-subunits, fewer interactions contributing to dimer association in mammalian tetramers than in their *E. coli* counterpart [16].

The regulatory β-subunit shows no structural relationship with α-subunits and has been classified as a member of the oxidoreductase PFAM 04321 family. As such, the β-subunit contains a FAD^+/$NADP^+$ Rossman fold, where $NADP^+$ has been crystallized. Binding of the cofactor induces ordering into three regions (A-C) of the subunit, two of them (A and B) directly involved in $NADP^+$ binding [6]. The MAT II hetero-oligomer interface is proposed to involve regions A and C of the β-subunit, together with the central domain of α2-subunits [6].

3. METHIONINE ADENOSYLTRANSFERASES IN PATHOLOGY

Most studies on the pathophysiological role of MAT isoenzymes have been carried out in liver (recently reviewed in [17]), where ~50% of the ingested methionine is metabolized [18]. However, alterations in expression of MAT isoenzymes and AdoMet levels have been reported in a variety of pathologies that include hepatocellular carcinoma (HCC), diabetes, and Alzheimer's or Wilson's disease [2,3,19]. HCC studies showed changes in promoter methylation of *MAT1A* (hypermethylated) and *MAT2A* (hypomethylated), leading to an expression switch towards reduced MAT1A and increased MAT2A mRNA levels, that is followed by enhanced *MAT2B* expression [17,20]. This expression switch correlates with a reduction in hepatic AdoMet levels, as expected from the exchange from high V_{max} isoenzymes to low V_{max} MAT II [2,3,17,21](Fig. 1). The link between HCC and AdoMet levels has been confirmed

using two knockout mouse models that exhibited either low (*MAT1A* KO) or high (glycine N-methyltransferase, *GNMT* KO) hepatic AdoMet concentrations and that spontaneously developed HCC [22,23]. This relationship has been strengthened by knocking down microRNAs (miR-664, miR-485-3p, miR-495) that are highly expressed in HCC and reduce *MAT1A* expression [24]. A large amount of work has been devoted to elucidating the signaling cascades relating changes in AdoMet synthesis and disease (reviewed in [17]). Among them are those involving NFκB and AP-1, for which *MAT2A* and *MAT2B* promoters include binding sites and that also induce iNOS expression, thus enhancing nitric oxide (NO) production leading to MAT I/III inhibition (see below).

In contrast, most human *MAT1A* mutations are asymptomatic, and only in a few individuals demyelination has been reported [25]. These neurological symptoms were described for patients with alterations in the C-terminal end of the α1-subunit, but only in one case was exogenous AdoMet used to obtain an improvement in myelination [25,26]. Importantly, a mutant form with an alteration at the C-terminal end, analogous to those causing human demyelination, showed nuclear accumulation in living cells [7]. The process by which demyelination occurs is unknown, although an anomalous α1/α2 oligomerization was postulated to reduce AdoMet synthesis [27]. The availability of the structures for MAT subunits, data showing expression of *MAT1A* in extrahepatic tissues, and the demonstration that MATα1 localizes mainly to the nucleus in most cell types except hepatocytes, allowed reexamination of this hetero-oligomerization hypothesis in 2011 [28].

4. METHIONINE ADENOSYLTRANSFERASES AND REDOX STRESS

Several disease models, including treatments with carbon tetrachloride [29] or lipopolysaccharide [30], buthionine sulfoximine intoxication [31], and Long Evans Cinnamon rats (LEC; Wilson's disease model)[19], have shown a relationship between redox stress and MAT function. In these models reductions in glutathione synthesis, alterations in expression of MAT genes, lower levels of MATα1 and/or inhibition of MAT I/III activity were observed. Results derived from these studies, together with *in vitro* experiments carried out in parallel, demonstrated MAT I/III inhibition by GSSG and modulation of this activity by the GSH/GSSG ratio [32,33]. Redox stress also altered the association state of MAT I/III, leading to either the accumulation of inactive dimers or even monomers [32,34]. Moreover, C121, the cysteine located at the active site loop of MAT I/III, was modified by NO and hydroxyl radicals in some of these *in vivo* models, leading to enzyme inactivation [35-37]. Therefore C121 nitrosylation was proposed to force the loop into a position that blocks substrate admission into the catalytic site, thus precluding AdoMet synthesis.

The key role of cysteine residues in activity and oligomerization of MAT I/III was also demonstrated using chemical modification or mutagenesis [38-40]. The C69S mutant accumulated as α1-dimers and elimination of cysteines between positions 35-105 altered MAT I/III ratios [40]. However, none of these individual mutations fully inactivates MAT I/III [16,40]. Additionally, *in vitro* production of the C35-C61 intrasubunit disulfide bond blocked dimer-tetramer interconversion, without affecting activity [16]. This disulfide in the central domain was a normal feature of the purified hepatic α1-oligomers [41], and the conformational changes it induced were observed as subtle changes in SDS-PAGE mobility of MATα1 upon thiopropyl Sepharose purification [32].

