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The ABC of Solute Carriers

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The *SLC16* gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond

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Abstract The monocarboxylate cotransporter (MCT) family now comprises 14 members, of which only the first four (MCT1–MCT4) have been demonstrated experimentally to catalyse the proton-linked transport of metabolically important monocarboxylates such as lactate, pyruvate and ketone bodies. *SLC16A10* (T-type amino-acid transporter-1, TAT1) is an aromatic amino acid transporter whilst the other members await characterization. MCTs have 12 transmembrane domains (TMDs) with intracellular N- and C-termini and a large intracellular loop between TMDs 6 and 7. MCT1 and MCT4 require a monotopic ancillary protein, CD147, for expression of functional protein at the plasma membrane. Lactic acid transport across the plasma membrane is fundamental for the metabolism of and pH regulation of all cells, removing lactic acid produced by glycolysis and allowing uptake by those cells utilizing it for gluconeogenesis (liver and kidney) or as a respiratory fuel (heart and red muscle). The properties of the different MCT isoforms and their tissue distribution and regulation reflect these roles.

Keywords Lactate · Intracellular pH · Glycolysis · monocarboxylate transporter (MCT)

Historical perspective

Originally thought to be via non-ionic diffusion of the free acid, it was only after the demonstration that lactate and pyruvate transport into human erythrocytes is strongly inhibited by α-cyano-4-hydroxycinnamate (CHC) and organomercurials that a specific monocarboxylate transport mechanism was recognised (see [13, 55]). The different characteristics of monocarboxylate transporter observed after extensive characterisation in erythrocytes, cardiac myocytes and hepatocytes led to the proposal that a family of MCTs might exist [55]. The molecular identity of the first member of this family, MCT1, was established by parallel studies in two laboratories. In this laboratory, specific labelling studies in rat and rabbit erythrocytes [54] followed by purification and N-terminal sequencing [56] identified a 40- to 50-kDa protein. The N-terminus of this protein is identical to a putative 12-TMD transporter (MEV) of unknown function previously cloned by the group of Goldstein and Brown from a mutated Chinese hamster ovary cell line that exhibited enhanced mevalonate uptake [30]. Subsequently they demonstrated that the wild-type protein catalysed proton-linked pyruvate and lactate transport activity and named it monocarboxylate transporter-1 (MCT1) [33]. They proceeded to clone human MCT1 [18] and then, by screening a rat liver cDNA library, a related protein, MCT2, that is strongly expressed in the liver [19]. During investigations on X-chromosome inactivation, gene sequencing revealed another MCT family member, originally called XPCT [for X-linked, proline, glutamic acid, serine, threonine (PEST)-containing transporter] and now renamed MCT8 [35]. MCT3 was identified in the chicken retinal pigment epithelium [22, 70] whilst, in this laboratory, four more members of the MCT family, now named MCT4, MCT5, MCT6 and MCT7, were identified [58]. Very recently, a sodium- and proton-independent aromatic amino acid transporter, TAT1, was identified as a member of the MCT superfamily [31, 32]. MCT9 was identified purely from analysis of the human genomic expressed sequence tag (EST) databases [24] and here we report the existence of four new family members, identified in a similar manner, which we will refer to as MCT11, MCT12, MCT13 and MCT14. It should be noted that because of early confusions in the nomenclature, the MCT and *SLC16A* numbering of the family do not coincide but are correctly annotated in Table 1. The topology of the MCT

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Table 1 *SLC16*—the monocarboxylate transporter family (*C* cotransporter, *E* exchanger, *F* facilitated transporter, *O* orphan transporter *G* genetic defect)

