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Identification of ABC transporters acting in vitamin B_{12} metabolism in *Caenorhabditis elegans*

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ABSTRACT

Vitamin B_{12} (cobalamin, Cbl) is a micronutrient essential to human health. Cbl is not utilized as is but must go through complex subcellular and metabolic processing to generate two cofactor forms: methyl-Cbl for methionine synthase, a cytosolic enzyme; and adenosyl-Cbl for methylmalonyl-CoA mutase, a mitochondrial enzyme. Some 10–12 human genes have been identified responsible for the intracellular conversion of Cbl to cofactor forms, including genes that code for ATP-binding cassette (ABC) transporters acting at the lysosomal and plasma membranes. However, the gene for mitochondrial uptake is not known. We hypothesized that ABC transporters should be candidates for other uptake and efflux functions, including mitochondrial transport, and set out to screen ABC transporter mutants for blocks in Cbl utilization using the nematode roundworm *Caenorhabditis elegans*. Thirty-seven mutant ABC transporters were screened for the excretion of methylmalonic acid (MMA), which should result from loss of Cbl transport into the mitochondria. One mutant, *wht-6*, showed elevated MMA excretion and reduced [^{14}C]-propionate incorporation, pointing to a functional block in methylmalonyl-CoA mutase. In contrast, the *wht-6* mutant appeared to have a normal cytosolic pathway based on analysis of cystathionine excretion, suggesting that cytosolic methionine synthase was functioning properly. Further, the MMA excretion in *wht-6* could be partially reversed by including vitamin B_{12} in the assay medium. The human ortholog of *wht-6* is a member of the G family of ABC transporters. We propose *wht-6* as a candidate for the transport of Cbl into mitochondria and suggest that a member of the corresponding ABCG family of ABC transporters has this role in humans. Our ABC transporter screen also revealed that *mrp-1* and *mrp-2* mutants excreted lower MMA than wild type, suggesting they were concentrating intracellular Cbl, consistent with the cellular efflux defect proposed for the mammalian MRP1 ABC transporter.

1. Introduction

Vitamin B_{12} (cobalamin, Cbl) is an essential nutrient that functions as a cofactor in intermediary metabolism. It has critical roles in the maintenance of the brain and nervous system and the integrity of red blood cells. Investigations of Cbl utilization in human cells have led to the delineation of a complex pathway toward its assimilation into physiologically active cofactor forms, a pathway that is widely shared across phyla, allowing human gene identification by probing orthologs among diverse species, including bacteria [1,2], archaea [3–5] and, important to the present study, the nematode roundworm *Caenorhabditis elegans*[6–8]. Following cellular uptake, Cbl is delivered to the lysosome and proceeds through processing steps to generate methylcobalamin (MeCbl) in the cytosol and adenosylcobalamin (AdoCbl) in the mitochondrion and attachment to their cognate enzymes, methionine synthase (MTR) and methylmalonyl-CoA mutase (MCM), respectively [9–11]. To achieve these outcomes, some 10–12 genes encoding enzymes, transporters and chaperones have been identified that,

Abbreviations: Cbl, cobalamin; MMA, methylmalonic acid; MeCbl, methylcobalamin; AdoCbl, adenosylcobalamin; MTR, methionine synthase; MCM, methylmalonyl-CoA mutase; PCC, propionyl-CoA carboxylase; ABC, ATP-binding cassette; TCA, trichloroacetic acid; NBF, nucleotide binding fold; TMD, transmembrane domain

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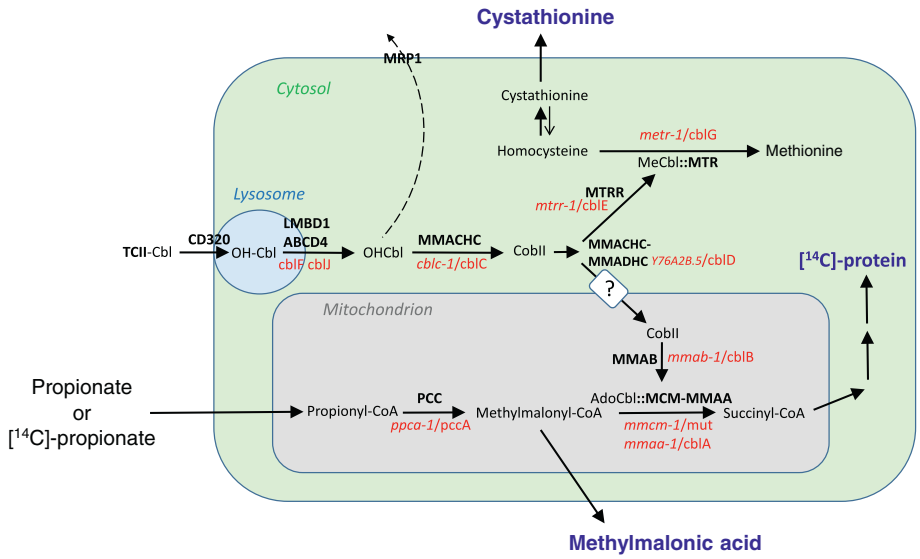

