Genetic disorders of vitamin B$_{12}$ metabolism: eight complementation groups – eight genes

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Vitamin B$_{12}$ (cobalamin, Cbl) is an essential nutrient in human metabolism. Genetic diseases of vitamin B$_{12}$ utilisation constitute an important fraction of inherited newborn disease. Functionally, B$_{12}$ is the cofactor for methionine synthase and methylmalonyl CoA mutase. To function as a cofactor, B$_{12}$ must be metabolised through a complex pathway that modifies its structure and takes it through subcellular compartments of the cell. Through the study of inherited disorders of vitamin B$_{12}$ utilisation, the genes for eight complementation groups have been identified, leading to the determination of the general structure of vitamin B$_{12}$ processing and providing methods for carrier testing, prenatal diagnosis and approaches to treatment.

Vitamin B$_{12}$, also known as cobalamin (Cbl), is a micronutrient that is synthesised only by microorganisms, yet is essential to human health. Cobalamin was first isolated by Smith (Ref. 1) and Rickes (Ref. 2), after Minot and Murphy (Ref. 3) showed that pernicious anaemia could be treated with oral liver extract. Later, vitamin B$_{12}$ deficiency as a result of genetic disease was described despite adequate vitamin intake (Ref. 4). Some patients responded successfully to very high doses of vitamin B$_{12}$, suggesting blocks in vitamin processing. These patients had homocystinuria and/or methylmalonic aciduria, implicating dysfunctional methionine synthase (MS) and/or methylmalonyl-CoA mutase (MUT or MCM). We now know that blocks in the intracellular processing of cobalamin into cofactor forms, methylcobalamin (MeCbl) for MS and adenosylcobalamin (AdoCbl) for MCM, or in the functional activity of MS or MCM result in inborn errors. These genetic blocks may be devastating in newborns or in early childhood. Understanding the genes, gene products and subcellular transport of vitamin B$_{12}$ is important for minimising the disease burden from these disorders. This review outlines the present knowledge of cobalamin metabolism, with a focus on steps related to the intracellular human pathway and the initial cataloguing of cobalamin-utilisation disorders into complementation groups and biochemically distinct classes. Key to these discoveries have

This manuscript is dedicated to the memory of Aarif Edoo.

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been the hundreds of patients who have been the source of cell cultures and DNA samples that have given us our current understanding of vitamin B_{12} utilisation in humans.

Vitamin B_{12} structure

The structure of cobalamin was first solved by Hodgkin (Ref. 5) using x-ray crystallography. It is a large organometallic molecule, ~1300–1500 Da in size, and is the most chemically complex vitamin known. The focal point of vitamin B_{12} is the central cobalt atom, which has up to six ligands bound to it. Four of the ligands are the nitrogen atoms of the planar corrin ring that surround the cobalt atom (Fig. 1). The α-axial ligand, extending below the corrin ring, is a nitrogen of the 5,6-dimethylbenzimidazole (DMB) phosphoribosyl moiety that also attaches back to the corrin ring through one of its propionamide side chains. The upper or β-axial ligand varies, depending on the modification state of cobalamin (R-group in Fig. 1a). Functional β-axial ligands are methyl (MeCbl) or 5′-deoxyadenosyl (AdoCbl) groups. Additionally, a hydroxyl group (OHCbl) or a cyano group (CNCbl) can be bound as physiologically relevant β-axial ligands.

There are three important, inter-related factors that contribute to cobalamin reactivity and function: the oxidation state of cobalt; whether the DMB is coordinated to cobalt in the lower axial position; and the identity of the R-group bound in the upper axial position. The cobalt atom of cobalamin may exist in the +3 [cob(III)alamin], +2 [cob(II)alamin] or +1 [cob(I)alamin] oxidation state. AdoCbl, MeCbl, CNCbl and OHCbl, all of which are cob(III)alamins, prefer to adopt a configuration where the DMB nitrogen base is coordinated to the cobalt in the lower axial position (referred to as 'base-on') (Fig. 1). Some enzymes, however, are able to shift these cob(III)alamins to the 'base-off' configuration. Interestingly, MS and MCM, which use MeCbl and AdoCbl as cofactors, respectively, bind the cobalamin so that the DMB nitrogen is displaced from the cobalt and replaced by a histidine of the enzyme (Fig. 1b). This type of binding is considered 'base-off/His-on' and is important for the catalytic activity of the enzymes. Cob(II)alamin generally has no R-group bound, binding only DMB as the lower axial ligand to make its preferred five-coordinate state. However, in the

presence of ATP, MMAB, the human adenosyltransferase (ATR) enzyme, is able to bind cob(II)alamin in a novel four-coordinate state, where neither axial position is occupied (Ref. 51). Cob(I)alamin usually has no axial ligand. It is a highly reactive nucleophile that very easily oxidises to cob(II)alamin (Ref. 52). The challenge to the cell is how to productively produce cob(I)alamin, as in the reaction cycle of MS, without exposing the local environment to nucleophillic attack and risk of free-radical damage. The nature of the axial ligands also affects the ease with which the central cobalt is reduced. Strongly coordinating ligands stabilise cobalt against reduction, whereas weakly coordinating ligands allow cobalt to be reduced more easily (Ref. 53). Base-on cobalamin falls into the former category, protecting the cobalt from reduction because DMB has greater electron-donating character than the H_2O molecule that binds in its absence (Ref. 54).

Vitamin B_{12} origins

Vitamin B_{12} is an extremely old molecule in evolution. It has even been suggested that B_{12} was synthesised prebiotically (Ref. 55). Accordingly, vitamin B_{12} utilisation is dispersed throughout evolution, occurring in both Eukaryota and Prokaryota, perhaps having been passed on from the 'breakthrough organism' – the last organism to use RNA as the sole genetically encoded catalyst (Ref. 56). Interestingly, although cobalamin utilisation is distributed widely among phyla, cobalamin synthesis seems limited to only a select few Archaea and Eubacteria. Perhaps this is because the synthesis of cobalamin is so complex – involving in excess of 25 steps, which can proceed either aerobically (*cob* genes) or anaerobically (*cbi* genes) (Ref. 57). Therefore, although mammals and other higher organisms require vitamin B_{12} for life, they ostensibly acquire it from their prokaryotic counterparts. Another interesting facet of the evolution of cobalamin is that vitamin B_{12} users seem more scattered than logically spread out through evolution, with whole phyla sometimes gaining or losing vitamin B_{12} dependency (Ref. 58). Additionally, although mammals and other higher eukaryotes are restricted to two cobalamin-dependent enzymes, MS and MCM, prokaryotes use a plethora of enzymes requiring this cofactor. These enzymes include three
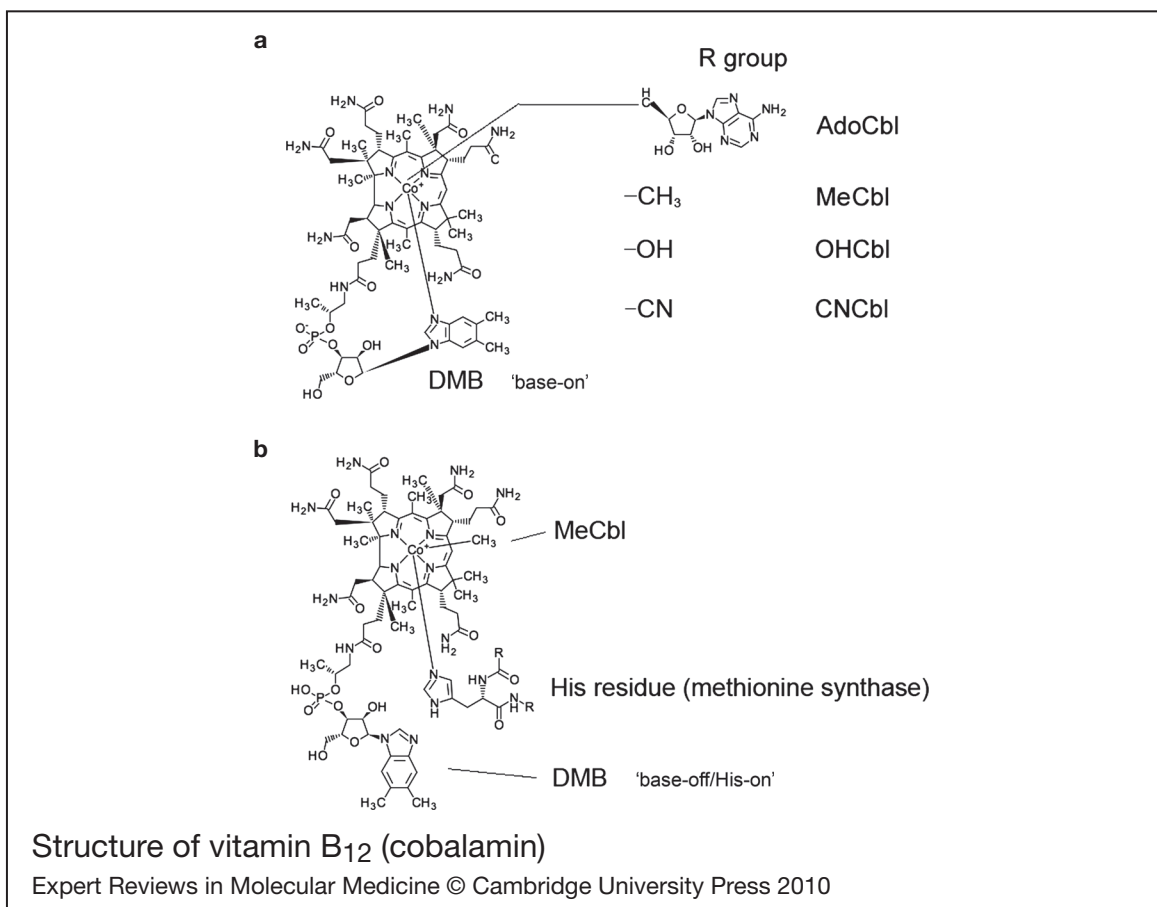
2

Structure of vitamin B$_{12}$ (cobalamin)

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Figure 1. Structure of vitamin B$_{12}$ (cobalamin). (a) The 'R group' corresponds to substitutions at the upper or β-axial ligand (5′-deoxyadenosyl-, methyl-, hydroxo-, cyano-). The dimethylbenzimidazole constituent (DMB) is shown coordinated to the cobalt in the lower α-axial position ('base-on' structure). DMB is linked to the corrin ring through a phosphoribosyl attached to a propionamide side chain. (b) Structure of methylcobalamin (MeCbl) with DMB displaced from the cobalt by a histidine residue in methionine synthase (MS; the 'base-off/His-on' structure). A similar configuration is observed for adenosylcobalamin (AdoCbl) bound to methylmalonyl-CoA mutase. Structures are from http://www.genome.jp using the 'SIMCOMP Search' utility (query C00576, vitamin B$_{12}$; C06410, MeCbl-MS).

classes of AdoCbl-dependent mutases, the isomerases (e.g. MCM, ribonucleotide reductase, glutamate mutase), the eliminases (e.g. diol dehydratase) and the aminomutases (e.g. D-lysine-5,6-aminomutase), as well as the MeCbl-dependent methyltransferases (e.g. MS) and the vitamin B$_{12}$-dependent reductive dehalogenases (e.g. 3–6 chloro-4-hydroxybenzoate dehalogenase) (Refs 59, 60). Therefore, because higher eukaryotes share common vitamin B$_{12}$ ancestry with prokaryotes, but have apparently limited use of the vitamin and no biosynthesis, prokaryotes in general and a few specific bacteria and *Archaea* in particular have proved to be very

useful models to understand vitamin B$_{12}$ metabolism.

