4

Biochemical and Biological Functions of Isoprenylcysteine Carboxyl Methyltransferase

HELEN COURT[a] • KALUB HAHNE[b] • MARK R. PHILIPS[a] • CHRISTINE A. HRYCYNA[b]

[a]*Departments of Medicine, Cell Biology and Pharmacology and NYU Cancer Institute*
NYU Langone School of Medicine
New York, USA

[b]*Department of Chemistry and the Purdue Center for Cancer Research*
Purdue University, West Lafayette
Indiana, USA

I. Abstract

Many eukaryotic proteins are initially synthesized with a ''CaaX motif'' or CXC residues at the C-terminus and undergo a series of sequential posttranslational modifications that result in an isoprenylated and methylated cysteine residue at their C-terminus. Isoprenylcysteine carboxyl methyltransferase (Icmt) is the sole enzymatic component responsible for the carboxyl methylation of these proteins. As many CaaX- and CXC-containing proteins require membrane localization for their function, these modifications are thought to work in concert to promote membrane association. In particular, methyl esterification of the prenylcysteine residue neutralizes the negative charge at the C-terminus. In addition to promoting membrane localization, methyl esterification may also facilitate necessary protein–protein interactions with other cellular proteins. The requirement of methylation for localization or function varies greatly for

DOI: 10.1016/B978-0-12-415922-8.00004-5

different prenylcysteine-containing substrates and more work is required to fully assess how methylation affects the function of these different proteins. This review covers both the biochemistry of Icmt and cellular roles of isoprenylcysteine carboxyl methylation.

II. Introduction

Many eukaryotic proteins are initially synthesized with a C-terminal ''CaaX motif,'' where C is cysteine, ''a'' is generally an aliphatic residue, and X is one of a number of amino acids [1]. This motif signals a series of sequential posttranslational modifications that include isoprenylation of the cysteine residue by either farnesyltransferase (FTase) or geranylgeranyltransferase I (GGTase I), followed by endoproteolysis of the aaX sequence and last, methyl esterification of the newly exposed cysteine residue by Icmt. Over 300 proteins have been found to contain a CaaX motif and are therefore putative substrates for this processing pathway [2]. The confirmed substrates for these modifications include the **a**-factor yeast-mating peptide, Ras proteins and closely related GTPases, Rho family GTPases, Rheb, the γ subunits of heterotrimeric G proteins, the nuclear lamins, and a few enzymes such as rhodopsin kinase. Thus, CaaX proteins that are substrates for Icmt include a broad range of signaling molecules that regulate, among other processes, cell growth, the actin cytoskeleton, nuclear architecture, and vision. Rab proteins are small GTPases that regulate vesicular trafficking and, with 70 members, represent the largest family of Ras-related proteins. Rab proteins ending with a CC or CXC motif, where the X amino acid is an Ala, Ser, or Gly, are also substrates for prenylation although by a distinct prenyltransferase, geranylgeranyltransferase II (GGTase II). CXC Rab proteins are modified with two geranylgeranyl lipids and have been found to be methyl esterified by Icmt [3–5]. Icmt also methylates the few Rab proteins that contain a CaaX motif [6].

The methyl esterification of the α-carboxyl group of a C-terminal prenylated cysteine residue of a protein was first identified in the peptidyl sex hormones of the jelly fungi *Tremella mesenterica* and *Tremella brasiliensis* [7,8] and shortly afterward in the **a**-factor-mating peptide of *Saccharomyces cerevisiae* [9]. In these fungal species, a haploid yeast cell of one mating type responds to the presence of mating peptides released from a nearby haploid yeast cell of the opposite mating type by undergoing a cell cycle arrest and forming a polarized mating projection or conjugation tube. The two haploid cells then fuse to generate a diploid cell. Mutations in any of the genes involved in the molecular pathways of yeast mating can result in a sterile phenotype [10–12]. Many of the mating peptides from several different

fungal species contain a C-terminal CaaX motif and are therefore substrates for the posttranslational processing pathway that includes prenylation, cleavage of the aaX, and the subsequent methylation of the C-terminal prenylcysteine. The addition of the C-terminal methyl group, which occurs as the final step in the CaaX protein posttranslational processing pathway, was found to be absolutely required for the biological activity of these mating pheromones [8,9,13–15].

Subsequently, methyl esterification has been found to occur on a range of proteins containing prenylated cysteine residues at their C-termini, notably the Ras proto-oncoprotein [16,17]. Since the methyl ester was found to be essential for the function of the fungal-mating pheromones, the contribution of methyl esterification to the biology of Ras and other substrates is therefore of particular interest, especially in the field of cancer biology, where the ability to target the activity of Ras pharmacologically could have a great impact on tumor growth and human health. This chapter describes the genetic, biochemical, and cell biological studies carried out to elucidate the requirement of methyl esterification for the function of different prenylated proteins and the characterization of the enzyme that catalyzes this modification.

III. Icmt: Structure and Biological Function

To date, the only enzyme identified that catalyzes the C-terminal methyl esterification of prenylated cysteine residues is Icmt. An ortholog of this gene was first identified in *S. cerevisiae* [18,19]. Using an *in vitro* assay to detect methyltransferase activity, it was established that the methyltransferase resided in enriched *S. cerevisiae* membrane fractions and that methyltransferase activity was completely lacking in membranes isolated from a *Δste14* deletion strain [18]. The *STE14* gene was subsequently cloned and found to encode the methyltransferase that mediates the C-terminal methylation of both the **a**-factor-mating peptide and Ras proteins [20]. It was found that methylation of the **a**-factor-mating peptide by Ste14p was required for its export by the ABC transporter Ste6p [21] and for interaction with the **a**-factor receptor on α-cells [14]. Mutation of the *STE14* gene, therefore, resulted in the loss of biological activity of the **a**-factor-mating peptide and a sterile phenotype. However, the loss of *STE14* did not compromise cellular viability. Since *RAS* genes are required for viability in *S. cerevisiae*, it was deduced that the function of Ras proteins did not require methylation under normal conditions. Viability notwithstanding, *Δste14* strains had less Ras associated with membrane than did wild-type strains, indicating that although methyl esterification of Ras may

not be required for function, it did play a role in membrane association. Given the differences between **a**-factor and Ras, it is apparent that the role of methylation may vary greatly for different substrates.

A mammalian methyltransferase with activity toward prenylated cysteine-containing substrates was first detected in rat liver microsomal membranes [22,23]. The human ortholog of the *STE14* gene was later cloned from myeloid cells and called prenylcysteine carboxyl methyltransferase (pcCMT) [24], which later became human isoprenylcysteine carboxyl methyltransferase (hIcmt). The deduced amino acid sequence of the hIcmt protein predicted that the structure would be that of a multiple membrane-spanning protein. The hIcmt protein was found to be homologous to Ste14p (26% identical) except for a N-terminal extension consisting of 65 amino acids and two hydrophobic stretches, which had homology (36% identical) to amino acids 750–821 of the human band 3 anion transporter. Consistent with this observation, Ste14p was found to contain six membrane-spanning domains [25], while hIcmt contains eight [26]. It has been hypothesized that the N-terminal extension of hIcmt may have a regulatory function. Hydropathy analysis of both hIcmt and Ste14p suggested that the N- and C-termini are disposed toward the cytosol, and that both proteins contain a conserved helix-turn-helix comprising transmembrane segments 5–6 in Ste14p (Figure 4.1) and 7–8 in hIcmt. Epitope tagging and fluorescence experiments on both yeast Ste14p and hIcmt confirmed these predicted topologies [26,28].