Fig. 1. Metabolic pathway showing the vitamin B_{12}-dependent processing of homocysteine (cytosolic pathway) and propionate (mitochondrial pathway). Metabolites are in black; proteins are in black-bold; complementation groups are in red; *C. elegans* genes are in red-italics; accumulated or excreted metabolites or protein, as observed in this study, are in blue-bold. Key cobalamin (Cbl)-dependent enzymes are methionine synthase (MTR) which is methylcobalamin (MeCbl)-dependent and methylmalonyl-CoA mutase (MCM) which is adenosylcobalamin (AdoCbl)-dependent. The pathway of vitamin B_{12} utilization is shown with identified Cbl genes of *C. elegans* followed by the complementation groups of humans shown at each step (note: TCII-dependent uptake of Cbl is not replicated in *C. elegans*). The dashed arrow through MRP1 reflects proposed removal of excess Cbl through efflux protein MRP1. The "?" on a white background is the site for mitochondrial transport of Cbl and the subject of this study. Also shown are the consequences of metabolic blocks in MCM or MTR resulting from a failure to generate the required Cbl co-factors. *C. elegans* with a mutant MTR (*metr-1*) is blocked at homocysteine and can be shown to excrete cystathionine and with a mutant MCM (*mmcm*-1 or *mmaa-1*) is blocked at methylmalonyl-CoA and can be shown to excrete methylmalonic acid, which is exacerbated in high propionate medium. Also shown is the outcome of incubating worms in a medium containing [^{14}C]-propionate which is metabolized through the propionate pathway to generate [^{14}C]-labelled macromolecules (notably protein). This fails in the presence of blocks in the propionate pathway. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increasingly, are being described as components of protein complexes that serve not only to facilitate the enzymatic modifications of Cbl, but also to prevent the release of this highly reactive molecule into the cellular milieu [12]. Yet, despite the many advances made, the transport mechanism that delivers Cbl into the mitochondrion remains elusive.

Most of the cellular pathway has been established through the investigation of patients with metabolic blocks in the utilization of Cbl (Fig. 1, Table 1). Some of these defects involve blocks in transport across membranes. Initial cellular uptake from the circulation occurs by receptor-mediated endocytosis of the transcobalamin-Cbl complex via the transcobalamin (CD320) receptor and delivery into the lysosome [13,14]. In the acid milieu of the lysosome, Cbl is released and transported across the lysosomal membrane by a protein complex comprised of LMBD1 and ABCD4, the latter an ATP-binding cassette (ABC) transporter [15,16]. Patients assigned to the cblF or cblJ complementation groups of Cbl disorders have mutations in LMBD1 or ABCD4, respectively, and metabolically have methylmalonic aciduria and homocystinuria owing to the inability to generate functional forms of either of the Cbl-dependent enzymes.

In the cytosol, the MMACHC (methylmalonic aciduria and homocystinuria type C) protein acts as a carrier protein and Cbl processing

Table 1
Comparison of genes in cellular cobalamin and propionate metabolism in *C. elegans* and humans.

Protein name/function	Protein acronym	Human gene	Human complementation group	Human metabolic phenotype[a]	*C. elegans* gene	*C. elegans* similarity to human ortholog[b]	*C. elegans* strain name (knockout allele)
Lysosomal transport	LMBD1/ ABCD4	*LMBDR1*	cblF	Hcy, MMA	None identified		
Lysosomal transport	LMBD1/ ABCD4	*ABCD4*	cblJ	Hcy, MMA	*pmp-3*	39% 4e-147 58%	RB1108(ok1087)
Cbl carrier/reductase	MMACHC	*MMACHC*	cblC	Hcy, MMA	*cblc-1*	35% 3e-35 52%	
Cytoplasmic/mitochondrial sorting protein	MMADHC	*MMADHC*	cblD	Hcy &/or MMA	Y76A2B.5	34% 2e-36 51%	
Methionine synthase reductase	MTRR	*MTRR*	cblE	Hcy	*mtrr-1*	31% 3e-94 50%	VC536 (ok718)
Methionine synthase	MTR	*MTR*	cblG	Hcy	*metr-1*	63% e 0.0 80%	RB755 (ok521)
ATP:Cob(I)alamin adenosyltransferase	MMAB	*MMAB*	cblB	MMA	*mmab-1*	44% 7e-38 57%	
AdoCbl protective protein	MMAA	*MMAA*	cblA	MMA	*mmaa-1*	52% 7e-124 70%	RB1926 (ok2512)
Methylmalonyl-CoA mutase	MCM	*MUT*	mut	MMA	*mmcm-1*	69% e 0.0 82%	RB1434 (ok1637)
Propionyl-CoA carboxylase	PCC	*PCCA*	ppcA	PPA	*pcca-1*	59% e 0.0 75%	RB1774 (ok2282)
Cbl efflux protein	MRP1	*MRP1*	none	none	*mrp-1*	47% e 0.0 63% (to MRP1)	NL147(pk89)
Probable Cbl efflux protein	MRP2	*MRP1*-type	none	none	*mrp-2*	46% e 0.0 62% (to MRP1)	RB1713(ok2157)
Unassigned	WHT-6	*ABCG*-type	none	unknown	*wht-6*	31% 3e-78 52% (to ABCG2)	VC629 (ok882)

[a] Indicates presence of homocystinuria (Hcy), methylmalonic aciduria (MMA), or propionic aciduria (PPA) in affected patients of the indicated complementation group.
[b] Alignment scores: % identity; Expect Score; % similarity.