Hepatic copper accumulation in LEC rats induces redox stress, the *MAT1A* to *MAT2A* expression switch, inhibition of MAT I/III activity, and decreased AdoMet levels [19]. In contrast, *MAT2B* expression is almost suppressed. The consequence is that MAT II oligomers in these livers must be mainly composed by α2-subunits with higher $S_{0.5}^{Met}$ than MAT II hetero-oligomers [2]. Moreover, copper altered folding of α1 and α2-subunits in the test tube, suggesting a potential folding problem *in vivo*. The lack of cysteines equivalent to C121 and C61 in the MATα2 sequence precludes inhibition by NO or intrasubunit disulfide production [5,15], and no reports have shown whether MATα2 activity can be modulated by GSH/GSSG ratios. Therefore, a redox regulatory mechanism similar to that described for MAT I/III seemed unlikely for MAT II. Our recent report demonstrating increased affinity of $NADP^+$-bound β-subunit for α2-dimers [6], suggests that high levels of this cofactor (*e.g.*, during drug detoxification) would favor production of MAT II. These data open the possibility that redox regulation is exerted on different subunits and with opposite results for AdoMet production in each type of MAT isoenzyme.

5. PROPOSED HYPOTHESIS

All the data summarized in the previous sections suggest a redox regulatory mechanism controlling mammalian MAT isoenzymes in a concerted way (Fig. 2). This redox mechanism directs AdoMet production at several levels including MAT expression, activity, oligomerization, and potentially subcellular localization. Only some of the models studied to date showed MAT expression changes induced by oxidative stress (reviewed in [2,17]). Lack of consensus sequences for metal (MREs) or antioxidant (AREs) response elements in the corresponding promoters precludes direct effects through binding of transcription factors to such elements, as previously

discussed [19]. Other options include promoter binding of redox stress-regulated transcription factors (*e.g.*, NFκB in *MAT2B* and *MAT2A*)[2,42], changes in promoter methylation status directly or indirectly involving redox regulation of demethylases or methyltransferases [43,44], or changes in other epigenetic modifications controlled by redox stress (*e.g.*, sirtuin binding of NAD^+) (Fig. 2A). Additionally, MAT1A and MAT2A mRNAs can be regulated by 3'-UTR binding of AUF1 or meHuR/HuR, respectively [45], an AdoMet-dependent process that could be modulated by redox mechanisms.

Under diverse redox conditions distinct subunit associations can be produced that may differ among subcellular compartments. Thus, fully reduced recombinant subunits appear in a tetramer/dimer concentration-dependent equilibrium [46,47] (Fig. 2B; step 1). However, in liver cytosol MATα1 homo-oligomers are stable, a fact ascribed to the presence of the C35-C61 intrasubunit disulfide (step 2)[16,41,46]. Under redox stress the GSH/GSSG ratio is reduced (<50) and different oxidation degrees could be produced favoring dissociation into dimers (step 4; observed in cirrhotic livers) or even into inactive α1-monomers (step 5)[32]. Moreover, *in vivo* C121 nitrosylation can be the initial oxidation event, followed by the GSSG effects (steps 4 and 5). Altogether these changes result in reduction of cytosolic MAT I/III activity during redox stress, and hence of AdoMet levels available for methylation.

Confocal microscopy demonstrated the presence of nuclear MATα1-EGFP in living cells [7], but the mechanisms that govern its subcellular localization are unknown. Among the possibilities, protein-protein interactions may induce direct or indirect binding of the homo-oligomer to importins or exportins (step 6), a step that might be favored by the presence of post-translational modifications on MATα1. Redox stress is a putative mechanism controlling subcellular localization, but there is no

available information concerning the redox state of the nuclear MATα1 forms. Therefore, our proposal includes transport of fully reduced or partially oxidized MATα1 oligomers through the nuclear pore (step 6), and oxidation/reduction of these species if nuclear GSH/GSSG ratios change (step 7). Given that MAT activity is detected in nuclear fractions [7], nuclear MAT I species must be active to synthesize AdoMet, and nuclear monomers may arise from tetramer dissociation (step 8). Again, the reduced/oxidized character of these monomers is unknown, but they may associate into MAT I when higher nuclear levels of AdoMet are needed for epigenetic remodeling. Truncation of the C-terminal end of MATα1 induced nuclear accumulation [7]; hence, modifications in residues in this area of the molecule, such as T342 phosphorylation [48] or others that have been detected by proteomic approaches (PhosphoSite Plus), may play a role in subcellular localization.

Several cell culture experiments and MATα2 and β purification from mammalian tissues demonstrated the existence of MAT II hetero-oligomers (Fig. 2C)[6]. In bacterial cytosol, a reduced environment, both recombinant subunits appear in equilibrium-dependent associations, dimers and tetramers for α2 (step 1) and monomers and dimers for β-subunits (step 9)[6]. *In vitro*, β-monomers can bind $NADP^+$ (step 10), but both free- and $NADP^+$-bound subunits associate into stable MAT II hetero-trimers (steps 11 and 12). NO production, drug detoxification and GSSG reduction enhance $NADP^+$ levels (Fig. 1). Under these conditions the affinity of $NADP^+$-β for α2-dimers is increased (steps 10 and 12), and MAT II association (with the lower V_{max}) is favored leading to reduced AdoMet levels in the cytosol. However, not all the splicing forms of the β-subunit can bind $NADP^+$, thus providing selectivity to this regulation mechanism [6,42].