Like all methyltransferases, the Icmt family of methyltransferases use *S*-adenosyl-L-methionine (SAM, AdoMet) as the methyl donor. However, Icmts do not share a conserved SAM factor-binding motif found in many known protein, DNA, and/or RNA methyltransferases, suggesting parallel evolution of two distinct SAM-binding domains [21,29]. Sequence alignment of Icmt orthologs has identified high interspecies sequence homology among the members of this family. This alignment revealed 42 identical residues and 34 similar residues between 15 species, including a number of conserved residues known to result in a loss of function when mutated in Ste14p (G31E, L81F, G132R, S148F, P173L, E213D, E213Q, E214D, and L217S) [25].

The sequence alignment also revealed a highly conserved C-terminus with two recognizable binding motifs, motif A and motif B. Motif A (LVxxGxYxxxRHPxYxG) comprises residues L161 through G177 in Ste14p (L198 to G214 in hIcmt) followed by a hydrophobic stretch of amino acids (transmembrane helices 5 and 6 of Ste14p and 7 and 8 of hIcmt) [25] (Figure 4.1). Motif B (xRxxxEExxLxxxFGxxxxEYxxxVxxxxP) spans from residues R209 to P237 in Ste14 (R245 to P274 in hIcmt) and contains the enzymatically essential glutamate residues (E213 and E214). Alanine-scanning mutagenesis of these highly conserved regions has

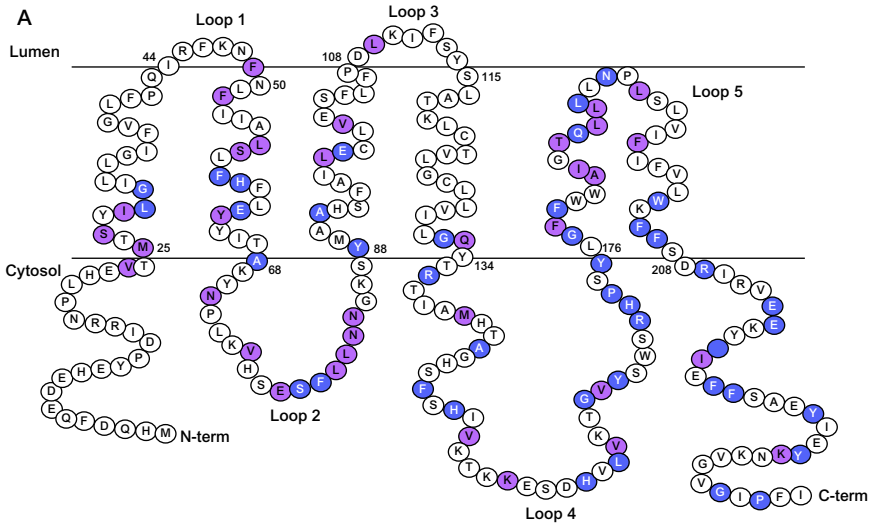

FIG. 4.1. Topology, conservation, and RHP motif of the Ste14p of *S. cerevisiae*. (A) Hydropathy plots predict six transmembrane segments (TMs). In this model, the N-and C-termini are disposed toward the cytosol. TM 5 and 6 are proposed to form a helix-turn-helix helical hairpin within the membrane [25]. Fifteen unique Icmt protein sequences were aligned using ClustalW 2.0.1.1 [27]. The blue residues denote amino acid identity and the magenta residues denote amino acid similarity. The C-terminal portion of the enzyme (136–239) contains the majority of the identical amino acids. (B) Sequence of the RHP motif, a C-terminal consensus sequence common to Icmt enzymes, a number of bacterial open reading frames, and two phosphatidylethanolamine methyltransferases. Numbers denote the amino acid position in Ste14p.

identified 31 loss-of-function mutants in Ste14p that retain less than 5% of activity compared to the wild-type enzyme (Hrycyna laboratory, unpublished data). Additionally, mutational analysis of conserved residues in hIcmt identified four residues (E107, F124, W241, and E251) that, when mutated to alanine, resulted in less than 30% of the activity of the wild-type enzyme [26]. The E251 residue corresponds to E213 of Ste14p, which is critical for function [25].

Recent data from chemical cross-linking, co-immunoprecipitation, and co-purification experiments suggest that Ste14p may function as a homodimer or higher order oligomer [30]. A tandem ^{31}GXXXGXXXG^{39} motif was identified in the first transmembrane segment of Ste14p. This GXXXG motif is highly conserved and has been found to be important in the dimerization of a number of integral single and multiple membrane-spanning

proteins by allowing close packing of two interacting alpha helices [31]. Interestingly, expression of functionally inactive mutants of Ste14p, such as the E213Q mutant, resulted in a dominant-negative effect on methyltransferase activity when co-purified with wild-type Ste14p. These data suggest that dimerization or oligomerization may be required for Ste14p function. The GXXXG motif is also found in the third transmembrane segment of human Icmt, although it remains to be determined if dimerization is a conserved feature of Icmt orthologs.

Both hIcmt and *S. cerevisiae* Ste14p are localized in the membranes of the endoplasmic reticulum (ER) [24,28]. Although this result was initially surprising because the target membrane of many of the substrates of Icmt is the plasma membrane, this finding helped to elucidate the trafficking pathway of some of these substrates to the plasma membrane via the ER and Golgi, including the Ras proteins [32] (Figure 4.2). Interestingly, the protease Ras-converting enzyme 1 (Rce1) that carries out the endoproteolysis event [34–36] (see accompanying Chapter 10), like Icmt, is also a polytopic membrane protein localized to the ER [24,37]. At present, it is unclear whether these enzymes form a processing complex in the ER membrane.

The catalytic mechanism of Icmt is proposed to be an ordered bi–bi kinetic reaction [38,39]. In this mechanism, the methyl donor, SAM, binds to the active site first, followed by binding of the prenylcysteine substrate. Once the transfer reaction is complete, the methylated product is released followed by dissociation of *S*-adenosyl-L-homocysteine (AdoHcy, SAH). Further biochemical and biophysical experiments are underway to further elucidate the mechanism of methyl donor and acceptor binding and catalysis of these unique enzymes.

IV. ICMT Substrate Specificity

The activity of Icmt was first studied using isoprenylated analogs of the synthetic peptide, LARYKC [22]. These studies demonstrated that only *S*-farnesyl-LARYKC and *S*-geranylgeranyl-LARYKC were substrates for Icmt, whereas the *S*-geranyl-LARYKC and *S*-alkyl-LARYKC derivatives were not [22]. It was also determined that the small molecules AFC (*N*-acetyl-*S*-farnesyl-L-cysteine), AGGC (*N*-acetyl-*S*-geranylgeranyl-L-cysteine), and FTP (*S*-farnesylthiopropionic acid) are also substrates of rat and yeast Icmt [40,41]. Further, it was determined that Icmt recognizes and turns over both AFC and AGGC equally [40,42,43]. Together, these initial results suggested that a farnesyl moiety attached to a sulfur atom

