HIGHLIGHTS

- Protein interactions allow functional regulation of homocysteine metabolism
- Homocysteine metabolism establishes pathway interplays through protein interactions
- Intermolecular interactions within homocysteine metabolism may support substrate channeling
- Homocysteine metabolism interaction networks are altered in oncogenesis
- Proteins of homocysteine metabolism interact with oncogenes for gene regulation

PROTEIN-PROTEIN INTERACTIONS INVOLVING ENZYMES OF THE MAMMALIAN **METHIONINE AND** HOMOCYSTEINE METABOLISM

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ABBREVIATIONS: AdoMet, S-adenosylmethionine; AdoHcy, S-
adenosylhomocysteine; AHCY, S-adenosylhomocysteine hydrolase; AP, affinity

purification; BHMT and BHMT2, betaine homocysteine S-methyltransferases 1 and 2; CBS, cystathionine β -synthase; CTH, cystathionine γ -lyase; GSH and GSSG, glutathione reduced and oxidized forms; Hcy, homocysteine; MAT, methionine adenosyltransferase; MS, mass spectrometry; MTR, methionine synthase; NNMT, nicotinamide N-methyltransferase; PDRG1, p53 and DNA damage-regulated gene 1.

ABSTRACT

Enzymes of the methionine and homocysteine metabolism catalyze reactions belonging to the methionine and folate cycles and the transsulfuration pathway. The importance of the metabolites produced through these routes (e.g. S-adenosylmethionine, homocysteine) and their role in e.g. epigenetics or redox mechanisms makes their tight regulation essential for a correct cellular function. Pharmacological or pathophysiological insults induce, among others, changes in activity, oligomerization, protein levels, subcellular localization and expression of these enzymes. The abundance of these proteins in liver has made this organ the preferred system to study their regulation. Nevertheless, knowledge about their putative protein-protein interactions is limited in this and other tissues and cell types. High-throughput methods, including immunoprecipitation, affinity purification coupled to mass spectrometry and yeast two-hybrid have rendered the identification of a number of protein-protein interactions involving these enzymes in several systems. Validation by coimmunoprecipitation and/or pull-down has been made, mainly, after coexpression of bait and prey, but few of the interactions have been confirmed. Additionally, information concerning the role of these interactions in the regulation of this pathway and other cellular processes is scarce. Here, we review the current knowledge on mammalian protein-protein interactions involving methionine adenosyltransferases, S-adenosylhomocysteine hydrolase, betaine

homocysteine S-methyltransferases, methionine synthase and cystathionine β -synthase, although references to data obtained in other organisms are also made. Moreover, the verified or putative implication of these interactions in the regulation of **methionine and homocysteine metabolism**, its interplay with other metabolic pathways and its putative link to pathophysiological processes, such as oncogenesis, is discussed.

KEYWORDS

Methionine cycle, S-adenosylmethionine synthesis, oncogene interactions, posttranslational modifications, metabolic interplay, redox regulation.

1. INTRODUCTION

Protein-protein interactions are one of the mechanisms by which the function of a protein is exerted or regulated [1]. They can be transient or stable, and their strength varies within a wide range. Moreover, stable interactions can be established between identical subunits, leading to homo-oligomers, or with additional proteins rendering hetero-oligomers or large macromolecular complexes. Transient interactions can take place during e.g. posttranslational modification (PTM) of proteins, and the presence or absence of a particular PTM may either favor the interaction with other protein partners to form an oligomer or a larger complex, preclude further associations, or act as a signal for this subunit to dissociate or for its degradation/stabilization [2]. Association at any of these levels (monomer, oligomers and macromolecular complexes) may offer the opportunity for a single protein to participate in different cellular processes in which its function may include moonlighting activities [3]. Additionally, the subcellular localization of a protein can be determined by its **oligomerization state (e.g. monomers vs homo-tetramers)** and the presence of alternative interaction partners (**hetero-oligomerization**), among other

possibilities. Regulation of methionine and homocysteine (Hcy) metabolism includes most of the aforementioned cases as will be explained in the next sections of this review.

Enzymes of methionine and Hcy metabolism are at the crossroad of three key metabolic pathways: the methionine and folate cycles and transsulfuration (Fig. 1)[4-7]. Most of these proteins require oligomerization to attain activity and their pathophysiological regulation has been shown to take place through the control of gene expression, their association state or their subcellular localization [6, 8]. Hcy synthesis occurs in the methionine cycle using the essential amino acid methionine as the input substrate for the pathway. Methionine, which is acquired in the diet, is first converted by methionine adenosyltransferase (MAT¹) isoenzymes into S-adenosylmethionine (AdoMet) that is used, in turn, by methyltransferases (MTases) to render a large variety of methylated substrates (X-CH₃) and S-adenosylhomocysteine (AdoHcy)[9-11]. MAT subunits are codified by three genes, *MAT1A* and *MAT2A* that encode the catalytic subunits MAT α 1 and MAT α 2, respectively, and *MAT2B* that codifies for the regulatory MAT β subunit [9, 10]. *MAT1A* exhibits preferential hepatic expression², whereas that of *MAT2A* and *MAT2B* is higher in many other mammalian tissues, in fetal liver and in hepatic diseases [6, 12].

Methylation reactions serve to connect the methionine cycle with several pathways that include, among others, phosphatidylcholine and neurotransmitter synthesis and epigenetic regulation [11, 13](Fig. 1). AdoHcy is a potent inhibitor of many MTases, and hence its levels are controlled by hydrolysis into Hcy and adenosine, a reversible reaction catalyzed by AdoHcy hydrolase, also named adenosylhomocysteinase

¹ Throughout the whole text, italic capital letters will be used for the human genes, whereas capital letters will denote the proteins, unless the original report used a different format.

² Expression patterns have been summarized from data of the Human Protein Atlas and the Expression Atlas

(AHCY)[13, 14]. This protein is codified by the *AHCY* gene, which is ubiquitously expressed. Hcy catabolism occurs through the transsulfuration pathway (reverse transsulfuration in non-mammalian organisms), in which this sulfur amino acid is first conjugated with either serine or cysteine by cystathionine β -synthase (CBS) to render cystathionine and H_2O or H_2S , respectively [15](Fig. 1). The CBS protein, which has been detected mainly in liver and pancreas, is codified by the *CBS* gene, for which two splicing forms have been described [6]. Cystathionine γ -lyase (CTH), also named cystathionase and encoded by the *CTH* gene, then excises cystathionine into cysteine, α -ketobutyrate and H_3N [16]. Importantly, CTH is also able to produce the gasotransmitter H_2S using cysteine and Hcy as substrates [16]. The CTH protein has been also detected in a limited set of tissues, the liver exhibiting the highest levels. Cysteine synthesized through this route supplies nearly 50% of that required for hepatic glutathione synthesis [17, 18], the remaining being obtained from the diet.

Alternatively, Hcy can be remethylated back into methionine, if there is a need of the latter, whereas Hcy excess is exported to the plasma (Fig. 1). Hcy remethylation can take place through three reactions that use different methyl donors and enzymes: i) 5-methyltetrahydrofolate and methionine synthase (MTR), which catalyzes a reaction that serves as the crossroad with the folate cycle [8]; ii) betaine and betaine homocysteine S-methyltransferase (BHMT), which serves as the link with choline oxidation [5]; and iii) S-methylmethionine and BHMT2 [19]. These proteins are codified by the ubiquitously expressed *MTR* gene, and the *BHMT* and *BHMT2* genes, which have a preferential hepatic expression.

2. CHARACTERISTICS AND MAIN MECHANISMS REGULATING HOMOCYSTEINE METABOLISM ENZYMES

Most of the enzymes involved in this pathway are homo-tetramers, although there are some exceptions (Fig. 1)[8]. These include, monomeric MTR, the homo-dimer MAT III composed by catalytic MAT α 1 subunits, and the hetero-trimer MAT II that is made by a dimer of catalytic MAT α 2 subunits and one regulatory MAT β monomer. The size of most monomers is between 40-48 kDa (BHMT2 < MAT α subunits < CTH ~ BHMT < AHCY), although smaller (MAT β) and larger subunits (CBS and MTR) exist, being MTR with 140.5 kDa the largest [8]. As mentioned previously, the highest expression levels of all these proteins are detected in hepatocytes, where most of the ingested methionine is processed. Liver pathology, induced by e.g. galactosamine or acetaminophen intoxication or tumorigenesis (hepatoma), tends to reduce the expression of proteins involved in methionine and Hcy metabolism [20, 21]. Exceptions to this rule are MAT α 2 and MAT β , which increase their levels, leading to an expression switch in MAT isoenzymes [13], in turn decreasing AdoMet production.

Oligomerization is required for activity, except for the MTR monomer [6, 8] (Fig. 1). Changes in the number of subunits involved may lead to differences in activity and substrate affinity, as occurs for homo-dimers (MAT III isoenzyme) and homo-tetramers (MAT I isoenzyme) of the MAT α 1 catalytic subunit [9, 10](Fig. 1). Nevertheless, additional differences in AdoMet production by MATs are also due to the existence of two catalytic subunits, MAT α 1 and MAT α 2. The sigmoidal kinetic behavior of MATs suggests cooperativity and the $S_{0.5}^{\text{Met}}$ (equivalent to the K_m of hyperbolic kinetics) of the three isoenzymes is in the low micromolar range for the hetero-trimer MAT II ($\alpha_2\beta$), close to 100 μM for MAT α 2 homo-oligomers (MAT α_2 or MAT α_4) and MAT I (MAT α_4), and in the low millimolar range for MAT III (MAT α_2) [10]. In contrast, the V_{max} of these isoenzymes follows the opposite pattern. Cellular methionine concentrations are 50-80 μM , which are enough to allow most MAT oligomers to work

at 50-100% of their $V_{\max}$; the exception would be MAT III, which may reach a 10% of its $V_{\max}$. Therefore, the possibility exists that MAT III acts as reservoir of dimers for the rapid generation of MAT I as required. Differences in MAT II versus MAT α 2 homo-oligomer kinetics are induced by association with the regulatory MAT β subunit [10, 22, 23], which is a non-related protein of the oxidoreductase family (PFAM 04321)(Fig. 1). Binding of NADP⁺ to MAT β increases its affinity for MAT α 2 homo-dimers [24], thus enhancing the effects on kinetics for AdoMet synthesis. MAT II is, therefore, the first example of the influence of protein-protein interactions in the behavior of enzymes of this metabolic pathway, as will be explained below.

The existence of splicing forms for proteins in the methionine and Hcy metabolism has been reported for most of the enzymes, except for MAT α 1 [8, 25]. Their presence may influence the type of oligomers formed, and hence the activity, regulation and interactions of the enzyme involved. A good example of this impact is provided by MAT II and the four MAT β splicing forms described in hepatoma cells [25]. Elimination of diverse sequence stretches in each of these splicing forms may result in structural changes that affect directly or indirectly the NADP⁺ site or cofactor binding. In turn, the affinity of each MAT β splicing form for MAT α 2 homo-dimers can be different, leading to modifications in the kinetics of the resulting MAT II variant and their AdoMet production. Subsequent identification of the existence of two MAT α 2 splicing forms [26], further complicated the number and variety of associations between this catalytic subunit and MAT β monomers and, consequently the diversity of changes in AdoMet production that could be expected.

The preferred subcellular localization of methionine and Hcy metabolism enzymes is the cytoplasm, but several recent reports have shown most of them also in the cell nucleus [12, 20, 27-30], and MAT α 1 even in the mitochondria [31]. In a few cases

(MAT α 1 and BHMT), the subcellular distribution has been analyzed in different tissues, where high and low protein levels correlate with mainly cytoplasmic and nuclear localization, respectively [12, 29]. In contrast to the association states found in the cytoplasm, nuclear MAT α 1 is mainly monomeric, although low MAT I homo-tetramer amounts are also found [12]. Liver damage alters the distribution of MAT α 1 and BHMT, causing a decrease in their cytoplasmic levels, while increasing those in the nuclear compartment [20, 29]; of note, AHCY levels increase in both subcellular compartments [20]. Nuclear accumulation associates with a decrease in the GSH/GSSG ratio, and is prevented by compounds that serve as glutathione sources (e.g. N-acetylcysteine). Moreover, nuclear MAT I homo-tetramer levels increase in the pathological state, suggesting a higher need of AdoMet synthesis in this subcellular compartment. In fact, this nuclear accumulation correlated with increased levels of the H3K27me3 repression mark [20]. In other cases, changes in subcellular localization have been related to PTMs, as for CBS that appears sumoylated in the nucleus [30].

Several PTMs have been identified, especially by high-throughput methods, in enzymes of methionine and Hcy metabolism, but their influence in the regulation of these proteins remains in many cases unknown. Among others, phosphorylation, acetylation, methylation and sumoylation have been detected (reviewed in [6]), all of which require a transient interaction with the proteins catalyzing these modifications. Despite the difficulty, some of these transient interactions have been identified and confirmed (e.g. BHMT-Prkaca [32]). Other PTMs identified in methionine and Hcy metabolic enzymes are related to redox stress, a main mechanism involved in a large variety of pathologies and aging. Redox stress is probably the best-known mechanism regulating methionine and Hcy metabolism at several levels, ranging from expression to activity [6, 33]. CBS is one example of this regulation, acting as redox sensor through its heme group [15], but

also as a target for glutathionylation that increases its activity [34]. Moreover, changes induced by this type of stress on GSH/GSSG, NADPH/NADP⁺ and/or NADH/NAD⁺ ratios may control the activity and association state of MAT I/III, MAT II and AHCY, respectively [24, 35, 36], and provide additional links of methionine and Hcy metabolism with glutathione synthesis, the polyol and pentose phosphate pathways and NAD⁺ synthesis.

