Vitamin B 12 transport from food to the body's cells – A sophisticated, multistep pathway

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Vitamin B_{12} transport from food to the body's cells—a sophisticated, multistep pathway

Marianne J. Nielsen, Mie R. Rasmussen, Christian B. F. Andersen, Ebba Nexø and Søren K. Moestrup

Abstract | Vitamin B_{12} (B_{12}; also known as cobalamin) is a cofactor in many metabolic processes; deficiency of this vitamin is associated with megaloblastic anaemia and various neurological disorders. In contrast to many prokaryotes, humans and other mammals are unable to synthesize B_{12}. Instead, a sophisticated pathway for specific uptake and transport of this molecule has evolved. Failure in the gastrointestinal part of this pathway is the most common cause of nondietary-induced B_{12} deficiency disease. However, although less frequent, defects in cellular processing and further downstream steps in the transport pathway are also known culprits of functional B_{12} deficiency. Biochemical and genetic approaches have identified novel proteins in the B_{12} transport pathway—now known to involve more than 15 gene products—delineating a coherent pathway for B_{12} trafficking from food to the body's cells. Some of these gene products are specifically dedicated to B_{12} transport, whereas others embrace additional roles, which explains the heterogeneity in the clinical picture of the many genetic disorders causing B_{12} deficiency. This Review describes basic and clinical features of this multistep pathway with emphasis on gastrointestinal transport of B_{12} and its importance in clinical medicine.

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Introduction

Vitamin B_{12} (B_{12} [also known as cobalamin]; the abbreviation B_{12} covers all forms of cobalamins and not only cyanocobalamin, which is the vitamin B_{12}) is a water-soluble molecule that functions as an essential coenzyme for two enzymes in the human body: cytoplasmic methionine synthase, which catalyzes methylation of homocysteine to methionine; and methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the mitochondrion (Figure 1). The methionine synthase reaction, which also involves folate (vitamin B_9) is essential for a high number of methyl-transfer reactions and is also, therefore, indirectly involved in nucleotide synthesis. The methylmalonyl-CoA mutase reactions are involved in digestion of different organic compounds, including branched amino acids and odd-chain fatty acids

Worldwide, moderate B_{12} deficiency is common, especially in developing nations in which individuals have a low intake of animal products (the sole source of the vitamin).[1] Moderate B_{12} deficiency is also common amongst the elderly population, which is possibly due to age-dependent changes in the gastric epithelium and inability to effectively absorb food-bound B_{12}.[2] Overt B_{12} deficiency, which most frequently occurs as a result of failure to uptake the vitamin, is a severe disease characterized by megaloblastic anaemia and/or neurological disorders; if left untreated, these disorders can lead to irreversible damage and eventually death.[3]

Once referred to as "nature's most beautiful cofactor",[4] the red-coloured B_{12} is a tetrapyrrole that occurs in several active and inactive forms (Figure 1).[5–10] As the complex 30-step pathway of B_{12} biosynthesis is confined to certain prokaryotes, humans are completely dependent upon a dietary source of the vitamin.[11] The ingested vitamin passes to the terminal ileum for absorption and once in the blood, it is distributed to all cells of the body. Owing to efficient enterohepatic circulation, as well as reuptake in the kidney, the vitamin is retained in the body for long periods, and therefore an insufficient dietary intake has to last for years in adults to cause clinical symptoms of deficiency. By contrast, deficiency can occur much faster during early life and in patients lacking the capacity to absorb the vitamin. B_{12} deficiency not caused by an insufficient dietary supply is commonly due to autoimmune destruction of gastric parietal cells (leading to classic pernicious anaemia), but many other acquired or genetic conditions can also result in lack of the vitamin, including molecular defects in the intestinal absorption machinery (Tables 1 and 2).

The absorption of B_{12} and its subsequent distribution in the body is mediated by a complex set of carrier proteins, receptors and transporters, and it is now possible to describe a coherent pathway of B_{12} from food to the body's cells. In this Review, we describe the trafficking mechanisms of B_{12}, together with disorders resulting from molecular defects in these transport pathways, with a special focus on the intestinal uptake of B_{12}.

B_{12} carriers in extracellular fluids

Transport of B_{12} in extracellular fluids is dependent on three homologous carrier proteins: intrinsic factor, transcobalamin (also known as transcobalamin II),

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Key points

- A coherent vitamin B_{12} (B_{12}) transport pathway from food to the body's cells has now been delineated; the pathway includes an ABC transporter for cellular B_{12} efflux and a receptor for uptake of B_{12}-bound transcobalamin
- More than 15 gene products are involved in B_{12} transport and/or processing; several new genes encoding intracellular proteins (including a potential lysosomal transporter of B_{12}) have been identified
- Gastrointestinal uptake of B_{12} is via cubam, the complex of cubilin and amnionless
- Novel genetic causes of B_{12} deficiency disease have been clarified; many of the new proteins have been identified by positional cloning of the genes harbouring the disease-causing mutations
- New diagnostic assays for B_{12} deficiency are being developed; plasma level of holo-transcobalamin is a promising biomarker in combination with existing markers

and haptocorrin (also known as the R-protein or transcobalamin I). These proteins share the same overall structural scaffold and each carries a single B_{12} molecule (Figure 2).[12,13]