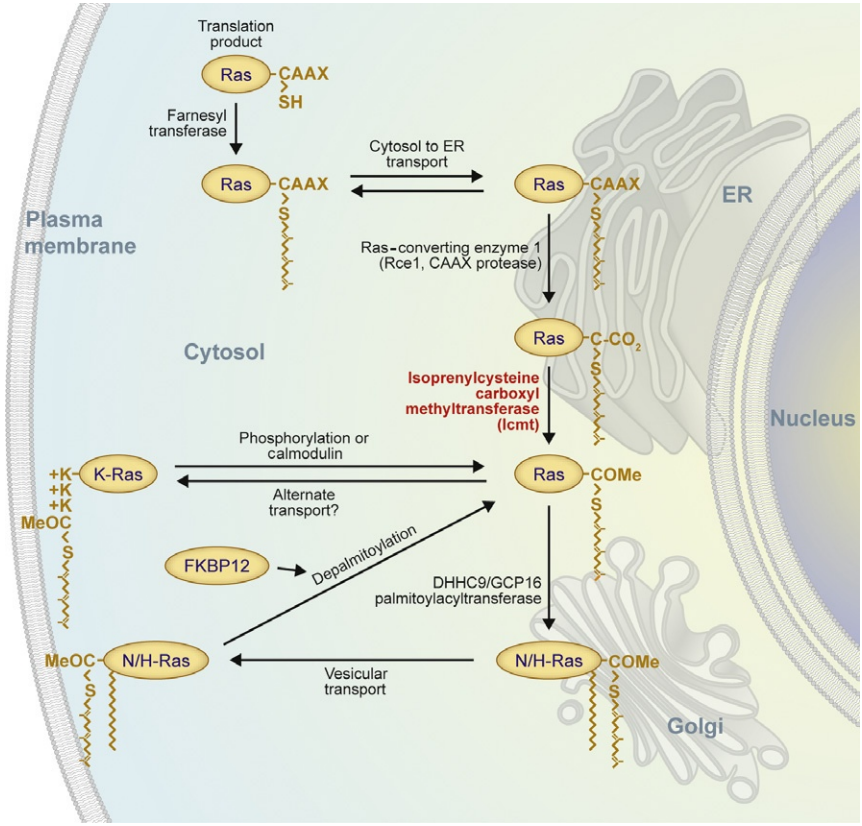

FIG. 4.2. Ras processing and trafficking. Ras and other CaaX proteins are translated in the cytosol on free polysomes. Immediately following translation, they become substrates for one of two cytosolic prenyltransferases. Prenylation targets the proteins to the ER where they encounter the subsequent CaaX processing enzymes, Rce1 and Icmt. Once CaaX processing is complete, the pathways used by the various isoforms diverge. K-Ras is sent to the plasma membrane via an uncharacterized pathway and can be returned to the endomembrane following phosphorylation of the hypervariable region or through the action of calcium/calmodulin. In contrast, N-Ras and H-Ras are further processed on the Golgi by a palmitoylacyltransferase and then sent to the plasma membrane via vesicular transport. Retrograde traffic of N-Ras and H-Ras back to the Golgi occurs following depalmitoylation. In the case of H-Ras, this process is regulated by the peptidyl-prolyl isomerase FKBP12. Adapted with permission from Ref. [33].

represents the minimal recognition unit for substrate activity [23,42,44,45]. More recent studies have shown that maintenance of the first isoprene group is especially important for substrate activity [46] (Hrycyna and Gibbs laboratories, unpublished data).

The sulfur atom present in AFC, FTP, and presumably other substrates is of great importance for Icmt enzymatic function. AFC or FTP analogs in which the sulfur atom was replaced with oxygen, selenium, sulfoxide, or an amide were found to be poor substrates of Icmt [45]. A desthio carbon for sulfur analog of AFC was also not a substrate of Icmt, again suggesting an essential role for the sulfur in recognition by the enzyme [47]. More recently, an AFC analog was synthesized in which the essential sulfur atom was replaced with a triazole moiety and determined to be a poor substrate for Icmt, again suggesting that the sulfur atom is indispensable for substrate recognition and the enzymatic function of Icmt [48].

To date, only one member of the Icmt family, Ste14p, has been purified and functionally reconstituted into proteoliposomes [43]. Upon solubilization by 1% dodecyl maltoside (DDM), Ste14p retained 90% of wild-type enzymatic activity as compared to crude membrane fractions [43]. Purified Ste14p was then functionally reconstituted into liposomes made from *Escherichia coli* lipids and activity was demonstrated using AFC, AGGC, and partially purified Ras2p as substrates. Further kinetic analysis determined that purified Ste14p has no preference for either AFC or AGGC as demonstrated by their nearly identical specificity constants ($K_{cat}/K_{m(app)}$) of 0.99×10^{-4} and $1.0 \times 10^{-4}\ M^{-1}\ s^{-1}$ for AFC and AGGC, respectively [43]. These data along with earlier studies on a TrpE-Ste14p fusion protein show that Icmt is the sole enzymatic component necessary to methyl esterify the α-carboxyl of isoprenylated cysteine residues [43,49]. Unfortunately, despite significant efforts, hIcmt has proven refractory to functional purification and reconstitution. Active hIcmt could not be extracted with detergent from leukocyte membranes in active form nor could its enzymatic activity be restored upon reconstitution into liposomes [23].

V. Effect of Methylation by Icmt on Substrate Function: *In Vitro* Studies

The role of methylation on substrate function remains incompletely understood due to the large number of substrates and the wide range of functions carried out by these substrates. However, many genetic and biochemical studies have identified important roles for Icmt in the function of specific substrates. Early studies utilized a pig liver esterase to demethylate the βγ subunit of transducin, the retinal heterotrimeric G protein (T) [50]. These studies demonstrated a two-fold reduction in the level of GTPγS exchange by the unmethylated $T_{\beta\gamma}$-OH subunit in the presence of T_α and photoactivated membrane-bound rhodopsin compared to the methylated $T_{\beta\gamma}$-OCH_3 form [51,52], and a 10-fold reduction in the ability to activate

phosphatidylinositol-specific phospholipase C (PIPLC) and phosphoinositide 3-kinase (PI3K) [53]. However, there was no difference in the activity of $T_{\beta\gamma}$-OH and $T_{\beta\gamma}$-OCH_3 in detergent extracts [51]. This result suggests that methylation of the $T_{\beta\gamma}$ subunit increases the activity of the G protein by increasing the membrane affinity of the $T_{\beta\gamma}$ subunit and thus facilitating the interaction with T_α and rhodopsin [54].

Methylation has also been found to enhance the membrane affinity of a number of different Icmt substrates. Of particular interest has been the effect of methylation on the biology of the proto-oncoprotein Ras. In an early study, K-Ras produced by *in vitro* translation was found to be farnesylated, but further modification including proteolysis and methylation required incubation with intracellular membranes. Unmethylated K-Ras produced by *in vitro* translation in the presence of pancreatic microsomes and an inhibitor of methylation, methylthioadenosine (MTA), was found to associate less efficiently with P100 membrane fractions from COS cells than the fully modified protein [55]. In other *in vitro* studies, farnesylated peptides corresponding to the C-terminus of Ras had 20-fold higher affinity for liposomes when methylated [56].

Subsequent studies benefited immensely from the generation of Icmt knockout MEFs [57] and conditional (“floxed”) knockout MEFs [58]. These $Icmt^{-/-}$ MEFs were utilized to study the localization of a variety of fluorescently tagged Icmt substrates. GFP-K-Ras [57], GFP-N-Ras, and GFP-H-Ras were found to be mislocalized to varying degrees from the plasma membrane to the cytosol in $Icmt^{-/-}$ MEFs [59,60] and found less abundantly in membrane fractions (Figure 4.3).