3. HIGH-THROUGHPUT METHODS FOR THE IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS

In order to obtain the maximum information regarding protein-protein interactions taking place in a cell or tissue several procedures have been implemented, mainly based on affinity purification/capture (AP) of the targets (preys) that bind to a protein of interest (bait), coupled to different identification methods [37]. For this purpose, baits are normally overexpressed with tags or captured/immunoprecipitated with specific antibodies for AP. In some instances, several tags are added to the bait's sequence to allow several purification steps, in what is called tandem affinity purification (TAP). Identification of preys is later performed by methods such as mass spectrometry (MS), western blot (WB) or luminescence and the combined procedures named AP-MS or TAP-MS, AP-WB, AP-luminescence, etc. Samples used in these procedures include whole extracts or subcellular fractions, the latter providing information about putative subcellular localization of the interactions detected. Many of these methods have been used to identify protein-protein interactions of methionine and Hcy metabolism as will be explained in section 4.

Alternatively, yeast two-hybrid screening allows identification of preys from a tissue cDNA library, in which their sequences are fused to the DNA-binding domain of a

transcription factor, using a bait fused to the activation domain of the same factor (e.g. GAL4)[38]. Interaction of bait and prey allows reconstitution of the transcription factor, in turn, allowing yeast growth in restricted culture media. High levels of false positive colonies are normally obtained when dealing with homo-oligomeric proteins, as is the case for most enzymes of methionine and Hcy metabolism [32, 39, 40]. However, their presence serves as a control of the correct folding of the expressed subunits, which is required for their association and activity. Although traditional yeast two-hybrid systems are more suitable for identification of nuclear interactions [38], new systems for detection in other subcellular compartments have been reported but, to date, they have not been used for detection of putative interactions of methionine and Hcy metabolism enzymes. Information obtained by these high-throughput methods requires verification by additional assays to confirm specificity and biological relevance of the interactions identified. Among these complementary methods, we can mention coimmunoprecipitation followed by WB, pull-down, proximity ligation assays and activity measurements on reconstituted complexes, most of which have been utilized to confirm interaction with proteins of methionine and Hcy metabolism. Protein-protein interactions can be detected by additional methods (e.g. bioID) that have not been used yet to detect interaction targets for enzymes of methionine and Hcy metabolism.

Pathway analysis is the last step carried out once a set of interaction partners has been uncovered. For this purpose, several tools have been developed and can be accessed online. Among them, we can mention STRING (<https://string-db.org/>), KEGG (<https://www.genome.jp/kegg/pathway.html>) or PIANA (<http://sbi.imim.es/piana/>), some of which have been used to analyze networks involving methionine and Hcy metabolic enzymes and their interaction targets.

4. PROTEIN-PROTEIN INTERACTIONS INVOLVING ENZYMES OF METHIONINE AND HOMOCYSTEINE METABOLISM

Several high-throughput studies using mainly AP-MS, AP-WB, AP-luminescence, but also yeast two-hybrid, cofractionation or proximity ligation-MS assays, have provided the identification of a large number of putative interaction targets for methionine and Hcy metabolism enzymes, which can be found in BioGRID (<https://thebiogrid.org/>) and IntAct (<https://www.ebi.ac.uk/intact/>) databases [41-50]. However, only a very limited set of these potential partners has been verified by additional methods, and its functional relevance evaluated. Therefore, we will refer only to those protein-protein interactions that have been identified by at least two independent methods, whereas for the complete list of potential partners the reader is referred to the already mentioned databases and original reports [41-50].

For some enzymes, few interaction partners have been described in mammalian cells, but additional identifications that deserve mention have been carried out in other organisms. This is the case of interactions with plant virus proteins, such as the interaction of MAT with the CLCuMuV C4 protein that inhibits AdoMet synthesis [51], that of AHCY with the coat protein of crinivirus [52] or the interaction of AHCY with betasatellite-encoded protein β C1 that inhibits AdoHcy hydrolysis [40]. The functional role of these interactions seems to be directed to decrease methylation events involved in transcriptional and/or posttranscriptional gene silencing during viral infection to evade antiviral responses. Additionally, AHCY interacts in plants with adenosine kinase (ADK1)[53], which is involved in adenosine elimination, and with mRNA cap methyltransferase in the nucleus of *Xenopus laevis* oocytes [28]. These data suggest that similar interactions may occur in mammalian cells to favor viral infection or the elimination of AdoHcy during mRNA processing.

4.1. Interaction Targets Identified for Methionine Adenosyltransferase Subunits

The importance of attaining adequate AdoMet levels for a correct cellular function, together with the correlation between impairments in AdoMet synthesis and a variety of diseases, has attracted a lot of attention to MATs since their discovery [10]. Pathological changes in AdoMet levels, mainly reductions in concentration and, in turn in the AdoMet/AdoHcy ratio (the methylation index), have been especially explored in tumor cells [13]. Indeed, most of the available data derive from studies in hepatoma cells, where the *MAT1A/MAT2A* expression switch is produced which, given the lower $V_{\max}$ of MAT α 2-containing isoenzymes [9, 10], results in decreased AdoMet synthesis. Currently, information regarding MATs in other pathologies and tumor cells is increasing, but it is still limited compared to the accumulated knowledge about hepatic enzymes and liver diseases. Nevertheless, the identification of protein-protein interactions involving MATs, even in liver, is one aspect that needs further development.

4.1.1. *The first MAT α interaction target described: the regulatory MAT β subunit*

The MAT β -MAT α 2 interaction was initially identified in lymphocytes [22, 54], and later found in high-throughput experiments carried out, among others, by AP-MS, yeast two-hybrid or cofractionation [41, 42, 45, 46, 49, 55, 56]. Recent analysis of the MAT β -MAT α 2 interaction, by isothermal titration calorimetry (ITC) [24] and X-ray crystallography [57, 58], demonstrated that the MAT II oligomer is a hetero-trimer with 2:1 stoichiometry ($\alpha_2\beta$). ITC data also showed that the interaction of the catalytic dimer with the regulatory subunit exhibits high binding affinity ($2.7 \times 10^8 \text{ M}^{-1}$) [24]. Moreover, as mentioned in section 2, this interaction leads to kinetic differences between MAT α 2 homo-oligomers and MAT II [6], which decrease the AdoMet synthesis capacity of the latter. No such information is available regarding the MAT β -MAT α 1 interaction, which

has been also reported [23, 41, 42], but remains uncharacterized. MAT β structure contains a FAD/NADP⁺ Rossmann fold, where NADP⁺ is bound with 1:1 stoichiometry and high affinity [24]. Cofactor binding increases the affinity of the MAT β -(MAT α 2)₂ interaction, due to structural changes induced on MAT β at three regions of the protein, two of which interact with NADP⁺ [24].

Few data are available regarding putative effects on oligomerization, activity and cellular AdoMet levels as a consequence of the existence of MAT β and MAT α 2 splicing forms [25, 26]. MAT α 2 splicing forms differ in the retention or absence of intron 8 sequence [26]. In contrast, main MAT β splicing forms, V1 and V2, differ in their N-terminal region due to the use of a different exon 1 during transcription, and minor V2a and V2b forms also lose internal sequence stretches of various lengths near the C-terminal end or in the middle of the protein, respectively [25]. All MAT β splicing forms preserve two of the regions that suffer structural changes upon NADP⁺ binding, regions A (F60-A77) and C (D325-F333), whereas region B (A95-N113) is lacking in the V2b form [24]. Regions A and B interact directly with the cofactor, thus differences in NADP⁺ binding at least for the V2b form can be expected. In fact, MAT β mutants lacking shorter stretches of the N-terminal than V2 forms already exhibit kinetic changes [24]. Altogether, a gradation of MAT II activities could be expected from the association of different MAT β splicing forms in the hetero-trimer.

Interaction targets for MAT β V1 and V2 forms have been identified in tumor cells, including: SIRT1 [59]; GIT1, MEK1 and ERK2 [60]; B-Raf, cRaf and Ras [61]; MAT α 2, DEAD box polypeptide 1 (DDX1), splicing factor 3b subunit 3 (SF3B3), pre-mRNA cleavage factor 1 (CPSF7) and several heat shock proteins [62]. Besides, specific partners for MAT β V1 include the HuR mRNA stabilizing protein (ELAVL1), asparaginyl-tRNA synthetase (NARS) and cleavage and polyadenylation specificity factor 6 (CPSF6),

whereas stem cell growth factor precursor (CLEC11A) and ATP-dependent RNA helicase (DDX3) are specific for MAT β V2 [62]. Among these interaction targets several are nuclear proteins, suggesting a nucleocytoplasmic distribution for MAT β that was verified using several methods and cell lines [62]. Resveratrol favors the tripartite complex MAT β -SIRT1-HuR, through which the stability of the three proteins is increased [59], whereas the MAT β -GIT1 interaction is needed for MEK1/ERK activation [60] and, together with the interactions with Ras and Raf, contributes to cell growth [61]. Transient interactions with MAT β are expected to be responsible of introducing the PTMs identified to date in this protein. In this line, MEK, ERK1/2 and B-Raf phosphorylate MAT β , generally leading to its stabilization in hepatic stellate cells [63]. Special interest was paid to the MAT β -HuR interaction, which was further verified by overexpression and immunoprecipitation from hepatoma Huh7 cells. Differences in V1 and V2 expression levels affected cytoplasmic HuR content, which increased upon their overexpression [62]. These subcellular distribution changes influence cell cycle progression, as HuR is involved in mRNA stabilization of several cyclins (e.g. cyclins D1 and A) and *MAT2A* [62, 64]. Conversely, in normal liver, low *MAT2B* expression together with high AdoMet levels, will counteract these effects through HuR methylation that, in turn, contributes to *MAT2A* mRNA destabilization [64].

Immunoprecipitation followed by LC-MS/MS identified MAT β interaction with Bcl2-associated athanogene 3 (BAG3). This target was also confirmed using proteome microarrays, in which GST-preys were used for incubation with purified BAG3 and the interaction detected with anti-BAG3 [65]. In agreement, KEGG pathway analysis detected an enrichment in the cysteine and methionine metabolism among the BAG3 interactome. The role played by this interaction remains unclear, but it could be related to the functions of BAG3 as co-chaperone or in mediating adaptive responses to stress.

4.1.2. MAT α 2 protein interaction targets

The nucleocytoplasmic distribution of MAT α 2 allows its interaction in different compartments with a variety of targets. [66]. In cell lysates, Cullin 3 (CUL3), a protein that serves as a scaffold for the assembly of ubiquitin ligase complexes (e.g. BCR), has been identified as a MAT α 2 interaction target using AP-MS and AP-WB assays and their binding confirmed in reconstitution experiments [47, 67]. The functional role of the CUL3-MAT α 2 interaction is to downregulate MAT α 2 stability by facilitating its ubiquitination and its subsequent degradation by the proteasome (Fig. 2)[67]. MAT α 2 ubiquitination is favored by acetylation, interaction with CUL3 and folate deprivation, altogether suggesting an interesting interplay between PTMs (i.e. acetylation **vs** phosphorylation that induces stabilization [63]), nutrients and protein-protein interactions to regulate cell growth.

Nuclear MAT α 2 was found as an interaction partner for the oncoprotein MafK, during a study that used TAP-MS and mouse X63/0 plasmacytoma cells [27]. This work and others have specifically uncovered more than a hundred potential MAT α 2 targets in several cell types [27, 66], including: MAT β , histone lysine **MTases** (G9a/Ehmt2, Ehmt1, ALL1/KMT2A, SETDB1 and Suv39h1), as well as subunits of complexes involved in repression (PRC1), chromatin remodeling (Swi/Snf) and DNA repair (PARP1). The role of MAT α 2 in these interactions has been elucidated in just two cases, **where it is related to gene repression. MAT α 2 acts as a corepressor of the MAF BZIP transcription factor K (MafK) during its binding to antioxidant response elements (AREs) on the promoter of the heme oxygenase-1 (HO-1) gene [27], and allows** recruitment of SETDB1 to Maf elements on the *COX-2* gene [66](Fig. 2).

Reconstitution and pull-down experiments also demonstrated the interaction of MAT α 2 homo-oligomers and MAT II with p53 and DNA damage-regulated gene 1

(PDRG1)[39](Fig. 2). Interaction of PDRG1 with MAT II seems to displace MAT β from the complex, therefore suggesting that PDRG1 and MAT β may share the same interaction site on MAT α 2. Moreover, pull-down experiments using truncated forms showed the involvement of the PDRG1 C-terminal region in its binding to MAT α 2 [39]. This part of PDRG1 sequence contains stretches predicted to participate in DNA or protein-protein interactions [68]. Nevertheless, the interaction with MAT α 2 should not preclude other PDRG1 interactions involving the prefoldin “hook” and that are established with prefoldin-like proteins such as URI or Art27/UXT or with the R2TP prefoldin complex [69-71]. As will be further explained in subsection 4.1.3., the PDRG1-MAT α 2 interaction causes a ~50% reduction in AdoMet synthesis *in vitro* and a trend towards DNA hypomethylation in cells [39](Fig. 2), putatively contributing to impair methylation events during disease.