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/coupling ions	Tissue distribution and cellular/subcellular expression	Link to disease[#]	Human gene locus	Sequence accession ID	Splice variants and their specific features
SLC16A1	MCT1		Lactate, pyruvate, ketone bodies	C/H^+ or E/monocarboxylate	Ubiquitous	Muscle weakness (exercise intolerance) G	1p13.2	NM_003051	
SLC16A2	MCT8	XPCT (*was MCT7)	T3, T4 (unpublished)	F	Liver, heart, brain, thymus, intestine, ovary, prostate, pancreas, placenta		Xq13.2	NM_006517	
SLC16A3	MCT4	(*was MCT3)	Lactate, pyruvate, ketone bodies	C/H^+	Skeletal muscle, chondrocytes, leukocytes, testis, lung, placenta, heart		17q25.3	NM_004207	
SLC16A4	MCT5	(*was MCT4)	O		Brain, muscle, liver, kidney, lung, ovary, placenta, heart		1p13.3	NM_004696	
SLC16A5	MCT6	(*was MCT5)	O		Kidney, muscle, brain, heart, pancreas, prostate, lung, placenta		17q25.1	NM_004695	
SLC16A6	MCT7	(*was MCT6)	O		Brain, pancreas, muscle		17q24.2	NM_004694	
SLC16A7	MCT2		Pyruvate, lactate, ketone bodies	C/H^+	Kidney, brain		12q14.1	NM_004731	
SLC16A8	MCT3	REMP	Lactate	C/H^+ (pH dependent but cotransport not confirmed experimentally)	Retinal pigment epithelium, choroid plexus		22q13.1	NM_013356	
SLC16A9	MCT9			O	Endometrium, testis, ovary, breast, brain, kidney, adrenal, retina		10q21.2	BN000144	
SLC16A10	TAT1		Aromatic amino acids (W, Y, F, L-DOPA)	F	Kidney, intestine, muscle, placenta, heart		6q21-q22	NM_018593	
SLC16A11	MCT11			O	Skin, lung, ovary, breast, lung, pancreas, retinal pigment epithelium, choroid plexus		17p13.2	NM_153357	
SLC16A12	MCT12			O	Kidney		10q23.3	ENSG00000152779	
SLC16A13	MCT13			O	Breast, bone marrow stem cells		17p13.1	BN000145	
SLC16A14	MCT14			O	Brain, heart, ovary, breast, lung, pancreas retinal pigment epithelium, choroid plexus		2q36.3	BN000146	

* Prior to publication of [66]
[#] Acquired defect

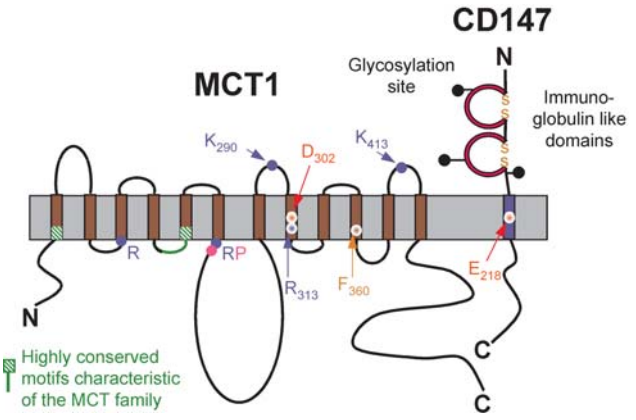

Fig. 1 The proposed topology of the monocarboxylate transporter (*MCT*) family members. CD147, the ancillary protein that associates with MCT1 and MCT4, is also shown. The N- and C-termini and the large loop between TMDs 6 and 7 show the greatest variation between family members, whilst the TMDs themselves are highly conserved. Critical residues identified in MCT1 and two highly conserved motifs characteristic of the MCT family are included, and further discussed in the section "Biochemical and structural aspects"

family is shown in Fig. 1 and discussed in detail below (section "Biochemical and structural aspects").

Functional characteristics of the family

MCT1 catalyses either net transport of one monocarboxylate with one proton or the exchange of one carboxylate for another [13]. Detailed kinetic analysis (see [55]) suggests that an ordered binding to the carrier of H^+ (K_m 0.2 μM, equivalent to a pK of 6.7 for the accepting group) followed by L-lactate (K_m 4–7 mM) precedes a conformational change that translocates the substrate across the membrane. This is followed by release and the return of the empty substrate binding site to the external surface. This last step is rate-limiting for net lactic acid transport across the membrane, as illustrated by trans-stimulation experiments [13]. The translocation cycle is freely reversible, with the kinetic parameters for lactic acid influx and efflux thermodynamically constrained according to the Haldane equation $(V_{max}/K_m)_{influx}=(V_{max}/K_m)_{efflux}$. Transport can be stimulated by decreasing the pH from 8 to 6 on the *cis*-side (primarily through a decrease in the K_m for lactate) or by raising the pH on the opposite side of the membrane (via an increase in the V_{max} of transport that stimulates the rate at which the unloaded carrier re-orientates in the membrane) [55].