Indirect immunofluorescence detected nuclear MAT II, where α2-subunits interact with the MafK transcription factor, acting as corepressors [10]. The mechanisms of nucleocytoplasmic transport of MAT II remain also unknown, although again protein-protein interactions or post-translational modifications might be involved (step 13). These putative modifications may result from oxidative stress, a point that deserves investigation. Moreover, $NADP^+$ binding may favor one or another subcellular localization (step 13), the cofactor being lost once the adequate compartment is reached (step 14). Additional modifications in α2 and β-subunits that may have a role in localization include those identified by mass spectrometry (PhosphoSite Plus) [49,50,51]. Despite the similarity among C-terminal ends of MATα1 and MATα2, the small differences detected may allow differential regulation of their subcellular localization [7].

All these association changes render species differing in their V_{max}, and hence in AdoMet production. When liver function is normal the high V_{max} MAT isoenzymes are prevalent, synthesizing enough AdoMet to meet cell requirements. However, when hepatic function is challenged, the defense or drug detoxification mechanisms induce production of NO, GSSG and/or increased $NADP^+$ levels (Fig. 1). All these metabolic changes led to inhibition of the normal hepatic isoenzymes (MAT I/III) and increased levels of MAT II, the net result being a reduction of the AdoMet levels available for methylation reactions (*e.g.*, DNA hypomethylation). These concerted actions guarantee AdoMet synthesis in the cytosol under a minimum supply of methionine, but whether the same effects can be extrapolated to nuclear behavior should be explored.

In summary, redox stress data suggest a fundamental role for this mechanism in AdoMet homeostasis. Negative effects on MAT I/III would be exerted at different levels from expression to activity of the α1-subunit, whereas compensatory/positive

effects on MAT II would occur through the regulatory β-subunit. The putative role of these mechanisms in governing subcellular localization of MATs and the relationship between changes in localization and pathology remain unexplored.

ACKNOWLEDGMENTS

The authors wish to thank N. Mantei for his suggestions. This work was supported by grants of the Ministerio de Economía y Competitividad (BFU2008-00666 and BFU2009-08977 to M.A.P., and SAF2009-11642 and SAF2012-36519 to D.P.S.) and Instituto de Salud Carlos III (RETICS RD12/0013/0008 to D.P.S. and FIS PI12/01196 to L.A.).

REFERENCES

[1] Cantoni GL. (1975) Biological methylation: selected aspects. Annu Rev Biochem 44:435-451.
[2] Pajares MA, Markham GD. (2011) Methionine adenosyltransferase (S-adenosylmethionine synthetase). Adv Enzymol Relat Areas Mol Biol 78:449-452.
[3] Mato JM, et al. (1997) S-adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol Ther 73:265-280.
[4] Cantoni GL. (1951). Activation of methionine for transmethylation. J Biol Chem 189:745-754.
[5] Markham GD, Pajares MA. (2009) Structure-function relationships in methionine adenosyltransferases. Cell Mol Life Sci 66:636-648.
[6] Gonzalez B, et al. (2012) NADP(+) Binding to the Regulatory Subunit of Methionine Adenosyltransferase II Increases Intersubunit Binding Affinity in the Hetero-Trimer. PLoS One 7:e50329.
[7] Reytor E, et al. (2009) Conformational signals in the C-terminal domain of methionine adenosyltransferase I/III determine its nucleocytoplasmic distribution. FASEB J 23:3347-3360.
[8] Chen NC, et al. (2010) Regulation of homocysteine metabolism and methylation in human and mouse tissues. FASEB J 24:2804-2817.
[9] Pajares MA, et al. (1992). How is rat liver S-adenosylmethionine synthetase regulated? FEBS Lett 309:1-4.
[10] Katoh Y, et al. (2011) Methionine Adenosyltransferase II Serves as a Transcriptional Corepressor of Maf Oncoprotein. Mol Cell 41:554-566.