Consistent with this observation, $Icmt^{flx/flx}$ MEFs that had been previously infected with adenovirus expressing *Cre* recombinase to mediate the deletion of the floxed segment of the *Icmt* gene ($Icmt^{-/-}$) were found to be resistant to oncogenic transformation by K-Ras and B-Raf in an *in vitro* soft agar assay and in nude mice [58]. These results were attributed to an increased expression of the cell cycle inhibitor p21 and to an increased binding of p21 to its inhibitory target cyclin A in the $Icmt^{-/-}$ MEFs expressing oncogenic K-Ras compared to wild type because oncogenic K-Ras was able to transform MEFs deficient in Icmt in the absence of p21. The $Icmt^{-/-}$ MEFs also had a reduced cell growth rate *in vitro*. However, despite the evidence that oncogenic Ras signaling was impaired in the absence of Icmt, no reduction in EGF-stimulated Erk or Akt phosphorylation was observed in the $Icmt^{-/-}$ MEFs. This result suggests that either sufficient endogenous Ras is associated with membranes even in the absence of Icmt to allow signaling downstream of receptor tyrosine kinases to Erk and Akt or that, in the absence of Icmt, cells upregulate alternative pathways to phosphorylate Erk and Akt that bypass the requirement for membrane-bound Ras. It was also shown

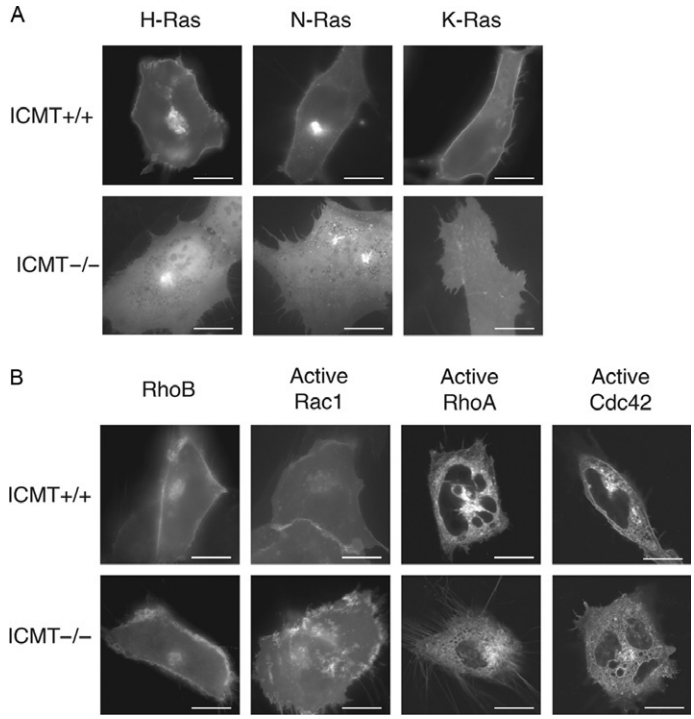

FIG. 4.3. Farnesylated CaaX proteins are more dependent on carboxylmethylation by Icmt for correct membrane localization than are geranylgeranylated CaaX proteins. (A) The indicated GFP-tagged Ras isoform was expressed in MEFs deficient in Icmt (−/−) or wild-type MEFs from littermates (+/+). All three Ras isoforms showed marked mislocalization with accumulation of GFP-Ras in the fluid phase cytosol. (B) GFP-tagged Rho proteins were expressed in the same MEFs as in (A). Activated forms of Rac1, RhoA, and Cdc42 were utilized for clarity since these forms do not bind to the cytosolic chaperone RhoGDI but instead associate with the cellular membranes to which their C-termini are targeted. In contrast to farnesylated Ras proteins, geranylgeranylated Rho proteins are localized with similar patterns in both +/+ and −/− MEFs.

that expression of constitutively active K-Ras retained the ability to stimulate the phosphorylation of S6K in the *Icmt*$^{-/-}$ MEFs [60], suggesting that either K-Ras retained sufficient plasma membrane localization even in the absence of methylation, or K-Ras was able to signal to downstream effectors from its mislocalized position within the cell.

One way that carboxyl methylation might influence the behavior of prenylated proteins is by promoting protein–protein interactions. Evidence for such a mechanism comes from the *S. cerevisiae*-mating pathway where **a**-factor interacts with its transporter, Ste6p, and its receptor on α-cells only

when methylated [14,21]. Further, in mammalian cells, carboxyl methylation of K-Ras was found to be absolutely required for its interaction with microtubules in an *in vitro* assay [61]. Interestingly, farnesylated Rheb proteins were also found to be mislocalized in *Icmt*$^{-/-}$ MEFs [60,62]. GFP-Rheb1 and GFP-Rheb2 were found on the ER and Golgi in wild-type MEFs, but in *Icmt*$^{-/-}$ MEFs, the localization was indistinguishable from GFP expressed alone. However, constitutively active Rheb mutants retained the ability to stimulate S6 kinase phosphorylation in the *Icmt*$^{-/-}$ MEFs to a similar degree as wild-type MEFs. Further, knockdown of the Rheb GAP TSC2 also stimulated S6K phosphorylation in *Icmt*$^{-/-}$ MEFs.

Another group of Icmt substrates of particular interest are the nuclear lamina proteins lamin A, B1, and B2 because defects in the posttranslational processing of these proteins are associated with human diseases such as Hutchinson-Gilford progeria and other laminopathies [63]. Icmt has been shown to also localize to the inner nuclear membrane, and this is the compartment on which postprenylation CaaX processing of nuclear lamins takes place [64]. It has been suggested that methylation of lamin B1 may allow its interaction with a novel carboxyl methylation-dependent lamin receptor in the nuclear envelope and may allow organization of lamin B1 into subdomains of the nuclear lamina [65]. A NLS-YFP-C40 construct containing the last 40 amino acids of lamin B1 was found to localize to the nuclear envelope in wild-type MEFs but not *Icmt*$^{-/-}$ MEFs, and morphological changes in the nuclear lamina were observed in *Icmt*$^{-/-}$ MEFs.

Interestingly, whereas Icmt appears to be required for efficient association of farnesylated proteins with cell membranes, many geranylgeranylated proteins do not require carboxyl methylation for proper localization. This difference is believed to be due to the fact that the geranylgeranyl moiety is larger and more hydrophobic than the farnesyl moiety [66]. Early evidence showed that unlike the farnesylated $T_{\beta\gamma}$, the function of the geranylgeranylated $\beta_1\gamma_2$ subunit of a heterotrimeric G protein was only mildly impaired in the absence of methylation [67]. However, the data on the localization of geranylgeranylated Rho proteins in *Icmt*$^{-/-}$ MEFs remain contradictory. In one study using the *Icmt*$^{-/-}$ MEFs, GFP-tagged fusions of constitutively active Rac1, Cdc42, RhoA, and wild-type RhoB localized in a pattern indistinguishable from wild type [59] (Figure 4.3). While these Rho proteins were confirmed to be substrates for Icmt *in vivo*, no defect could be observed in *Icmt*$^{-/-}$ MEFs in the formation of membrane ruffles in response to constitutively active Rac1 or oncogenic Dbl expression. Further, no change in filopodia formation in response to constitutively active Cdc42 or actin remodeling in response to contact with fibronectin coated beads was observed. Similarly, the localization of the

CaaX-containing, geranylgeranylated EGFP-Rab8a, EGFP-Rab18, and EGFP-Rab23 was found to be unaffected in *Icmt*$^{-/-}$ MEFs [6]. However, another study demonstrated that Icmt was required for the correct localization of RhoA and several other Rho proteins, both farnesylated and geranylgeranylated [68], and it was found that the conditional knockout *Icmt*$^{-/-}$ MEFs contained only 5–10% of the levels of RhoA and GTP-bound RhoA compared to wild-type MEFs due to accelerated turnover of the RhoA protein [58].