Intrinsic factor has a crucial function in transporting B_{12} to the intrinsic factor–B_{12} receptor, cubam, which is expressed on enterocytes in the ileum and is responsible for the absorption of the vitamin by means of receptor-mediated endocytosis. Intrinsic factor is also essential for the actual uptake process as cubam recognizes only the intrinsic factor–B_{12} complex and neither intrinsic factor nor free B_{12} alone.[14,15] In humans, intrinsic factor is synthesized and secreted by parietal cells of the stomach, and only small amounts have been detected outside the gastrointestinal tract.[16] This carrier protein is highly glycosylated, which, as well as its specific amino acid sequence, is thought to protect it from digestion by intestinal enzymes.[17]

Structurally, intrinsic factor features a two-domain architecture where B_{12} binds at the interface between the two domains.[12] A similar mode of binding of B_{12} at the interface of two domains is reiterated not only for transcobalamin and haptocorrin,[13] but also in the B_{12}-dependent enzymes methionine synthase[18] and methylmalonyl-CoA mutase.[19]

Owing to the critical role of intrinsic factor in B_{12} absorption, deficiency of this protein (caused by autoimmune attack of the parietal cells or rare inborn errors of synthesis) leads to severe B_{12} avitaminosis and classic pernicious anaemia.[20,21] Intrinsic factor was discovered by Castle as the 'intrinsic factor' lacking in patients suffering from pernicious anaemia despite normal supply of the 'extrinsic factor' (that is B_{12}).[22]

Circulating transcobalamin has an essential role in transporting B_{12} absorbed in the ileum to cells of the body. The importance of transcobalamin is obvious in the small number of children with inborn errors of transcobalamin synthesis. The affected child displays few symptoms at birth, but within months a severe deficiency develops and, if left untreated, it leads to lifelong impairments due to neurological damage.[23–27] Several different kinds of mutations leading to a lack of transcobalamin have been identified, including deletions and mutations resulting in erroneous RNA editing.[23–27]

Haptocorrin is heavily glycosylated and is expressed in many, but not all, mammals.[28] In humans, haptocorrin is

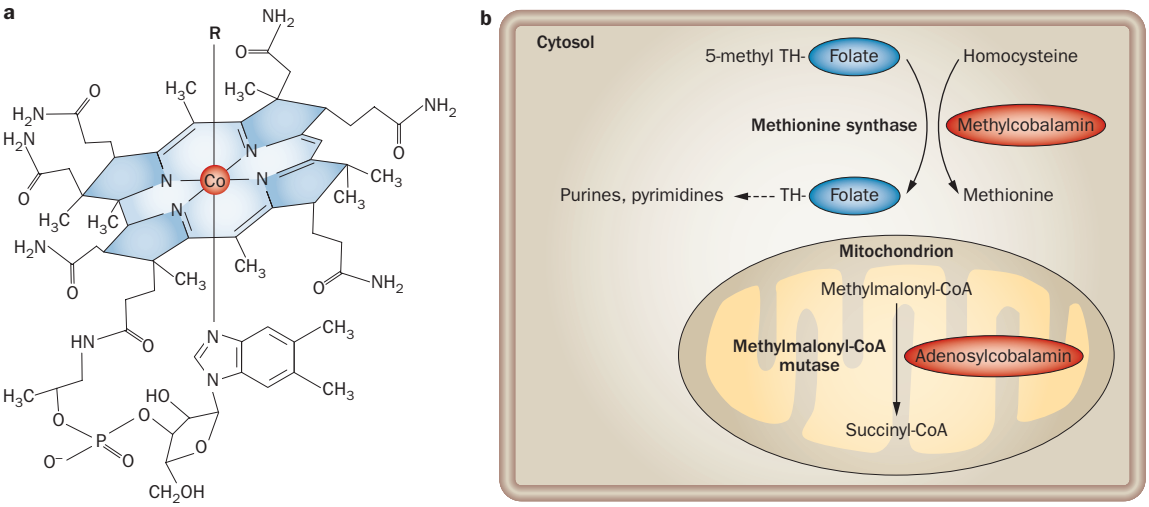

Figure 1 | B_{12} structure and coenzyme function. **a** | B_{12} structure. The core of B_{12} consists of a corrin ring that encircles a central cobalt ion. The latter is linked to four nitrogen atoms from the corrin ring, as well as to a nitrogen atom from a 5,6-dimethylbenzimidazole ribonucleotide moiety positioned below the plane of the corrin ring and a variable group (R) positioned above the plane of the ring.[5–8,10] The variable group can be occupied by several ligands, including a hydroxyl, cyano, methyl, or 5'-deoxyadenosyl group. The enzymatically active cofactor carries either a methyl or a 5'-deoxadenosyl group at this position. In this Review, the term B_{12} refers to all variants of the vitamin, unless otherwise stated. **b** | Coenzyme function. B_{12} serves as a coenzyme in two distinct enzymatic processes: the conversion of homocysteine to methionine by cytosolic methionine synthase and the conversion of methylmalonyl-CoA to succinyl-CoA by mitochondrial methylmalonyl-CoA mutase. The former reaction is linked to folate metabolism because the methyl group transferred to homocysteine is provided by the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate. Tetrahydrofolate is essential for the production of purines and pyrimidines. Prolonged B_{12} deficiency results in accumulation of 5-methyl tetrahydrofolate with impaired DNA synthesis as a result. This scenario is known as the methyl-folate-trap. Abbreviation: TH, tetrahydro-.