Furthermore, the variety of PTMs that have been detected on MAT α 2 suggest its transient interaction with a diversity of modifying proteins. Among them, mitogen-activated protein kinase kinase 1 (MEK) and ubiquitin conjugating enzyme E2I (UBE2I/Ubc9) phosphorylate and sumoylate MAT α 2, respectively, enhancing its stability [63, 72]. Moreover, sumoylation favors MAT α 2 interaction with the apoptosis regulator Bcl2 [72], this interaction mediating the protective role exerted by Ubc9 in this process. In contrast, MAT α 2 acetylation by P300 favors its ubiquitination by UBR4 and further proteasomal degradation [73](Fig. 2), whereas deacetylation is carried out by histone deacetylase 3 (HDAC3)[73].

4.1.3. MAT α 1 protein interaction partners

Several studies devoted to the identification of the interactome for some proteins or tissues yielded MAT α 1 as a potential interaction target. Among them, we can first highlight, the identification of the brain interactome for amyloid beta precursor protein

(APP), after transcardiac formaldehyde perfusion [74]. This APP-MAT α 1 interaction seemed quite specific, as binding of MAT α 1 to amyloid beta precursor-like proteins APLP1 and APLP2 was not found. Second, the interactome of nicotinamide N-methyltransferase (NNMT) obtained by immunoprecipitation-MS from mouse livers overexpressing this enzyme, which included MAT α 1, BHMT and AHCY, interactions that were confirmed by reverse immunoprecipitation (Fig. 3)[75]. Additionally, this work showed concomitant reductions in hepatic AdoMet levels and the methylation index in this mouse model.

Specific studies to identify the MAT α 1 hepatic interactome have been also performed using proteomic or yeast two-hybrid approaches. Surprisingly, in the proteomic study carried out using liver lysates from wild-type and *Mat1a*^{-/-} mice, nearly a half of the proteins identified were mitochondrial and, among them, cytochrome P450 2E1 (CYP2E1) was detected [31](Fig. 3). Immunogold electron microscopy confirmed MAT α 1 localization to the mitochondrial matrix, where the CYP2E1-MAT α 1 interaction occurs; this interaction was also described in the endoplasmic reticulum [31]. Moreover, CYP2E1-MAT α 1 binding was also detected in alcohol liver disease (ALD). The functional role of the CYP2E1-MAT α 1 interaction is to favor R379 methylation on the cytochrome, for which AdoMet channeling from MAT α 1 to a MTase would be required. [31](Fig. 3). Methylation of CYP2E1 has a destabilizing effect inducing proteasomal degradation, as confirmed by the increased CYP2E1 levels detected in two settings where MAT α 1 expression is diminished, namely, the *Mat1a*^{-/-} knockout mice and human ALD [31]. For MAT α 1 to reach the mitochondria either a localization signal or a piggyback mechanism are needed. No such signal was identified in MAT α 1 sequence, but its interaction with heat shock proteins HSP70A8 and HSP90 β that could aid in this transport

was demonstrated [31](Fig. 3). Analogous mechanisms of transport into other subcellular compartments and the proteins that could be implicated have not been described yet.

Analysis of the MAT α 1 interactome using yeast-two hybrid and a rat liver cDNA library allowed identification of 23 putative interaction targets, **mostly MAT α 1 itself, but among the remaining targets PDRG1 was found [39](Fig. 3). Both proteins exhibit nucleocytoplasmic distribution [39, 76], but coimmunoprecipitation only captured the complex (~360 kDa) in nuclear fractions [39]. Information derived from the construction of a structural homology model of the PDRG1 core (K27-Q106) using prefoldin helped to design truncated forms of the protein, which aided into the identification of the interaction site [39]. Pull-down experiments confirmed the PDRG1-MAT α 1 interaction and suggested the PDRG1 core as the region of interaction with MAT α 1 [39]. Interestingly, this interaction site differs from that suggested by similar experiments using MAT α 2 (84% identical).**

Information regarding PDRG1 is limited and mainly related to its isolation in several macromolecular complexes, including the RNA polymerase II, the U12-type spliceosome, the R2TP prefoldin complex, as well as others involved in chromatin remodeling (e.g. INO80) and nutrient sensing [76]. Colocalization of PDRG1 and MAT α 1 with the nuclear speckles marker SC-35 [12, 39], further suggests their involvement in complexes such as the spliceosome. PDRG1 is considered an oncogene and its expression down-regulated by p53. RTqPCR showed that *Pdrg1* mRNA levels are higher in extrahepatic rat tissues than in liver [39]; in fact, its expression **pattern is analogous to** that of *Mat2a*, which is nearly opposite to that of *Mat1a* [12]. This finding initially shed doubts over the feasibility of PDRG1-MAT α 1 interaction. However other evidences are in favor of the existence of this interaction, for example, the preferential nuclear localization of MAT α 1 in extrahepatic tissues [12], the nuclear accumulation of

MAT α 1 in liver disease [20, 77], or the increased hepatic *Pdrg1* expression in the same liver diseases. Initial insights about the putative functional role of the PDRG1-MAT α 1 interaction were obtained in CHO cells, where DNA hypermethylation induced by MAT α 1 overexpression was precluded by coexpression with PDRG1 [39]. This effect suggested a decrease in AdoMet availability, which was further supported by the DNA hypomethylation trend found during overexpression of PDRG1 in these cells [39], which mainly express MAT α 2. *In vitro* assays confirmed the PDRG1 role in decreasing AdoMet production by MATs and demonstrated a concentration-dependent effect [39]. Thus, under liver damage the presence of increased PDRG1 and MAT α 2 levels, together with nuclear accumulation of both PDRG1 and MAT α 1 [20, 39, 77], may favor formation of PDRG1-MAT α 1 and PDRG1-MAT α 2 complexes and, therefore a significant reduction in nuclear AdoMet synthesis as reflected by DNA hypomethylation.

In normal liver, nuclear MAT α 1 interaction targets also include Max and Mnt [78](Fig. 3). In contrast, in cholestatic and cholangiocarcinoma tissues, MAT α 1 prefers cMyc, cMaf and MafG as partners instead of Mnt, while the MAT α 1-Max interaction is maintained [78]. Examination of the functional role of these complexes has shown their binding to promoter E-boxes to regulate the expression of their own genes (i.e. *MAT1A*, *c-MYC*, *c-MAF*, *MAFG*) (Fig. 3). All these nuclear interactions involving MAT α 1 require its traffic from and into this compartment. However, lack of effects upon leptomycin (exportin 1 inhibitor) treatment suggest that nuclear export does not include MAT α 1-CRM1 exportin 1 interaction, as initially suggested by studies on ancient metazoan [12, 49].

Identification of MAT α 1 phosphorylation in high-throughput studies, together with its modification *in vitro* by PKC [79], suggested transient interactions with this and

other kinases that have not been confirmed yet. Nevertheless, MAT α 1 phosphorylation on T342 was found in both types of experiments **and *in vitro* effects** on activity and on the MAT I/III association state **were detected** [79]. Interestingly, this threonine is near the areas involved in MAT α 1 subcellular distribution [12], **suggesting** a putative role of this PTM in the localization changes described to date. **Additional** interactions within the **methionine and Hcy** metabolism, involving MAT α 1, MAT α 2 and BHMT **have been found** in studies that will be described in section 4.5. Nevertheless, putative MAT α 1 interaction targets are more numerous than those mentioned in this section but, as occurs for other **methionine and Hcy** metabolism enzymes, most of them have been found only by one high-throughput method and their functional relevance remains unknown [41, 42, 48-50].

4.2 Protein interaction targets for S-adenosylhomocysteine hydrolase

C1orf50 [41, 42, 44-46], ankyrin repeat domain-containing protein 40 (ANKRD40) [41, 42, 44, 46], anterior gradient protein 2 homolog (AGR2), AHCY-like 1 (AHCYL1) [41, 42] and NNMT [75] have been identified as AHCY interaction partners by AP-MS, yeast-two-hybrid and/or proximity ligation-MS **(Fig. 3-5)**. Moreover, a variation of the bimolecular fluorescence complementation (BiFC) method adapted for high-throughput detected galectin-3 and pseudouridylate synthase 7-like (PUS7L) as additional **AHCY** interactions partners, as well as AHCYL1 [80]. **The** functional relevance of most of these interactions has not been studied, except for the AHCY-AHCYL1 interaction **(Fig. 5)**. AHCYL1 contains IRBIT and AHCY domains; **the first binds the inositol 1,4,5-triphosphate receptor and the second has more than 50% sequence identity to AHCY**. Its known functions encompass from the control of Ca²⁺ levels to the inhibition of ribonucleotide reductase, but also include modulation of mRNA

polyadenylation [81, 82]. Despite AHCY and AHCYL1 similarity, no AdoHcy hydrolytic activity has been detected for the latter, although its binding to AHCY was expected and detected by coimmunoprecipitation [83]. Results using fusion proteins also suggested that their binding occurs through their C-terminal ends [80]. BiFC assays provided evidence for the AHCY-AHCYL1 interaction in the cytoplasm, nucleus and endoplasmic reticulum of several cell types and nuclear accumulation of AHCY was observed in *AHCYL1* silencing experiments [84], thus suggesting a role for the interaction in regulating AHCY subcellular distribution.

Regarding AHCY and galectin-3, both proteins are involved in mRNA processing when located in the nuclear compartment, the former in mRNA capping and the latter in pre-mRNA splicing [28, 85, 86](Fig. 5). However, AHCY-galectin-3 association was identified predominantly in intracellular vesicles (i.e. early, recycling and late endosomes, lysosomes and Golgi), where its role remains unknown. Nevertheless, location into vesicles could be expected, given the known role of galectin-3 in intracellular trafficking and nuclear protein transport. Once more, the identification of a variety of PTMs in AHCY suggests transient interactions with modifier proteins (e.g. kinases, MTases) that have not been identified and/or verified.

4.3. Identification of protein interaction partners for cystathionine β -synthase

CBS modification by phosphorylation [87], glutathionylation [34] and sumoylation [30, 88] requires in most cases transient interactions with the modifier proteins (e.g. kinases). Among them, interaction with the sumoylation machinery UBE2I (containing SUMO-1) has been detected by yeast two-hybrid [30, 46] and in activity assays [30]. This interaction allows sumoylation on the K211 residue, leading to CBS localization at the nuclear matrix [30]. Sumoylation of CBS is inhibited in the presence

of its substrates, but increased by addition of the polycomb protein hPc2 [88], which was also detected in the same screenings (Fig. 5). Functionally, this modification decreases the enzyme activity, an effect potentiated when hPc2 is included in the assay, in turn suggesting hPc2 role as a SUMO-E3 ligase for CBS [88]. In contrast, S227 phosphorylation on CBS, after carbachol stimulation of the cGMP/PKG route, suggested its interaction with PKG, an event that has not been verified yet [87]. This phosphorylation stimulates H₂S production by CBS, and hence the use of cysteine instead of serine for Hcy catabolism.

The search for CBS protein-protein interactions retrieved mostly references reporting interactions of CBS domain-containing proteins of diverse origins [89-93]. The wide distribution of these CBS domains in proteins with a variety of functions allows a broad impact of their mutations in physiology, as reflected in humans by the number of hereditary diseases associated with these mutations [94]. In this context, we can highlight two examples of interactions involving CBS domains. First, the interaction of inosine-5'-phosphate dehydrogenase (IMPDH2) through its CBS domain with sagliffehrin-bound cyclophilin A to modulate cell growth [92] and, second, the interaction of MAT with CBS domain-containing protein chloroplastic-like in wheat under stress [93]. Both examples of CBS-domain mediated interactions open the possibility that other CBS interactions may take place within the methionine and Hcy metabolism or that a crosstalk may be established with many other pathways in different organisms.

4.4 Protein interactions involving methionine synthase

More than 40 protein interaction targets have been identified for MTR, mainly by a single method such as AP-MS [41, 42, 95-97], as stated in the BioGRID database. Interestingly, prohibitin (PHB) is found among the protein targets detected [97].

Expression of PHB1, one form of this mitochondrial chaperone, mirrors that of MAT α 1 in normal liver (high) and HCC (low) [98, 99]. Both, PHB1 and MAT α 1 have cMyc and MAX as a common interaction targets, interaction with either of this partners results in different complexes that bind to *PHB1* and *MAT1A* promoter E-boxes to induce or decrease their expression [98]. Additionally, interaction of nuclear PHB1 with p53 allows subsequent connections with MAT and AHCY regulation through PDRG1 and AGR2, respectively [41, 42, 100]. Hence, additional effects of PHB on MTR suggest several layers of control on the methionine cycle of special importance during carcinogenesis, where both *PHB* and *MAT1A* expression levels are reduced and increased proliferation is produced.