[11] Xia M, et al. (2010) Novel function and intracellular localization of methionine adenosyltransferase 2{beta} splicing variants. J Biol Chem 285:20015-20021.
[12] Gibson BA, Kraus WL. (2011) Small molecules, big effects: a role for chromatin-localized metabolite biosynthesis in gene regulation. Mol Cell 41, 497-499.
[13] Gonzalez B, et al. (2000) The crystal structure of tetrameric methionine adenosyltransferase from rat liver reveals the methionine-binding site. J Mol Biol 300:363-375.
[14] Gonzalez B, et al. (2003) Crystal structures of methionine adenosyltransferase complexed with substrates and products reveal the methionine-ATP recognition and give insights into the catalytic mechanism. J Mol Biol 331:407-416.
[15] Sanchez-Perez GF, et al. (2004) Methionine adenosyltransferase as a useful molecular systematics tool revealed by phylogenetic and structural analyses. J Mol Biol 335:693-706.
[16] Sanchez-Perez GF, et al. (2003) Role of an intrasubunit disulfide in the association state of the cytosolic homo-oligomer methionine adenosyltransferase. J Biol Chem 278:7285-7293.
[17] Lu SC, Mato JM. (2012) S-adenosylmethionine in liver health, injury, and cancer. Physiol Rev 92:1515-1542.
[18] Zeisel SH, Wurtman RJ. (1979) Dietary intake of methionine: Influence of brain S-adenosylmethionine. In Transmethylation (Usdin E, Borchardt RT, Creveling CR, eds), pp. 59-68. Elsevier, New York.
[19] Delgado M, et al. (2008) Early effects of copper accumulation on methionine metabolism. Cell Mol Life Sci 65:2080-2090.
[20] Torres L, et al. (2000) Liver-specific methionine adenosyltransferase MAT1A gene expression is associated with a specific pattern of promoter methylation and histone acetylation: implications for MAT1A silencing during transformation. FASEB J 14:95-102.
[21] Cai J, et al. (1996) Changes in S-adenosylmethionine synthetase in human liver cancer: molecular characterization and significance. Hepatology 24:1090-1097.
[22] Lu SC, et al. (2001) Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci 98:5560-5565.
[23] Martinez-Chantar ML, et al. (2008) Loss of the glycine N-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology 47:1191-1199.
[24] Yang H, et al. (2013) MicroRNAs regulate methionine adenosyltransferase 1A expression in hepatocellular carcinoma. J Clin Invest 123:285-298.
[25] Mudd SH. (2011) Hypermethioninemias of genetic and non-genetic origin: A review. Am J Med Genet C Semin Med Genet 157, 3-32.
[26] Chamberlin ME, et al. (2000) Methionine adenosyltransferase I/III deficiency: novel mutations and clinical variations. Am J Hum Genet 66:347-355.
[27] Hazelwood S, et al. (1998) Normal brain myelination in a patient homozygous for a mutation that encodes a severely truncated methionine adenosyltransferase I/III. Am J Med Genet 75:395-400.
[28] Pajares MA, Pérez C. (2011) The relationship between human MAT1A mutations and disease: a folding and association problem? In Protein Folding (Walters EC, ed), pp. 261-287. Nova Science Publishers, New York.

[29] Corrales F, et al. (1992) S-adenosylmethionine treatment prevents carbon tetrachloride-induced S-adenosylmethionine synthetase inactivation and attenuates liver injury. Hepatology 16:1022-1027.
[30] Avila MA, et al. (1997) Regulation of rat liver S-adenosylmethionine synthetase during septic shock: role of nitric oxide. Hepatology 25:391-396.
[31] Corrales F, et al. (1991) Inhibition of glutathione synthesis in the liver leads to S-adenosyl-L-methionine synthetase reduction. Hepatology 14:528-533.
[32] Pajares MA, et al. (1992) Modulation of rat liver S-adenosylmethionine synthetase activity by glutathione. J Biol Chem 267:17598-17605.
[33] Martinez-Chantar ML, Pajares MA. (1996) Role of thioltransferases on the modulation of rat liver S-adenosylmethionine synthetase activity by glutathione. FEBS Lett 397:293-297.
[34] Cabrero C, et al. (1988) Specific loss of the high-molecular-weight form of S-adenosyl-L-methionine synthetase in human liver cirrhosis. Hepatology 8:1530-1534.
[35] Avila MA, et al. (1998) Specific interaction of methionine adenosyltransferase with free radicals. Biofactors 8:27-32.
[36] Sanchez-Gongora E, et al. (1997) Interaction of liver methionine adenosyltransferase with hydroxyl radical. FASEB J 11:1013-1019.
[37] Ruiz F, et al. (1998) Nitric oxide inactivates rat hepatic methionine adenosyltransferase In vivo by S-nitrosylation. Hepatology 28:1051-1057.
[38] Pajares MA, et al. (1991) The role of cysteine-150 in the structure and activity of rat liver S-adenosyl-L-methionine synthetase. Biochem J 274:225-229.
[39] Corrales F, et al. (1990) Inactivation and dissociation of S-adenosylmethionine synthetase by modification of sulfhydryl groups and its possible occurrence in cirrhosis. Hepatology 11:216-222.
[40] Mingorance J, et al. (1996) Site-directed mutagenesis of rat liver S-adenosylmethionine synthetase. Identification of a cysteine residue critical for the oligomeric state. Biochem J 315:761-766.
[41] Martinez-Chantar ML, Pajares MA (2000) Assignment of a single disulfide bridge in rat liver methionine adenosyltransferase. Eur J Biochem 267:132-137.
[42] Yang H, et al. (2008) Expression pattern, regulation, and functions of methionine adenosyltransferase 2beta splicing variants in hepatoma cells. Gastroenterology 134:281-291.
[43] Chen CC, et al. (2012) The mammalian de novo DNA methyltransferases DNMT3A and DNMT3B are also DNA 5-hydroxymethylcytosine dehydroxymethylases. J Biol Chem 287:33116-33121.
[44] Zawia NH, et al. (2009) Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 46:1241-1249.
[45] Vazquez-Chantada M, et al. (2010) HuR/Methyl-HuR and AU-Rich RNA Binding Factor 1 Regulate the Methionine Adenosyltransferase Expressed During Liver Proliferation, Differentiation, and Carcinogenesis. Gastroenterology 138:1943-1953.
[46] Mingorance J, et al. (1997) Recombinant rat liver S-adenosyl-L-methionine synthetase tetramers and dimers are in equilibrium. Int J Biochem Cell Biol 29:485-491.
[47] Lopez-Vara MC, et al. (2000) Refolding and characterization of rat liver methionine adenosyltransferase from Escherichia coli inclusion bodies. Protein Expr Purif 19:219-226.