Table 1 | Gene products with essential functions in human B_{12} homeostasis (genetic evidence)

Gene product (gene)	Function or proposed function of gene product	Principal location of function
Intrinsic factor	Binds B_{12}	Small intestine
Cubilin (*CUBN*)	The intrinsic factor–B_{12}-binding subunit of cubam	Apical surface of the brush border epithelial cells in the terminal ileum
AMN (*AMN*)	The transmembrane cubam subunit that accounts for internalization of B_{12}	Apical surface of the brush border epithelial cells in the terminal ileum
Transcobalamin	Binds B_{12}	Blood
Cobalamin A protein (*MMAA*)	Ensures that the cofactor form bound by methylmalonyl-CoA mutase is adenosylcobalamin	Mitochondrion
Cobalamin B protein (*MMAB*)	Catalyses adenosylation of B_{12}	Mitochondrion
Cobalamin C protein (*MMACHC*)	Catalyses decyanation of cyanocobalamin and dealkylation of alkylcobalamins	Cytosol
Cobalamin D protein (*MMADHC*)	Binds intracellular B_{12} and directs it to the mitochondrial or cytosolic pathway	Cytosol
Cobalamin E protein (*MTRR*)	Catalyses methylation of B_{12}	Cytosol
LMBD1/cobalamin F protein	Transports B_{12} from the lysosome to the cytosol	Lysosomal membrane
Methionine synthase/ cobalamin G protein	Catalyses methylation of homocysteine to form methionine	Cytosol
Methylmalonyl-CoA mutase (mut protein)	Catalyses the conversion of methylmalonyl-CoA to succinyl-CoA	Mitochondrion
CD320	Binds transcobalamin–B_{12}	Plasma membrane

Information collated from various sources.[21,23–27,48,62,65,67,70,73–75]

Table 2 | Acquired causes of vitamin B_{12} deficiency[2]

Cause	Comments
Inadequate dietary intake of B_{12}	An important cause of B_{12} deficiency, especially in vegans, some vegetarians and in some developing nations
Destruction of gastric parietal cells	Autoimmune disorder leading to classic pernicious anaemia; the autoantibodies might also be directed towards intrinsic factor itself
Gastric atrophy	Typically caused by persistent infection with *Helicobacter pylori*; alternatively, gastric atrophy can be autoimmune in origin
Intestinal infection with the tapeworm *Diphyllobothrium latum*	The parasite competes with the host for B_{12}
Crohn's disease	The disease causes villous atrophy, which interferes with the absorption of nutrients
Coeliac disease	Intake of gliadin initiates an inflammatory reaction that causes villous atrophy, hence interfering with the absorption of nutrients
Chronic pancreatitis	B_{12} remains sequestered by haptocorrin in the intestine owing to inefficient degradation of haptocorrin by the pancreatic enzymes in the intestinal juice
Gastric bypass surgery	Inadequate secretion of intrinsic factor from the bypassed stomach might result in B_{12} malabsorption
Treatment with antacids	Prolonged treatment with antacids might counteract release of B_{12} from dietary protein
Metformin intake	Mechanism undefined

present in many body fluids, including saliva, breastmilk and plasma.[29] In addition to escorting B_{12} down the upper part of the gastrointestinal tract (see below), haptocorrin also binds a substantial amount of the vitamin in plasma and breastmilk. Haptocorrin differs from intrinsic factor and transcobalamin in its ability to bind not only B_{12}, but also the inactive forms of the vitamin, the so-called analogues of B_{12}. For many years the origin of B_{12} analogues remained unsolved. Cobanimide and six other types of analogues have been discovered in faeces, indicating production by the colonic microflora, although the colonic absorption of the analogues is not shown.[30] Studies have also demonstrated that analogues are present in cord blood (Hardlei *et al.*, unpublished data), thereby supporting the idea that analogues are formed within the body. In addition, the patterns of analogues in blood differ from the patterns observed in faeces.[30,31] In conclusion, haptocorrin binds cobalamin analogues, but the physiological role of these analogues is a matter of continuing debate.[32,33]

As only the transcobalamin-bound B_{12} in plasma, and not the fraction bound to haptocorrin, seems to be available to the body's cells, the level of B_{12}-bound transcobalamin (holo-transcobalamin) in plasma is thought