MCT2, MCT3 and MCT4 also transport monocarboxylates with a proton [7], but the detailed kinetic mechanisms of these isoforms have not been studied. TAT1 has been shown to transport aromatic amino acids in a sodium-independent manner that also appears to be independent of protons [31]. To date, there are no published data on other MCT isoforms.

Distinctive feature of members of the MCT family

MCT1 (*SLC16A1*)

MCT1 is found in the great majority of tissues of all species studied, with no evidence for splice variants. Characterisation of endogenous MCT1 in erythrocytes [13], and MCT1 expressed in *Xenopus* oocytes [9, 40], has revealed that it transports short chain (C-2 to C-5) unbranched aliphatic monocarboxylates such as acetate (K_m 3.5 mM) and propionate (K_m 1.5 mM). Substitutions on C2 and C3 (excluding amino- and amido-) are tolerated or even preferred (e.g. pyruvate (K_m 0.7 mM), L-lactate (K_m 3–5 mM), acetoacetate (K_m 4–6 mM) and D-β-hydroxybutyrate (K_m 10–12 mM). Formate is a very poor substrate whilst bicarbonate, dicarboxylates, tricarboxylates and sulphonates are not transported. The carrier is stereoselective for 2-hydroxy-substituted monocarboxylates (e.g. L- over D-lactate >10-fold) but not for 2-chloropropionate and β-hydroxybutyrate.

Inhibitors of MCT1 can be divided into three categories:

(i) Bulky or aromatic monocarboxylates are competitive inhibitors, including 2-oxo-4-methylpentanoate, phenyl-pyruvate and derivatives of α-cyanocinnamate such as α-cyano-4-hydroxycinnamate (CHC). These are the most potent inhibitors of this class with K_i values of 50–500 μM. It is important to note that although CHC is often thought of as a specific inhibitor of MCT1, it also inhibits the mitochondrial pyruvate transporter with a K_i of <5 μM, as well as the anion exchanger AE1.

(ii) A range of amphiphilic compounds of widely divergent structure act as potent inhibitors ($K_{0.5}$ 1–10 μM) although they also inhibit AE1 and other membrane transport processes. These include bioflavanoids (e.g. quercetin and phloretin) and anion transport inhibitors such as 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) and niflumic acid.

(iii) Some 4,4′-substituted stilbene-2,2′-disulphonates [such as 4,4′-diisothiocyanostilbene-2,2′-disulphonate (DIDS) and 4,4′-dibenzamidostilbene-2,2′-disulphonate (DBDS)] act as reversible inhibitors of MCT1 in erythrocytes, although with much lower affinity than for AE1. Inhibition by DIDS eventually becomes irreversible on prolonged incubation, reflecting covalent modification of the transporter [53]. MCT1 is inhibited irreversibly by a range of thiol and amino reagents and is especially sensitive to the organomercurial thiol reagent *p*-chloromercuribenzene sulphonate (pCMBS). More extensive data on the substrate and inhibitor specificity may be found elsewhere [13, 55].

MCT2 (*SLC16A7*)

MCT2 appears to demonstrate substantial species differences in both its amino acid sequence and tissue distribution [27], although some of the reported differences may reflect lack of specificity exhibited by commercial antibodies [3]. Northern blot analysis and inspection of the human EST database suggests that MCT2 is expressed poorly, if at all, in major human tissues [58], whilst in mouse, rat and hamster Northern and Western blot analysis and immunofluorescence microscopy have demonstrated MCT2 expression in liver, kidney, brain and sperm tails both and in hamster also in skeletal muscle and heart [19, 27]. Where MCT2 is expressed together with MCT1 its exact location within the tissue is different [3, 19, 20, 51, 52], suggesting a unique functional role as discussed below (see Physiological implications). There is no evidence for splice variants of the protein although alternative splicing in the 5′- and 3′-untranslated region (UTR) has been shown [27, 39]. Like MCT1, MCT2 (expressed in *Xenopus* oocytes) catalyses the proton-linked transport of a range of monocarboxylates, but with a considerably higher affinity than MCT1. K_m values for pyruvate and L-lactate are about 0.1 and 0.7 mM respectively [7, 39]. MCT2 is also more sensitive than MCT1 to inhibition by a range of inhibitors including CHC, DBDS and DIDS, but is insensitive to pCMBS [7, 19].