Human vitamin B$_{12}$ ingestion and absorption

Because vitamin B$_{12}$ is made by only a few microorganisms, it is acquired through dietary uptake in animals. Human dietary sources include milk, eggs, fish and meat in quantities in excess of a few micrograms a day (Ref. 61). In humans, the absorption, transport and cellular uptake of cobalamin is complex. Food-bound cobalamin is released in the stomach with the help of peptic activity, where it is subsequently

bound by haptocorrin (HC) (Ref. 62). In the small intestine, cobalamin is released from HC by pancreatic protease digestion and bound by intrinsic factor (IF) to form an IF–Cbl complex. IF is very specific for cobalamin (i.e. for forms that have the lower DMB intact) and presumably acts as an early screening mechanism to prevent degraded cobalamins from intracellular access (Ref. 63). The IF–Cbl complex passes through the small intestine, where it is bound on the apical surface of ileal epithelial cells by a receptor composed of a heterodimer of amnionless and cubilin, called cubam, which aids in the endocytosis of IF–Cbl (Refs 64, 65). Once inside the cell, IF is degraded in the lysosomes and cobalamin is released into the cytosol (Ref. 66), where it is transported across the ileal receptor cell and released into the bloodstream, possibly by the recently identified (with respect to vitamin B_{12} transport) multidrug resistance protein MRP1 (Ref. 67). In the bloodstream, cobalamin binds to either HC or transcobalamin (TC) (Ref. 68). Although HC binds the bulk of plasma cobalamin (75–90%), it is not involved in cellular cobalamin uptake apart from uptake in hepatocytes (Ref. 68). Therefore, individuals who have deficient or absent HC have serum cobalamin values in the deficient range, but show no sign of cobalamin deficiency (Ref. 61). Although TC binds only a minor fraction of circulating cobalamins (10–25%), it is the protein responsible for facilitating the uptake of cobalamin by cells (Refs 69, 70). Mutations in the gene encoding TC (*TCN1*) result in severe tissue cobalamin insufficiency, megaloblastic anaemia, failure to thrive and often neurological complications, despite normal plasma cobalamin concentrations (Refs 71, 72). Additionally, TC acts as a final screening mechanism because, like IF, TC is very specific for cobalamin forms that have the lower DMB intact (Refs 73, 74). Treatment of TC deficiency requires very high serum cobalamin levels, ranging from 1000 to 10 000 pg/ml, achieved by oral or intramuscular delivery of 0.5–1.0 mg of CNCbl or HOCbl once or twice weekly (Refs 60, 75). There is some evidence that at sufficiently high concentrations, at least some tissues are capable of taking up unbound cobalamin (Ref. 61). From the bloodstream, cobalamin is taken up into cells through receptor-mediated endocytosis as a complex of Cbl–TC bound to the TC receptor (TCblR)

(Refs 76, 77, 78). A mutation in the gene encoding TCblR (*CD320*) was recently identified in asymptomatic newborns whose fibroblasts showed decreased Cbl uptake, where restoration of the missing codon by site-directed mutagenesis (c.262–264) resulted in normal TCblR function (Ref. 79). In the lysosome, the Cbl–TC complex is digested to create free cobalamin, which is subsequently transported into the cytosol probably as a mixture of cob(III)alamin and cob(II)alamin. Once in the cytosol, cobalamin is processed by many proteins, some known, others perhaps still unknown, to produce the cofactors MeCbl and AdoCbl. Failure to produce the cobalamin cofactors results in a lack of functional enzymes and causes the constellation of biochemical, developmental and neurological manifestations associated with intracellular pathway defects.

Complementation analysis for cataloguing the intracellular pathway of vitamin B_{12} disorders

The considerable range of clinical and biochemical heterogeneity observed in patients with vitamin B_{12} pathway disorders led to a need to sort them into genetically defined groups. The question of whether severe and mild disease or B_{12}-responsive and -unresponsive forms could be explained by mutations in different genes was addressed early on by complementation analysis. This is a powerful technique that permits the identification of specific genes through their expression in fibroblast heterokaryons, multinucleate cells produced by the fusion of fibroblast strains from different patients, which could then be tested for restoration of function. To examine complementation, the incorporation of [^{14}C]propionate or [^{14}C]methyltetrahydrofolate [or [^{14}C]formate to methionine and serine (Ref. 22)] into trichloroacetic acid (TCA)-precipitable material was monitored by autoradiography of cells in situ or by direct scintillation counting of the TCA precipitate (Refs 80, 81). Initially, four distinct complementation groups were identified, *cblA–cblC* and *mut.* However, over the years, the method came to be used diagnostically with hundreds of cell lines being analysed, mainly in the McGill University laboratory of David Rosenblatt, which became a dominant diagnostic centre using these techniques. So far,

4

eight complementation groups, *cblA–cblG* and *mut*, have been described that have blocks in the production or utilisation of MeCbl, AdoCbl or both cofactors. Although all the genes corresponding to these disorders have now been described, many of their functions remain unclear. Figure 2 illustrates the known or predicted functional location of the protein products of these genes. Three complementation groups – *cblF*, *cblC* and *cblD* – correspond to blocks in steps that are common to the synthesis of both cofactors with resulting deficiency of MS and MCM activities. Patients from these groups have combined homocystinuria and methylmalonic aciduria. Three groups, *cblD* variant 1, *cblE* and *cblG*, have blocks in the cytosolic pathway leading to MeCbl synthesis or apo-MS and result in deficient MS activity and

Intracellular processing of vitamin B_{12} showing sites of defects in complementation groups

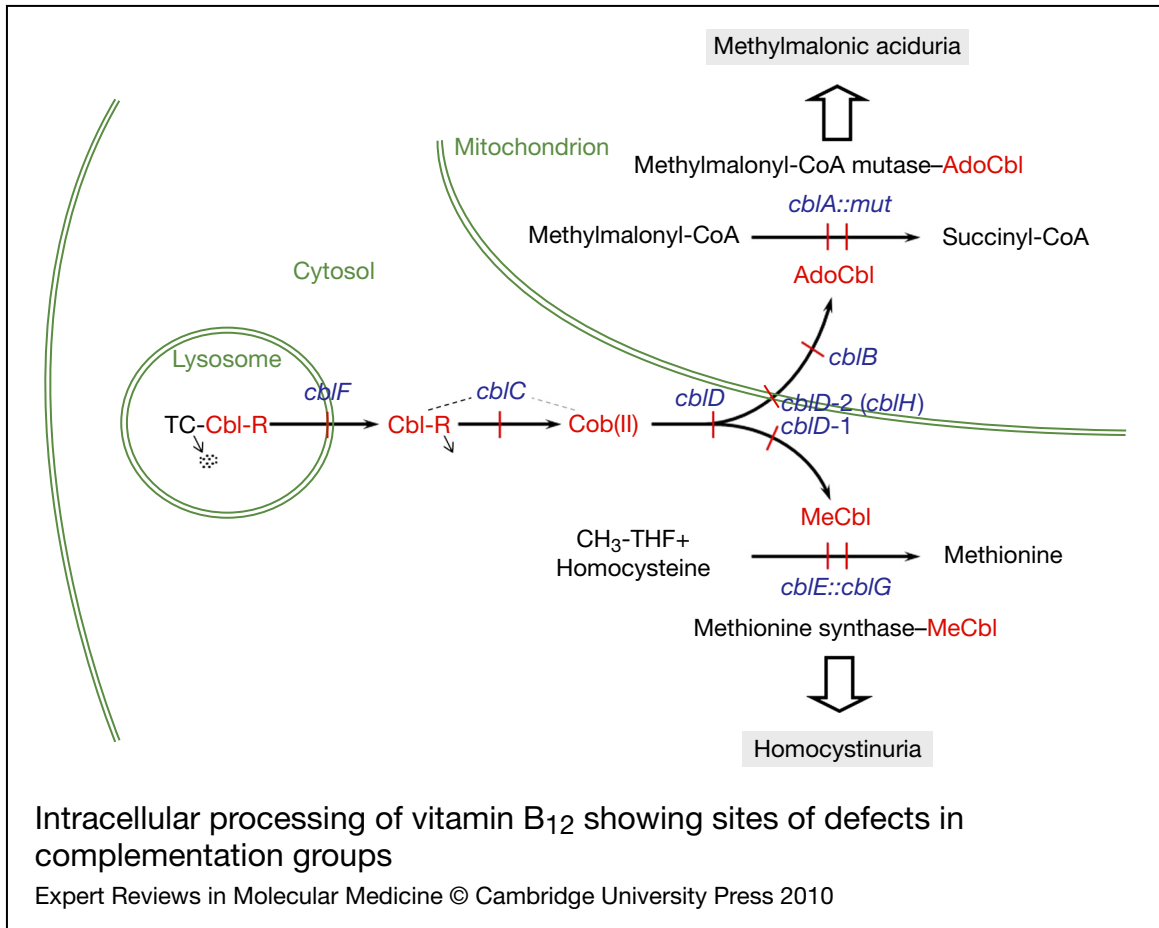Expert Reviews in Molecular Medicine © Cambridge University Press 2010

Figure 2. Intracellular processing of vitamin B_{12} showing sites of defects in complementation groups. Complementation groups are in blue and are positioned at sites of metabolic blocks (shown in red). Cobalamin intermediates are in red. Excreted metabolites due to genetic defects are in shaded boxes. Pathway details are described in the text. In the lysosome, cobalamin is released from transcobalamin (TC) through its degradation (arrow pointing to dots). In the cytosol, R groups are released by the *cblC* protein with the cob(II)alamin [Cob(II)] product remaining bound (dotted line emanating from the *cblC* protein denotes complex with cobalamin forms). The three versions of the *cblD* protein (*cblD*, *cblD-1*, *cblD-2*) illustrate the role of the protein in directing cobalamin to the mitochondrial or cytosolic pathway. In the mitochondrion, the *cblB* protein adds the 5′-deoxyadenosyl group, generating the active cofactor [adenosylcobalamin (AdoCbl)], which is transferred to the *mut* [methylmalonyl-CoA mutase (MCM)] protein. The cblA protein is proposed to act as a gatekeeper to ensure that the cofactor form that is accepted and retained by MCM is AdoCbl. In the cytosolic pathway, cob(II)alamin is bound to the *cblG* [methionine synthase (MS)] protein. The *cblE* [methionine synthase reductase (MSR)] protein catalyses generation of the active cofactor, methylcobalamin (MeCbl), or its regeneration if oxidised to cob(II)alamin during reaction cycles.

homocystinuria. The final groups, *cblD* variant 2, *cblA*, *cblB* and *mut*, affect steps occurring in the mitochondrion leading to AdoCbl synthesis or apo-MCM and result in deficient MCM activity and methylmalonic aciduria.