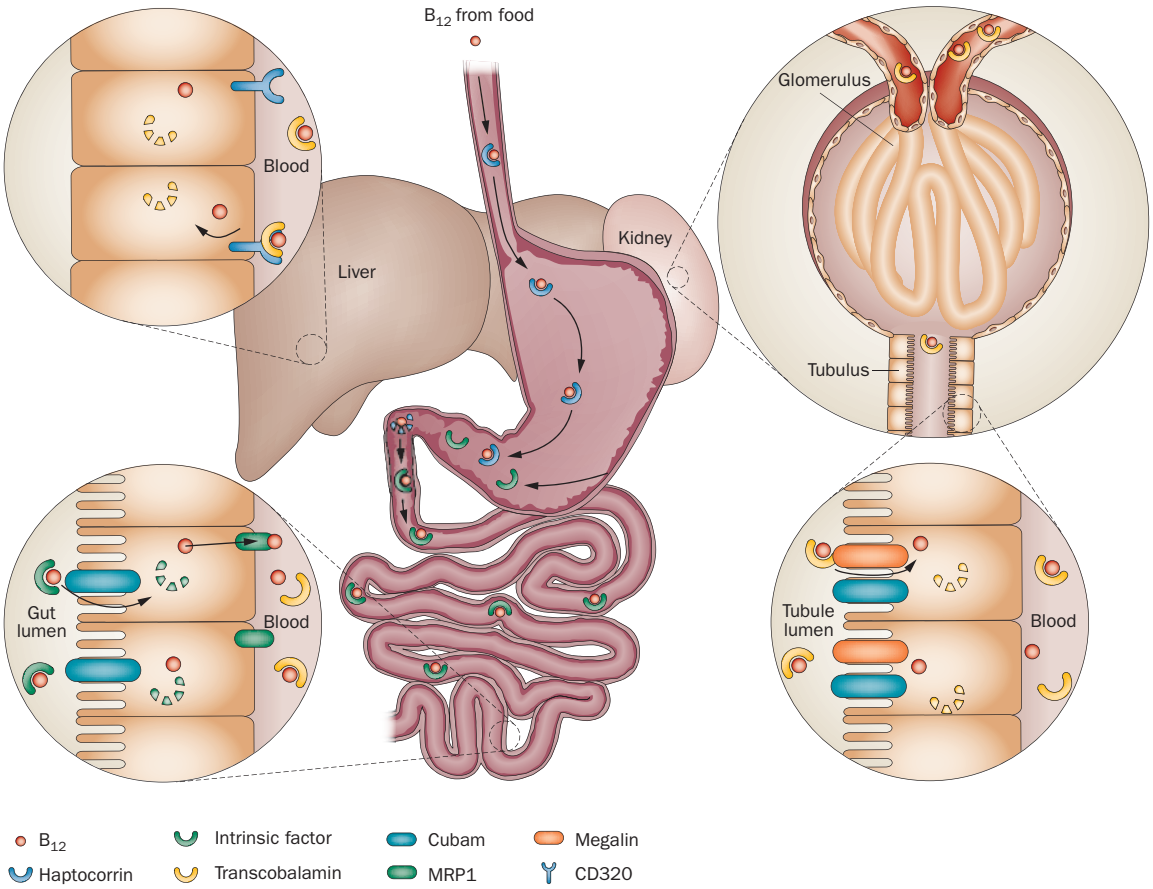

Figure 2 | Schematic overview of uptake and transport of B_{12} in humans. In the upper gastrointestinal tract, B_{12} is released from food components and is bound by haptocorrin, a protein present in saliva and gastric fluids. On reaching the duodenum, haptocorrin is degraded by enzymes and B_{12} is captured by intrinsic factor secreted by parietal cells of the mucosa of the gastric wall. In the terminal ileum, intrinsic-factor-bound B_{12} is endocytosed by a receptor complex between cubilin and amnionless (AMN), termed cubam. Inside the ileal enterocyte, intrinsic factor is degraded and B_{12} is released to plasma from the basolateral side of the cell by the ABC transporter MRP1. In plasma, B_{12} is bound to haptocorrin or transcobalamin. The latter is responsible for delivery of B_{12} to cells of peripheral tissues. In the liver and other tissues, transcobalamin-dependent cellular B_{12} uptake is mediated by the receptor CD320, whereas the receptor megalin mediates renal reabsorption in the kidney. Abbreviation: ABC, ATP-binding cassette.

to serve as an indicator of the overall B_{12} status in an individual.[34] Currently, measurement of plasma holotranscobalamin levels is considered a valuable supplement to old tests for diagnosing B_{12} deficiency, such as assessment of total plasma B_{12} or measurement of methylmalonic acid and/or homocysteine, both of which accumulate in affected patients (Box 1).[34–38]

Uptake of B_{12} in the intestine

During the ingestion of food in the upper gastrointestinal tract, B_{12} is captured by haptocorrin, and this mechanism is thought to shield the vitamin from hydrolysis by the acidic environment in the stomach.[39] In the duodenum, haptocorrin is degraded by enzymes secreted from the pancreas and the released vitamin is then bound by intrinsic factor, which is resistant to proteolytic attack by these enzymes.[17,39] In the terminal ileum, intrinsic factor–B_{12} is absorbed by endocytosis, which is mediated by the cubam receptor complex (Figure 3).[14,15,40–44]

The cubam complex consists of two collaborating molecules: cubilin (which is a peripheral membrane protein that binds intrinsic factor–B_{12}) and amnionless (AMN; a transmembrane, endocytic protein). The 460 kDa cubilin and the 48 kDa AMN are coexpressed in the apical membrane of absorptive epithelia such as the ileum, the proximal tubules of the kidney, and the visceral yolk sac.[41,44–48] Cubilin can roughly be divided into three regions: an amino-terminal region that trimerizes the protein; eight epidermal growth factor domains; and 27 so-called CUB domains.[15,42] CUB domains 5–8 are required for intrinsic factor–B_{12} binding,[49,50] whereas the remaining CUB modules are thought to comprise binding sites for additional cubilin ligands identified in nonintestinal tissues such as the kidney.[51–53] The intracellular region of AMN harbours two endocytic motifs that convey internalization of the receptor complex and its bound ligands.[54]