Computational studies carried out with STRING predicted MTR interactions, which were restricted to the methionine cycle (MAT α 1, MAT α 2, AHCY), the folate pathway (MTHFD1, MTHFD1L, MTHFR, MTRR, SHMT1) and transsulfuration (CBS, CTH), although most of them have not been verified experimentally [101]. Nevertheless, the MTR-MTRR interaction has been confirmed by several studies and justified by the need of a continuous reactivation of the methylcobalamin cofactor for MTR activity, a role carried out by the flavin-dependent enzyme MTR reductase (MTRR)(Fig. 5). Interactions between different redox forms of these proteins exert diverse effects on MTR behavior *in vitro* [102]. MTRR increases MTR stability and facilitates cobalamin uptake, whereas oxidized MTRR is unable to reactivate cob(II)alamin MTR, leading to a decrease in its Hcy methylation activity [102]. Additionally, MTR interaction with methylmalonic aciduria and homocystinuria type C protein (MMACHC) has also been described and inactive MTR forms shown to decrease MMACHC activity [103]. Analysis of the MTR interactome using hepatoma HepG2 cells and coimmunoprecipitation revealed binding of this protein with methylmalonic aciduria and homocystinuria type D protein

(MMADHC), as well as MTRR interactions with both MMACHC and MMADHC [104](Fig.5). Furthermore, proximity ligation assays demonstrated interactions involving the four proteins, MTR, MTRR, MMACHC and MMADHC and silencing experiments showed the need of MTRR for MTR-MMACHC and MTR-MMADHC interactions [104]. Altogether, these data indicate the existence of a network of interactions taking place between enzymes of cobalamin metabolism, putatively forming a multiprotein complex that would facilitate cobalamin uptake by MTR, allowing adequate Hcy remethylation and flow through the folate cycle.

4.5 Protein-protein interaction targets for betaine homocysteine S-methyltransferases

Interactions of BHMT, or some of its fragments, with tubulin [105], the hepatitis B virus [106, 107], tissue-type transglutaminases [108], the prion protein PrP [109], high-mobility group box 1 (HMGB1)[110], NNMT [75], and within a complex formed by 10-formyltetrahydrofolate dehydrogenase (Aldh1l1) and carbamoyl phosphate synthetase 1 [111] have been reported using diverse samples types (e.g. taxol-treated liver and ischemia/reperfusion samples). Thus, differences exist among the interaction targets identified in those studies. Some treatments may lead to changes in subcellular distribution that contribute to favor BHMT binding to targets in distinct subcellular compartments; this could be the case of ischemia/reperfusion and the BHMT-HMGB1 interaction [32, 110]. Additionally, high-throughput studies aimed to establish the whole human interactome have also identified several putative BHMT interaction partners [41, 42]. Moreover, other works devoted to the identification of PTMs targets also detected BHMT [112-116], thus implicitly suggesting the existence of transient interactions with modifiers that in most cases remain unknown. Many of these interactions could not be

verified by additional methods. Of notice, discovery of the BHMT counterpart BHMT2 (73% identical) was made during the work that identified interaction of BHMT fragments with PrP [109]. However, BHMT2 did not coimmunoprecipitate with the copper-binding protein PrP [109].

More recently, two complementary high-throughput methods were used for the identification of the BHMT interactome in rat liver [32]. On one hand, rat liver cytosol and AP-MS, utilizing three consecutive chitin-based affinity columns that differed in the proteins bound to the beads (none, intein and intein-BHMT). This strategy detected more than 130 putative interactions, including several partners previously described, many of which exhibited high non-specific binding to chitin and/or intein-chitin columns (e.g. tubulin). On the other, a parallel yeast two-hybrid screening of a rat liver cDNA library further enriched the list with 15 additional partners [32]. Analysis of these targets using STRING and KEGG allowed the classification of a limited subset of these proteins into four main pathways, namely: metabolic pathways (16), spliceosome (7), biosynthesis of amino acids (7) and cysteine and methionine metabolism (5).

BHMT targets identified in that study were considered validated when the interaction was verified by two independent methods [32]. This was the case of proteins belonging to the methionine cycle such as BHMT2, which was identified independently by both AP-MS and yeast-two hybrid [32], and previously found in other high-throughput studies [41, 42](Fig. 4). Production of a BHMT-BHMT2 hetero-oligomer could be an advantage for Hcy recycling under certain circumstances. Indeed, remethylation of Hcy by BHMT2 uses S-methylmethionine as methyl donor, thus rendering 2 mol of methionine per mol of Hcy utilized as substrate, the double than the same reaction catalyzed by BHMT using betaine. Thus, a BHMT₂-BHMT₂ tetramer should produce 50% more methionine than a BHMT tetramer (6 mol vs 4 mol). Furthermore, the AP-MS

assays also identified the two catalytic MAT α 1 and MAT α 2 subunits as BHMT partners and their interactions were confirmed by coimmunoprecipitation after overexpression in Cos7 cells [32]. The existence of BHMT-MAT α interactions suggests channeling of the methionine recovered by Hcy remethylation to guarantee its supply for AdoMet synthesis (Fig. 4). This channeling would be favored, most probably, under low methionine or AdoMet levels, but this hypothesis has not been experimentally demonstrated yet.

Additional BHMT targets also validated by coimmunoprecipitation include the catalytic subunit of protein kinase A (Prkaca), aldolase B (Aldob) and 4-hydroxyphenylpyruvic acid dioxygenase (HPD)[32](Fig. 4). Several high-throughput studies have detected a variety of PTMs on BHMT, including phosphorylation; thus, its interaction with Prkaca and other kinases was expected. Moreover, C-terminal transglutamination of BHMT, as well as crosslinked multimers, have been detected *in vitro* as a result of its interaction with transglutaminases [108]. However, the impact of these PTMs on the enzyme behavior, activity, oligomerization or subcellular localization remains largely unknown. Regarding BHMT binding to Aldob, we can only speculate that the BHMT-Aldob interaction may regulate ATP supply from glycolysis for AdoMet synthesis. In contrast, the BHMT-HPD interaction may be involved in an indirect mechanism to control production of fumaryl acetoacetate (MAT inhibitor) from homogentisate. Finally, AP-MS experiments have identified BHMT2 interaction partners, such as INPPL1 and tubulin-specific chaperone E (TBCE)[41, 42]. However, there are no clues about their functional role or the possible impact of macromolecular complexes containing the four proteins (BHMT2, BHMT, INPPL1 and TBCE).

5. PATHWAY INTERPLAY ESTABLISHED THROUGH PROTEIN-PROTEIN INTERACTIONS

The current knowledge about protein-protein interactions concerning enzymes of methionine and Hcy metabolism is still limited to allow an integrated view of the protein networks involved. Nevertheless, attempts such as the hu.MAP (<http://proteincomplexes.org>) have integrated MS protein-protein interaction data to develop a map of human complexes, some of which include proteins of methionine and Hcy metabolism [117](Table 1). However, the hu.MAP does not include most of the interactions mentioned in this review that take place within the methionine and Hcy metabolism and that have been confirmed experimentally by at least two methods. The exceptions are BHMT-BHMT2 and MAT β -MAT α interactions (Fig. 5). Moreover, the map does not specify the subcellular localization of the complexes nor their differential presence in diverse cell types (e.g. normal vs tumor cells), thus giving the erroneous impression that all the interactions listed are taking place in all settings simultaneously. Differences in spatial distribution are of great importance as shown for MAT α 1-PDRG1 and MAT α 1-CYP2EI interactions, which occur in the nucleus and in the mitochondrial matrix, respectively [31, 39]. Moreover, some interactions may occur or be favored in tumorigenesis, where the expression of proteins of methionine and Hcy metabolism, their subcellular localization and/or oligomeric state is in most cases altered, and analogous changes can be expected for their interaction targets (reviewed in [5, 6, 8]). Again, this is the case for several interactions involving MAT α subunits, due to the *MAT1A/MAT2A* expression switch detected in hepatoma, the increased *MAT2A* expression in other type of tumors and nuclear accumulation of MAT α 1. Specific interactions restricted to certain compartments also exert a variety of effects directly or indirectly related to AdoMet production. This is the case of the decreased MAT activity induced by the nuclear MAT α 1-PDRG1 interaction [39], the methylation of CYP2EI in the mitochondria

favored by MAT α 1-CYP2E1 binding [31], and the MAT α 1 interactions involving e.g. Max or cMyc that control gene expression (e.g. *PHB1*, *MAT1A*) [78, 98].

TABLE 1

Complexes involving homocysteine metabolism proteins according to the hu.MAP

Protein	Complex	Other components of the complex
MATα1	3320	Rho-related GTP-binding protein RhoC
	3705	MAT2A MAT2B
MATα2	3705	MAT2A MAT2B
	3267	Heterogeneous nuclear ribonucleoprotein H (HNRNPH1) Heterogeneous nuclear ribonucleoprotein F (HNRNPF)
MATβ	2267	NFX1-type zinc finger-containing protein 1 (ZNFX1)
	3705	MAT2A MAT2B
AHCY	1074	Fumarate hydratase (FH)
	2061	C1orf50
	4583	C1orf50 Nucleoside diphosphate kinase B (NME2) Peptidyl-prolyl cis-trans isomerase (FKBP1B) 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) tubulin-specific chaperone A (TBCA)
BHMT	25	BHMT2
	2283	BHMT2 tubulin-specific chaperone E (TBCE)
BHMT2	25	BHMT
	2283	BHMT tubulin-specific chaperone E (TBCE)
	4416	tubulin-specific chaperone E (TBCE)
MTR	2396	Protein O-linked N-acetylglucosaminidase (MGEA5)
CBS	-	
PDRG1	188	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2, (PAPSS2) Prefoldin subunit 3 (VBP1) Prefoldin subunit 2 (PFDN2)
	2184	WD repeat-containing protein 92 (WDR92) PIH1 domain-containing protein 1 (PIH1D1) RNA polymerase II-associated protein 3 (RPAP3) Leucine-rich repeat and calponin homology domain-containing protein 3 (LRCH3) UXT Unconventional prefoldin RPB5 interactor 1 (URI1)

To date, interactions confirmed by several methods show that most of the protein-protein interactions established, within the methionine and Hcy metabolism and with related pathways, seem to be directly or indirectly involved in the tight control of AdoMet synthesis. In this line, BHMT interaction with MAT α subunits [32] or the MTR-MTRR interaction [104] seem devoted to guarantee channeling and methionine production for AdoMet synthesis (Fig. 5). Conversely, the already mentioned PDRG1-MAT α interaction decreases nuclear AdoMet production and, in turn, epigenetic methylations [39]. Additionally, channeling is also suggested by the NNMT-MAT α 1 and NNMT-AHCY interactions, which may supply the AdoMet methyl donor and eliminate the AdoHcy demethylated product, respectively [75]. Crosstalks established with routes such as glycolysis and the polyol pathway (e.g. BHMT-Aldob) would allow additional levels of regulation of AdoMet synthesis through ATP and/or NADP⁺ levels, which may be complementary to interactions controlling production of MAT inhibitors (e.g. BHMT-HPD)(Fig. 5)[32]. Other interactions uncovered are, however, dedicated to the transport of proteins of the methionine and Hcy metabolism to subcellular compartments other than the cytoplasm (e.g. HSPs-MAT α 1)[31]. Lack of classical localization signals in the sequence of methionine and Hcy metabolism proteins suggests either the existence of conformational signals for this purpose (e.g. MAT α 1 [12]), or the use of transport mechanisms involving other proteins containing the required signals (i.e. piggyback). However, knowledge about the identity of these proteins is still scarce.

Another regulatory level is provided by nuclear interactions that link methionine and Hcy metabolism to the control of gene expression, splicing or translation. These interactions have been identified for MAT α subunits and their role is only partially established. For example, MAT α 2 serves as corepressor of MafK for *HO-1* expression, whereas MAT α 1 is part of complexes, including e.g. Max, that bind to promoter E-boxes

during oxidative stress to regulate among others *PHB1* or its own expression [78, 98, 118]. Moreover, the PDRG1-MAT α 1 interaction may allow crosstalk of methionine metabolism with a variety of macromolecular complexes containing PDRG1 (Fig. 5)[76]. Subsequent PDRG1 interactions, or their preclusion by MAT α binding, may modulate apoptosis, through the U12-type spliceosome protein PDCD7 [119], or RNA polymerase II assembly, through proteins included in the prefoldin complex (URI and Art27/UXT)[69-71, 120]. RNA processing and DNA replication could be also modulated through the AHCY-AHCYL1 interaction, which may impact the subcellular localization of FIP1L1, PAPOLA and RRM1 (subunit of ribonucleotide reductase) activities, and hence mRNA polyadenylation and dNTP pools.