MCT3 (*SLC16A8*)

MCT3 has a unique distribution, being confined to the basal membrane of the retinal pigment epithelium and choroid plexus epithelia [50, 70], in contrast to the apically located MCT1 [2]. No splice variants of MCT3 are known, but in the case of chicken MCT3 there are two different mRNAs, resulting from different promoter usage. These are involved in temporal rather than spatial regulation [69]. When expressed in yeast, MCT3 transports L-lactate with a K_m of about 6 mM and is insensitive to CHC, phloretin and pCMBS, but detailed information on the substrate and inhibitor specificity is lacking [22]. It should be noted that MCT4 (below) was originally called MCT3 [58] on the basis of its sequence homology rather than function or localisation [50, 66].

MCT4 (*SLC16A3*)

MCT4 is expressed widely both at the mRNA and protein levels and particularly strongly in glycolytic tissues such as white skeletal muscle fibres, astrocytes, white blood cells, chondrocytes and some mammalian cell lines [4, 14, 43, 58, 66]. This has led us to propose that it may be of particular importance in tissues that rely on high levels of glycolysis to meet their energy needs [24, 66]. Indeed, in the rat MCT4 is expressed in the neonatal heart, which is more glycolytic in its energy metabolism than the adult heart where MCT4 is absent [25, 66]. MCT4 is also expressed strongly in placenta, which exports lactic acid rapidly from the foetal to the maternal circulation [58]. MCT4 expressed in *Xenopus* oocytes exhibits a much lower affinity for most substrates and inhibitors than MCT1, with K_m and K_i values some 5–10 times higher [14, 40]. Thus K_m values for L-lactate and pyruvate are 28 and 150 mM respectively and little inhibition by DIDS or CHC is observed at concentrations giving >50% inhibition of MCT1.

TAT1 (*SLC16A10*)

TAT1 is expressed strongly in intestine (basolateral membrane of the epithelial cells), placenta and liver in rat, whereas in humans skeletal muscle and kidney are the major sites of expression with lower amounts in heart, placenta and intestine [31, 32]. When expressed in *Xenopus* oocytes, both human and rat TAT1 transport aromatic amino acids in a sodium- and proton-independent manner with K_m values (millimolar, with rat values in parentheses) for L-phenylalanine, D-phenylalanine, L-tyrosine, L-tryptophan and L-DOPA of 0.75 (7.0), 2.3, 0.64 (2.6), 0.45 (3.7) and 1.21 (6.4) respectively. Monocarboxylates such as lactate and pyruvate are not substrates. No data are available on the sensitivity of TAT1 to inhibitors.

Other MCT family members

No data are available on the properties of MCT5, MCT6 and MCT7 (*SLC16A4–SLC16A6*) although their distribution in human tissues has been subject to Northern blot analysis [58]. Recently, in collaboration with Theo Visser's laboratory, we have demonstrated that, when expressed in *Xenopus* oocytes, MCT8, the MCT isoform most closely related to TAT1, transports thyroid hormone (T4 and T3) in a sodium- and proton-independent manner with a K_m of about 1 μM. Neither aromatic amino acids nor lactate are transported. Northern blot analysis of human tissues has shown MCT8 to be widely expressed, but most strongly in liver and heart. An interesting feature of MCT8 is that its predicted N-terminal sequence is extended by a 75-amino acid sequence that contains the PEST motif indicative of rapid degradation [63]. The significance of this is currently unknown. MCT9 (*SLC16A9*, Accession No. BN000144) was identified by searching the human genomic and EST databases [24] and, in collaboration with Theo Visser we have used the same technique to identify four new MCT family members which we will refer to as MCT11 (*SLC16A11*, NM_153357), MCT12 (*SLC16A12*, ENSG00000152779), MCT13 (*SLC16A13*, BN000145) and MCT14 (*SLC16A14*, BN000146). These are included in Table 1 but no information is available on their properties or function.