The identity and physiological importance of cubam has been consolidated by biochemical studies showing the mutual dependence of cubilin and AMN for the processing and folding of the cubam complex,[43,45,47,55–59] as well as by genetic studies of Imerslund–Gräsbeck

syndrome (IGS), a rare, juvenile disorder leading to B_{12} deficiency.[60,61] Patients suffering from this condition carry mutations in the genes encoding cubilin[62] or AMN.[48] Furthermore, the same syndrome has been described in a dog with a spontaneous *AMN* gene defect.[63] In addition to megaloblastic anaemia and/or neurological symptoms, IGS is often accompanied by pronounced B_{12}-resistant proteinuria, which is explained by the additional function of cubam in the renal reabsorption of filtered proteins such as albumin, apolipoprotein A-I and transferrin.[51–53] Patients with IGS but who do not have proteinuria are known, and in some cases this finding might be explained by mutations affecting only intrinsic factor–B_{12} binding. One example of such a mutation in the *CUBN* gene is the so-called Finnish mutation 1 (FM1), a missense mutation that is found in the majority of the known IGS cases in Finland and is known to disrupt cubilin binding to intrinsic factor–B_{12}.[64] In these cases, the clinical picture resembles that of hereditary juvenile B_{12} deficiency caused by mutations in the gene encoding intrinsic factor.[21]

Transport of B_{12} in the cell

Upon cubam-mediated internalization of intrinsic factor–B_{12}, intrinsic factor is degraded in the lysosome, which contains proteases that can attack the carrier protein (unlike the intestinal proteases). The liberated B_{12} then traverses the lysosomal membrane to enter the cytoplasm, a process that probably involves the protein LMBD1 (Figure 4).[65] At any rate, using a combination of genome-wide linkage analysis and cDNA transfection rescue experiments, Rutsch *et al.*[65] have demonstrated that mutations in the gene encoding LMBD1 are responsible for the rare cblF defect, an inborn error of B_{12} metabolism that is characterized by defective lysosomal release of B_{12}.[66] The consequent trapping of the vitamin within the lysosome hinders its use as a coenzyme for methionine synthase and methylmalonyl-CoA mutase.

LMBD1 is a 61 kDa lipocalin receptor-like protein that is predicted to harbour nine transmembrane helices and has been shown to locate to the lysosomal membrane with the C-terminus facing the cytoplasm.[65] In the same study, transfection of cultured cblF patient fibroblasts with cDNA encoding wild-type LMBD1 resulted in a substantial increase in the synthesis of B_{12}, which in turn was reflected in enhanced enzymatic activity of methionine synthase and methylmalonyl-CoA mutase. Altogether, these data support the hypothesis that LMBD1 is involved in the lysosomal export of B_{12}.

Exactly how B_{12} is handled within the cell from the point of lysosomal exit until its usage as a coenzyme or its export from the cell is still largely unknown. However, as previously experienced with other aspects of B_{12} trafficking and/or metabolism, the cooperative effect of genetic, biochemical and clinical studies seems to constitute a platform for filling in many of the missing pieces. Accordingly, eight genes underlying inborn errors in B_{12} metabolism have now been characterized. Each of these genes represents one of the eight known complementation groups that have defects in intracellular

Box 1 | Blood parameters indicating B_{12} deficiency

- Decreased B_{12} levels in plasma
- Increased methylmalonic acid levels in plasma
- Increased homocysteine levels in plasma (also increased in patients with vitamin B_6 and folate [vitamin B_9] deficiency)
- Decreased holo-transcobalamin levels in plasma

B_{12} trafficking and metabolism as revealed by *in vitro* somatic complementation analysis of patient fibroblast cells (Table 1).[67] Depending on the location within the intracellular B_{12} pathway, the defects might block the production or utilization of methyl-B_{12}, adenosyl-B_{12}, or both, thus resulting in homocystinuria, methylmalonic aciduria, or both (Figure 1). Although much is still to be learned about the proteins encoded by these eight genes, the overall principles of intracellular B_{12} transport and processing are now slowly emerging (reviewed in detail elsewhere[67,68]).

In short, current knowledge indicates that B_{12} exited from the lysosome is bound by the cytosolic cobalamin C protein, which is involved in decyanation of