Complementation groups affecting both methionine synthase and methylmalonyl-CoA mutase

cblF

cblF was initially assigned to describe an infant with developmental delay and mild methylmalonic aciduria who was vitamin B_{12} responsive (Ref. 82). Her fibroblasts were found to accumulate free cobalamin, failing to attach to MS or MCM. By electron microscopy and subcellular fractionation, it was shown that most of the cobalamin was trapped in the lysosome, with only a small portion reaching the cytosol or mitochondria (Ref. 83). Complementation studies confirmed the genetically distinct disorder (Ref. 84). It was proposed that *cblF* represents a defect in the export of cobalamin out of the lysosome into the cytosol (Ref. 82). The gene responsible for *cblF* was very recently cloned by homozygosity mapping and microcell-mediated chromosome transfer and was found to correspond to *LMBRD1*, which encodes the lysosomal membrane protein LMBD1 (Ref. 27). LMBD1 shares homology with a family of membrane proteins that internalise lipocalins, which are carriers of small hydrophobic molecules such as steroids and lipids, leading to the suggestion that a lipocalin-like molecule may bind vitamin B_{12} in the lysosome on its release following TC degradation (Ref. 27). Transfection of *cblF* fibroblasts with intact cDNA corrected intracellular cobalamin processing and restored functional MS and MCM. The nature of the protein, with nine predicted transmembrane domains, and localisation of the protein to the lysosomal membrane, is consistent with a role in the export of cobalamin from the lysosome. Only 13 patients have been described with *cblF* (Refs 27, 28). Six mutations have been identified, and, interestingly, all of them have been chain-terminating frameshift mutations, with one, 1056delG, accounting for 18 of 26 analysed alleles (Fig. 3). Clinically, it is a highly variable disorder. Most patients show failure to thrive in infancy and mild to severe developmental delay, but respond to vitamin B_{12} therapy. Although most patients presented with homocystinuria and methylmalonic aciduria, the index case, mentioned above, showed no evidence of megaloblastic anaemia or homocystinuria. This individual was reportedly asymptomatic on B_{12} therapy in adulthood, despite being homoallelic for the 1056delG mutation (Ref. 27).

cblC

The *cblC* complementation group originally corresponded to the first set of patients who failed to produce either AdoCbl or MeCbl (Ref. 85). Since then, approximately 400 patients have been described with *cblC*, making it the most common disorder of intracellular vitamin B_{12} metabolism (Ref. 14). The gene responsible for the *cblC* group, called *MMACHC*, was identified by homozygosity mapping in 2006 (Ref. 15). More than 50 different disease-causing mutations have been identified and are summarised in Ref. 14 (Fig. 3). The most common is the c.271dupA mutation, which causes a frameshift truncation, accounting for 42% of pathogenic alleles (Ref. 14). Additionally, the c.394C > T (R132X) and c.331C > T (R111X) mutations are found commonly, at 20% and 5% of alleles, respectively. Although all *cblC* patients have combined homocystinuria and methylmalonic aciduria and often have haemotological, neurological and ophthalmic abnormalities to some degree, they tend to fall into either of two distinct phenotypes correlating with age of onset (Ref. 86). Early-onset patients present in the first year of life with severe disease and rarely respond clinically to treatment, whereas late-onset patients present in childhood to adulthood, are more likely to have less severe symptoms, and usually respond better to treatment (Ref. 86). A strong genotype–phenotype correlation can be found with some mutations: the c.271dupA and c.331C > T (R111X) mutations usually cause the much more prevalent early-onset disease, whereas some missense mutations [e.g. c.482G > A (R161Q)] and, bewilderingly, the c.394C > T (R132X) nonsense mutation usually result in late-onset disease (Refs 14, 15). The *cblC* protein (MMACHC) was predicted to have a vitamin B_{12} binding site and a TonB-like domain; the latter is a protein associated with bacterial cobalamin uptake (Ref. 15). Although initial studies suggested that MMACHC had base-on CNCbl binding (Ref. 87), it was later
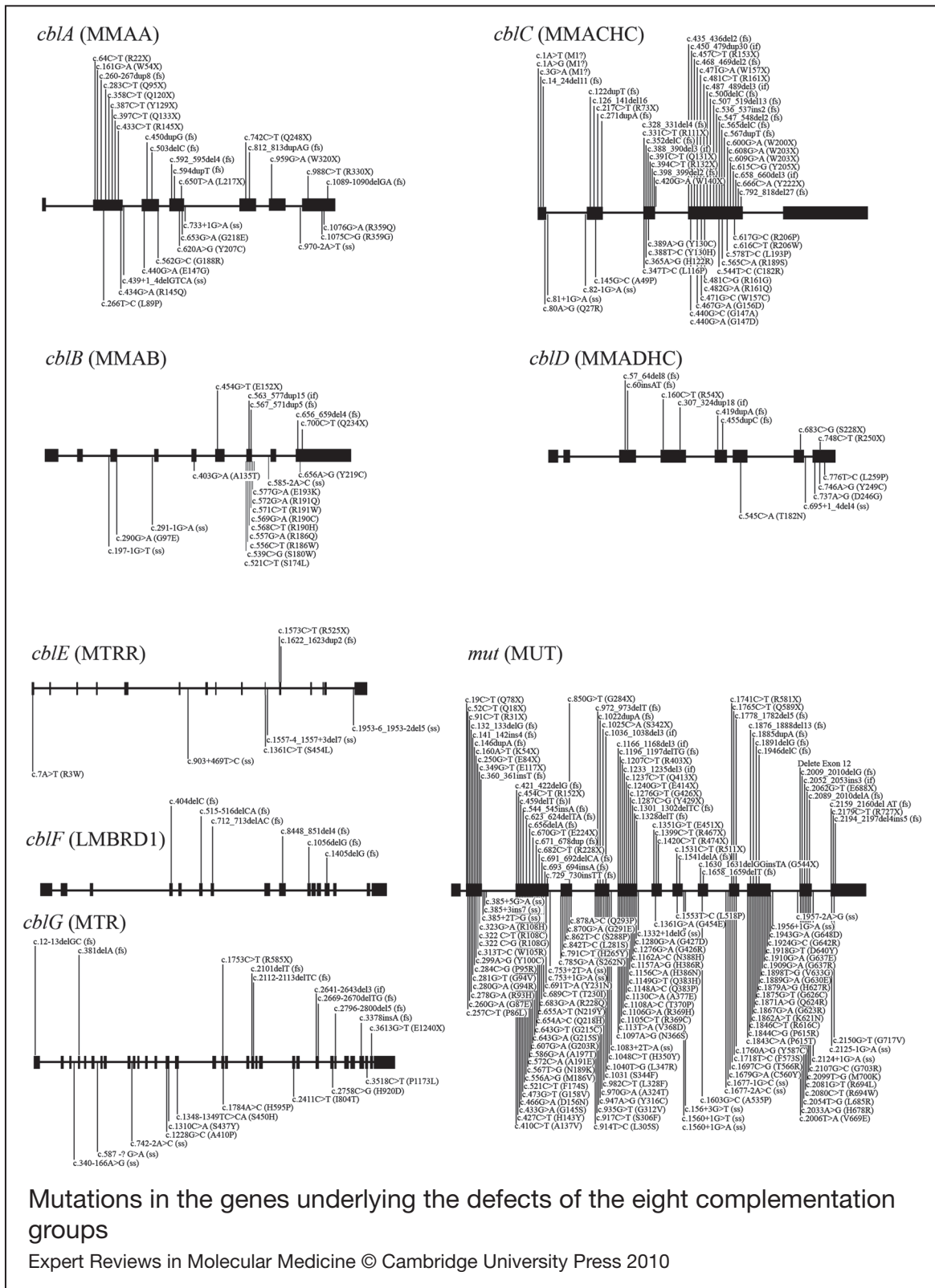
Genetic disorders of vitamin B_{12} metabolism: eight complementation groups – eight genes

6

Genetic disorders of vitamin B$_{12}$ metabolism: eight complementation groups – eight genes

Mutations in the genes underlying the defects of the eight complementation groups

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Figure 3. Mutations in the genes underlying the defects of the eight complementation groups. (*See next page for legend.*)

Genetic disorders of vitamin B₁₂ metabolism: eight complementation groups – eight genes

Figure 3. Mutations in the genes underlying the defects of the eight complementation groups. (*See previous page for figure.*) For each complementation group, the gene name is given in brackets. Mutations are shown as cDNA position with corresponding amino-acid change in brackets. The numbering for each is based on the cDNA sequence: +1 corresponds to the A of the ATG translation initiation codon. Nonsense and frameshift (fs) mutations are displayed above the gene whereas missense and possible splice site or cryptic splice site (ss) mutations are displayed below. Mutations are based on *cblA* (Refs 6, 7, 8, 9, 10), *cblB* (Refs 11, 12, 13), *cblC* (Refs 14, 15, 16, 17, 18, 19), *cblD* (Refs 20, 21), *cblE* (Refs 22, 23, 24, 25, 26), *cblF* (Refs 27, 28), *cblG* (Refs 29, 30, 31, 32) and *mut* (Refs 9, 13, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50).

demonstrated to bind CNCbl in the base-off conformation (Ref. 88) and to reductively cleave the CN group to form MMACHC-bound cob(II)alamin (Ref. 87). It has also been shown to catalyse glutathione-dependent dealkylation of cobalamins containing C2–C6 alkanes, Ado-, or Me- as the upper axial ligand (Ref. 89). These results suggest that MMACHC might be involved in intracellular cobalamin transport and reductive dealkylation or decyanation, perhaps interacting with the *cblF* protein for export of cobalamin out of the lysosome and beginning the initial processing of cobalamin, yielding cob(II)alamin, before passing it along for distribution to the rest of the pathway (Refs 14, 62) (Fig. 2).