Physiological implications of MCT family members

Rapid transport of lactic acid transport across the plasma membrane is of fundamental importance to all mammalian cells under hypoxic conditions [24] when they become glycolytic. Lactic acid is exported by MCT1 (Fig. 2A) to prevent a fall in cytosolic pH (pH_i) and inhibition of continued glycolysis [55]. However, for those cells that rely on glycolysis for their normal energy metabolism MCT4 appears to be the major isoform. The high K_m of MCT4 for pyruvate ensures that this metabolite is not lost from the cell but is reduced to lactic acid, regenerating NAD^+ and so allowing glycolysis to continue. The high K_m of MCT4 for L-lactate is intriguing and may explain why lactic acid accumulates in muscle during exercise, causing the decrease in pH_i that is responsible for fatigue. This probably represents an important physiological mechanism whereby the fatigue prevents exercise continuing to the point at which lactic acid would overload the buffering capacity of the blood and cause damaging systemic acidosis [29, 40]. In other tissues, lactic acid is transported into the cell to supply gluconeogenesis and lipogenesis (liver and kidney: Fig. 2B) or, together with ketone bodies, for oxidation as a major respiratory fuel (heart, skeletal muscle, brain: Fig. 2A) [24]. MCT1 fulfils this role in skeletal muscle and heart, whilst in liver, kidney and brain either MCT1 or MCT2 may be used, the latter providing a higher-affinity lactate uptake mechanism [24]. There is one tissue with no detectable MCT activity; the β-cells of the Islets of Langerhans in the endocrine pancreas [72]. This, together with low levels of lactate dehydrogenase, ensures that pyruvate is fully oxidised via the citric acid cycle, rather than being converted to lactate, so ensuring ATP production and thus insulin secretion accurately reflects glucose levels.

In many tissues more than one MCT isoform is found, which would appear to correlate with either the influx or efflux of lactic acid into different cell types. Two well-documented examples are illustrated in Fig. 2C. In skeletal muscle, the white fibres are glycolytic and contain primarily MCT4 that catalyses the efflux of lactic acid. This is then taken up and oxidised by the red fibres that express primarily MCT1 (and perhaps a little MCT2 in some species). Similarly, glial cells in the brain, which contain MCT1 and MCT4, can export lactic acid to be oxidised by the neurons that contain MCT1 and MCT2. MCT2 is especially concentrated in the post synaptic density which is rich in mitochondria and thus likely to be a major site of lactate oxidation, whilst the endothelial cells of the blood vessels contain only MCT1 [3, 4, 51].