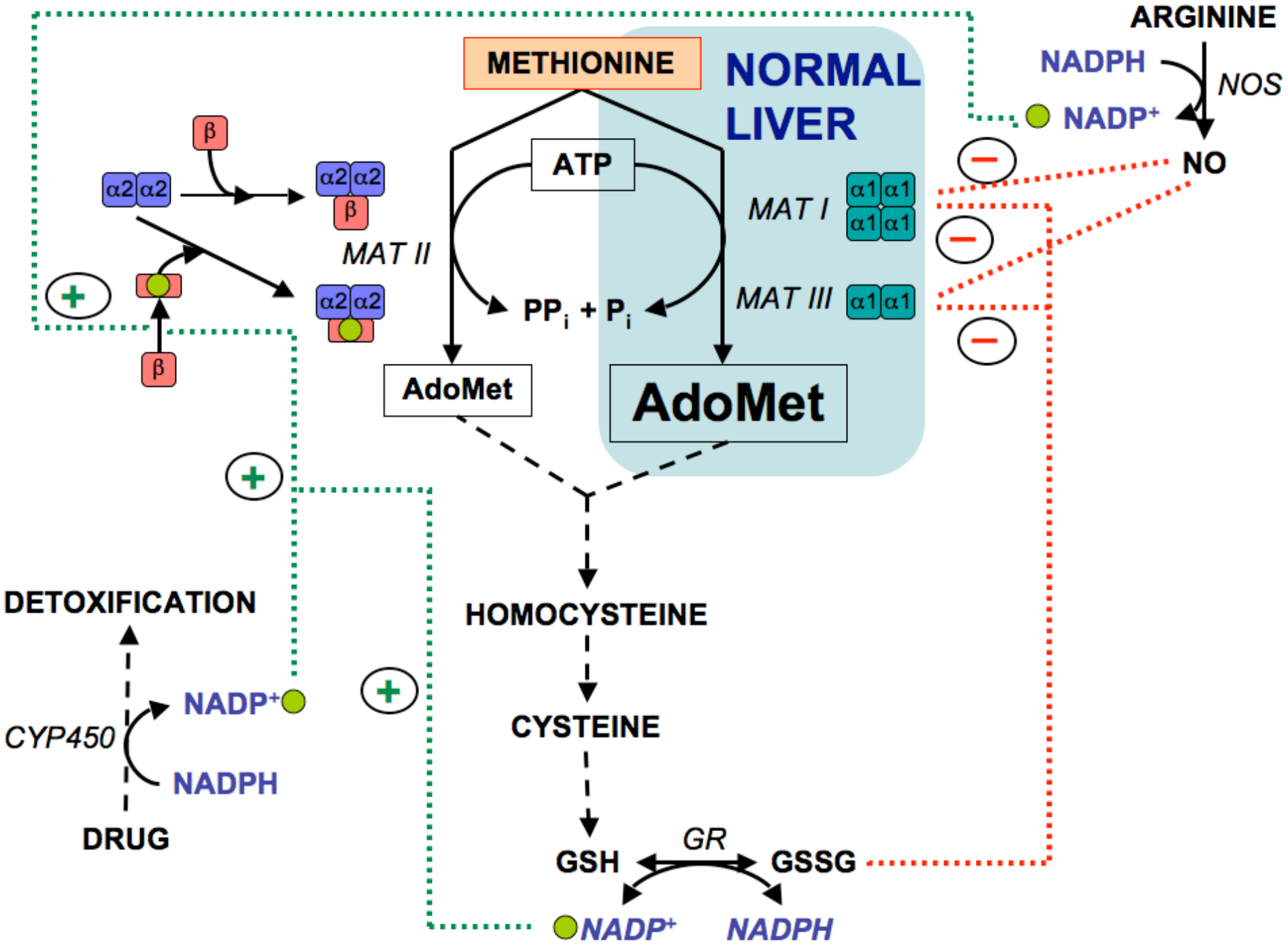
[48] Pajares MA, et al. (1994) Protein kinase C phosphorylation of rat liver S-adenosylmethionine synthetase: dissociation and production of an active monomer. Biochem J 303:949-955.
[49] Choudhary C, et al. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325:834-840.
[50] Kim W, et al. (2011) Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol Cell 44:325-340.
[51] Moritz A, et al. (2010) Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. Sci Signal 3:ra64.