Late-onset *cblC* patients are cobalamin responsive but, as observed initially in fibroblasts and later in patients, they respond poorly to CNCbl compared with OHCbl, a phenomenon that is unique among the vitamin B₁₂ disorders (Refs 86, 90, 91, 92). Investigations of cobalamin binding by wild-type and mutant protein, the latter containing the R161Q mutation commonly associated with OHCbl responsiveness in *cblC* patients, revealed reduced binding of CNCbl but not OHCbl by the mutant protein compared with the wild type (Ref. 93). Further, thermolability studies showed that MMACHC protein is strongly stabilised by cobalamin binding, whereas mutant protein was much less stabilised and only minimally or not at all with CNCbl (Ref. 87). These results suggest that OHCbl responsiveness in patients with the R161Q mutation is due to a combination of better affinity for OHCbl than CNCbl and a much better stabilisation of the mutant protein by OHCbl. It may well be that the high-dose OHCbl treatment generally used with *cblC* patients protects the protein from degradation in vivo. An interesting outcome of these studies was the finding that the cobalamin cofactors, AdoCbl and MeCbl, were far more

protective of the mutant protein than OHCbl. Although treatment with AdoCbl or MeCbl has been tried before, it was found that they are not incorporated directly as cofactors of their cognate enzymes but are dealkylated and processed anew, results that have been confirmed in vitro with MMACHC (Refs 89, 94). However, the increased stabilisation of mutant protein afforded by the cofactors suggests that their use should be re-examined in some cases.

cblD

The *cblD* complementation group was first described in two siblings with combined homocystinuria and methylmalonic aciduria and deficiency of MCM and MS activities (Ref. 95), although the designation '*cblD*' was not given until many years later (Ref. 81). For over 25 years, they remained the only *cblD* patients described, and biochemical analysis revealed that only this complementation group seemed to behave in a manner similar to *cblC*, but with less severe defects (Ref. 91). However, in 2004, Suormala et al. (Ref. 96) described three new cases of *cblD:* two had only MS deficiency (called *cblD* variant 1) and one had only MCM deficiency (*cblD* variant 2). These results suggested that the *cblD* protein might be responsible for branching of the cobalamin metabolism pathways to the cytosolic or mitochondrial compartments (Fig. 2). The same group cloned the *cblD* gene 4 years later (Ref. 20), naming it *MMADHC*. With an additional four patients they showed a clear genotype–phenotype relationship, whereby truncation mutations in the 5′ region resulted in only methylmalonic aciduria (MCM deficiency), truncation mutations in the middle and 3′ regions resulted in combined methylmalonic aciduria and homocystinuria (MCM and MS deficiency), and missense mutations in the 3′ region resulted in only homocystinuria (MS deficiency) (Fig. 3). Transfection experiments

8

demonstrated correction of the defect in mutant fibroblasts. In particular, a cDNA constructed with a 5′-truncation mutation could correct the synthesis of MeCbl, suggesting that an internal translation initiation site is probably functional. Additionally, they demonstrated that the *cblH* complementation group, which had been previously described for one patient with unidentified methylmalonic aciduria (Ref. 97), was actually an example of *cblD* variant 2 (Fig. 2). MMADHC has a predicted mitochondrial leader sequence and a putative vitamin B_{12} binding sequence and shows limited homology to a bacterial ABC transporter (Ref. 20). Although no functional or biochemical data are yet available for MMADHC, it is currently speculated to interact with MMACHC as part of a chaperone role to present cobalamin to the cytosolic or mitochondrial pathways (Refs 20, 62).

Complementation groups affecting only methionine synthase

In 1984, Schuh and colleagues (Ref. 98) described an infant with homocystinuria, megaloblastic anaemia and developmental delay who, although treatable with cobalamin, showed no evidence of methylmalonic aciduria, a novel outcome at the time. This suggested that the infant had a unique block in MS or the synthesis of MeCbl. Subsequently, two other patients were described with the same symptoms (Refs 99, 100). Broken cell extracts from the original patient revealed that with added reducing agents, MS worked perfectly. However, this was not the case for MS from the next two patients. Their cell extracts were defective regardless of additions. Ultimately, Watkins and Rosenblatt (Ref. 101) used complementation analysis to show that the original patient, designated as *cblE*, had a block in a separate genetic locus to the other two patients, which they designated as *cblG* (Fig. 2).

cblG

The gene responsible for *cblG* was cloned by three separate groups based on the identification of human sequences homologous to the *E. coli* vitamin-B_{12}-dependent MS, encoded by the *metH* gene, and other bacterial and *Caenorhabditis elegans* sequences (Refs 29, 102, 103). The human gene is designated *MTR*, for methyltransferase, as it was

named when initially mapped to human chromosome 1 (Ref. 91), or more formally as 5-methyltetrahydrofolate:homocysteine methyltransferase. Twenty different mutations have been identified in *MTR* (Fig. 3). The most common is c.3518C > T (P1173L), which is present in 16 of 24 *cblG* cell lines surveyed (Ref. 30). The clinical disease is highly variable, with onset ranging from neonatal to adulthood, although most patients present with homocystinuria, hypomethioninaemia, megaloblastic anaemia and developmental delay (Refs 101, 104, 105, 106). In addition to its importance in protein synthesis, MS is a key enzyme of the methionine cycle, which maintains the cellular level of the methionine derivative, *S*-adenosylmethionine, the methyl donor in a wide array of cellular processes including DNA, RNA and protein methylation. It is also uniquely involved in the folate cycle, because it is the only mammalian enzyme to use 5-methyltetrahydrofolate as a methyl donor (Ref. 30). MS catalyses the methylation of homocysteine to form methionine in a reaction that requires the presence of enzyme-bound MeCbl for activity (Refs 107, 108). The reaction proceeds by methyl transfer from 5-methyltetrahydrofolate to MS-bound cob(I)alamin to form MeCbl, followed by transfer of the methyl group to homocysteine to form methionine and regeneration of cob(I)alamin (Refs 109, 110). Mammalian MS and *Escherichia coli* MetH are 55% identical (Ref. 103). The sequence homology extends to the domain structure of MetH in which linearly arrayed domains of the enzyme contain binding sites for the various substrates and cofactors (Refs 111, 112, 113). These domains seem to be faithfully maintained in mammalian MS.

cblE

Because the reaction catalysed by MS regenerates cob(I)alamin in every reaction cycle, the cofactor risks occasional oxidation to cob(II)alamin with consequent inactivation of the enzyme (Ref. 114). In *E. coli*, the restoration of a functional cofactor is dependent on two flavoproteins, flavodoxin and flavodoxin reductase, containing flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) prosthetic groups, respectively (Refs 115, 116). The corresponding human reducing system proved to be encoded by a single gene, *MTRR*,

which is mutated in *cblE* patients (Fig. 3); it encodes a single protein containing FMN- and FAD/NADPH-binding sites (Ref. 117). This enzyme, named methionine synthase reductase (MSR), is a linear array of 'flavodoxin' at the N-terminus, an intervening linker sequence in the middle and 'flavodoxin reductase' at the C-terminus. It restores MS activity by catalysing the reductive methylation of cob(II)alamin on the inactivated MS in which *S*-adenosylmethionine is the source of the methyl group (Refs 118, 119). Additionally, MSR has been shown to catalyse the reduction of free B_{12} [as aquocob(III)alamin] to cob(II)alamin (Ref. 120), making it an aquocobalamin reductase and possibly functioning as such in the cytosolic pathway. MSR has also been suggested to function as a cob(II)alamin reductase in the mitochondrial pathway. This hypothesis was based on a potential mitochondrial leader sequence found by alternative splicing in the *MTRR* gene, as well as in vitro results demonstrating the production of cob(I)alamin through physical interaction with the mitochondrial MMAB protein (Refs 117, 121). However, evidence demonstrating MSR expression only in the cytosol (Ref. 122) and separate evidence suggesting that MMAB does not require a distinct cobalamin reductase (Ref. 123) have strongly countered this argument. The clinical presentation of *cblE* patients is similar to that of *cblG* patients. It is usually impossible to separate the two disorders, except for one patient who unexpectedly also had methylmalonic aciduria (Ref. 105), which remains unexplained.

Complementation groups affecting only methylmalonyl-CoA mutase

Patients with methylmalonic aciduria without elevated homocysteine or abnormalities of circulating vitamin B_{12} have defects in the mitochondrial pathway of AdoCbl synthesis and functional MCM. Clinically, patients have the following common features: failure to thrive, lethargy, vomiting of protein feeds, dehydration, respiratory distress and hypotonia (Ref. 124). Early on, three genes were implicated in the mitochondrial pathway, corresponding to complementation groups *cblA*, *cblB* and *mut* (Refs 80, 85) (Fig. 2). A detailed understanding of the mitochondrial processing of vitamin B_{12} is only now beginning to emerge, based

largely on studies completed on bacterial model systems.

cblA

cblA was initially the designation for patient fibroblasts that failed to accumulate AdoCbl in intact cells, but showed restored AdoCbl synthesis in a broken-cell assay with OHCbl, ATP and a reducing system (Ref. 85). This block was hypothesised to correspond to a defect in mitochondrial cob(II)alamin reductase because of the ability of an external reducing system to alleviate the block (Ref. 85). The human gene, named *MMAA*, was identified in a search for genes clustered in proximity to MCM (*mut*) in microbial genomes and while searching for orthologous sequences in the human genome (Ref. 6). Examination of the sequence of *MMAA*, however, revealed that it did not encode a reductase, but rather a protein that belonged to the G3E family of P-loop GTPases, a group of proteins that participate in the assembly or function of the metal centres in metalloenzymes (Ref. 6). More than 30 *cblA* patient mutations have been described in the *MMAA* gene, with most of them corresponding to nonsense or frameshift mutations (Fig. 3). Although most *cblA* patients present in infancy or childhood with methylmalonic aciduria and potentially life-threatening acidotic crises (Ref. 104), they often respond to vitamin B_{12} therapy despite the severity of mutations (Refs 104, 124, 125). Reasons for this are becoming clearer because of our increased understanding of the role of MMAA. Studies have focused on the bacterial orthologues of MMAA, including key research by Banerjee and colleagues working with MeaB, the MMAA orthologue from *Methylobacterium extorquens*. Initial bacterial studies showed that MMAA orthologues form a complex with MCM and that GTP binding and hydrolysis contribute to cobalamin processing (Refs 126, 127, 128). Further, it was shown that MeaB protects MCM from inactivation and that the state of MeaB (apo, GDP or GTP bound) alters the affinity of MCM for AdoCbl (Ref. 129). Finally, recent studies suggest that MeaB acts as a regulator of MCM cofactor binding and ejection, where the binding and hydrolysis of GTP by MeaB are important in the discrimination of MCM binding to AdoCbl versus cob(II)alamin, and promotes ejection of the latter, inactive cofactor

from MCM (Ref. 130). These studies are summarised below in the proposed model for cobalamin processing.