Further conflicting evidence exists regarding the role of methylation in Rac1 function. One study suggests that Icmt is required for a TNFα-induced increase in Rac1 association with detergent-insoluble membranes. This increase was found to be diminished in *Icmt*$^{-/-}$ MEFs and in human aortic endothelial cells (ECs) treated with the Icmt competitive inhibitor AFC [69]. In this study, Rac1 was mislocalized from the plasma membrane in *Icmt*$^{-/-}$ MEFs and AFC-treated ECs, and there was a reduction in GTP-Rac1 levels, and phosphorylation of p38 in response to TNFα. Data from this study also suggested that in the absence of methylation, the interaction between Rac1 and RhoGDI was enhanced. Icmt was also reported to be required for the TNFα-dependent induction of VCAM-1 expression [70]. These data imply that the methylation of Rac1 is required for its role in the redox-sensitive signaling pathway downstream of TNFα that upregulates EC adhesion molecules to enhance the interaction of ECs with leukocytes in response to inflammatory cytokines.

Icmt has also been investigated in EC function, where it was found to regulate endothelial monolayer permeability due to its effects on RhoA function [71,72]. Methylation can be inhibited by treatment of cells with adenosine and homocysteine, which diminish the cellular pools of the methyl donor SAM [73]. Inhibition of methylation with adenosine plus homocysteine or inhibition of Icmt with the competitive inhibitor AGGC was found to decrease EC permeability, as determined by an increase in electrical resistance across confluent monolayers of bovine pulmonary artery ECs [71,72]. In contrast, overexpression of Icmt-GFP increased endothelial cell permeability by disrupting adherens junctions, as shown by an increase in intercellular gaps and decreases in VE-cadherin and β-catenin at cell–cell junctions [71]. The effects of overexpression of Icmt-GFP were reversed by expression of dominant-negative RhoA. These results suggest that overexpression of Icmt increases the methylation and the activity of RhoA. Aside from this result, there is little evidence in the literature of overexpression of Icmt having an effect on the activity of its substrates, consistent with the prevailing view that CaaX processing occurs constitutively and rapidly following translation [74]. However, considering that methylation is the only step in the process that is reversible, it is

tantalizing to hypothesize that cycles of methylation and demethylation could dynamically regulate substrate function.

Inhibition of Icmt by adenosine and homocysteine or AGGC has also been proposed to induce apoptosis in pulmonary artery ECs by two distinct mechanisms involving Ras and RhoA [75,76]. The first proposed mechanism was the attenuation of Ras-stimulated Erk and Akt signaling. Evidence for this consisted of the ability of active H-Ras to overcome the effects of adenosine and homocysteine, as well as the ability of dominant-negative H-Ras to induce apoptosis in the absence of adenosine and homocysteine [75]. The second mechanism proposed was that inhibition of Icmt induces apoptosis by causing dysfunction of the ER unfolded protein response (UPR), potentially due to a subcellular relocalization, aggregation, and decrease in protein level of the ER molecular chaperone GRP94. This apoptotic phenotype could be rescued upon expression of active RhoA [76]. Overexpression of Icmt-GFP was also found to protect ECs from apoptosis induced by UV, TNFα, and adenosine and homocysteine [75].

Although several studies have demonstrated that many substrates are mislocalized in the absence of Icmt [57,59], conclusive evidence of altered signaling from unmethylated substrates is lacking. One potential problem is the reliance on either knockout MEFs or inhibitors for these studies. The knockout MEFs are selected for survival and are likely to contain compensatory mutations and alterations in signaling pathways. Studies that rely on the pharmacological inhibition of Icmt are also fraught with ambiguity because many of the agents employed to date are known to have significant off-target activities [77,78]. Preliminary studies using siRNA have shown that EGF-stimulated Erk and Akt signaling depends to some extent on Icmt (Helen Court and Mark R. Philips, unpublished data). More comprehensive gene silencing studies in the future will likely help to clarify the discrepancies in the literature and provide conclusive evidence for a functional role of Icmt.

VI. ICMT Effect on Substrate Function: *In Vivo* Studies

Because *ste14* yeast that lack Icmt activity have little if any growth defect, Icmt was long considered an unimportant target with regard to anti-Ras drug discovery. This view changed with the discovery that the *Icmt* gene is essential for mouse development. $Icmt^{-/-}$ knockout embryos die at mid-gestation between E10.5 and E11.5 [79,80]. In retrospect, it should not have been surprising that *Icmt* is an essential gene given the large number of substrates for this methyltransferase that are themselves essential and given the conservation of Icmt through evolution. The embryonic lethality associated with Icmt deficiency is believed to be caused by a

defect in liver development characterized by a delay in albumin induction and failed hepatocyte outgrowth [80]. *Icmt*$^{-/-}$ cells in chimeric mice that were otherwise wild type were unable to contribute to the development of certain organs (e.g., liver and brain), while they retained the ability to contribute to other organs (e.g., skeletal muscle) [79]. Due to the lethal phenotype of the knockout, a conditional (floxed) allele of Icmt was created in order to determine the role of methylation of prenylated cysteines in organ development and oncogenic transformation *in vivo*.

To study the role of Icmt in the development of a Ras-driven malignancy, mice containing the Icmt floxed allele were crossed with mice containing a latent, *Cre*-inducible allele of oncogenic KrasG12D (*LSL-KRAS*) and an interferon-inducible Mx1-*Cre* allele [81]. In adult mice containing these alleles, injection with the double-stranded RNA analog polyinosinic–polycytidylic acid (pI–pC) induces interferon expression in bone marrow cells. This in turn induces expression of *Cre* recombinase, which simultaneously allows expression of oncogenic KrasG12D and causes the excision of the floxed section of the *Icmt* gene, resulting in loss of Icmt expression. Mx1-*Cre;LSL-KRAS* mice that were wild type for Icmt [82,83] were found to develop a fatal myeloproliferative disorder with 100% penetrance after injection with pI–pC. Mice lacking a functional copy of Icmt in hematopoietic cells (*Icmt*$^{flx/flx}$), however, had a reduced white cell count and reduced numbers of immature myeloid cells in peripheral blood 13–14 weeks after injection with pI–pC compared to control mice with wild-type Icmt and had markedly reduced splenomegaly and infiltration of myeloid cells into the liver [81]. In addition, isolated Icmt-deficient bone marrow cells and splenocytes from pI–pC injected Mx1-*Cre;LSL-KRAS* mice showed a reduction in phenotypes associated with malignant transformation *in vitro*, such as growth factor-independent colony growth, compared to cells with wild-type Icmt. Despite the apparent amelioration of the myeloproliferative phenotype, the mice deficient in Icmt had a reduced median survival rate compared to wild type, obfuscating the interpretation of the results.