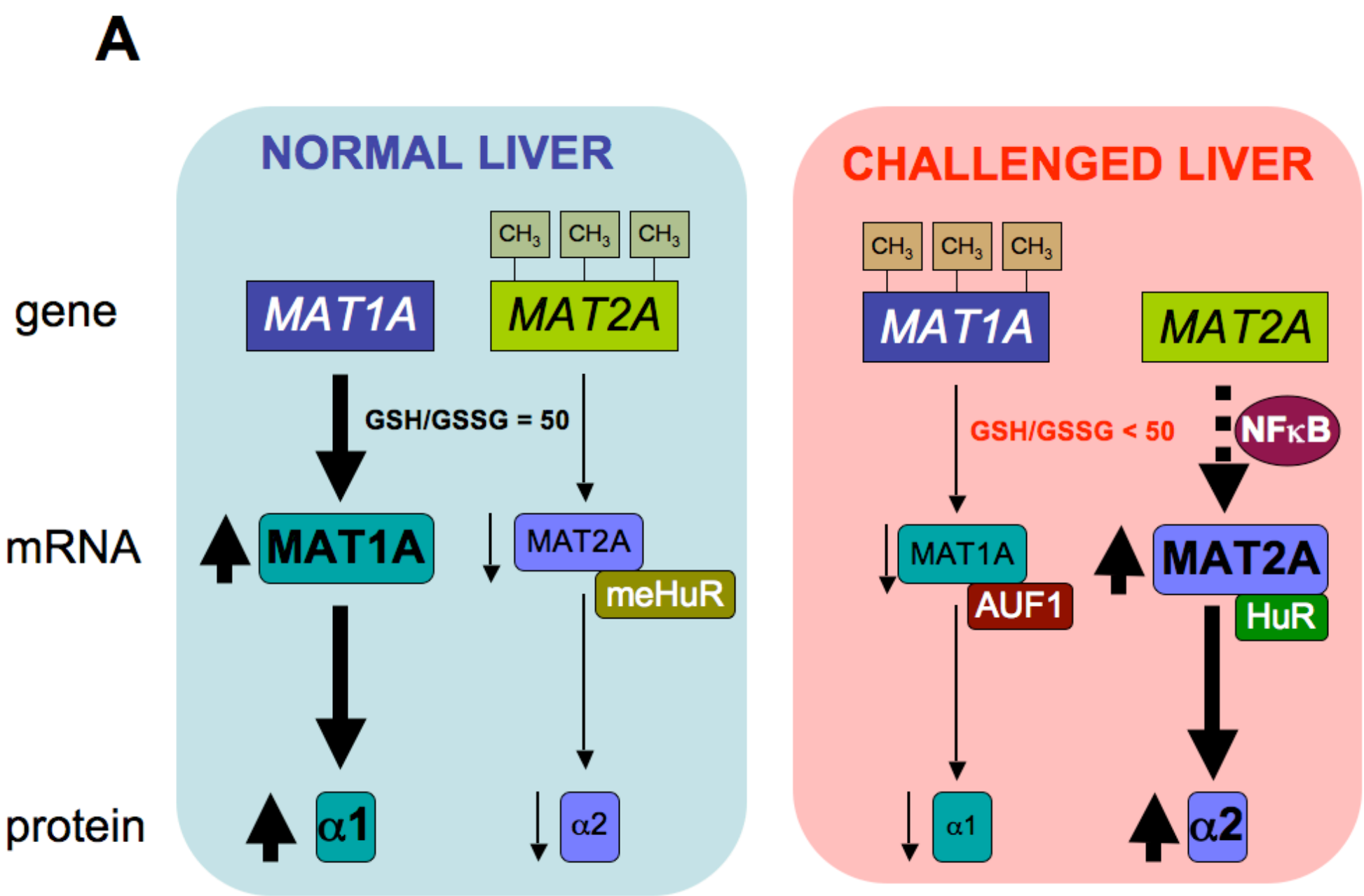
FIGURE LEGENDS

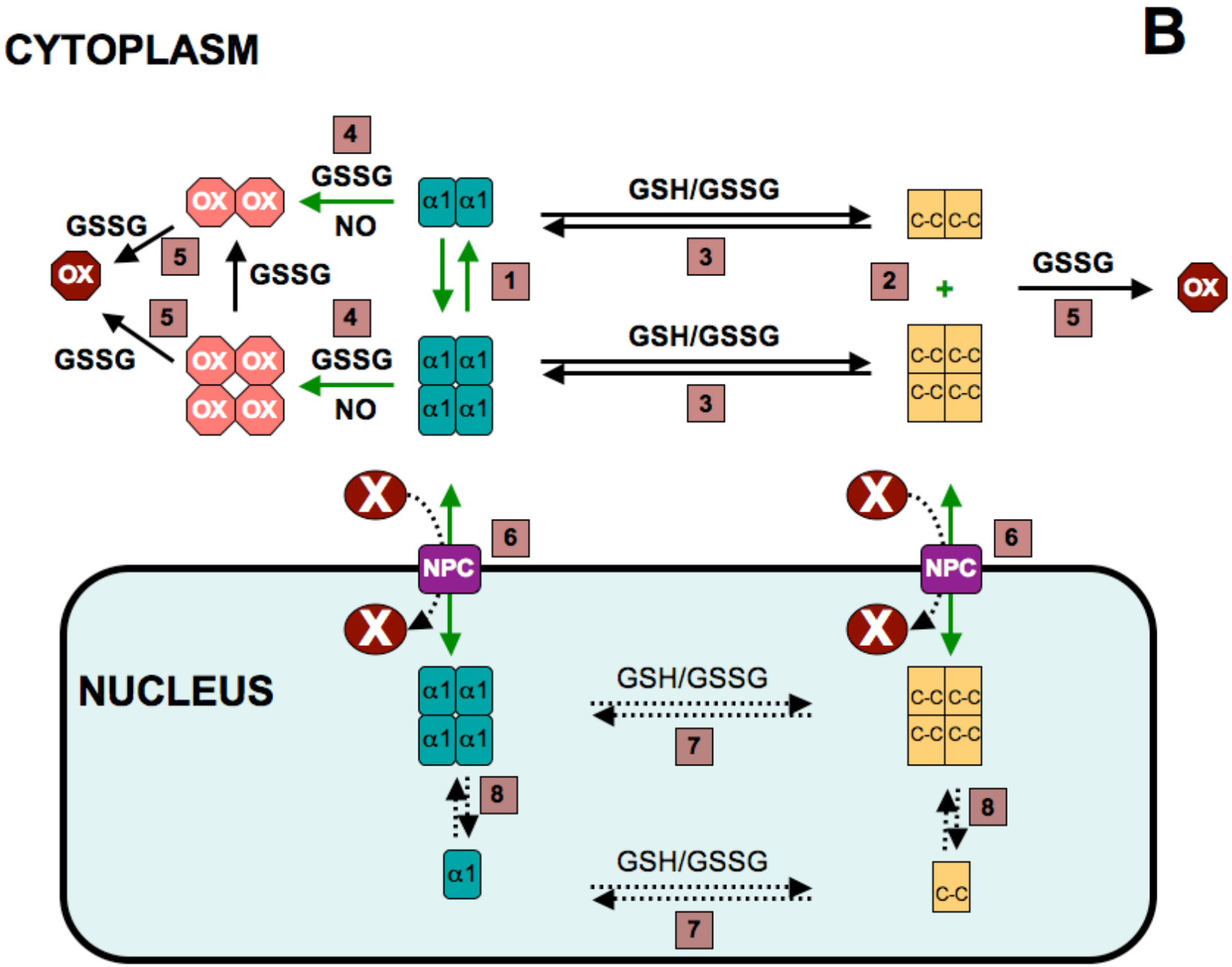
Figure 1. Metabolic links between redox stress and AdoMet synthesis. A schematic representation of the reactions linking AdoMet and glutathione synthesis (black dashed lines) is shown, together with actions of glutathione, nitric oxide (NO) and $NADP^+$ (green circles) levels on methionine adenosyltranferases. MATα1 subunits (aquamarine) appear as homotetramers (MAT I) and homodimers (MAT III) that are inhibited by GSSG or NO (red dashed lines). MATα2 subunits (blue) appear as homodimers or heterotrimers (MAT II) with the β-subunit (red). Reactions involved in $NADP^+$ synthesis and the increased β/α2-dimer affinity produced by cofactor binding (green dashed lines) are shown.

Figure 2. Scheme of redox regulation. Experimental changes affecting MATs are shown (thick arrows), together with putative additional points of redox-regulation (dotted arrows). Effects on transcription (A), including promoter methylation and putative binding of redox-regulated transcription factors, are depicted together with factors affecting mRNA stability. Thick lines denote results concerning MAT I/III (B) or MAT II (C) proteins in animal models and living cells (green) or *in vitro* (black), and