Oncogenesis is a process in which important changes in gene expression are induced due to alterations in epigenetic modifications and/or promotor binding of different factors, generation of splicing forms, effects on the half-life of mRNA and/or proteins, anomalous subcellular distributions and/or association states and/or protein-protein interactions. Some of these effects involve proteins of methionine and Hcy metabolism and their interaction partners, among which oncogenes (e.g. PDRG1, cMyc) are included [76, 78, 98], although in many cases these relationships can be only inferred. In other opportunities, down-regulation of one of the interaction partners has been used to block/reduce the interaction of interest to analyze its functional effects at different levels (e.g. PDRG1, CYP2E1 or MAT α 1) [31, 39, 121]. Downregulation of *Pdrg1* in rat hepatoma cells does not alter expression of its MAT α partners, but has an important impact on the expression profile of these cells (e.g. response to starvation or lipid metabolism), including the downregulation of several genes commonly upregulated in tumors (e.g. *Sema3c*, *Id1*, *Cxcl1*, *Ctgf*)[39, 121]. Conversely, tumor cells/tissues have been employed for the analysis of effects derived from upregulation of some interaction

targets, which normally correlate with DNA hypomethylation and a diversity of histone methylation patterns. In this line, upregulation of *PDRG1* in human tumors associates with DNA hypomethylation [119, 122, 123], but with increased H3K27me3 levels in other settings [39]. This behavior is connected to down-regulation of p53 and miRNA-214, both of which control *PDRG1* expression [76], as well as to enhanced EZH2 methyltransferase levels [124]. This MTase regulates DNA methylation through its involvement in Polycomb repressive complexes [125]. Additional connections with p53 could be obtained through the AHCY-AGR2 interaction, which may act as a trap for the latter avoiding its p53 inhibition [126].

Besides expression effects, changes in subcellular localization of the proteins may take place during transformation. Nuclear MAT α 1 accumulation in hepatoma [77] may be an initially response to guarantee AdoMet supply for epigenetic events. However, this effect could be counteracted by the increased PDRG1 levels in transformed cells, favoring its nuclear interaction with MAT α subunits, and the subsequent reduction in local AdoMet synthesis. Effects on subcellular localization also affect HuR through its interaction with MAT β and, subsequently, *MAT2A* mRNA stability. Additional interaction targets within methionine and Hcy metabolism, such as NNMT, are also upregulated in tumors, correlating with decreased histone methylation levels [75, 127]. This MTase acts in nutrient metabolism through the SIRT1 pathway, stabilizing SIRT1, and its suppression affects glucose and cholesterol metabolism [127]. The NNMT/SIRT1 connection may allow an additional interplay with MATs through the interaction of the V1 and V2 MAT β splicing forms with SIRT1. This tripartite interaction, or the putative interference of NNMT in SIRT1-MAT β binding, and the impact of the NADP⁺ cofactor in either case remains unexplored. Finally, the increased levels of Ubc9, Bcl2 and

MAT α 2 found in tumor cells and the Bcl2-MAT α 2 interaction provide two ways to control apoptosis.

6. CONCLUSIONS

The number of studies uncovering protein-protein interactions of methionine and Hcy metabolism has increased lately. However, most works used only one experimental method for the identification of the potential interaction targets, highlighting the need to confirm many of the interactions reported by additional procedures. Moreover, in many opportunities the functional relevance of the interactions described remains unknown or has been inferred from the available literature. Defining a general methionine and Hcy metabolism interaction network using the current knowledge seems unrealistic, since most data have been obtained in a disparity of systems, where some interactions may be favored or precluded due to the specific characteristics of the samples used (e.g. normal vs tumor tissue or different cells of diverse origins). Importantly, subcellular localization may vary according to the sample type utilized in the study, and we already know that methionine and Hcy metabolism enzymes distribute between several subcellular compartments, this distribution is cell type-specific and can be pathophysiologically altered (e.g. by redox stress). Altogether, the available data have just started to uncover interesting interplays between methionine and Hcy metabolism, several metabolic pathways and regulatory mechanisms that deserve further research efforts. Additionally, more detailed studies are needed to decipher the role of many of the protein-protein interactions already identified, the role of PTMs in their regulation and the putative moonlighting activities that may derive from the association into different types of complexes. Finally, interaction networks should be established for normal and a variety

of pathological states in different tissues to allow full comprehension of the specific events involved in the evolution of each disease.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

This work was supported by grants of the Ministerio de Ciencia, Innovación y Universidades (BFU2005-00050, BFU2008-00666, BFU2009-08977 to MAP; BFU2008-04285 to FP; BIO2015-67580-P and PGC2018-097019-B-I00 to JV), the Instituto de Salud Carlos III-Fondo de Investigación Sanitaria PRB3 (IPT17/0019-ISCIIS-SGEFI/ERDF, ProteoRed to JV), Fundació Marató TV3 (122/C/2015 to JV) and La Caixa Banking Foundation (HR17-00247 to JV). The CNIC is supported by the Instituto de Salud Carlos III (ISCIIS), the Ministerio de Ciencia, Innovación y Universidades (MCNU) and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (SEV-2015-0505). The funders had no role in study design, collection, analysis and interpretation of data, in writing the manuscript and the decision to publish.

AUTHOR CONTRIBUTIONS

F. Portillo, J. Vázquez and M. A. Pajares prepared the draft and figures; M. A. Pajares wrote the final version of the paper.

ACKNOWLEDGEMENTS

The authors wish to thank Dolores Pérez-Sala for critical reading of the manuscript.

REFERENCES

- [1] I.M. Nooren, J.M. Thornton, Diversity of protein-protein interactions, *EMBO J*, 22 (2003) 3486-3492.
- [2] G. Duan, D. Walther, The roles of post-translational modifications in the context of protein interaction networks, *PLoS Comput Biol*, 11 (2015) e1004049.
- [3] C.J. Jeffery, Protein moonlighting: what is it, and why is it important?, *Phil Trans R Soc B*, 373 (2017) 20160523.
- [4] J.M. Mato, L. Alvarez, P. Ortiz, M.A. Pajares, S-adenosylmethionine synthesis: molecular mechanisms and clinical implications, *Pharmacology & therapeutics*, 73 (1997) 265-280.
- [5] M.A. Pajares, D. Perez-Sala, Betaine homocysteine S-methyltransferase: just a regulator of homocysteine metabolism?, *Cell Mol Life Sci*, 63 (2006) 2792-2803.
- [6] M.A. Pajares, D. Perez-Sala, Mammalian Sulfur Amino Acid Metabolism: A Nexus Between Redox Regulation, Nutrition, Epigenetics, and Detoxification, *Antioxidants & redox signaling*, 29 (2018) 408-452.
- [7] M.S. Field, E. Kamynina, J. Chon, P.J. Stover, Nuclear Folate Metabolism, *Annu Rev Nutr*, 38 (2018) 219-243.
- [8] D. Pérez-Sala, O.H. Martínez-Costa, J.J. Aragón, M.A. Pajares, Alterations in Nucleocytoplasmic Localization of the Methionine Cycle Induced by Oxidative Stress During Liver Disease, in: V.B. Patel, R. Rajendram, V.R. Preedy (Eds.) *The Liver: Oxidative Stress and Dietary Antioxidants*, Academic Press 2018, pp. 21-42.
- [9] G.D. Markham, M.A. Pajares, Structure-function relationships in methionine adenosyltransferases, *Cell Mol Life Sci*, 66 (2009) 636-648.
- [10] M.A. Pajares, G.D. Markham, Methionine adenosyltransferase (s-adenosylmethionine synthetase), *Adv Enzymol Relat Areas Mol Biol*, 78 (2011) 449-521.
- [11] G.L. Cantoni, Biological methylation: selected aspects, *Annu Rev Biochem*, 44 (1975) 435-451.
- [12] E. Reytor, J. Perez-Miguelsanz, L. Alvarez, D. Perez-Sala, M.A. Pajares, Conformational signals in the C-terminal domain of methionine adenosyltransferase I/III determine its nucleocytoplasmic distribution, *FASEB J*, 23 (2009) 3347-3360.
- [13] S.C. Lu, J.M. Mato, S-adenosylmethionine in liver health, injury, and cancer, *Physiol Rev*, 92 (2012) 1515-1542.
- [14] D. Kloor, H. Osswald, S-Adenosylhomocysteine hydrolase as a target for intracellular adenosine action, *Trends Pharmacol Sci*, 25 (2004) 294-297.

- [15] S. Taoka, B.W. Lepore, O. Kabil, S. Ojha, D. Ringe, R. Banerjee, 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 (2002) 10454-10461.
- [16] Q. Sun, R. Collins, S. Huang, L. Holmberg-Schiavone, G.S. Anand, C.H. Tan, S. van-den-Berg, L.W. Deng, P.K. Moore, T. Karlberg, J. Sivaraman, Structural basis for the inhibition mechanism of human cystathionine gamma-lyase, an enzyme responsible for the production of H₂S, *J Biol Chem*, 284 (2009) 3076-3085.
- [17] E. Mosharov, M.R. Cranford, R. Banerjee, The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes, *Biochemistry*, 39 (2000) 13005-13011.
- [18] V. Vitvitsky, S. Dayal, S. Stabler, Y. Zhou, H. Wang, S.R. Lentz, R. Banerjee, Perturbations in homocysteine-linked redox homeostasis in a murine model for hyperhomocysteinemia, *Am J Physiol Regul Integr Comp Physiol*, 287 (2004) R39-46.
- [19] S.S. Szegedi, C.C. Castro, M. Koutmos, T.A. Garrow, Betaine-homocysteine S-methyltransferase-2 is an S-methylmethionine-homocysteine methyltransferase, *J Biol Chem*, 283 (2008) 8939-8945.
- [20] M. Delgado, F. Garrido, J. Perez-Miguelsanz, M. Pacheco, T. Partearroyo, D. Perez-Sala, M.A. Pajares, Acute liver injury induces nucleocytoplasmic redistribution of hepatic methionine metabolism enzymes, *Antioxid Redox Signal*, 20 (2014) 2541-2554.
- [21] M.A. Avila, C. Berasain, L. Torres, A. Martin-Duce, F.J. Corrales, H. Yang, J. Prieto, S.C. Lu, J. Caballeria, J. Rodes, J.M. Mato, Reduced mRNA abundance of the main enzymes involved in methionine metabolism in human liver cirrhosis and hepatocellular carcinoma, *J Hepatol*, 33 (2000) 907-914.
- [22] A.B. Halim, L. LeGros, A. Geller, M. Kotb, Expression and functional interaction of the catalytic and regulatory subunits of human methionine adenosyltransferase in mammalian cells, *J Biol Chem*, 274 (1999) 29720-29725.
- [23] H.L. LeGros, Jr., A.B. Halim, A.M. Geller, M. Kotb, Cloning, expression, and functional characterization of the beta regulatory subunit of human methionine adenosyltransferase (MAT II), *J Biol Chem*, 275 (2000) 2359-2366.
- [24] B. Gonzalez, F. Garrido, R. Ortega, M. Martinez-Julvez, A. Revilla-Guarinos, Y. Perez-Pertejo, A. Velazquez-Campoy, J. Sanz-Aparicio, M.A. Pajares, NADP⁺ binding to the regulatory subunit of methionine adenosyltransferase II increases intersubunit binding affinity in the hetero-trimer, *PLoS One*, 7 (2012) e50329.
- [25] H. Yang, A.I. Ara, N. Magilnick, M. Xia, K. Ramani, H. Chen, T.D. Lee, J.M. Mato, S.C. Lu, Expression pattern, regulation, and functions of methionine adenosyltransferase 2beta splicing variants in hepatoma cells, *Gastroenterology*, 134 (2008) 281-291.
- [26] K.E. Pendleton, B. Chen, K. Liu, O.V. Hunter, Y. Xie, B.P. Tu, N.K. Conrad, The U6 snRNA m6A Methyltransferase METTL16 Regulates SAM Synthetase Intron Retention, *Cell*, 169 (2017) 824-835 e814.
- [27] Y. Katoh, T. Ikura, Y. Hoshikawa, S. Tashiro, T. Ito, M. Ohta, Y. Kera, T. Noda, K. Igarashi, Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein, *Mol Cell*, 41 (2011) 554-566.
- [28] N. Radomski, C. Kaufmann, C. Dreyer, Nuclear accumulation of S-adenosylhomocysteine hydrolase in transcriptionally active cells during development of *Xenopus laevis*, *Mol Biol Cell*, 10 (1999) 4283-4298.