These *in vivo* data suggest that methylation by Icmt may be required for the transforming capability of K-Ras. This conclusion was strengthened by an additional study of *Icmt*$^{flx/flx}$;*LSL-KRAS* mice crossed with a *Cre* mouse. In this study, *Cre* expression was driven off a lysozyme M promoter, which is expressed in myeloid cells and type 2 pneumocytes [84]. *LSL-KRAS*;lysozyme M-*Cre* mice develop rapidly progressing lung tumors and have to be sacrificed at 3 weeks of age due to respiratory failure [85]. In contrast, *Icmt*$^{flx/flx}$;*LSL-KRAS*;lysozyme M-*Cre* animals had a median survival of 50 days, and upon necropsy, the lung weight was reduced by 33% compared to controls. These *in vivo* results suggest that Icmt is a promising therapeutic target for the treatment of

Ras-driven malignancies. However, further studies in other more relevant models and organ systems are required before any definitive conclusions can be drawn.

VII. Conclusions

Knockout of Icmt leads to embryonic lethality in mice [79]. Despite this essential function *in vivo*, the *in vitro* evidence describing a definitive function for Icmt is somewhat limited. Further study is required to determine how different substrates of Icmt can function in the absence of methylation and how this affects downstream signaling pathways. Curiously, the loss of either Icmt or the protease Rce1 (discussed in Chapter 10) resulted in strikingly different patterns of substrate localization [60,68]. As proteolysis and methylation are hypothesized to work in concert to promote membrane localization and substrate function, these discrepancies highlight how further insight into the role of postprenylation processing is needed to understand the implications of interfering with this pathway pharmacologically as a treatment for human disease.

Interestingly, carboxyl methylation is the only reversible posttranslational modification in the CaaX processing pathway. It is intriguing to conceive of a cycle of dynamic regulation of protein localization, function, and interactions modulated by Icmt and a methylesterase [86]. To date, however, a methylesterase that specifically catalyzes the reverse reaction has not been identified for any substrate. Several studies have identified a polyisoprenylated methylated protein methyl esterase (PMPMEase) that could function as the esterase in the pathway [87–89]. However, to date, there are no studies demonstrating a role for this enzyme in cells. If a specific esterase were identified, this could greatly help our understanding of the importance of methylation by Icmt in cells.

References

1. Fu, H.W., and Casey, P.J. (1999). Enzymology and biology of CaaX protein prenylation. *Recent Prog Horm Res* 54:315–342, discussion 342–313.
2. Reid, T.S., Terry, K.L., Casey, P.J., and Beese, L.S. (2004). Crystallographic analysis of CaaX prenyltransferases complexed with substrates defines rules of protein substrate selectivity. *J Mol Biol* 343:417–433.
3. Smeland, T.E., Seabra, M.C., Goldstein, J.L., and Brown, M.S. (1994). Geranylgeranylated Rab proteins terminating in Cys-Ala-Cys, but not Cys-Cys, are carboxyl-methylated by bovine brain membranes in vitro. *Proc Natl Acad Sci USA* 91:10712–10716.

4. Farnsworth, C.C., Kawata, M., Yoshida, Y., Takai, Y., Gelb, M.H., and Glomset, J.A. (1991). C terminus of the small GTP-binding protein smg p25A contains two geranylgeranylated cysteine residues and a methyl ester. *Proc Natl Acad Sci USA* 88:6196–6200.
5. Svensson, A.W., Casey, P.J., Young, S.G., and Bergo, M.O. (2006). Genetic and pharmacologic analyses of the role of Icmt in Ras membrane association and function. *Methods Enzymol* 407:144–159.
6. Leung, K.F., Baron, R., Ali, B.R., Magee, A.I., and Seabra, M.C. (2007). Rab GTPases containing a CAAX motif are processed post-geranylgeranylation by proteolysis and methylation. *J Biol Chem* 282:1487–1497.
7. Sakagami, Y., Yoshida, M., Isogai, A., and Suzuki, A. (1981). Peptidal sex hormones inducing conjugation tube formation in compatible mating-type cells of Tremella mesenterica. *Science* 212:1525–1527.
8. Ishibashi, Y., Sakagami, Y., Isogai, A., and Suzuki, A. (1984). Structures of tremerogens A-9291-I and A-9291-VIII: peptidal sex hormones of Tremella brasiliensis. *Biochemistry* 23:1399–1404.
9. Anderegg, R.J., Betz, R., Carr, S.A., Crabb, J.W., and Duntze, W. (1988). Structure of Saccharomyces cerevisiae mating hormone a-factor. Identification of S-farnesyl cysteine as a structural component. *J Biol Chem* 263:18236–18240.
10. Mackay, V., and Manney, T.R. (1974). Mutations affecting sexual conjugation and related processes in Saccharomyces cerevisiae. I. Isolation and phenotypic characterization of nonmating mutants. *Genetics* 76:255–271.
11. Mackay, V., and Manney, T.R. (1974). Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae*. II. Genetic analysis of nonmating mutants. *Genetics* 76:273–288.
12. Hartwell, L.H. (1980). Mutants of Saccharomyces cerevisiae unresponsive to cell division control by polypeptide mating hormone. *J Cell Biol* 85:811–822.
13. Fujino, M., Kitada, C., Sakagami, Y., Isogai, A., Tamura, S., and Suzuki, A. (1980). Biological activity of synthetic analogs of tremerogen A-10. *Naturwissenschaften* 67:406–408.
14. Marcus, S., Caldwell, G.A., Miller, D., Xue, C.B., Naider, F., and Becker, J.M. (1991). Significance of C-terminal cysteine modifications to the biological activity of the *Saccharomyces cerevisiae* a-factor mating pheromone. *Mol Cell Biol* 11:3603–3612.
15. Davey, J. (1992). Mating pheromones of the fission yeast *Schizosaccharomyces pombe*: purification and structural characterization of M-factor and isolation and analysis of two genes encoding the pheromone. *EMBO J* 11:951–960.
16. Clarke, S., Vogel, J.P., Deschenes, R.J., and Stock, J. (1988). Posttranslational modification of the Ha-ras oncogene protein: evidence for a third class of protein carboxyl methyltransferases. *Proc Natl Acad Sci USA* 85:4643–4647.
17. Gutierrez, L., Magee, A.I., Marshall, C.J., and Hancock, J.F. (1989). Post-translational processing of p21ras is two-step and involves carboxyl-methylation and carboxy-terminal proteolysis. *EMBO J* 8:1093–1098.
18. Hrycyna, C.A., and Clarke, S. (1990). Farnesyl cysteine C-terminal methyltransferase activity is dependent upon the STE14 gene product in *Saccharomyces cerevisiae*. *Mol Cell Biol* 10:5071–5076.
19. Marr, R.S., Blair, L.C., and Thorner, J. (1990). Saccharomyces cerevisiae STE14 gene is required for COOH-terminal methylation of a-factor mating pheromone. *J Biol Chem* 265:20057–20060.
20. Hrycyna, C.A., Sapperstein, S.K., Clarke, S., and Michaelis, S. (1991). The *Saccharomyces cerevisiae* STE14 gene encodes a methyltransferase that mediates C-terminal methylation of a-factor and RAS proteins. *EMBO J* 10:1699–1709.