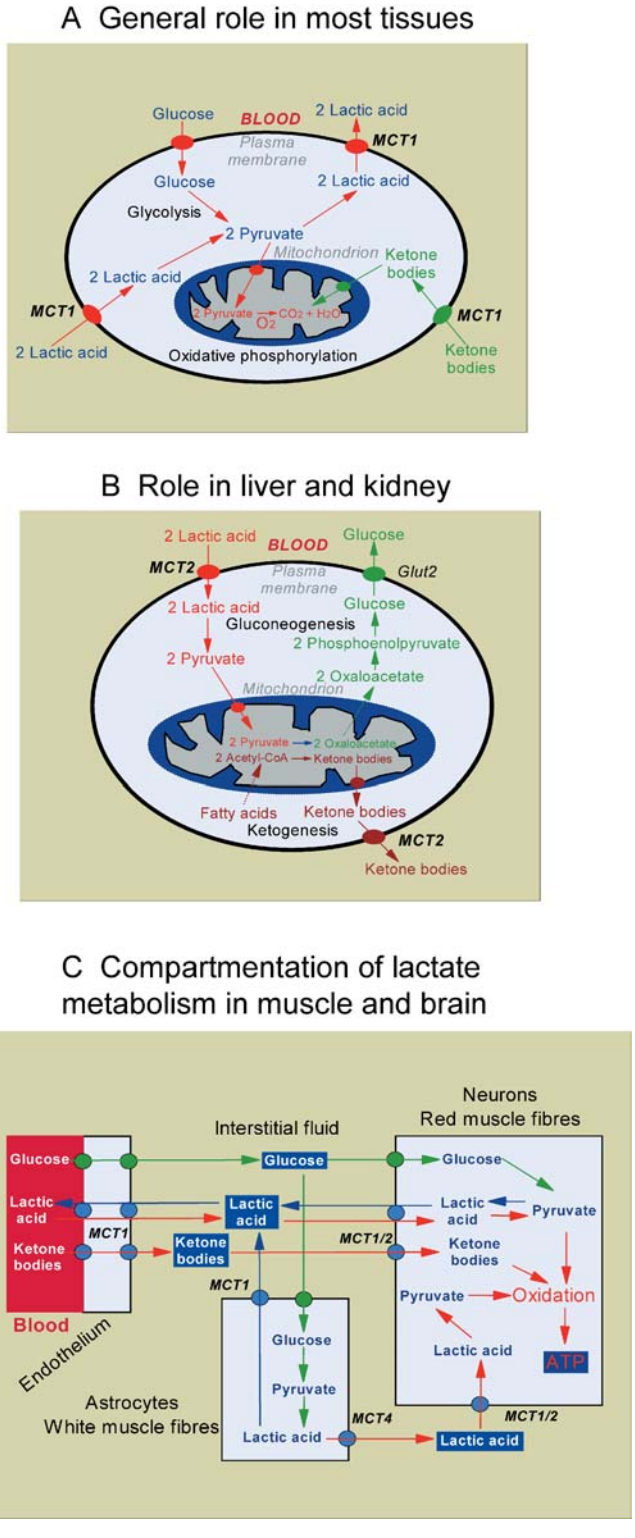

Fig. 2A–C MCT isoforms play a critical role in the metabolism of all cells. **A** Most cells oxidise glucose to CO_2 and water to produce energy, but some, including white skeletal muscle, red blood cells and tumour cells, are primarily glycolytic, converting glucose to two lactic acids that must leave the cell. All cells must rely on glycolysis when oxygen supplies are low (hypoxia/ischaemia). Some tissues like the heart, red muscle, spermatozoa and brain can oxidise lactic acid. **B** The liver and kidney import lactic acid for gluconeogenesis. **C** In some tissues, intercellular cellular compartmentation of lactate metabolism occurs and this is reflected in the distribution of different MCT isoforms. Thus in both the brain and mixed muscle fibres, some cells (neurons and red fibres) oxidise the lactic acid produced by other cells (glia and white fibres). MCTs are also responsible for the transport of ketone bodies into and out of cells that produce them (liver) or utilize them (red muscle, heart and brain)

There is also evidence that lactate metabolism in the retina is subject to a complex interplay between the retinal pigment epithelium (RPE), photoreceptor cells (which oxidise lactate), other neurons and glial cells (Müller cells) which export lactate through MCT4. MCT3 is located exclusively on the basolateral surface of the RPE and is responsible for lactate efflux into the choroidal blood supply, whereas MCT1 is exclusively located on the apical surface of the RPE [20, 49, 50, 70]. MCT1 has been proposed to play an additional role in regulating the volume of the sub-retinal space by also transporting water with the lactate/protons [71]. An accumulation of lactate within the subretinal space would cause osmotic swelling, resulting in the retina becoming detached from the RPE, which could be prevented by an ability of MCT1 (in association with MCT3) to rapidly transport both lactic acid and water across the RPE and into the blood.

Although L-lactate is quantitatively by far the most important substrate of MCT1-MCT4, these isoforms also catalyse the transport of other metabolically important monocarboxylates including the ketone bodies acetoacetate and β-hydroxybutyrate, and the branched-chain keto-acids such as α-ketoisocaproate formed from transamination of amino acids [24, 55]. In the colon, MCT1 may also be important for the uptake of short-chain fatty acids such as acetate and butyrate [12], although these are capable of entering most cells by free diffusion [55]. It has also been proposed that the MCTs may play an important role in communicating information on the redox state between cells [55]. The ratio of lactate to pyruvate reflects the cytosolic NADH/NAD^+ ratio through equilibration of the lactate dehydrogenase reaction. MCTs enable these metabolites to be transported between tissues and this may provide some harmonization of cytosolic redox potential. Similar arguments may apply to the mitochondrial NADH/NAD^+ ratio through equilibration of β-hydroxybutyrate dehydrogenase and the transport of β-hydroxybutyrate and acetoacetate between tissues.

Regulation of MCT isoforms

In skeletal muscle, MCT1 is up-regulated in response to chronic stimulation or exercise in rats and humans (see [6, 8, 21, 29]). Conversely MCTs are down-regulated in response to denervation of muscle or spinal injury [29]. MCT1 expression also increases in the heart following surgical ligation of a major branch of the left coronary artery, presumably reflecting the greater energy demands of the remaining functional hypertrophied left ventricle [28]. In adipose tissue, heart and skeletal muscle MCT1 protein expression has been reported to be reduced in streptozotocin (STZ)-induced diabetes [23, 15], although others have not observed the effect in skeletal muscle [61]. A decrease in MCT4 expression has been observed in skeletal muscle from STZ-induced diabetic rats [15] and obese rats, in which MCT1 expression is also decreased [60]. There is also evidence for changes in MCT isoform expression during development in heart and muscle [25], the inner ear [48] and brain [37], whilst the transition to malignancy is accompanied by changes in the level of MCT1 expression in the colon [36] and brain [17]. In these two tissues there is evidence for up-regulation of MCT1 expression mediated by butyrate and ketone bodies respectively [12, 38]. It has also been shown that MCT4 expression is up-regulated in skeletal muscle of a patient with a mitochondrial myopathy [1].