cblB

Patients in the *cblB* group are also deficient in AdoCbl synthesis and are metabolically similar to *cblA* (Ref. 86). However, *cblB* patients tend to present earlier, respond more poorly to vitamin B_{12} and, consequently, may produce a more severe clinical course with more profound neurological and metabolic complications (Refs 124, 125). The *cblB* disorder was separated biochemically from *cblA* by the failure to synthesise AdoCbl in broken cell extracts containing a reducing system (Ref. 85). On the basis of this early work, *cblB* had long been expected to correspond to a defect in the ATP:cob(I)alamin ATR. Like *MMAA*, the gene was identified in the survey of MCM-containing gene clusters in microbial genomes (see the previous section) and was named *MMAB* (Ref. 11). It was shown to correspond to an ATR based on sequence and functional similarity with a class of bacterial ATRs called PduO (Refs 11, 131). The MMAB protein catalyses the transfer of the 5′-deoxyadenosyl group of ATP to cob(I)alamin to form AdoCbl. To assess function, cob(II)alamin is the usual cobalamin added in vitro, with a reducing system, often MSR and NADPH, added to facilitate the reaction. Unexpectedly, in the presence of ATP, MMAB binds cob(II)alamin in an unusual base-off, four-coordinate state (Ref. 51). This unusual binding elevates the redox potential of cob(II)alamin to the physiological range of possible reduction by reduced flavin, perhaps in the form of an electron transfer protein, thus obviating the need for a specific cobalamin reductase to generate the reactive cob(I)alamin intermediate (Ref. 123). Most of the mutations identified in *cblB* disease were found to cluster in exon 7, which encodes the active site of the enzyme (Refs 12, 132) (Fig. 3). Several mutations have been modelled in human and microbial ATRs, identifying defects in substrate or cofactor binding, active site functions or protein dynamics (Refs 132, 133, 134, 135, 136). Interestingly, two of the mutations (R186W, E193K) resulted in absent protein in western blots of patient cell extracts, suggesting protein instability as a major contributor to disease phenotype (Ref. 137). Human MMAB

has been crystallised in the presence of ATP (Ref. 132). It was found to exist as a trimer with three active sites, only two of which contained ATP. Crystallisation in the presence of both ATP and cob(II)alamin has been accomplished for the *Lactobacillus reuteri* ATR (*Lr*PduO) (Ref. 135). The two enzymes are highly similar and, interestingly, AdoCbl was detected in *Lr*PduO crystals, underscoring the capacity of reduced flavin, generated in the incubation mix, to drive the four-coordinate cob(II)alamin intermediate to cob(I)alamin and the formation of AdoCbl.

mut

The *mut* complementation group is representative of mutations in the *MUT* (MCM) gene. MCM is important for the metabolism of branched-chain amino acids, odd-chain fatty acids and cholesterol (Ref. 104). It catalyses the reversible isomerisation of L-methylmalonyl-CoA to L-succinyl-CoA (Fig. 2), which is important for the breakdown of propionate and for replenishing the tricarboxylic acid cycle. The *MUT* gene was the earliest of the human B_{12} pathway genes to be cloned, accomplished by antibody screening of a human liver λgt11 expression library (Ref. 138). Nearly 200 disease-causing mutations have been identified in *MUT* (Ref. 33) (Fig. 3). Two distinct classes of mutations have been described: mut^- ('mut-minus'), when there is residual enzyme activity or detectable [^{14}C] propionate incorporation by mutant fibroblasts; and mut^0 ('mut-zero'), when protein or enzyme activity is not detected, as found, for example, in frameshift or chain-terminating mutations and some amino-acid substitutions (Refs 139, 140). Unsurprisingly, these two groups separate patients clinically. mut^0 patients have a higher occurrence of morbidity, mortality and neurological complications than mut^-, and mut^- patients are more responsive to B_{12} therapy (Ref. 124). MCM from *Proprionibacterium shermanii* has been crystallised in the presence of AdoCbl and substrate (PDB accession number 4REQ) (Refs 141, 142, 143). The human enzyme (PDB accession number 3BIC) is structurally similar to the *P. shermanii* enzyme, with which it shares 60% identity in the α-subunit (Ref. 144). Human MCM exists as a homodimer in the mitochondrial matrix with 1 mol of AdoCbl bound per subunit (Refs 145, 146). Studies of the *Methylobacterium extorquens* enzyme, as described above, suggest that human MCM does not exist alone but

functions as a complex with other proteins, notably MMAA and possibly MMAB.

Model for the intracellular processing of vitamin B_{12}

The genes and proteins corresponding to all eight complementation groups defined in patients have provided most of the elements required to describe the intracellular processing of vitamin B_{12} (Fig. 2). Cobalamin, generally as OHCbl or CNCbl, is taken into the lysosome as a Cbl–TC complex, where digestion of the transcobalamin releases free cobalamin. The cobalamin is transported into the cytosol through the LMBD1 protein, possibly drawn through the lysosomal transporter by interaction of the cytosol face of LMBD1 with the MMACHC protein. It appears that cobalamins are bound to MMACHC in the base-off state, poised for cleavage of the upper axial ligand if one is present. MMACHC may act as an intracellular cobalamin carrier for delivery of the cofactor to the MMADHC protein for targeting to the cytosolic (MS) or mitochondrial (MCM) pathways. Evidence of interaction of MMACHC with either LMBD1 or MMADHC has yet to be demonstrated. In the cytosolic compartment, cob(II)alamin is expected to be bound to MS, where it is reductively methylated by MSR, using NADPH as an electron donor, to generate the MeCbl active cofactor of MS. MMADHC also participates in the targeting of cobalamin to the mitochondrial pathway, although the specific transporter has yet to be identified. Cob(II)alamin, on entry into the mitochondrial matrix, is bound by MMAB for the generation of AdoCbl, with the reducing equivalents probably coming from an electron transfer protein rather than a cobalamin reductase, as had been previously anticipated. The subsequent transfer of AdoCbl to MCM is predicted to be an exquisitely complicated process involving a complex of MMAA and MCM and possibly MMAB.

First, the complex of MCM:MMAA–GTP prevents the binding of cob(II)alamin, which would otherwise inactivate MCM. Second, AdoCbl is transferred directly from MMAB to the MCM–MeaB–GTP complex in a process requiring ATP binding to MMAB and GTP hydrolysis by MMAA. Third, in reaction cycles in which the radical 5′deoxyadenosyl intermediate is lost, leaving MCM with an inactive cob(II)alamin cofactor, the GTP-bound MMAA causes displacement of the cofactor, making the enzyme available for renewed AdoCbl binding. The proposed role for MMAA derives principally from studies of MeaB by Padovani and Banerjee (Refs 127, 129, 130). The crystal structure of MeaB has been determined and carries the expected nucleotide-binding domains and domains predicted to be involved in MCM binding at the N-terminus and a dimerisation domain at the C-terminus (PDB accession number 4REQ) (Ref. 147). The recently crystallised human MMAA (PDB accession number 2WWW), although it has a similar overall structure, seems to adopt a slightly different mode of assembly, which may have implications for the three-way interaction with MMAB and MCM.

Research in progress and outstanding research questions

Although all eight genes predicted through complementation analysis have been identified, additional genes are anticipated. Most notably, the mechanism of the mitochondrial transport of B_{12} remains unknown. The involvement of *cblD* defects in both the cytosolic and mitochondrial pathways suggests that the MMADHC protein is an accessory to the mitochondrial uptake of vitamin B_{12}. The lysosomal delivery of vitamin B_{12} to the cytosol might also involve additional genes. The similarity of LMBD1 to the lipocalin family of membrane receptors predicts the involvement of a lipocalin-like molecule that might act as a vitamin B_{12} carrier after digestion of the Cbl–TC complex in the lysosome. Although a mitochondrial cobalamin reductase cannot be fully ruled out, studies on PduO-type ATRs argue against such a protein in human cells. Finally, if confirmed in human cells, the MMAA–MCM or MMAA–MCM–MMAB complex predicted by studies on MeaB proteins might account for the gene set required for mitochondrial cobalamin processing and utilisation. All these suggestions recognise the absence of additional disease states among human vitamin B_{12} processing disorders. The general view is that unidentified genes would probably not tolerate mutation (and therefore be lethal embryonically), might be associated with shared functions (and therefore might not reveal a vitamin B_{12} disease phenotype) or might be redundant with genes encoding proteins of similar function (allowing a bypass of a genetic

Genetic disorders of vitamin B_{12} metabolism: eight complementation groups – eight genes

defect). Therefore, completion of the human pathway requires either finding new genes or demonstrating that the pathway is fully functional without them. One approach might be to investigate the pathway in a model eukaryotic organism carrying orthologues of most (or all) the human genes, as suggested in studies of the methylmalonate pathway in *C. elegans*, an organism with knockout mutants of several vitamin B_{12} pathway genes (Refs 148, 149, 150).

Although we have gained much insight into the pathway of vitamin B_{12} metabolism, the goal of the medical geneticist has been to gain insight into managing the vitamin B_{12} disorders, providing access to carrier testing and prenatal diagnosis, and, in the best of outcomes, preventing or successfully treating symptomatic disease. The remarkable feature of vitamin B_{12} utilisation disorders has been their potential for treatment. The discovery that high-dose vitamin B_{12} can overcome pathway deficits in some patients has given new life to individuals with an otherwise potentially severe or fatal disease. The early discovery that OHCbl is effective in the treatment of *cblC* disorder while CNCbl is not is a powerful illustration of the complexity of vitamin B_{12} biochemistry. The more recent finding that AdoCbl or MeCbl may have a significant stabilising effect on MMACHC protein, despite ultimately being hydrolysed to cob(II)alamin, reminds us that there is still much to be learned on behalf of the patient. Strikingly, the most recent success with gene therapy to treat mice with knockout of the *Mut* gene (Ref. 151) has opened up a new avenue for treatment that might ultimately benefit patients with metabolically 'unresponsive' disorders. The application of widespread newborn screening for homocysteine and methylmalonate underscores the opportunity to identify and treat these patients before the onset of potentially irreversible disease.