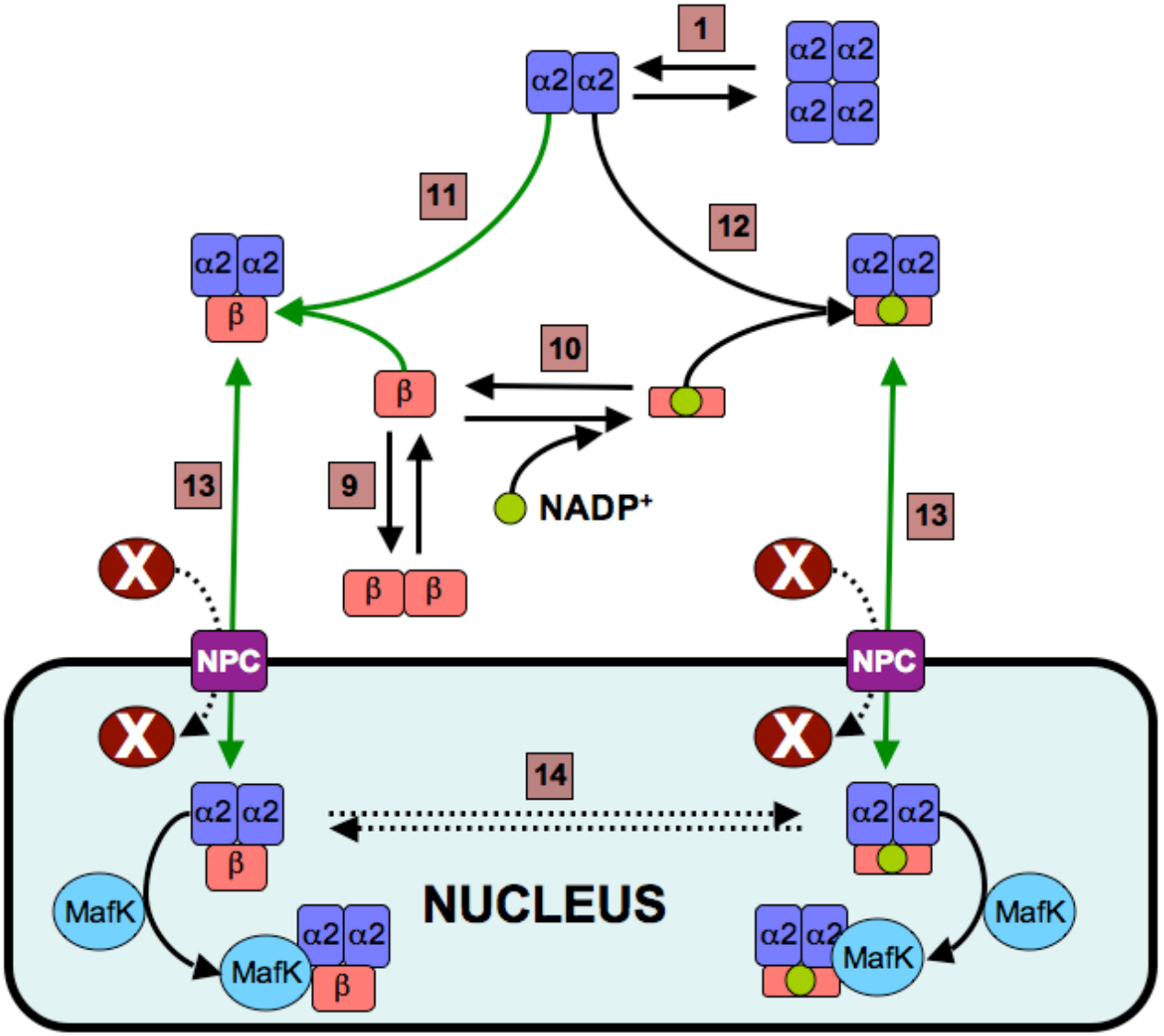
putative reactions appear as dotted lines. Fully reduced MATα1 (aquamarine) and MATα2 (blue), appear in a concentration-dependent equilibrium between tetramers and dimers (1). Partially oxidized MATα1 (orange), containing the C35-C61 intrasubunit disulfide (C-C), associate into stable oligomers (2-3). NO and GSSG inhibition (4) and full oxidation and dissociation (5) are also depicted. Putative redox-regulated steps of MAT transport through the nuclear pore complex (NPC) and nuclear function are indicated, considering also putative interactions with unidentified X-proteins (6-8). Free β-subunits (red) appear as monomers and dimers (9) to which $NADP^+$ (green circles) binds (10), and which can associate with α2-dimers into heterotrimers (11-12). Putative redox-regulated steps for MAT II transport and function in the nucleus, including association with MafK transcription factor, are indicated (13-14).


METHIONINE
NORMAL LIVER
ATP
PPi + Pi
AdoMet
AdoMet
MAT I
MAT III
MAT II
α2 α2
β
α1 α1
ARGININE
NADPH
NOS
NADP+
NO
HOMOCYSTEINE
CYSTEINE
GSH
GR
GSSG
NADP+
NADPH
DETOXIFICATION
CYP450
NADP+
NADPH
DRUG


A
NORMAL LIVER
CHALLENGED LIVER
gene
mRNA
protein
CH3
CH3
CH3
MAT1A
MAT2A
GSH/GSSG = 50
GSH/GSSG < 50
NFκB
MAT1A
MAT2A
meHuR
AUF1
HuR
α1
α2


CYTOPLASM
B
4
GSSG
NO
OX OX
GSSG
5
OX
GSSG
α1 α1
1
GSH/GSSG
3
C-C C-C
2
+
GSSG
5
OX
5
GSSG
4
GSSG
NO
OX OX
OX OX
α1 α1
α1 α1
GSH/GSSG
3
C-C C-C
C-C C-C
X
6
NPC
X
NUCLEUS
α1 α1
α1 α1
GSH/GSSG
7
8
α1
GSH/GSSG
7
X
6
NPC
X
C-C C-C
C-C C-C
8
C-C

C


CYTOPLASM
1
α2 α2
α2 α2
α2 α2
11
12
α2 α2
β
α2 α2
10
β
13
9
NADP+
13
β β
NPC
NPC
14
α2 α2
β
α2 α2
β
MafK
NUCLEUS
α2 α2
β
MafK
α2 α2
MafK