- [29] J. Perez-Miguelsanz, N. Vallecillo, F. Garrido, E. Reytor, D. Perez-Sala, M.A. Pajares, Betaine homocysteine S-methyltransferase emerges as a new player of the nuclear methionine cycle, *Biochim Biophys Acta*, 1864 (2017) 1165-1182.
- [30] O. Kabil, Y. Zhou, R. Banerjee, Human cystathionine beta-synthase is a target for sumoylation, *Biochemistry*, 45 (2006) 13528-13536.
- [31] B. Murray, H. Peng, L. Barbier-Torres, A.E. Robinson, T.W.H. Li, W. Fan, M.L. Tomasi, R.A. Gottlieb, J. Van Eyk, Z. Lu, M.L. Martinez-Chantar, S. Liangpunsakul, N.J. Skill, J.M. Mato, S.C. Lu, Methionine Adenosyltransferase alpha1 Is Targeted to the Mitochondrial Matrix and Interacts with Cytochrome P450 2E1 to Lower Its Expression, *Hepatology*, (2019).
- [32] F. Garrido, M. Pacheco, R. Vargas-Martinez, R. Velasco-Garcia, I. Jorge, H. Serrano, F. Portillo, J. Vazquez, M.A. Pajares, Identification of hepatic protein-protein interaction targets for betaine homocysteine S-methyltransferase, *PLoS One*, 13 (2018) e0199472.
- [33] M.A. Pajares, L. Alvarez, D. Perez-Sala, How are mammalian methionine adenosyltransferases regulated in the liver? A focus on redox stress, *FEBS letters*, 587 (2013) 1711-1716.
- [34] W.N. Niu, P.K. Yadav, J. Adamec, R. Banerjee, S-glutathionylation enhances human cystathionine beta-synthase activity under oxidative stress conditions, *Antioxid Redox Signal*, 22 (2015) 350-361.
- [35] M.A. Pajares, C. Duran, F. Corrales, M.M. Pliego, J.M. Mato, Modulation of rat liver S-adenosylmethionine synthetase activity by glutathione, *J Biol Chem*, 267 (1992) 17598-17605.
- [36] M.A. Turner, X. Yang, D. Yin, K. Kuczera, R.T. Borchardt, P.L. Howell, Structure and function of S-adenosylhomocysteine hydrolase, *Cell Biochem Biophys*, 33 (2000) 101-125.
- [37] W.H. Dunham, M. Mullin, A.C. Gingras, Affinity-purification coupled to mass spectrometry: basic principles and strategies, *Proteomics*, 12 (2012) 1576-1590.
- [38] A. Bruckner, C. Polge, N. Lentze, D. Auerbach, U. Schlattner, Yeast two-hybrid, a powerful tool for systems biology, *Int J Mol Sci*, 10 (2009) 2763-2788.
- [39] C. Perez, F.J. Perez-Zuniga, F. Garrido, E. Reytor, F. Portillo, M.A. Pajares, The Oncogene PDRG1 Is an Interaction Target of Methionine Adenosyltransferases, *PLoS One*, 11 (2016) e0161672.
- [40] X. Yang, Y. Xie, P. Raja, S. Li, J.N. Wolf, Q. Shen, D.M. Bisaro, X. Zhou, Suppression of methylation-mediated transcriptional gene silencing by betaC1-SAHH protein interaction during geminivirus-betasatellite infection, *PLoS Pathog*, 7 (2011) e1002329.
- [41] E.L. Huttlin, L. Ting, R.J. Bruckner, F. Gebreab, M.P. Gygi, J. Szpyt, S. Tam, G. Zarraga, G. Colby, K. Baltier, R. Dong, V. Guarani, L.P. Vaites, A. Ordureau, R. Rad, B.K. Erickson, M. Wuhr, J. Chick, B. Zhai, D. Kolippakkam, J. Mintseris, R.A. Obar, T. Harris, S. Artavanis-Tsakonas, M.E. Sowa, P. De Camilli, J.A. Paulo, J.W. Harper, S.P. Gygi, The BioPlex Network: A Systematic Exploration of the Human Interactome, *Cell*, 162 (2015) 425-440.
- [42] E.L. Huttlin, R.J. Bruckner, J.A. Paulo, J.R. Cannon, L. Ting, K. Baltier, G. Colby, F. Gebreab, M.P. Gygi, H. Parzen, J. Szpyt, S. Tam, G. Zarraga, L. Pontano-Vaites, S. Swarup, A.E. White, D.K. Schweppe, R. Rad, B.K. Erickson, R.A. Obar, K.G. Guruharsha, K. Li, S. Artavanis-Tsakonas, S.P. Gygi, J.W. Harper, Architecture of the human interactome defines protein communities and disease networks, *Nature*, 545 (2017) 505-509.

- [43] K. Tiemann, C. Garri, S.B. Lee, P.D. Malihi, M. Park, R.M. Alvarez, L.P. Yap, P. Mallick, J.E. Katz, M.E. Gross, K. Kani, Loss of ER retention motif of AGR2 can impact mTORC signaling and promote cancer metastasis, *Oncogene*, 38 (2019) 3003-3018.
- [44] N. Sahni, S. Yi, M. Taipale, J.I. Fuxman Bass, J. Coulombe-Huntington, F. Yang, J. Peng, J. Weile, G.I. Karras, Y. Wang, I.A. Kovacs, A. Kamburov, I. Krykbaeva, M.H. Lam, G. Tucker, V. Khurana, A. Sharma, Y.Y. Liu, N. Yachie, Q. Zhong, Y. Shen, A. Palagi, A. San-Miguel, C. Fan, D. Balcha, A. Dricot, D.M. Jordan, J.M. Walsh, A.A. Shah, X. Yang, A.K. Stoyanova, A. Leighton, M.A. Calderwood, Y. Jacob, M.E. Cusick, K. Salehi-Ashtiani, L.J. Whitesell, S. Sunyaev, B. Berger, A.L. Barabasi, B. Charlotteaux, D.E. Hill, T. Hao, F.P. Roth, Y. Xia, A.J.M. Walhout, S. Lindquist, M. Vidal, Widespread macromolecular interaction perturbations in human genetic disorders, *Cell*, 161 (2015) 647-660.
- [45] H. Yu, L. Tardivo, S. Tam, E. Weiner, F. Gebreab, C. Fan, N. Svrvikapa, T. Hirozane-Kishikawa, E. Rietman, X. Yang, J. Sahalie, K. Salehi-Ashtiani, T. Hao, M.E. Cusick, D.E. Hill, F.P. Roth, P. Braun, M. Vidal, Next-generation sequencing to generate interactome datasets, *Nat Methods*, 8 (2011) 478-480.
- [46] T. Rolland, M. Tasan, B. Charlotteaux, S.J. Pevzner, Q. Zhong, N. Sahni, S. Yi, I. Lemmens, C. Fontanillo, R. Mosca, A. Kamburov, S.D. Ghiassian, X. Yang, L. Ghamsari, D. Balcha, B.E. Begg, P. Braun, M. Brehme, M.P. Broly, A.R. Carvunis, D. Convery-Zupan, R. Corominas, J. Coulombe-Huntington, E. Dann, M. Dreze, A. Dricot, C. Fan, E. Franzosa, F. Gebreab, B.J. Gutierrez, M.F. Hardy, M. Jin, S. Kang, R. Kiros, G.N. Lin, K. Luck, A. MacWilliams, J. Menche, R.R. Murray, A. Palagi, M.M. Poulin, X. Rambout, J. Rasla, P. Reichert, V. Romero, E. Ruysinck, J.M. Sahalie, A. Scholz, A.A. Shah, A. Sharma, Y. Shen, K. Spirohn, S. Tam, A.O. Tejada, S.A. Trigg, J.C. Twizere, K. Vega, J. Walsh, M.E. Cusick, Y. Xia, A.L. Barabasi, L.M. Iakoucheva, P. Aloy, J. De Las Rivas, J. Tavernier, M.A. Calderwood, D.E. Hill, T. Hao, F.P. Roth, M. Vidal, A proteome-scale map of the human interactome network, *Cell*, 159 (2014) 1212-1226.
- [47] E.J. Bennett, J. Rush, S.P. Gygi, J.W. Harper, Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics, *Cell*, 143 (2010) 951-965.
- [48] H. An, L. Yang, C. Wang, Z. Gan, H. Gu, T. Zhang, X. Huang, Y. Liu, Y. Li, S.J. Chang, J. Lai, Y.B. Li, S. Chen, F.L. Sun, Interactome Analysis Reveals a Novel Role for RAD6 in the Regulation of Proteasome Activity and Localization in Response to DNA Damage, *Mol Cell Biol*, 37 (2017) e00419-00416.
- [49] C. Wan, B. Borgeson, S. Phanse, F. Tu, K. Drew, G. Clark, X. Xiong, O. Kagan, J. Kwan, A. Bezginov, K. Chessman, S. Pal, G. Cromar, O. Papoulas, Z. Ni, D.R. Boutz, S. Stoilova, P.C. Havugimana, X. Guo, R.H. Maltby, M. Sarov, J. Greenblatt, M. Babu, W.B. Derry, E.R. Tillier, J.B. Wallingford, J. Parkinson, E.M. Marcotte, A. Emili, Panorama of ancient metazoan macromolecular complexes, *Nature*, 525 (2015) 339-344.
- [50] P.C. Havugimana, G.T. Hart, T. Nepusz, H. Yang, A.L. Turinsky, Z. Li, P.I. Wang, D.R. Boutz, V. Fong, S. Phanse, M. Babu, S.A. Craig, P. Hu, C. Wan, J. Vlasblom, V.U. Dar, A. Bezginov, G.W. Clark, G.C. Wu, S.J. Wodak, E.R. Tillier, A. Paccanaro, E.M. Marcotte, A. Emili, A census of human soluble protein complexes, *Cell*, 150 (2012) 1068-1081.
- [51] A. Ismayil, Y. Haxim, Y. Wang, H. Li, L. Qian, T. Han, T. Chen, Q. Jia, A. Yihao Liu, S. Zhu, H. Deng, R. Gorovits, Y. Hong, L. Hanley-Bowdoin, Y. Liu, Cotton Leaf

- Curl Multan virus C4 protein suppresses both transcriptional and post-transcriptional gene silencing by interacting with SAM synthetase, *PLoS Pathog*, 14 (2018) e1007282.
- [52] M.C. Canizares, R. Lozano-Duran, T. Canto, E.R. Bejarano, D.M. Bisaro, J. Navas-Castillo, E. Moriones, Effects of the crinivirus coat protein-interacting plant protein SAHH on post-transcriptional RNA silencing and its suppression, *Mol Plant Microbe Interact*, 26 (2013) 1004-1015.
- [53] S. Lee, A.C. Doxey, B.J. McConkey, B.A. Moffatt, Nuclear targeting of methyl-recycling enzymes in *Arabidopsis thaliana* is mediated by specific protein interactions, *Mol Plant*, 5 (2012) 231-248.
- [54] M. Kotb, N.M. Kredich, S-Adenosylmethionine synthetase from human lymphocytes. Purification and characterization, *The Journal of biological chemistry*, 260 (1985) 3923-3930.
- [55] M.L. Martinez-Chantar, E.R. Garcia-Trevijano, M.U. Latasa, A. Martin-Duce, P. Fortes, J. Caballeria, M.A. Avila, J.M. Mato, Methionine adenosyltransferase II beta subunit gene expression provides a proliferative advantage in human hepatoma, *Gastroenterology*, 124 (2003) 940-948.
- [56] A.R. Kristensen, J. Gsponer, L.J. Foster, A high-throughput approach for measuring temporal changes in the interactome, *Nat Methods*, 9 (2012) 907-909.
- [57] B. Murray, S.V. Antonyuk, A. Marina, S.M. Van Liempd, S.C. Lu, J.M. Mato, S.S. Hasnain, A.L. Rojas, Structure and function study of the complex that synthesizes S-adenosylmethionine, *IUCrJ*, 1 (2014) 240-249.
- [58] B. Murray, S.V. Antonyuk, A. Marina, S.C. Lu, J.M. Mato, S.S. Hasnain, A.L. Rojas, Crystallography captures catalytic steps in human methionine adenosyltransferase enzymes, *Proc Natl Acad Sci U S A*, 113 (2016) 2104-2109.
- [59] H. Yang, Y. Zheng, T.W. Li, H. Peng, D. Fernandez-Ramos, M.L. Martinez-Chantar, A.L. Rojas, J.M. Mato, S.C. Lu, Methionine adenosyltransferase 2B, HuR, and sirtuin 1 protein cross-talk impacts on the effect of resveratrol on apoptosis and growth in liver cancer cells, *J Biol Chem*, 288 (2013) 23161-23170.
- [60] H. Peng, L. Dara, T.W. Li, Y. Zheng, H. Yang, M.L. Tomasi, I. Tomasi, P. Giordano, J.M. Mato, S.C. Lu, MAT2B-GIT1 interplay activates MEK1/ERK 1 and 2 to induce growth in human liver and colon cancer, *Hepatology*, 57 (2013) 2299-2313.
- [61] H. Peng, T.W. Li, H. Yang, M.P. Moyer, J.M. Mato, S.C. Lu, Methionine adenosyltransferase 2B-GIT1 complex serves as a scaffold to regulate Ras/Raf/MEK1/2 activity in human liver and colon cancer cells, *Am J Pathol*, 185 (2015) 1135-1144.
- [62] M. Xia, Y. Chen, L.C. Wang, E. Zandi, H. Yang, S. Bermanian, M.L. Martinez-Chantar, J.M. Mato, S.C. Lu, Novel function and intracellular localization of methionine adenosyltransferase 2{beta} splicing variants, *J Biol Chem*, 285 (2010) 20015-20021.
- [63] K. Ramani, S. Donoyan, M.L. Tomasi, S. Park, Role of methionine adenosyltransferase alpha2 and beta phosphorylation and stabilization in human hepatic stellate cell trans-differentiation, *J Cell Physiol*, 230 (2015) 1075-1085.
- [64] M. Vazquez-Chantada, D. Fernandez-Ramos, N. Embade, N. Martinez-Lopez, M. Varela-Rey, A. Woodhoo, Z. Luka, C. Wagner, P.P. Anglim, R.H. Finnell, J. Caballeria, I.A. Laird-Offringa, M. Gorospe, S.C. Lu, J.M. Mato, M.L. Martinez-Chantar, HuR/methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis, *Gastroenterology*, 138 (2010) 1943-1953.
- [65] Y. Chen, L.N. Yang, L. Cheng, S. Tu, S.J. Guo, H.Y. Le, Q. Xiong, R. Mo, C.Y. Li, J.S. Jeong, L. Jiang, S. Blackshaw, L.J. Bi, H. Zhu, S.C. Tao, F. Ge, Bcl2-associated