Parallel measurements of MCT1 and MCT2 mRNA and protein in several tissues suggest that both transcriptional and post-transcriptional mechanisms may be involved in regulating their expression at the plasma membrane [6, 8, 27, 28]. In addition, there is evidence for a novel intracellular pool of MCT1 associated with cisternae close to the t-tubules in hypertrophied left ventricles with increased expression of MCT1. This may indicate that de-novo synthesis and translocation to the sarcolemma occurs via this pool and act as a potential regulatory site [28]. Interestingly, MCT1 possesses two acidic clusters and an LL-motif in the C-terminus; these motifs are believed to be important in endosomal-lysosomal targeting of glucose uniporter-4 (GLUT-4) [64]. Another potential mechanism for post-transcriptional regulation is at the level of translation and this usually involves specific sequences and secondary structure in the 5′- or, frequently, the 3′-UTR with which initiation factors and regulatory factors interact to enhance or repress translation [59]. Thus it may be significant that the 3′-UTR of MCT1 is very long (some 1.2 kb longer than either MCT4 or MCT2 in rats) [24]. There are no convincing experimental data to support regulation of any MCT isoform by phosphorylation.

Biochemical and structural aspects of the MCT family

Hydropathy plots predict 10–12 α-helical TMDs for MCT family members, with the N- and C-termini located within the cytoplasm as illustrated in Fig. 1. This has been confirmed experimentally for MCT1 in erythrocytes [57]. The greatest sequence variation between isoforms is found in the long C-terminus and the large intracellular loop TMD 6–7 that varies substantially in length from 105 residues in MCT5 to only 29 residues in MCT4. The predicted phylogeny of MCT family members is shown in Fig. 3 as a radial tree. Theoretical predictions and experimental evidence indicate that no MCT family member is glycosylated [11, 24, 70]. Two highly conserved sequences can be identified as characteristic of the MCT family (including its non-mammalian members); these are [**D/E**]**G**[**G/S**][**W/F**][**G/A**]**W** which traverses the lead into TMD1 and **YF**xK[**R/K**][**R/L**]xLAx[**G/A**]xAxA**G** which leads into TMD5 [24] (residues in bold face are totally conserved whilst consensus residues are indicated in normal type). Site-directed mutagenesis has shown that the conservative change of Asp_{302} to Glu in TMD 8 of rat MCT1 leads to total loss of lactate transport activity [62], whilst conver-

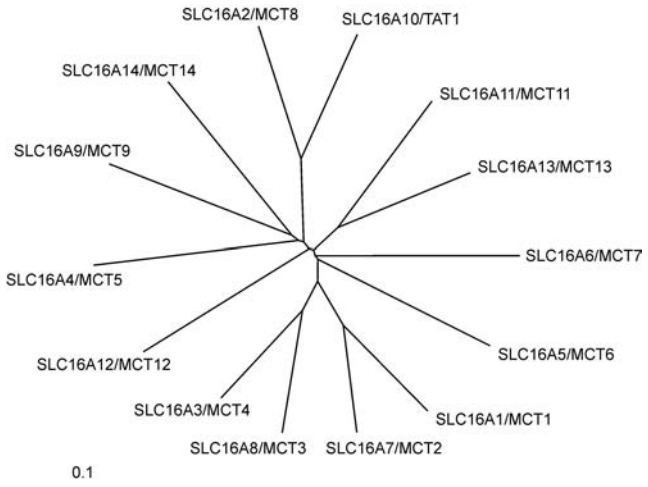

Fig. 3 The predicted phylogeny of MCT family members. Sequences were aligned using the Clustal W algorithm and are displayed as a *radial tree*, The *bar* indicates the number of substitutions per residue with 0.1 corresponding to a distance of 10 substitutions per 100 residues. The calculated, pairwise, percentage identity of MCT2, MCT3, MCT4, MCT5, MCT6, MCT7, MCT8, MCT9, TAT1, MCT11, MCT12, MCT13 and MCT14 to MCT1 are 58, 38, 42, 22, 27, 26, 22, 23, 22, 27, 28, 31, and 23 respectively; MCT4 is 55% and 44% identical to MCT3 and MCT2 respectively and TAT1 50% identical to MCT8