21. Sapperstein, S., Berkower, C., and Michaelis, S. (1994). Nucleotide sequence of the yeast STE14 gene, which encodes farnesylcysteine carboxyl methyltransferase, and demonstration of its essential role in a-factor export. *Mol Cell Biol* 14:1438–1449.
22. Stephenson, R.C., and Clarke, S. (1990). Identification of a C-terminal protein carboxyl methyltransferase in rat liver membranes utilizing a synthetic farnesyl cysteine-containing peptide substrate. *J Biol Chem* 265:16248–16254.
23. Pillinger, M.H., Volker, C., Stock, J.B., Weissmann, G., and Philips, M.R. (1994). Characterization of a plasma membrane-associated prenylcysteine-directed alpha carboxyl methyltransferase in human neutrophils. *J Biol Chem* 269:1486–1492.
24. Dai, Q., *et al.* (1998). Mammalian prenylcysteine carboxyl methyltransferase is in the endoplasmic reticulum. *J Biol Chem* 273:15030–15034.
25. Romano, J.D., and Michaelis, S. (2001). Topological and mutational analysis of Saccharomyces cerevisiae Ste14p, founding member of the isoprenylcysteine carboxyl methyltransferase family. *Mol Biol Cell* 12:1957–1971.
26. Wright, L.P., Court, H., Mor, A., Ahearn, I.M., Casey, P.J., and Philips, M.R. (2009). Topology of mammalian isoprenylcysteine carboxyl methyltransferase determined in live cells with a fluorescent probe. *Mol Cell Biol* 29:1826–1833.
27. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., *et al.* (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.
28. Romano, J.D., Schmidt, W.K., and Michaelis, S. (1998). The Saccharomyces cerevisiae prenylcysteine carboxyl methyltransferase Ste14p is in the endoplasmic reticulum membrane. *Mol Biol Cell* 9:2231–2247.
29. Kagan, R.M., and Clarke, S. (1994). Widespread occurrence of three sequence motifs in diverse S-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. *Arch Biochem Biophys* 310:417–427.
30. Griggs, A.M., Hahne, K., and Hrycyna, C.A. (2010). Functional oligomerization of the Saccharomyces cerevisiae isoprenylcysteine carboxyl methyltransferase, Ste14p. *J Biol Chem* 285:13380–13387.
31. Senes, A., Engel, D.E., and DeGrado, W.F. (2004). Folding of helical membrane proteins: the role of polar, GxxxG-like and proline motifs. *Curr Opin Struct Biol* 14:465–479.
32. Choy, E., *et al.* (1999). Endomembrane trafficking of ras: the CAAX motif targets proteins to the ER and Golgi. *Cell* 98:69–80.
33. Mor, Adam, and Philips, Mark R. (2006). Compartmentalized Ras/MAPK signaling. *Annu Rev Immunol* 24:771–800.
34. Boyartchuk, V.L., Ashby, M.N., and Rine, J. (1997). Modulation of Ras and a-factor function by carboxyl-terminal proteolysis. *Science* 275:1796–1800.
35. Freije, J.M., Blay, P., Pendas, A.M., Cadinanos, J., Crespo, P., and Lopez-Otin, C. (1999). Identification and chromosomal location of two human genes encoding enzymes potentially involved in proteolytic maturation of farnesylated proteins. *Genomics* 58:270–280.
36. Otto, J.C., Kim, E., Young, S.G., and Casey, P.J. (1999). Cloning and characterization of a mammalian prenyl protein-specific protease. *J Biol Chem* 274:8379–8382.
37. Schmidt, W.K., Tam, A., Fujimura-Kamada, K., and Michaelis, S. (1998). Endoplasmic reticulum membrane localization of Rce1p and Ste24p, yeast proteases involved in carboxyl-terminal CAAX protein processing and amino-terminal a-factor cleavage. *Proc Natl Acad Sci USA* 95:11175–11180.
38. Baron, R.A., and Casey, P.J. (2004). Analysis of the kinetic mechanism of recombinant human isoprenylcysteine carboxylmethyltransferase (Icmt). *BMC Biochem* 5:19.
39. Shi, Y.Q., and Rando, R.R. (1992). Kinetic mechanism of isoprenylated protein methyltransferase. *J Biol Chem* 267:9547–9551.

40. Volker, C., Lane, P., Kwee, C., Johnson, M., and Stock, J. (1991). A single activity carboxyl methylates both farnesyl and geranylgeranyl cysteine residues. *FEBS Lett* 295:189–194.
41. Volker, C., *et al.* (1991). Effects of farnesylcysteine analogs on protein carboxyl methylation and signal transduction. *J Biol Chem* 266:21515–21522.
42. Perez-Sala, D., Gilbert, B.A., Tan, E.W., and Rando, R.R. (1992). Prenylated protein methyltransferases do not distinguish between farnesylated and geranylgeranylated substrates. *Biochem J* 284(Pt. 3):835–840.
43. Anderson, J.L., Frase, H., Michaelis, S., and Hrycyna, C.A. (2005). Purification, functional reconstitution, and characterization of the *Saccharomyces cerevisiae* isoprenylcysteine carboxylmethyltransferase Ste14p. *J Biol Chem* 280:7336–7345.
44. Hasne, M.P., and Lawrence, F. (1999). Characterization of prenylated protein methyltransferase in Leishmania. *Biochem J* 342(Pt 3):513–518.
45. Tan, E.W., Perez-Sala, D., Canada, F.J., and Rando, R.R. (1991). Identifying the recognition unit for G protein methylation. *J Biol Chem* 266:10719–10722.
46. Anderson, J.L., Henriksen, B.S., Gibbs, R.A., and Hrycyna, C.A. (2005). The isoprenoid substrate specificity of isoprenylcysteine carboxylmethyltransferase: development of novel inhibitors. *J Biol Chem* 280:29454–29461.
47. Henriksen, B.S., Anderson, J.L., Hrycyna, C.A., and Gibbs, R.A. (2005). Synthesis of desthio prenylcysteine analogs: sulfur is important for biological activity. *Bioorg Med Chem Lett* 15:5080–5083.
48. Bergman, J.A., Hahne, K., Hrycyna, C.A., and Gibbs, R.A. (2011). Lipid and sulfur substituted prenylcysteine analogs as human Icmt inhibitors. *Bioorg Med Chem Lett* 21:5616–5619.
49. Hrycyna, C.A., Wait, S.J., Backlund, P.S., Jr., and Michaelis, S. (1995). Yeast STE14 methyltransferase, expressed as TrpE-STE14 fusion protein in Escherichia coli, for in vitro carboxylmethylation of prenylated polypeptides. *Methods Enzymol* 250:251–266.
50. Perez-Sala, D., Tan, E.W., Canada, F.J., and Rando, R.R. (1991). Methylation and demethylation reactions of guanine nucleotide-binding proteins of retinal rod outer segments. *Proc Natl Acad Sci USA* 88:3043–3046.
51. Parish, C.A., and Rando, R.R. (1994). Functional significance of G protein carboxymethylation. *Biochemistry* 33:9986–9991.
52. Fukada, Y., *et al.* (1994). Effects of carboxyl methylation of photoreceptor G protein gamma-subunit in visual transduction. *J Biol Chem* 269:5163–5170.
53. Parish, C.A., Smrcka, A.V., and Rando, R.R. (1995). Functional significance of beta gamma-subunit carboxymethylation for the activation of phospholipase C and phosphoinositide 3-kinase. *Biochemistry* 34:7722–7727.
54. Ohguro, H., Fukada, Y., Takao, T., Shimonishi, Y., Yoshizawa, T., and Akino, T. (1991). Carboxyl methylation and farnesylation of transducin gamma-subunit synergistically enhance its coupling with metarhodopsin II. *EMBO J* 10:3669–3674.
55. Hancock, J.F., Cadwallader, K., and Marshall, C.J. (1991). Methylation and proteolysis are essential for efficient membrane binding of prenylated p21K-ras(B). *EMBO J* 10:641–646.
56. Silvius, J.R., and l'Heureux, F. (1994). Fluorimetric evaluation of the affinities of isoprenylated peptides for lipid bilayers. *Biochemistry* 33:3014–3022.
57. Bergo, M.O., Leung, G.K., Ambroziak, P., Otto, J.C., Casey, P.J., and Young, S.G. (2000). Targeted inactivation of the isoprenylcysteine carboxyl methyltransferase gene causes mislocalization of K-Ras in mammalian cells. *J Biol Chem* 275:17605–17610.
58. Bergo, M.O., *et al.* (2004). Inactivation of Icmt inhibits transformation by oncogenic K-Ras and B-Raf. *J Clin Invest* 113:539–550.
59. Michaelson, D., *et al.* (2005). Postprenylation CAAX processing is required for proper localization of Ras but not Rho GTPases. *Mol Biol Cell* 16:1606–1616.