- athanogene 3 interactome analysis reveals a new role in modulating proteasome activity, *Mol Cell Proteomics*, 12 (2013) 2804-2819.
- [66] Y. Kera, Y. Katoh, M. Ohta, M. Matsumoto, T. Takano-Yamamoto, K. Igarashi, Methionine adenosyltransferase II-dependent histone H3K9 methylation at the COX-2 gene locus, *J Biol Chem*, 288 (2013) 13592-13601.
- [67] J. Wang, Z.H. Zhu, H.B. Yang, Y. Zhang, X.N. Zhao, M. Zhang, Y.B. Liu, Y.Y. Xu, Q.Y. Lei, Cullin 3 targets methionine adenosyltransferase IIalpha for ubiquitylation-mediated degradation and regulates colorectal cancer cell proliferation, *FEBS J*, 283 (2016) 2390-2402.
- [68] X. Luo, Y. Huang, M.S. Sheikh, Cloning and characterization of a novel gene PDRG that is differentially regulated by p53 and ultraviolet radiation, *Oncogene*, 22 (2003) 7247-7257.
- [69] P. Mita, J.N. Savas, S. Ha, N. Djouder, J.R. Yates, 3rd, S.K. Logan, Analysis of URI nuclear interaction with RPB5 and components of the R2TP/prefoldin-like complex, *PLoS One*, 8 (2013) e63879.
- [70] S. Boulon, B. Pradet-Balade, C. Verheggen, D. Molle, S. Boireau, M. Georgieva, K. Azzag, M.C. Robert, Y. Ahmad, H. Neel, A.I. Lamond, E. Bertrand, HSP90 and its R2TP/Prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II, *Mol Cell*, 39 (2010) 912-924.
- [71] M.E. Sardi, Y. Cai, J. Jin, S.K. Swanson, R.C. Conaway, J.W. Conaway, L. Florens, M.P. Washburn, Probabilistic assembly of human protein interaction networks from label-free quantitative proteomics, *Proc Natl Acad Sci U S A*, 105 (2008) 1454-1459.
- [72] M.L. Tomasi, M. Ryoo, K. Ramani, I. Tomasi, P. Giordano, J.M. Mato, S.C. Lu, Methionine adenosyltransferase alpha2 sumoylation positively regulate Bcl-2 expression in human colon and liver cancer cells, *Oncotarget*, 6 (2015) 37706-37723.
- [73] H.B. Yang, Y.Y. Xu, X.N. Zhao, S.W. Zou, Y. Zhang, M. Zhang, J.T. Li, F. Ren, L.Y. Wang, Q.Y. Lei, Acetylation of MAT IIalpha represses tumour cell growth and is decreased in human hepatocellular cancer, *Nat Commun*, 6 (2015) 6973.
- [74] Y. Bai, K. Markham, F. Chen, R. Weerasekera, J. Watts, P. Horne, Y. Wakutani, R. Bagshaw, P.M. Mathews, P.E. Fraser, D. Westaway, P. St George-Hyslop, G. Schmitt-Ulms, The in vivo brain interactome of the amyloid precursor protein, *Mol Cell Proteomics*, 7 (2008) 15-34.
- [75] S. Hong, B. Zhai, P. Pissios, Nicotinamide N-Methyltransferase Interacts with Enzymes of the Methionine Cycle and Regulates Methyl Donor Metabolism, *Biochemistry*, 57 (2018) 5775-5779.
- [76] M.A. Pajares, PDRG1 at the interface between intermediary metabolism and oncogenesis, *World J Biol Chem*, 8 (2017) 175-186.
- [77] H. Yang, M.E. Cho, T.W. Li, H. Peng, K.S. Ko, J.M. Mato, S.C. Lu, MicroRNAs regulate methionine adenosyltransferase 1A expression in hepatocellular carcinoma, *J Clin Invest*, 123 (2013) 285-298.
- [78] H. Yang, T. Liu, J. Wang, T.W. Li, W. Fan, H. Peng, A. Krishnan, G.J. Gores, J.M. Mato, S.C. Lu, Deregulated methionine adenosyltransferase alpha1, c-Myc, and Maf proteins together promote cholangiocarcinoma growth in mice and humans(double dagger), *Hepatology*, 64 (2016) 439-455.
- [79] M.A. Pajares, C. Duran, F. Corrales, J.M. Mato, Protein kinase C phosphorylation of rat liver S-adenosylmethionine synthetase: dissociation and production of an active monomer, *Biochem J*, 303 (Pt 3) (1994) 949-955.
- [80] A. Lepur, L. Kovacevic, R. Beluzic, O. Vugrek, Combining Unique Multiplex Gateway Cloning and Bimolecular Fluorescence Complementation (BiFC) for High-

- Throughput Screening of Protein-Protein Interactions, *J Biomol Screen*, 21 (2016) 1100-1111.
- [81] H. Ando, A. Mizutani, H. Kiefer, D. Tsuzurugi, T. Michikawa, K. Mikoshiba, IRBIT suppresses IP3 receptor activity by competing with IP3 for the common binding site on the IP3 receptor, *Mol Cell*, 22 (2006) 795-806.
- [82] A. Arnaoutov, M. Dasso, Enzyme regulation. IRBIT is a novel regulator of ribonucleotide reductase in higher eukaryotes, *Science*, 345 (2014) 1512-1515.
- [83] B. Devogelaere, E. Sammels, H. De Smedt, The IRBIT domain adds new functions to the AHCY family, *Bioessays*, 30 (2008) 642-652.
- [84] I. Grbesa, A. Kalo, R. Beluzic, L. Kovacevic, A. Lepur, F. Rokic, H. Hochberg, I. Kanter, V. Simunovic, P.M. Munoz-Torres, Y. Shav-Tal, O. Vugrek, Mutations in S-adenosylhomocysteine hydrolase (AHCY) affect its nucleocytoplasmic distribution and capability to interact with S-adenosylhomocysteine hydrolase-like 1 protein, *Eur J Cell Biol*, 96 (2017) 579-590.
- [85] M.E. Fernandez-Sanchez, T. Gonatopoulos-Pournatzis, G. Preston, M.A. Lawlor, V.H. Cowling, S-adenosyl homocysteine hydrolase is required for Myc-induced mRNA cap methylation, protein synthesis, and cell proliferation, *Mol Cell Biol*, 29 (2009) 6182-6191.
- [86] S.F. Dagher, J.L. Wang, R.J. Patterson, Identification of galectin-3 as a factor in pre-mRNA splicing, *Proc Natl Acad Sci U S A*, 92 (1995) 1213-1217.
- [87] R. d'Emmanuele di Villa Bianca, E. Mitidieri, F. Fusco, A. Russo, V. Pagliara, T. Tramontano, E. Donnarumma, V. Mirone, G. Cirino, G. Russo, R. Sorrentino, Urothelium muscarinic activation phosphorylates CBS(Ser227) via cGMP/PKG pathway causing human bladder relaxation through H2S production, *Sci Rep*, 6 (2016) 31491.
- [88] N. Agrawal, R. Banerjee, Human polycomb 2 protein is a SUMO E3 ligase and alleviates substrate-induced inhibition of cystathionine beta-synthase sumoylation, *PLoS One*, 3 (2008) e4032.
- [89] T. Schmidt-Rose, T.J. Jentsch, Reconstitution of functional voltage-gated chloride channels from complementary fragments of CLC-1, *J Biol Chem*, 272 (1997) 20515-20521.
- [90] G. Carr, N. Simmons, J. Sayer, A role for CBS domain 2 in trafficking of chloride channel CLC-5, *Biochem Biophys Res Commun*, 310 (2003) 600-605.
- [91] P.E. Lopez-Calcano, T.P. Howard, C.A. Raines, The CP12 protein family: a thioredoxin-mediated metabolic switch?, *Front Plant Sci*, 5 (2014) 9.
- [92] K.H. Pua, D.T. Stiles, M.E. Sowa, G.L. Verdine, IMPDH2 Is an Intracellular Target of the Cyclophilin A and Sanglifehrin A Complex, *Cell Rep*, 18 (2017) 432-442.
- [93] N. Zhang, L. Zhang, C. Shi, L. Zhao, D. Cui, F. Chen, Identification of Proteins Using iTRAQ and Virus-Induced Gene Silencing Reveals Three Bread Wheat Proteins Involved in the Response to Combined Osmotic-Cold Stress, *J Proteome Res*, 17 (2018) 2256-2281.
- [94] S. Ignoul, J. Eggermont, CBS domains: structure, function, and pathology in human proteins, *Am J Physiol Cell Physiol*, 289 (2005) C1369-1378.
- [95] R.M. Ewing, P. Chu, F. Elisma, H. Li, P. Taylor, S. Climie, L. McBroom-Cerajewski, M.D. Robinson, L. O'Connor, M. Li, R. Taylor, M. Dharsee, Y. Ho, A. Heilbut, L. Moore, S. Zhang, O. Ornatsky, Y.V. Bukhman, M. Ethier, Y. Sheng, J. Vasilescu, M. Abu-Farha, J.P. Lambert, H.S. Duewel, Stewart, II, B. Kuehl, K. Hogue, K. Colwill, K. Gladwish, B. Muskat, R. Kinach, S.L. Adams, M.F. Moran, G.B. Morin, T. Topaloglou, D. Figeys, Large-scale mapping of human protein-protein interactions by mass spectrometry, *Mol Syst Biol*, 3 (2007) 89.

- [96] M.Y. Hein, N.C. Hubner, I. Poser, J. Cox, N. Nagaraj, Y. Toyoda, I.A. Gak, I. Weisswange, J. Mansfeld, F. Buchholz, A.A. Hyman, M. Mann, A human interactome in three quantitative dimensions organized by stoichiometries and abundances, *Cell*, 163 (2015) 712-723.
- [97] Y. Xu, W. Yang, J. Shi, B.R. Zetter, Prohibitin 1 regulates tumor cell apoptosis via the interaction with X-linked inhibitor of apoptosis protein, *J Mol Cell Biol*, 8 (2016) 282-285.
- [98] W. Fan, H. Yang, T. Liu, J. Wang, T.W. Li, N. Mavila, Y. Tang, J. Yang, H. Peng, J. Tu, A. Annamalai, M. Nouredin, A. Krishnan, G.J. Gores, M.L. Martinez-Chantar, J.M. Mato, S.C. Lu, Prohibitin 1 suppresses liver cancer tumorigenesis in mice and human hepatocellular and cholangiocarcinoma cells, *Hepatology*, 65 (2017) 1249-1266.
- [99] K.S. Ko, M.L. Tomasi, A. Iglesias-Ara, B.A. French, S.W. French, K. Ramani, J.J. Lozano, P. Oh, L. He, B.L. Stiles, T.W. Li, H. Yang, M.L. Martinez-Chantar, J.M. Mato, S.C. Lu, Liver-specific deletion of prohibitin 1 results in spontaneous liver injury, fibrosis, and hepatocellular carcinoma in mice, *Hepatology*, 52 (2010) 2096-2108.
- [100] A.L. Theiss, S.V. Sitaraman, The role and therapeutic potential of prohibitin in disease, *Biochim Biophys Acta*, 1813 (2011) 1137-1143.
- [101] M. Desai, J.B. Chauhan, Predicting the functional and structural consequences of nsSNPs in human methionine synthase gene using computational tools, *Syst Biol Reprod Med*, 65 (2019) 288-300.
- [102] K.R. Wolthers, N.S. Scrutton, Cobalamin uptake and reactivation occurs through specific protein interactions in the methionine synthase-methionine synthase reductase complex, *FEBS J*, 276 (2009) 1942-1951.
- [103] M.B. Fofou-Caillierez, N.T. Mrabet, C. Chery, N. Dreumont, J. Flayac, M. Pupavac, J. Paoli, J.M. Alberto, D. Coelho, J.M. Camadro, F. Feillet, D. Watkins, B. Fowler, D.S. Rosenblatt, J.L. Gueant, Interaction between methionine synthase isoforms and MMACHC: characterization in cblG-variant, cblG and cblC inherited causes of megaloblastic anaemia, *Hum Mol Genet*, 22 (2013) 4591-4601.
- [104] C. Bassila, R. Ghemrawi, J. Flayac, D.S. Froese, M.R. Baumgartner, J.L. Gueant, D. Coelho, Methionine synthase and methionine synthase reductase interact with MMACHC and with MMADHC, *Biochim Biophys Acta Mol Basis Dis*, 1863 (2017) 103-112.
- [105] C. Sandu, P. Nick, D. Hess, E. Schiltz, T.A. Garrow, R. Brandsch, Association of betaine-homocysteine S-methyltransferase with microtubules, *Biol Chem*, 381 (2000) 619-622.
- [106] G.Q. Bai, J. Cheng, S.L. Zhang, Y.P. Huang, L. Wang, Y. Liu, S.M. Lin, Screening of hepatocyte proteins binding to complete S protein of hepatitis B virus by yeast-two hybrid system, *World J Gastroenterol*, 11 (2005) 3899-3904.
- [107] C.R. Liang, C.K. Leow, J.C. Neo, G.S. Tan, S.L. Lo, J.W. Lim, T.K. Seow, P.B. Lai, M.C. Chung, Proteome analysis of human hepatocellular carcinoma tissues by two-dimensional difference gel electrophoresis and mass spectrometry, *Proteomics*, 5 (2005) 2258-2271.
- [108] A. Ichikawa, Y. Ohashi, S. Terada, S. Natsuka, K. Ikura, In vitro modification of betaine-homocysteine S-methyltransferase by tissue-type transglutaminase, *Int J Biochem Cell Biol*, 36 (2004) 1981-1992.
- [109] L.H. Chadwick, S.E. McCandless, G.L. Silverman, S. Schwartz, D. Westaway, J.H. Nadeau, Betaine-homocysteine methyltransferase-2: cDNA cloning, gene sequence, physical mapping, and expression of the human and mouse genes, *Genomics*, 70 (2000) 66-73.