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References

1 Smith, E.L. (1948) Purification of anti-pernicious anaemia factors from liver. Nature 161, 638

2 Rickes, E.L. et al. (1948) Crystalline vitamin B12. Science 107, 396-397

3 Minot, G.R. and Murphy, W.P. (1926) Treatment of pernicious anemia by a special diet. 1926. Yale Journal of Biology and Medicine 74, 341-353

4 Rosenberg, L.E., Lilljeqvist, A. and Hsia, Y.E. (1968) Methylmalonic aciduria: metabolic block localization and vitamin B 12 dependency. Science 162, 805-807

5 Hodgkin, D.C. et al. (1956) Structure of vitamin B12. Nature 178, 64-66

6 Dobson, C.M. et al. (2002) Identification of the gene responsible for the cblA complementation group of vitamin B12-responsive methylmalonic acidemia based on analysis of prokaryotic gene arrangements. Proceedings of the National Academy of Sciences of the United States of America 99, 15554-15559

7 Lerner-Ellis, J.P. et al. (2004) Mutations in the MMAA gene in patients with the cblA disorder of vitamin B12 metabolism. Human Mutation 24, 509-516

8 Merinero, B. et al. (2008) Methylmalonic acidaemia: examination of genotype and biochemical data in 32 patients belonging to mut, cblA or cblB complementation group. Journal of Inherited Metabolic Disease 31, 55-66

9 Martinez, M.A. et al. (2005) Genetic analysis of three genes causing isolated methylmalonic acidemia: identification of 21 novel allelic variants. Molecular Genetics and Metabolism 84, 317-325

10 Yang, X. et al. (2004) Mutation analysis of the MMAA and MMAB genes in Japanese patients with vitamin B(12)-responsive methylmalonic acidemia: identification of a prevalent MMAA mutation. Molecular Genetics and Metabolism 82, 329-333

11 Dobson, C.M. et al. (2002) Identification of the gene responsible for the cblB complementation group of vitamin B12-responsive methylmalonic acidemia. Human Molecular Genetics 11, 3361-3369

12 Lerner-Ellis, J.P. et al. (2006) Mutation and biochemical analysis of patients belonging to the cblB complementation class of vitamin B12-dependent methylmalonic aciduria. Molecular Genetics and Metabolism 87, 219-225

13 Champattanachai, V. et al. (2003) Novel mutations in a Thai patient with methylmalonic acidemia. Molecular Genetics and Metabolism 79, 300-302

Genetic disorders of vitamin B_{12} metabolism: eight complementation groups – eight genes

13

14 Lerner-Ellis, J.P. et al. (2009) Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Human Mutation 30, 1072-1081

15 Lerner-Ellis, J.P. et al. (2006) Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nature Genetics 38, 93-100

16 Heil, S.G. et al. (2007) Marfanoid features in a child with combined methylmalonic aciduria and homocystinuria (CblC type). Journal of Inherited Metabolic Disease 30, 811

17 Thauvin-Robinet, C. et al. (2008) The adolescent and adult form of cobalamin C disease: clinical and molecular spectrum. Journal of Neurology, Neurosurgery, and Psychiatry 79, 725-728

18 Yuen, Y.P. et al. (2007) DNA-based diagnosis of methylmalonic aciduria and homocystinuria, cblC type in a Chinese patient presenting with mild developmental delay. Clinica Chimica Acta 375, 171-172

19 Nogueira, C. et al. (2008) Spectrum of MMACHC mutations in Italian and Portuguese patients with combined methylmalonic aciduria and homocystinuria, cblC type. Molecular Genetics and Metabolism 93, 475-480

20 Coelho, D. et al. (2008) Gene identification for the cblD defect of vitamin B12 metabolism. New England Journal of Medicine 358, 1454-1464

21 Miousse, I.R. et al. (2009) Clinical and molecular heterogeneity in patients with the cblD inborn error of cobalamin metabolism. Journal of Pediatrics 154, 551-556

22 Zavadakova, P. et al. (2002) CblE type of homocystinuria due to methionine synthase reductase deficiency: clinical and molecular studies and prenatal diagnosis in two families. Journal of Inherited Metabolic Disease 25, 461-476

23 Zavadakova, P. et al. (2005) cblE type of homocystinuria due to methionine synthase reductase deficiency: functional correction by minigene expression. Human Mutation 25, 239-247

24 Vilaseca, M.A. et al. (2003) CblE type of homocystinuria: mild clinical phenotype in two patients homozygous for a novel mutation in the MTRR gene. Journal of Inherited Metabolic Disease 26, 361-369

25 Wilson, A. et al. (1999) Molecular basis for methionine synthase reductase deficiency in patients belonging to the cblE complementation group of disorders in folate/cobalamin metabolism. Human Molecular Genetics 8, 2009-2016

26 Kahleova, R. et al. (2002) Essential hypertension in adolescents: association with insulin resistance and with metabolism of homocysteine and vitamins. American Journal of Hypertension 15, 857-864

27 Rutsch, F. et al. (2009) Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B12 metabolism. Nature Genetics 41, 234-239

28 Gailus, S. et al. (2010) A novel mutation in LMBRD1 causes the cblF defect of vitamin B(12) metabolism in a Turkish patient. Journal of Inherited Metabolic Disease 33, 17-24

29 Leclerc, D. et al. (1996) Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. Human Molecular Genetics 5, 1867-1874

30 Watkins, D. et al. (2002) Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. American Journal of Human Genetics 71, 143-153

31 Gulati, S. et al. (1996) Defects in human methionine synthase in cblG patients. Human Molecular Genetics 5, 1859-1865

32 Wilson, A. et al. (1998) Functionally null mutations in patients with the cblG-variant form of methionine synthase deficiency. American Journal of Human Genetics 63, 409-414

33 Lempp, T.J. et al. (2007) Mutation and biochemical analysis of 19 probands with mut0 and 13 with mut-methylmalonic aciduria: identification of seven novel mutations. Molecular Genetics and Metabolism 90, 284-290

34 Worgan, L.C. et al. (2006) Spectrum of mutations in mut methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. Human Mutation 27, 31-43

35 Cavicchi, C. et al. (2005) Mutational spectrum in ten Italian patients affected by methylmalonyl-CoA mutase deficiency. Journal of Inherited Metabolic Disease 28, 1175-1178

36 Adjalla, C.E. et al. (1998) Seven novel mutations in mut methylmalonic aciduria. Human Mutation 11, 270-274

37 Jansen, R. and Ledley, F.D. (1990) Heterozygous mutations at the mut locus in fibroblasts with mut0 methylmalonic acidemia identified by polymerase-chain-reaction cDNA cloning. American Journal of Human Genetics 47, 808-814

38 Qureshi, A.A. et al. (1994) Cloning and expression of mutations demonstrating intragenic

Genetic disorders of vitamin B$_{12}$ metabolism: eight complementation groups – eight genes

14

complementation in mut0 methylmalonic aciduria. Journal of Clinical Investigation 93, 1812-1819

39 Janata, J., Kogekar, N. and Fenton, W.A. (1997) Expression and kinetic characterization of methylmalonyl-CoA mutase from patients with the mut- phenotype: evidence for naturally occurring interallelic complementation. Human Molecular Genetics 6, 1457-1464

40 Crane, A.M. et al. (1992) Phenotype of disease in three patients with identical mutations in methylmalonyl CoA mutase. Human Genetics 89, 259-264

41 Crane, A.M. and Ledley, F.D. (1994) Clustering of mutations in methylmalonyl CoA mutase associated with mut- methylmalonic acidemia. American Journal of Human Genetics 55, 42-50

42 Raff, M.L. et al. (1991) Genetic characterization of a MUT locus mutation discriminating heterogeneity in mut0 and mut- methylmalonic aciduria by interallelic complementation. Journal of Clinical Investigation 87, 203-207

43 Acquaviva, C. et al. (2005) Molecular basis of methylmalonyl-CoA mutase apoenzyme defect in 40 European patients affected by mut(o) and mut-forms of methylmalonic acidemia: identification of 29 novel mutations in the MUT gene. Human Mutation 25, 167-176

44 Peters, H.L. et al. (2002) Molecular studies in mutase-deficient (MUT) methylmalonic aciduria: identification of five novel mutations. Human Mutation 20, 406

45 Fuchshuber, A. et al. (2000) mut0 methylmalonic acidemia: eleven novel mutations of the methylmalonyl CoA mutase including a deletion–insertion mutation. Human Mutation 16, 179

46 Ogasawara, M. et al. (1994) Identification of two novel mutations in the methylmalonyl-CoA mutase gene with decreased levels of mutant mRNA in methylmalonic acidemia. Human Molecular Genetics 3, 867-872

47 Acquaviva, C. et al. (2001) N219Y, a new frequent mutation among mut(degree) forms of methylmalonic acidemia in Caucasian patients. European Journal of Human Genetics 9, 577-582

48 Benoist, J.F. et al. (2001) Molecular and structural analysis of two novel mutations in a patient with mut(-) methylmalonyl-CoA deficiency. Molecular Genetics and Metabolism 72, 181-184

49 Berger, I. et al. (2001) Mutation analysis of the MCM gene in Israeli patients with mut(0) disease. Molecular Genetics and Metabolism 73, 107-110

50 Touraine, R.L. et al. (1995) A 13-bp deletion (1952 del 13) in the methylmalonyl CoA mutase gene of an affected patient. Human Mutation 5, 354-356

51 Stich, T.A. et al. (2005) Spectroscopic evidence for the formation of a four-coordinate Co2+ cobalamin species upon binding to the human ATP:cobalamin adenosyltransferase. Journal of the American Chemical Society 127, 7660-7661

52 Schrauzer, G.N., Deutsch, E. and Windgassen, R.J. (1968) The nucleophilicity of vitamin B12. Journal of the American Chemical Society 90, 2441-2442

53 Krautler, B. (2005) Vitamin B12: chemistry and biochemistry. Biochemical Society Transactions 33, 806-810

54 Lexa, D. and Saveant, J.M. (1983) The electrochemistry of vitamin B12. Accounts of Chemical Research 16, 235-243

55 Eschenmoser, A. (1988) Vitamin B12: experiments concerning the origin of its molecular structure. Angewandte Chemie (International Edition in English) 27, 5-39