60. Hanker, A.B., *et al.* (2010). Differential requirement of CAAX-mediated posttranslational processing for Rheb localization and signaling. *Oncogene* 29:380–391.
61. Chen, Z., Otto, J.C., Bergo, M.O., Young, S.G., and Casey, P.J. (2000). The C-terminal polylysine region and methylation of K-Ras are critical for the interaction between K-Ras and microtubules. *J Biol Chem* 275:41251–41257.
62. Takahashi, K., Nakagawa, M., Young, S.G., and Yamanaka, S. (2005). Differential membrane localization of ERas and Rheb, two Ras-related proteins involved in the phosphatidylinositol 3-kinase/mTOR pathway. *J Biol Chem* 280:32768–32774.
63. Broers, J.L., Ramaekers, F.C., Bonne, G., Yaou, R.B., and Hutchison, C.J. (2006). Nuclear lamins: laminopathies and their role in premature ageing. *Physiol Rev* 86:967–1008.
64. Barrowman, J., Hamblet, C., George, C.M., and Michaelis, S. (2008). Analysis of prelamin A biogenesis reveals the nucleus to be a CaaX processing compartment. *Mol Biol Cell* 19:5398–5408.
65. Maske, C.P., Hollinshead, M.S., Higbee, N.C., Bergo, M.O., Young, S.G., and Vaux, D.J. (2003). A carboxyl-terminal interaction of lamin B1 is dependent on the CAAX endoprotease Rce1 and carboxymethylation. *J Cell Biol* 162:1223–1232.
66. Black, S.D. (1992). Development of hydrophobicity parameters for prenylated proteins. *Biochem Biophys Res Commun* 186:1437–1442.
67. Parish, C.A., Smrcka, A.V., and Rando, R.R. (1996). The role of G protein methylation in the function of a geranylgeranylated beta gamma isoform. *Biochemistry* 35:7499–7505.
68. Roberts, P.J., *et al.* (2008). Rho Family GTPase modification and dependence on CAAX motif-signaled posttranslational modification. *J Biol Chem* 283:25150–25163.
69. Papaharalambus, C., *et al.* (2005). Tumor necrosis factor alpha stimulation of Rac1 activity. Role of isoprenylcysteine carboxylmethyltransferase. *J Biol Chem* 280:18790–18796.
70. Ahmad, M., Zhang, Y., Papharalambus, C., and Alexander, R.W. (2002). Role of isoprenylcysteine carboxyl methyltransferase in tumor necrosis factor-alpha stimulation of expression of vascular cell adhesion molecule-1 in endothelial cells. *Arterioscler Thromb Vasc Biol* 22:759–764.
71. Lu, Q., *et al.* (2004). Isoprenylcysteine carboxyl methyltransferase modulates endothelial monolayer permeability: involvement of RhoA carboxyl methylation. *Circ Res* 94:306–315.
72. Rounds, S., Lu, Q., Harrington, E.O., Newton, J., and Casserly, B. (2008). Pulmonary endothelial cell signaling and function. *Trans Am Clin Climatol Assoc* 119:155–167, discussion 167–159.
73. Pike, M.C., Kredich, N.M., and Snyderman, R. (1978). Requirement of S-adenosyl-L-methionine-mediated methylation for human monocyte chemotaxis. *Proc Natl Acad Sci USA* 75:3928–3932.
74. Backlund, P.S., Jr. (1997). Post-translational processing of RhoA. Carboxyl methylation of the carboxyl-terminal prenylcysteine increases the half-life of Rhoa. *J Biol Chem* 272:33175–33180.
75. Kramer, K., *et al.* (2003). Isoprenylcysteine carboxyl methyltransferase activity modulates endothelial cell apoptosis. *Mol Biol Cell* 14:848–857.
76. Lu, Q., Harrington, E.O., Newton, J., Jankowich, M., and Rounds, S. (2007). Inhibition of ICMT induces endothelial cell apoptosis through GRP94. *Am J Respir Cell Mol Biol* 37:20–30.
77. Ma, Y.T., Shi, Y.Q., Lim, Y.H., McGrail, S.H., Ware, J.A., and Rando, R.R. (1994). Mechanistic studies on human platelet isoprenylated protein methyltransferase: farnesylcysteine analogs block platelet aggregation without inhibiting the methyltransferase. *Biochemistry* 33:5414–5420.

78. Scheer, A., and Gierschik, P. (1993). Farnesylcysteine analogues inhibit chemotactic peptide receptor-mediated G-protein activation in human HL-60 granulocyte membranes. *FEBS Lett* 319:110–114.
79. Bergo, M.O., *et al.* (2001). Isoprenylcysteine carboxyl methyltransferase deficiency in mice. *J Biol Chem* 276:5841–5845.
80. Lin, X., Jung, J., Kang, D., Xu, B., Zaret, K.S., and Zoghbi, H. (2002). Prenylcysteine carboxylmethyltransferase is essential for the earliest stages of liver development in mice. *Gastroenterology* 123:345–351.
81. Wahlstrom, A.M., *et al.* (2008). Inactivating Icmt ameliorates K-RAS-induced myeloproliferative disease. *Blood* 112:1357–1365.
82. Braun, B.S., *et al.* (2004). Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc Natl Acad Sci USA* 101:597–602.
83. Chan, I.T., *et al.* (2004). Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. *J Clin Invest* 113:528–538.
84. Clausen, B.E., Burkhardt, C., Reith, W., Renkawitz, R., and Forster, I. (1999). Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res* 8:265–277.
85. Sjogren, A.K., *et al.* (2007). GGTase-I deficiency reduces tumor formation and improves survival in mice with K-RAS-induced lung cancer. *J Clin Invest* 117:1294–1304.
86. Philips, M.R., *et al.* (1993). Carboxyl methylation of Ras-related proteins during signal transduction in neutrophils. *Science* 259:977–980.
87. Tan, E.W., and Rando, R.R. (1992). Identification of an isoprenylated cysteine methyl ester hydrolase activity in bovine rod outer segment membranes. *Biochemistry* 31:5572–5578.
88. Oboh, O.T., and Lamango, N.S. (2008). Liver prenylated methylated protein methyl esterase is the same enzyme as Sus scrofa carboxylesterase. *J Biochem Mol Toxicol* 22:51–62.
89. Lamango, N.S., Duverna, R., Zhang, W., and Ablordeppey, S.Y. (2009). Porcine liver carboxylesterase requires polyisoprenylation for high affinity binding to cysteinyl substrates. *Open Enzyme Inhib J* 2:12–27.