- [110] T. Zhang, W. Wei, O. Dirsch, T. Kruger, C. Kan, C. Xie, O. Kniemeyer, H. Fang, U. Settmacher, U. Dahmen, Identification of Proteins Interacting with Cytoplasmic High-Mobility Group Box 1 during the Hepatocellular Response to Ischemia Reperfusion Injury, *Int J Mol Sci*, 18 (2017) 167.
- [111] H.S. Kim, J.M. Kim, K.B. Roh, H.H. Lee, S.J. Kim, Y.H. Shin, B.L. Lee, Rat liver 10-formyltetrahydrofolate dehydrogenase, carbamoyl phosphate synthetase 1 and betaine homocysteine S-methyltransferase were co-purified on Kunitz-type soybean trypsin inhibitor-coupled sepharose CL-4B, *J Biochem Mol Biol*, 40 (2007) 604-609.
- [112] J. Villen, S.A. Beausoleil, S.A. Gerber, S.P. Gygi, Large-scale phosphorylation analysis of mouse liver, *Proc Natl Acad Sci U S A*, 104 (2007) 1488-1493.
- [113] E.L. Huttlin, M.P. Jedrychowski, J.E. Elias, T. Goswami, R. Rad, S.A. Beausoleil, J. Villen, W. Haas, M.E. Sowa, S.P. Gygi, A tissue-specific atlas of mouse protein phosphorylation and expression, *Cell*, 143 (2010) 1174-1189.
- [114] S.A. Wagner, P. Beli, B.T. Weinert, C. Scholz, C.D. Kelstrup, C. Young, M.L. Nielsen, J.V. Olsen, C. Brakebusch, C. Choudhary, Proteomic analyses reveal divergent ubiquitylation site patterns in murine tissues, *Mol Cell Proteomics*, 11 (2012) 1578-1585.
- [115] A. Lundby, K. Lage, B.T. Weinert, D.B. Bekker-Jensen, A. Secher, T. Skovgaard, C.D. Kelstrup, A. Dmytriiev, C. Choudhary, C. Lundby, J.V. Olsen, Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns, *Cell Rep*, 2 (2012) 419-431.
- [116] B.T. Weinert, C. Scholz, S.A. Wagner, V. Iesmantavicius, D. Su, J.A. Daniel, C. Choudhary, Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation, *Cell Rep*, 4 (2013) 842-851.
- [117] K. Drew, C. Lee, R.L. Huizar, F. Tu, B. Borgeson, C.D. McWhite, Y. Ma, J.B. Wallingford, E.M. Marcotte, Integration of over 9,000 mass spectrometry experiments builds a global map of human protein complexes, *Mol Syst Biol*, 13 (2017) 932.
- [118] H. Yang, Z.Z. Huang, J. Wang, S.C. Lu, The role of c-Myb and Sp1 in the up-regulation of methionine adenosyltransferase 2A gene expression in human hepatocellular carcinoma, *FASEB J*, 15 (2001) 1507-1516.
- [119] L. Jiang, X. Luo, J. Shi, H. Sun, Q. Sun, M.S. Sheikh, Y. Huang, PDRG1, a novel tumor marker for multiple malignancies that is selectively regulated by genotoxic stress, *Cancer Biol Ther*, 11 (2011) 567-573.
- [120] A.P. Feinberg, B. Tycko, The history of cancer epigenetics, *Nat Rev Cancer*, 4 (2004) 143-153.
- [121] C. Perez, F.J. Perez-Zuniga, F. Garrido, E. Reytor, F. Portillo, M.A. Pajares, Correction: The Oncogene PDRG1 Is an Interaction Target of Methionine Adenosyltransferases, *PLoS One*, 11 (2016) e0163761.
- [122] S. Saigusa, K. Tanaka, Y. Toiyama, K. Matsushita, M. Kawamura, Y. Okugawa, J. Hiro, Y. Inoue, K. Uchida, Y. Mohri, M. Kusunoki, Gene expression profiles of tumor regression grade in locally advanced rectal cancer after neoadjuvant chemoradiotherapy, *Oncol Rep*, 28 (2012) 855-861.
- [123] J. Wang, X. Zhang, L. Wang, Y. Yang, Z. Dong, H. Wang, L. Du, C. Wang, MicroRNA-214 suppresses oncogenesis and exerts impact on prognosis by targeting PDRG1 in bladder cancer, *PLoS One*, 10 (2015) e0118086.
- [124] A. Derfoul, A.H. Juan, M.J. Difilippantonio, N. Palanisamy, T. Ried, V. Sartorelli, Decreased microRNA-214 levels in breast cancer cells coincides with increased cell proliferation, invasion and accumulation of the Polycomb Ezh2 methyltransferase, *Carcinogenesis*, 32 (2011) 1607-1614.

- [125] E. Vire, C. Brenner, R. Deplus, L. Blanchon, M. Fraga, C. Didelot, L. Morey, A. Van Eynde, D. Bernard, J.M. Vanderwinden, M. Bollen, M. Esteller, L. Di Croce, Y. de Launoit, F. Fuks, The Polycomb group protein EZH2 directly controls DNA methylation, *Nature*, 439 (2006) 871-874.
- [126] R. Hrstka, P. Bouchalova, E. Michalova, E. Matoulkova, P. Muller, P.J. Coates, B. Vojtesek, AGR2 oncoprotein inhibits p38 MAPK and p53 activation through a DUSP10-mediated regulatory pathway, *Mol Oncol*, 10 (2016) 652-662.
- [127] S. Hong, J.M. Moreno-Navarrete, X. Wei, Y. Kikukawa, I. Tzamelis, D. Prasad, Y. Lee, J.M. Asara, J.M. Fernandez-Real, E. Maratos-Flier, P. Pissios, Nicotinamide N-methyltransferase regulates hepatic nutrient metabolism through Sirt1 protein stabilization, *Nat Med*, 21 (2015) 887-894.

FIGURE LEGENDS

Fig. 1. Homocysteine metabolism. The figure shows the main reactions involved in homocysteine metabolism that belong to the methionine (pink) and the folate (yellow) cycles, as well as to the transsulfuration pathway (light grey). The enzymes are depicted in their oligomeric state, except for the methyltransferase family. Metabolites produced in the pathway appear in salmon boxes, whereas substrates provided by other routes are included in green boxes. The different substrates used by cystathionine β -synthase (CBS), and its corresponding products, appear in black or red font. Levels of S-adenosylmethionine production due to the existence of different methionine adenosyltransferase (MAT) isoenzymes are depicted with arrows of diverse size and color: high levels (blue) and low levels (orange). Abbreviations: AHCY, S-adenosylhomocysteine hydrolase; BHMT and BHMT2, betaine homocysteine S-methyltransferases 1 and 2; CBS, cystathionine β -synthase; CTH, cystathionase; DMG, dimethylglycine; α KB, α -ketobutyrate; MAT I and MAT III, homo-tetramer and homo-dimer composed by MAT α 1 subunits; MAT II, methionine adenosyltransferase composed by a MAT α 2 homo-dimer and one MAT β subunit; (MAT α 2)₄, homo-tetramer

of MAT α 2 subunits; MTases, methyltransferases; 5-MTHF, 5-methyl tetrahydrofolate; MTR, methionine synthase; PP_i, pyrophosphate; P_i, inorganic phosphate; THF, tetrahydrofolate; X, methylation substrate; X-CH₃, methylated product.

Fig. 2. Methionine adenosyltransferase MAT α 2 catalytic subunit interactions. The figure summarizes confirmed protein-protein interactions involving MAT α 2 and their functional role. Modifications by acetylation (Ac) or ubiquitination (Ub) are indicated. Transient interaction with histone lysine acetyltransferase P300 allows acetylation that favors cullin 3 binding, which then serves as a scaffold for ubiquitination by the BCR complex. Posttranslationally modified MAT α 2 is then degraded by the proteasome. Additional interactions are devoted to control AdoMet synthesis by modifying the affinity and/or V_{max} of MAT α 2, while others are involved in gene regulation. Abbreviations: ARE, antioxidant response element; BCR, BCR ubiquitination complex; Maf, MAF BZIP transcription factor; MafK, MAF BZIP transcription factor K; MAT β , regulatory methionine adenosyltransferase subunit; PDRG1, p53 and DNA damage-regulated gene 1; P300, histone lysine acetyltransferase P300; SETDB1, histone-lysine N-methyltransferase SETDB1.

Fig. 3. Protein interactions involving methionine adenosyltransferase MAT α 1 catalytic subunit. The figure shows protein-protein interactions confirmed for MAT α 1, which take place in three subcellular compartments: nucleus (grey) mitochondria (light blue) and cytoplasm (white). As an example of gene regulatory effects, binding to a promoter E-box of one of the complexes involving MAT α 1 in tumors is depicted. The functional role of the interactions verified or suggested (?) is indicated, as well as the existence of an unknown methyltransferase (MTase) for cytochrome methylation (Me). Abbreviations: AHCY, S-adenosylhomocysteine hydrolase; BHMT, betaine homocysteine S-methyltransferase; cMaf, MAF BZIP transcription factor; CYP2E1,

cytochrome P450 2E1; HSPs, heat shock proteins; MafG, MAF BZIP transcription factor G; MAT α 1, methionine adenosyltransferase MAT α 1 catalytic subunit; NNMT, nicotinamide N-methyltransferase; Max, MYC associated factor X; Mnt, MAX network transcriptional repressor; PDRG1, p53 and DNA damage regulated gene 1.

Fig. 4. Protein interaction partners for betaine homocysteine S-methyltransferase.

The figure shows interactions confirmed for betaine homocysteine S-methyltransferase (BHMT, boxed in red) and their functional implications; boxed in orange interaction targets of the methionine cycle and in light blue proteins belonging to other pathways. Putative functions inferred, but not experimentally proven, are indicated with dotted arrows or lines; possible activating or inhibiting effects are indicated within green or red circles. Channeling of methionine (Met) is indicated with white arrows within the proteins implicated and phosphorylation is denoted as P. Abbreviations: AHCY, S-adenosylhomocysteine hydrolase; Aldob, aldolase B; BHMT2, betaine homocysteine S-methyltransferase 2; HPD, 4-hydroxyphenylpyruvic acid dioxygenase; MAT α 1, methionine adenosyltransferase MAT α 1 catalytic subunit; MAT α 2, methionine adenosyltransferase MAT α 2 catalytic subunit; NNMT, nicotinamide N-methyltransferase; Prkaca, catalytic subunit of protein kinase A.

Fig. 5. Established interplays through protein-protein interactions. The figure shows colored boxes including enzymes of homocysteine metabolism (salmon), their interaction partners (dark blue) and indirect partners (light blue). A light green circle in the middle includes proteins of the methionine cycle, whereas transsulfuration and interplays established with additional routes/reactions are indicated within turquoise and light yellow circles, respectively. Colored double headed arrows indicate the interactions confirmed for: betaine homocysteine S-methyltransferase (BHMT) in red; methionine adenosyltransferase catalytic subunits (MAT α 1 and MAT α 2) in black; S-

adenosylhomocysteine hydrolase (AHCY) in green; methionine synthase (MTR) in orange; cystathionine β -synthase (CBS) in grey; and interactions of the direct partners with third parties in blue. Most interactions included in the figure are cytoplasmic, but others take place either in the nucleus or the mitochondria no such difference has been indicated for clarity. Abbreviations: AHCYL1, S-adenosylhomocysteine hydrolase-like 1; Aldob, aldolase B; Art27/UXT, protein UXT; Bcl2, apoptosis regulator Bcl2; CYP2E1, cytochrome P450 2E1; FIP1L1, pre-mRNA 3'-end-processing factor FIP1; GIT1, ARF GTPases-activating protein GIT1; hPc2, polycomb protein hPc2; HPD, 4-hydroxyphenylpyruvic acid dioxygenase; HSPs, heat shock proteins; HuR, ELAV-like protein 1; Max, MYC associated factor; Maf, MAF BZIP transcription factor; MafG and MafK, MAF BZIP transcription factors G and K; MMACHC and MMADHC methylmalonic aciduria and homocystinuria type C and D proteins; Mnt, MAX network transcriptional repressor; MTRR, methionine synthase reductase; NNMT, nicotinamide N-methyltransferase; PAPOLA, poly(A) polymerase alpha; PDCD7, programmed cell death protein 7; PDRG1, p53 and DNA damage-regulated gene 1; Prkaca, cAMP-dependent protein kinase catalytic subunit alpha RRMI, ribonucleotide reductase subunit; URI, unconventional prefoldin RPB5 interactor.