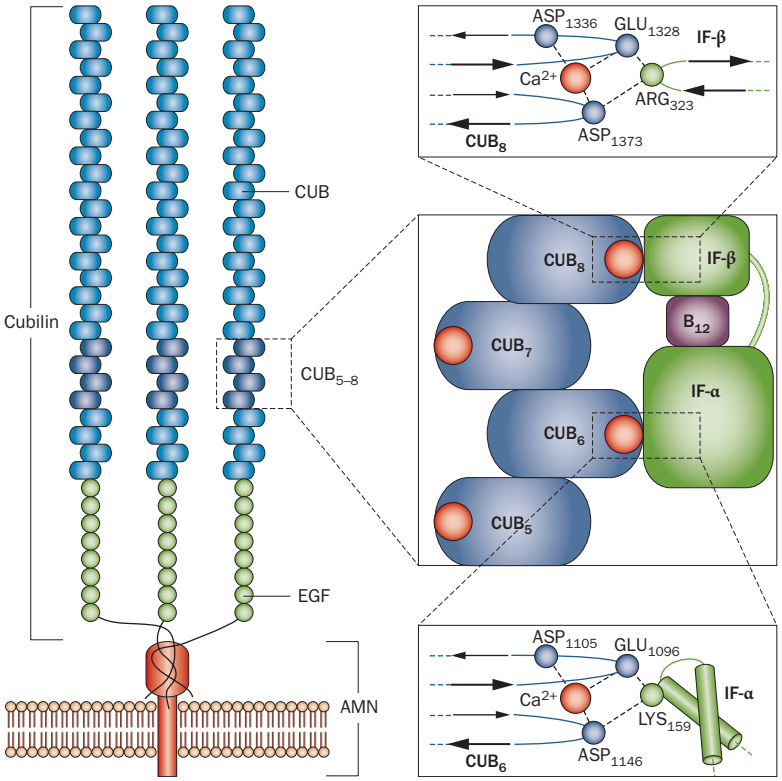

Figure 3 | Schematic representation of the cubam receptor complex and its binding to intrinsic factor–B_{12}. The transmembrane and integral cubam component AMN anchors the cubam receptor complex to the membrane. The 460 kDa cubilin is organized into three regions: an amino-terminal part containing a putative α-helix that trimerizes the protein, eight EGF domains, and 27 CUB domains, each featuring a β-barrel-like structure similar to those in immunoglobulins. CUB domains 5–8 are responsible for intrinsic factor–B_{12} binding. CUB domains 6 and 8 recognize intrinsic factor–B_{12} through a dual-point interaction with the α and β domains of intrinsic factor, respectively. At both interaction sites, Ca^{2+} is indirectly involved in ligand binding by positioning two negatively charged residues from cubilin for direct interaction with a positively charged residue from intrinsic factor. Abbreviations: EGF, epidermal growth factor; IF, intrinsic factor.

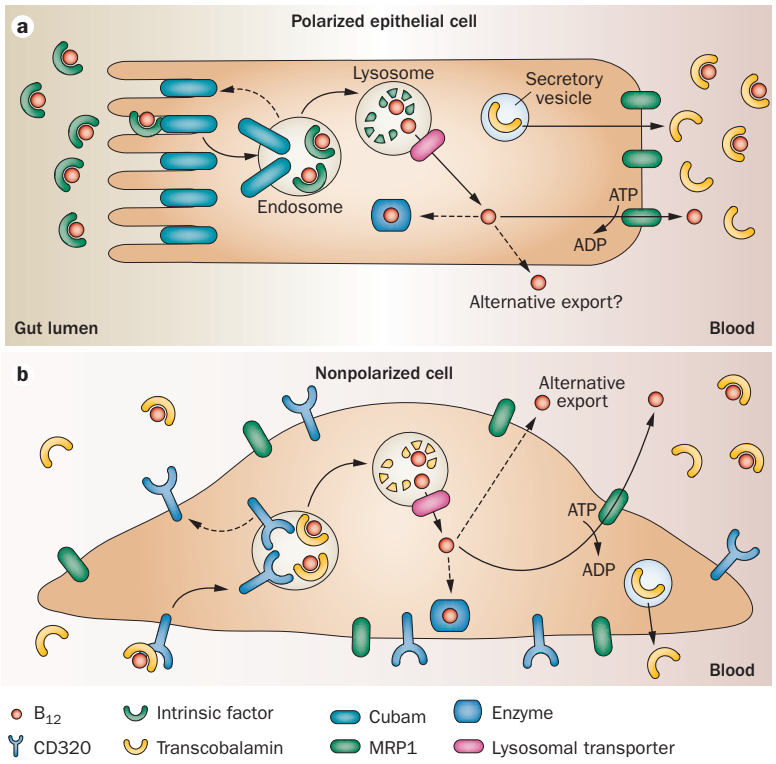

Figure 4 | Schematic model of pathways for B_{12} cellular uptake and exit. **a** | Pathway for cellular transport of B_{12} in a polarized cell, such as an enterocyte. Intrinsic factor–B_{12} in the gut is recognized by the receptor cubam present on the apical brush border of epithelial cells of the terminal ileum. Once internalized, intrinsic factor–B_{12} is liberated from cubam in endosomes and the complex is transferred to lysosomes. Cubam, on the other hand, recycles to the cell surface. In lysosomes, proteases are responsible for breakdown of the protein component of the intrinsic factor–B_{12} complex, and subsequent lysosomal exit of B_{12} involves the LMBD1 transporter. The exact details of B_{12} trafficking within the cell are not yet clarified but several proteins are known to be involved in intracellular processing and transport of B_{12}.[67,68] Ultimately, the vitamin can remain inside the cell to be used as a cofactor or it can be exported from the ileal cell by the basolateral ABC transporter MRP1 at the expense of ATP hydrolysis. Whether or not intracellular B_{12} processing and transport proteins are needed for cellular export by MRP1 remains to be determined. An alternative B_{12} export mechanism might also exist.[76] **b** | Pathway for transcellular transport of B_{12} in a nonpolarized cell. In nonpolarized cells, internalization of B_{12} occurs in a complex with transcobalamin via the receptor CD320. Abbreviation: ABC, ATP-binding cassette.

cyanocobalamin and dealkylation of alkylcobalamins.[69–72] The processed B_{12} is then probably passed on to the cytosolic cobalamin D protein,[73–75] which has been suggested to define the trafficking of B_{12} to apo-methionine synthase in the cytosol and apomethylmalonyl-CoA mutase in the mitochondrion. The B_{12} fraction entering the mitochondrion is modified by the cobalamin B protein to generate the active cofactor 5'-deoxyadenosylcobalamin, which is then used by methylmalonyl-CoA mutase (MUT). In this process, the cobalamin A protein might have an important role in ensuring that the cofactor remains in its active, adenosylated state. The B_{12} remaining in the cytosol is guided to the second of the two B_{12}-dependent enzymes, methionine synthase (cobalamin G protein). Here, the cobalamin E protein is thought to catalyse generation of methylcobalamin, the active cofactor form used by methionine synthase.[67]