56 Benner, S.A., Ellington, A.D. and Tauer, A. (1989) Modern metabolism as a palimpsest of the RNA world. Proceedings of the National Academy of Sciences of the United States of America 86, 7054-7058

57 Roth, J.R., Lawrence, J.G. and Bobik, T.A. (1996) Cobalamin (coenzyme B12): synthesis and biological significance. Annual Review of Microbiology 50, 137-181

58 Zhang, Y. et al. (2009) Comparative genomic analyses of nickel, cobalt and vitamin B12 utilization. BMC Genomics 10, 78

59 Brown, K.L. (2005) Chemistry and enzymology of vitamin B12. Chemical Reviews 105, 2075-2149

60 Banerjee, R. and Ragsdale, S.W. (2003) The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. Annual Review of Biochemistry 72, 209-47

61 Rosenblatt, D.S. and Fenton, W.A. (2001) Inherited disorders of folate and cobalamin transport and metabolism. In The Metabolic and Molecular Bases of Inherited Disease (Scriver C.R., Beaudet A.L., Valle D. and Sly W.S., eds), pp. 3897-3933, McGraw-Hill, New York, USA

62 Quadros, E.V. (2010) Advances in the understanding of cobalamin assimilation and metabolism. British Journal of Haematology 148, 195-204

63 Banerjee, R. (2006) B12 trafficking in mammals: a case for coenzyme escort service. ACS Chemical Biology 1, 149-159

64 Moestrup, S.K. et al. (1998) The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral

15

membrane protein with homology to
developmental proteins. Journal of Biological
Chemistry 273, 5235-5242

65 Fyfe, J.C. et al. (2004) The functional cobalamin
(vitamin B12)-intrinsic factor receptor is a novel
complex of cubilin and amnionless. Blood 103,
1573-1579

66 Kapadia, C.R. et al. (1983) Intrinsic factor-mediated
absorption of cobalamin by guinea pig ileal cells.
Journal of Clinical Investigation 71, 440-448

67 Beedholm-Ebsen, R. et al. (2010) Identification of
multidrug resistance protein 1 (MRP1/ABCC1) as a
molecular gate for cellular export of cobalamin.
Blood 115, 1632-1639

68 Morkbak, A.L. et al. (2006) Effect of vitamin B12
treatment on haptocorrin. Clinical Chemistry 52,
1104-1111

69 Hall, C.A. and Finkler, A.E. (1963) A second
vitamin B12-binding substance in human plasma.
Biochimica Biophysica Acta 78, 234-236

70 Finkler, A.E. and Hall, C.A. (1967) Nature of the
relationship between vitamin B12 binding and cell
uptake. Archives of Biochemistry and Biophysics
120, 79-85

71 Whitehead, V.M. (2006) Acquired and inherited
disorders of cobalamin and folate in children.
British Journal of Haematology 134, 125-136

72 Sennett, C., Rosenberg, L.E. and Mellman, I.S.
(1981) Transmembrane transport of cobalamin in
prokaryotic and eukaryotic cells. Annual Review of
Biochemistry 50, 1053-1086

73 Allen, R.H. (1975) Human vitamin B12 transport
proteins. Progress in Hematology 9, 57-84

74 Fedosov, S.N. et al. (2002) Comparative analysis of
cobalamin binding kinetics and ligand protection for
intrinsic factor, transcobalamin, and haptocorrin.
Journal of Biological Chemistry 277, 9989-9996

75 Cooper, B.A. and Rosenblatt, D.S. (1987) Inherited
defects of vitamin B12 metabolism. Annual Review
of Nutrition 7, 291-320

76 Youngdahl-Turner, P. et al. (1979) Protein mediated
vitamin uptake. Adsorptive endocytosis of the
transcobalamin II-cobalamin complex by cultured
human fibroblasts. Experimental Cell Research 118,
127-134

77 Youngdahl-Turner, P., Rosenberg, L.E. and Allen,
R.H. (1978) Binding and uptake of transcobalamin
II by human fibroblasts. Journal of Clinical
Investigation 61, 133-141

78 Quadros, E.V., Nakayama, Y. and Sequeira, J.M.
(2009) The protein and the gene encoding the
receptor for the cellular uptake of transcobalamin-
bound cobalamin. Blood 113, 186-192

79 Quadros, E.V. et al. (2010) Positive newborn screen
for methylmalonic aciduria identifies the first
mutation in TCblR/CD320, the gene for cellular
uptake of transcobalamin-bound vitamin B(12).
Human Mutation 31, 924-929

80 Gravel, R.A. et al. (1975) Genetic complementation
in heterokaryons of human fibroblasts defective in
cobalamin metabolism. Proceedings of the National
Academy of Sciences of the United States of
America 72, 3181-3185

81 Willard, H.F., Mellman, I.S. and Rosenberg, L.E.
(1978) Genetic complementation among inherited
deficiencies of methylmalonyl-CoA mutase
activity: evidence for a new class of human
cobalamin mutant. American Journal of Human
Genetics 30, 1-13

82 Rosenblatt, D.S. et al. (1985) Defect in vitamin B12
release from lysosomes: newly described inborn
error of vitamin B12 metabolism. Science 228,
1319-1321

83 Vassiliadis, A. et al. (1991) Lysosomal cobalamin
accumulation in fibroblasts from a patient with an
inborn error of cobalamin metabolism (cblF
complementation group): visualization by electron
microscope radioautography. Experimental Cell
Research 195, 295-302

84 Watkins, D. and Rosenblatt, D.S. (1986) Failure of
lysosomal release of vitamin B12: a new
complementation group causing methylmalonic
aciduria (cblF). American Journal of Human
Genetics 39, 404-408

85 Mahoney, M.J. et al. (1975)
Methylmalonicacidemia: biochemical
heterogeneity in defects of 5′-
deoxyadenosylcobalamin synthesis. Proceedings of
the National Academy of Sciences of the United
States of America 72, 2799-2803

86 Rosenblatt, D.S. et al. (1997) Clinical
heterogeneity and prognosis in combined
methylmalonic aciduria and homocystinuria
(cblC). Journal of Inherited Metabolic Disease
20, 528-538

87 Kim, J., Gherasim, C. and Banerjee, R. (2008)
Decyanation of vitamin B12 by a trafficking
chaperone. Proceedings of the National Academy
of Sciences of the United States of America 105,
14551-14554

88 Froese, D.S. et al. (2010) Thermolability of mutant
MMACHC protein in the vitamin B12-responsive
cblC disorder. Molecular Genetics and Metabolism
100, 29-36

89 Hannibal, L. et al. (2009) Processing of
alkylcobalamins in mammalian cells: a role for the

MMACHC (cblC) gene product. Molecular Genetics and Metabolism 97, 260-266

90 Mudd, S.H., Uhlendorf, B.W. and Hinds, K.R. (1970) Deranged B 12 metabolism: studies of fibroblasts grown in tissue culture. Biochemical Medicine 4, 215-239

91 Mellman, I. et al. (1979) Cobalamin coenzyme synthesis in normal and mutant human fibroblasts. Evidence for a processing enzyme activity deficient in cblC cells. Journal of Biological Chemistry 254, 11847-11853

92 Andersson, H.C. and Shapira, E. (1998) Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). Journal of Pediatrics 132, 121-124

93 Froese, D.S. et al. (2009) Mechanism of vitamin B12-responsiveness in cblC methylmalonic aciduria with homocysteinuria. Molecular Genetics and Metabolism 98, 338-343

94 Kim, J. et al. (2009) A human vitamin B12 trafficking protein uses glutathione transferase activity for processing alkylcobalamins. Journal of Biological Chemistry 284, 33418-33424

95 Goodman, S.I. et al. (1970) Homocystinuria with methylmalonic aciduria: two cases in a sibship. Biochemical Medicine 4, 500-515

96 Suormala, T. et al. (2004) The cblD defect causes either isolated or combined deficiency of methylcobalamin and adenosylcobalamin synthesis. Journal of Biological Chemistry 279, 42742-42749

97 Watkins, D., Matiaszuk, N. and Rosenblatt, D.S. (2000) Complementation studies in the cblA class of inborn error of cobalamin metabolism: evidence for interallelic complementation and for a new complementation class (cblH). Journal of Medical Genetics 37, 510-513

98 Schuh, S. et al. (1984) Homocystinuria and megaloblastic anemia responsive to vitamin B12 therapy. An inborn error of metabolism due to a defect in cobalamin metabolism. New England Journal of Medicine 310, 686-690

99 Hallam, L.J. et al. (1987) Vitamin B12-responsive neonatal megaloblastic anemia and homocystinuria with associated reduced methionine synthase activity. Blood 69, 1128-1133

100 Rosenblatt, D.S. et al. (1987) Vitamin B12 responsive homocystinuria and megaloblastic anemia: heterogeneity in methylcobalamin deficiency. American Journal of Medical Genetics 26, 377-383

101 Watkins, D. and Rosenblatt, D.S. (1988) Genetic heterogeneity among patients with methylcobalamin deficiency. Definition of two complementation groups, cblE and cblG. Journal of Clinical Investigation 81, 1690-1694

102 Li, Y.N. et al. (1996) Cloning, mapping and RNA analysis of the human methionine synthase gene. Human Molecular Genetics 5, 1851-1858

103 Chen, L.H. et al. (1997) Human methionine synthase. cDNA cloning, gene localization, and expression. Journal of Biological Chemistry 272, 3628-3634

104 Fenton, W.A. et al. (2001) Disorders of propionate and methylmalonate metabolism. In The Metabolic and Molecular Bases of Inherited Disease (Scriver C.R., Beaudet A.L., Sly W.S. and Valle D., eds), pp. 2165-2193, McGraw-Hill, New York, USA

105 Watkins, D. and Rosenblatt, D.S. (1989) Functional methionine synthase deficiency (cblE and cblG): clinical and biochemical heterogeneity. American Journal of Medical Genetics 34, 427-434

106 Harding, C.O. et al. (1997) Functional methionine synthase deficiency due to cblG disorder: a report of two patients and a review. American Journal of Medical Genetics 71, 384-390

107 Matthews, R.G. (1984) Methionine biosynthesis. In Folates and Pterins (Blakely R.L. and Bencovic S.J., eds), pp. 497-553, J. Wiley & Sons, Inc., New York, USA

108 Taylor, R.T. (1983) B12-dependent methionine biosynthesis. In B12 (Dolphin D., ed.), pp. 307-355, John Wiley & Sons, Inc., New York

109 Matthews, R.G. (1999) Cobalamin-dependent methionine synthase. In Chemistry and Biochemistry of B12 (Banerjee R., ed.), pp. 681-706, John Wiley & Sons, New York, USA