Exit of B_{12} from the cell

As an alternative to remaining inside the cell for use as a coenzyme, or possibly for storage, B_{12} can exit the cell to enter the bloodstream (or extracellular fluid). This role is particularly important in the intestine where the epithelial cells deliver B_{12} to plasma. Multidrug resistance protein 1 (MRP1) has been identified as one molecular gateway for B_{12} export in these cells.[76] MRP1 (also known as ABCC1) is ~190 kDa in size and belongs to the ATP-binding cassette (ABC) transporter family that couples ATP hydrolysis to the transport of substances against a concentration gradient.[77] MRP1 is a multifunctional protein and was originally characterized as a transporter capable of exporting various anticancer agents, thus contributing to resistance in some cancer forms.[78] The protein is expressed in basolateral membranes of polarized cells in addition to being present in most nonpolarized cells.[77,79,80]

Previously, B_{12} was assumed to leave the cells in complex with transcobalamin.[81–83] However, a series of cellular export experiments actually showed that B_{12} exit from cultured cells occurs by transmembrane transport of the vitamin in its 'free' (nonprotein-bound) form via MRP1.[76] Indeed, transport of the vitamin in its free form is also implicit by the fact that the Schilling test for diagnosing deficient B_{12} absorption uses the measurement of free radioactive B_{12} in the urine (after radioactive B_{12} has been orally administered and all circulating B_{12} binding proteins have been saturated by injection of unlabeled B_{12}).[84] In support of a physiological function of MRP1 in the epithelial basolateral efflux of B_{12}, *Mrp1*-knockout mice have a reduced concentration of B_{12} in plasma, as well as in the liver and kidney. Instead, the vitamin accumulates in the ileum and colon of these mice.[76] However, lack of Mrp1 only leads to partial inhibition of B_{12} efflux to plasma, and no morphological or metabolic abnormalities are observed in these mice.[76] Likewise, mutations in *MRP1* have not been identified in the genomes of B_{12}-deficient patients with yet unknown metabolic defects.[85] This observation suggests the existence of one or several alternative B_{12} export pathways that might compensate for the loss of *Mrp1* in knockout mice. Such redundant mechanisms for export might be carried out by other MRP family members as these proteins are known to have a broad substrate overlap.[86] However, a cellular B_{12} export assay[76] failed to prove B_{12} transport by the closest MRP1 homologues, ABCC3, ABCC5 and ABCC6, which are expressed in basolateral epithelia (M. R. Rasmussen and S. K. Moestrup, unpublished data).

Cellular uptake of B_{12} from plasma

Upon MRP1-mediated exit into the bloodstream, B_{12} is transported by transcobalamin.[87] B_{12} bound by transcobalamin is readily taken up by cells of the liver and other destinations in the body by an endocytic receptor whose identity has been revealed as the transmembrane CD320 protein, a ~58 kDa protein that, like megalin (see below), belongs to the LDL receptor family.[88] Several previous studies had described transcobalmin–B_{12} receptor activity but failed to link this activity to a protein with known primary structure.[33,89–91]

The extracellular region of CD320 harbours two LDL receptor type A domains and is heavily glycosylated.[88] CD320 is present on the cell surface[92–94] and in virtually all tissues.[94] In addition, a soluble form of the receptor is observed in the bloodstream.[95] Upon endocytosis, transcobalamin is degraded in the lysosome to liberate B_{12}, which might either be stored in the cell, used in B_{12}-dependent reactions, or leave the cell,[65,96,97] probably in exactly the same way as described for enterocytes (Figure 4). The role of CD320 in B_{12} uptake has been supported by the discovery of a deletion mutation in the *CD320* gene of a newborn with moderately increased levels of methylmalonic acid in the urine.[98] This mutation affecting the first LDL receptor domain of CD320 causes a marked decrease in transcobalamin–B_{12} binding and uptake by cells.[98]

Interestingly, CD320 expression seems to be tightly regulated according to the proliferative and differentiation status of the cell.[92] Increased expression of CD320 during cell proliferation is consistent with the high demand of dividing cells for nucleic acids, and hence for B_{12} (which has an important, indirect role in DNA synthesis). It is also tempting to speculate that the cellular level of B_{12} directly or indirectly regulates CD320 expression. This mechanism could perhaps explain the interesting observation that the rodent kidney accumulates more B_{12} than any other tissue during times of high levels of the vitamin.[99] In essence, if high levels of B_{12} downregulate receptor-mediated transcobalamin–B_{12} uptake in the tissues, a large fraction of the complex would be filtered in the kidney where it is taken up by the other known transcobalamin–B_{12} receptor, megalin (see below).[100] In contrast to transcobalamin, haptocorrin seems to have a less important role in B_{12} delivery to cells, although haptocorrin has been shown to bind to the asialoglycoprotein receptor.[101] The physiological relevance of this receptor interaction is unknown.

Reabsorption in the kidney

Uptake of B_{12} in the kidney is accounted for by renal filtration and reuptake of transcobalamin–B_{12} by the receptor megalin (Figure 2), a 600 kDa LDL-receptor family protein located on the apical membrane of proximal tubule cells.[100,102] Megalin has a high affinity for transcobalamin and prevents urinary loss of B_{12} present in the renal glomerular filtrate by internalizing the vitamin in complex with transcobalamin.[100] Once inside the renal cell, B_{12} is either bound by B_{12}-dependent enzymes, stored, or released from the cell.[103] On the basis of findings from animal studies, B_{12} seems to accumulate in lysosomes of the kidney, thus indicating a storage function for this particular organelle.[102,104] It remains to be clarified how B_{12} exits the renal tubule via the basolateral membrane, which apparently does not express MRP1.[77,79,80]

Interestingly, megalin coexpresses with cubam in the kidney and in several other absorptive epithelia, including the intestine. Both megalin and cubam are classified as multi-ligand receptors and, prior to the discovery of the AMN coreceptor function, megalin was thought to be essential for the endocytic activity of cubilin.[14,42,105,106] In fact, studies indicate that megalin has an important general function in the endocytic apparatus and in the regulation of cubilin and/or cubam expression.[107,108] Nevertheless, isolated expression of the proteins has now demonstrated that the cubam complex can function independently of megalin expression and vice versa.[43,54]

Box 2 | Clinical symptoms of B_{12} deficiency*

Megaloblastic anaemia
- Fatigue
- Tiredness
- Dizziness

Demyelinating central nervous disease
- Tingling or numbness in fingers and toes and walking/balance problems
- Irritability, depression, poor memory, and focus and concentration problems
- Psychiatric disorders including dementia, mania, psychosis and personality changes
- Optic atrophy

Gastrointestinal dysfunction
- Glossitis
- Malabsorption

Infertility

Vitiligo

*The list is not exhaustive and none of the symptoms are a prerequisite for the diagnosis of B_{12} deficiency.

Conclusions

Intensive research has identified a considerable number of genes and proteins involved in the transport of B_{12}. On the basis of these findings it is now possible to delineate the pathway of B_{12} from food to the body's cells. This knowledge explains and helps in the diagnosis of hitherto unknown inherited forms of B_{12} deficiency disease, which can result from defects in the genes involved in the pathway (Table 1).

This research on B_{12} trafficking in the body has also raised novel questions. For instance, the exact functions of many of the intracellular gene products important for B_{12} transport and metabolism are unknown, and with 15–20% of all cases of recessive hereditary B_{12} malabsorption unexplained,[85] some B_{12} regulating proteins might still await identification. Moreover, in contrast to the detailed information on the extracellular and transmembrane transport of B_{12} in the gastrointestinal system as described in the present Review, much is still to be learned about B_{12} transport in other organs. In particular, B_{12} transport mechanisms in the central nervous system (CNS) are largely unknown; in light of the neurological symptoms caused by B_{12} deficiency,[3] it seems highly relevant to study B_{12} transport here. Although entirely speculative at present, many of the proteins described in this Review might also carry out B_{12} transport in the CNS and across the blood–brain barrier. It will also be important to delineate feotal–maternal B_{12} transport in the placenta, as well as transport from the lactating mammary gland into milk, as both these trafficking systems are essential at the very early stages of life. The continued search for

B_{12} transporting proteins might, however, be complicated by redundancy in the transport pathways as seems to be the case in cellular B_{12} efflux.

In a clinical setting, the discovery of novel B_{12} transporters could be directly translated into new options for genetic testing and screening for the genetic causes of functional B_{12} deficiency. Furthermore, diagnosis of B_{12} deficiency can be guided by measurement of the active plasma fraction of transcobalamin (holotranscobalamin). This new marker seems to further strengthen the diagnosis of functional B_{12} deficiency that in borderline cases might be difficult to diagnose based solely on symptoms and results from currently used laboratory tests (Box 1 and 2).[109] Moreover, the newly developed assay for monitoring holo-transcobalamin levels in plasma upon oral administration of B_{12}[34,110] represents an efficient, indirect tool for evaluation of intestinal B_{12} absorption in patients with suspected B_{12} malabsorption. This diagnostic assay provides a modern alternative to the Schilling test, which, as mentioned, is based on oral administration of radioactive material.

In terms of therapy, the development of recombinant intrinsic factor–B_{12} for oral administration has been proposed[111] as a substitute for intramuscular B_{12} injections (with an interval of 1–3 months) or mega doses of oral B_{12}.[112] This administration form mimics normal physiological absorption of B_{12} and avoids both the discomfort of injections and the potential, as yet unknown, risks associated with a daily intake of >200-fold the physiological dose of the vitamin.

Drug-targeting via the B_{12} transport pathway has been investigated by several groups as an approach for the medical treatment of cancer. Although a therapy has not yet come to clinical studies, this technology is currently the subject of much attention.[113] This strategy for targeting B_{12}-consuming cancer cells includes direct coupling of cytotoxic drugs to B_{12} and the coupling of the cytotoxic saposin to an antibody that targets CD320.[114] In conclusion, research conducted at the interface between the basic mechanisms of B_{12} transport and clinical challenges holds promise for the future diagnosis and treatment of B_{12} deficiency disorders.

Review criteria

The Review is based on papers selected on reading and knowledge of the literature of vitamin B_{12} transport. We searched the PubMed database using the following terms: "vitamin B12" and "cobalamin". All papers were acquired as full-text papers via Aarhus University library access. Furthermore, papers were identified by searching reference lists of relevant written sources.

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Author contributions

M. J. Nielsen contributed to the writing and reviewing/editing of the manuscript. M. R. Rasmussen and C. B. F. Andersen researched data. E. Nexø researched data and contributed to reviewing/editing the manuscript. S. K. Moestrup contributed to all aspects of this manuscript.