110 Matthews, R.G. and Ludwig, M.L. (2001) Microbial modeling of human disease. In Homocysteine in Health and Disease (Carmel R. and Jacobsen D.W., eds), pp. 100-112, Cambridge University Press, Cambridge, UK

111 Goulding, C.W., Postigo, D. and Matthews, R.G. (1997) Cobalamin-dependent methionine synthase is a modular protein with distinct regions for binding homocysteine, methyltetrahydrofolate, cobalamin, and adenosylmethionine. Biochemistry 36, 8082-8091

112 Banerjee, R.V. et al. (1989) Cloning and sequence analysis of the Escherichia coli metH gene encoding cobalamin-dependent methionine synthase and isolation of a tryptic fragment containing the cobalamin-binding domain. Journal of Biological Chemistry 264, 13888-13895

113 Dixon, M.M. et al. (1996) The structure of the C-terminal domain of methionine synthase:

17

presenting S-adenosylmethionine for reductive methylation of B12. Structure 4, 1263-1275

114 Drummond, J.T. et al. (1993) Assignment of enzymatic function to specific protein regions of cobalamin-dependent methionine synthase from Escherichia coli. Biochemistry 32, 9290-9295

115 Fujii, K. and Huennekens, F.M. (1974) Activation of methionine synthetase by a reduced triphosphopyridine nucleotide-dependent flavoprotein system. Journal of Biological Chemistry 249, 6745-6753

116 Osborne, C., Chen, L.M. and Matthews, R.G. (1991) Isolation, cloning, mapping, and nucleotide sequencing of the gene encoding flavodoxin in Escherichia coli. The Journal of Bacteriology 173, 1729-1737

117 Leclerc, D. et al. (1998) Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. Proceedings of the National Academy of Sciences of the United States of America 95, 3059-3064

118 Olteanu, H. and Banerjee, R. (2001) Human methionine synthase reductase, a soluble P-450 reductase-like dual flavoprotein, is sufficient for NADPH-dependent methionine synthase activation. Journal of Biological Chemistry 276, 35558-35563

119 Wolthers, K.R. et al. (2003) Molecular dissection of human methionine synthase reductase: determination of the flavin redox potentials in full-length enzyme and isolated flavin-binding domains. Biochemistry 42, 3911-3920

120 Yamada, K. et al. (2006) Human methionine synthase reductase is a molecular chaperone for human methionine synthase. Proceedings of the National Academy of Sciences of the United States of America 20(103) 9476-9481

121 Leal, N.A. et al. (2004) Human ATP: Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. Journal of Biological Chemistry 279, 47536-47542

122 Froese, D.S. et al. (2008) Restricted role for methionine synthase reductase defined by subcellular localization. Molecular Genetics and Metabolism 94, 68-77

123 Mera, P.E. and Escalante-Semerena, J.C. (2010) Dihydroflavin-driven adenosylation of 4-coordinate Co(II) corrinoids: are cobalamin reductases enzymes or electron transfer proteins? Journal of Biological Chemistry 285, 2911-2917

124 Horster, F. et al. (2007) Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut0, mut-, cblA, cblB). Pediatric Research 62, 225-230

125 Matsui, S.M., Mahoney, M.J. and Rosenberg, L.E. (1983) The natural history of the inherited methylmalonic acidemias. New England Journal of Medicine 308, 857-861

126 Korotkova, N. and Lidstrom, M.E. (2004) MeaB is a component of the methylmalonyl-CoA mutase complex required for protection of the enzyme from inactivation. Journal of Biological Chemistry 279, 13652-13658

127 Padovani, D., Labunska, T. and Banerjee, R. (2006) Energetics of interaction between the G-protein chaperone, MeaB, and B12-dependent methylmalonyl-CoA mutase. Journal of Biological Chemistry 281, 17838-17844

128 Froese, D.S. et al. (2009) Sleeping beauty mutase (sbm) is expressed and interacts with ygfd in Escherichia coli. Microbiological Research 164, 1-8

129 Padovani, D. and Banerjee, R. (2006) Assembly and protection of the radical enzyme, methylmalonyl-CoA mutase, by its chaperone. Biochemistry 45, 9300-9306

130 Padovani, D. and Banerjee, R. (2009) A G-protein editor gates coenzyme B12 loading and is corrupted in methylmalonic aciduria. Proceedings of the National Academy of Sciences of the United States of America 106, 21567-21572

131 Leal, N.A. et al. (2003) Identification of the human and bovine ATP:Cob(I)alamin adenosyltransferase cDNAs based on complementation of a bacterial mutant. Journal of Biological Chemistry 278, 9227-9234

132 Schubert, H.L. and Hill, C.P. (2006) Structure of ATP-bound human ATP:cobalamin adenosyltransferase. Biochemistry 45, 15188-15196

133 Fan, C. and Bobik, T.A. (2008) Functional characterization and mutation analysis of human ATP:Cob(I)alamin adenosyltransferase. Biochemistry 47, 2806-2813

134 Saridakis, V. et al. (2004) The structural basis for methylmalonic aciduria. The crystal structure of archaeal ATP:cobalamin adenosyltransferase. Journal of Biological Chemistry 279, 23646-23653

135 St.Maurice, M. et al. (2007) Structural characterization of the active site of the PduO-type ATP:Co(I)rrinoid adenosyltransferase from Lactobacillus reuteri. Journal of Biological Chemistry 282, 2596-2605

136 Zhang, J. et al. (2009) Ligand-binding by catalytically inactive mutants of the cblB complementation group defective in human

18

ATP:cob(I)alamin adenosyltransferase. Molecular Genetics and Metabolism 98, 278-284

137 Zhang, J. et al. (2006) Impact of cblB mutations on the function of ATP:cob(I)alamin adenosyltransferase in disorders of vitamin B12 metabolism. Molecular Genetics and Metabolism 87, 315-322

138 Ledley, F.D. et al. (1988) Molecular cloning of L-methylmalonyl-CoA mutase: gene transfer and analysis of mut cell lines. Proceedings of the National Academy of Sciences of the United States of America 85, 3518-3521

139 Willard, H.F. and Rosenberg, L.E. (1980) Inherited methylmalonyl CoA mutase apoenzyme deficiency in human fibroblasts: evidence for allelic heterogeneity, genetic compounds, and codominant expression. Journal of Clinical Investigation 65, 690-698

140 Ledley, F.D. and Rosenblatt, D.S. (1997) Mutations in mut methylmalonic acidemia: clinical and enzymatic correlations. Human Mutation 9, 1-6

141 Mancia, F. et al. (1996) How coenzyme B12 radicals are generated: the crystal structure of methylmalonyl-coenzyme A mutase at 2 A resolution. Structure 4, 339-350

142 Mancia, F. and Evans, P.R. (1998) Conformational changes on substrate binding to methylmalonyl CoA mutase and new insights into the free radical mechanism. Structure 6, 711-720

143 Mancia, F., Smith, G.A. and Evans, P.R. (1999) Crystal structure of substrate complexes of methylmalonyl-CoA mutase. Biochemistry 38, 7999-8005

144 Thoma, N.H. and Leadlay, P.F. (1996) Homology modeling of human methylmalonyl-CoA mutase: a structural basis for point mutations causing methylmalonic aciduria. Protein Science 5, 1922-1927

145 Kolhouse, J.F., Utley, C. and Allen, R.H. (1980) Isolation and characterization of methylmalonyl-CoA mutase from human placenta. Journal of Biological Chemistry 255, 2708-2712

146 Fenton, W.A. et al. (1982) Purification and properties of methylmalonyl coenzyme A mutase from human liver. Archives of Biochemistry and Biophysics 214, 815-823

147 Hubbard, P.A. et al. (2007) Crystal structure and mutagenesis of the metallochaperone MeaB: insight into the causes of methylmalonic aciduria. Journal of Biological Chemistry 282, 31308-31316

148 Chandler, R.J. et al. (2006) Propionyl-CoA and adenosylcobalamin metabolism in Caenorhabditis elegans: evidence for a role of methylmalonyl-CoA epimerase in intermediary metabolism. Molecular Genetics and Metabolism 89, 64-73

149 Chandler, R.J. and Venditti, C.P. (2005) Genetic and genomic systems to study methylmalonic acidemia. Molecular Genetics and Metabolism 86, 34-43

150 Kuhnl, J. et al. (2005) Functional analysis of the methylmalonyl-CoA epimerase from Caenorhabditis elegans. FEBS Journal 272, 1465-1477

151 Carrillo-Carrasco, N. et al. (2010) Liver-directed rAAV gene delivery rescues a lethal mouse model of methylmalonic acidemia and provides long-term phenotypic correction. Human Gene Therapy 2010 Aug 2 [Epub ahead of print]

Genetic disorders of vitamin B$_{12}$ metabolism: eight complementation groups – eight genes

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Further reading, resources and contacts

Reviews

There are many excellent reviews that address different aspects of vitamin B$_{12}$ metabolism, the proteins involved and clinical correlates:

Banerjee, R., Gherasim, C. and Padovani, D. (2009) The tinker, tailor, soldier in intracellular B12 trafficking. Current Opinion in Chemical Biology 13, 484-491

Li, F., Watkins, D. and Rosenblatt, D.S. (2009) Vitamin B(12) and birth defects. Molecular Genetics and Metabolism 98, 166-172

Fowler, B., Leonard, J.V. and Baumgartner, M.R. (2008) Causes of and diagnostic approach to methylmalonic acidurias. Journal of Inherited Metabolic Disease 31, 350-360

Genetic disorders of vitamin B$_{12}$ metabolism: eight complementation groups – eight genes

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Further reading, resources and contacts *(continued)*

Dali-Youcef, N. and Andres, E. (2009) An update on cobalamin deficiency in adults. Quarterly Journal of Medicine 102, 17-28

Websites

The Organic Acidemia Association. A volunteer organisation for the provision of support and information on organic acidurias, including the methylmalonic acidurias:

http://www.oaanews.org/

National Institutes of Health: Office of Rare Diseases Research. A highly informative site covering basic information, patient advocacy, research and clinical trials, and research resources for the public and scientific community:

http://rarediseases.info.nih.gov/

National Center for Biotechnology Information: Online Mendelian Inheritance in Man. Compilation of gene and phenotype information on all known Mendelian genetic disorders searchable by disease or gene name:

http://www.ncbi.nlm.nih.gov/omim

Features associated with this article

Figures

Figure 1. Structure of vitamin B$_{12}$ (cobalamin).
Figure 2. Intracellular processing of vitamin B$_{12}$ showing sites of defects in complementation groups.
Figure 3. Mutations in the genes underlying the defects of the eight complementation groups.

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