sion of Phe_{360} in TM 10 to Cys enables MCT1 to transport mevalonate whilst reducing its ability to carry lactate and pyruvate [33]. The DIDS-binding site of MCT1 is in the C-terminal half of the transporter [57] and mutation of either Lys_{290} in the loop between TMDs 7 and 8 or Lys_{413} in the loop between TMDs 11 and 12 to glutamine, prevents irreversible inhibition [42]. An arginine in TM8 (Arg_{313} of human MCT1) is conserved in all the putative MCTs from higher eukaryotes except MCT5, and site-directed mutagenesis of this residue greatly reduces the affinity of MCT1 for lactate [62] whilst the arginine-specific reagent phenylglyoxal inhibits transport [55]. This arginine may act as the positively charged group that binds the COO^- anion much as it does in lactate dehydrogenase [10, 24, 55].

Ancillary proteins are required for MCT expression at the plasma membrane

MCT1 and MCT4 require CD147 [also known as OX-47, extracellular matrix metalloproteinase inducer (EMMPRIN), HT7 or basigin) or the related protein GP70 (Embigen) for proper cell surface expression and function. These are widely distributed cell surface glycoproteins with a single transmembrane span, two immunoglobulin-like domains in the extracellular region and a short C-terminal cytoplasmic tail. In contrast, MCT2 does not interact with CD147 although it does appear to require an, as yet unidentified, ancillary protein to be properly expressed at the cell surface [34]. In the red blood cell, DIDS cross-links MCT1 to GP70 whilst in a variety of cell lines antibodies against CD-147 co-immunoprecipitates MCT1 and MCT4, but not MCT2. Immunofluorescence microscopy has confirmed that CD147 co-localises with MCT1 or MCT4 in other tissues [34, 72]. We have confirmed the close association between CD147 and MCT1/MCT4 within the plasma membrane by co-expressing the two proteins with their C- and N-termini tagged with cyan- and yellow-fluorescent proteins. Fluorescence resonance energy transfer (FRET) between the two proteins could be demonstrated, but only if both were tagged on intracellular domains [67]. The interaction between MCT1/4 and CD147 appears to be essential for their correct expression at the cell surface. Only when CD147 is co-expressed with MCT1 or MCT4 are both proteins correctly targeted to the plasma membrane rather than accumulating in the endoplasmic reticulum/Golgi apparatus [34]. Furthermore, co-injection of *Xenopus* oocytes with MCT1 cRNA and an antisense oligonucleotide to a *Xenopus* homologue of CD147 reduced MCT1 expression and activity [41].

Pathological Implications

The critical importance of lactate transport for key metabolic processes such as glycolysis and gluconeogenesis suggest that any impairment in the activity of an MCT isoform is likely to have far-reaching consequences. These may not be compatible with life unless compensated for by changes in expression of another isoform. MCT1 has been implicated in one rare condition known as cryptic exercise intolerance. Apparently healthy sufferers of this condition suffer severe chest pain and muscle cramping on vigorous exercise, and it has been suggested that this may be due to a defect in MCT which prevents lactate efflux [16]. Measurements of lactate uptake by the erythrocytes of such patients showed a reduction in transport that was attributed to an MCT defect. More recently, RT-PCR of MCT1 from muscle biopsy identified a number of amino acid differences that were not attributable to polymorphisms and therefore could be affecting protein function [44, 45]. Of these, only a lysine-to-glutamate mutation in the large cytoplasmic loop between TMDs 6 and 7 was considered a likely candidate. However, we have expressed the K204E mutant in *Xenopus* oocytes and were unable to demonstrate any difference in its properties from wild type MCT1 (A.P. Halestrap, D. Meredith, unpublished data). Thus it remains unclear whether mutations in MCT1 are responsible for cryptic exercise intolerance or whether other factors (such as the interaction with CD147 or GP70) may be involved. No data are available on mutations in other MCT isoforms, although it is interesting to speculate whether unexplained muscle fatigue might be associated with impaired MCT4 expression.

Many tumour cells exhibit an absolute dependence on glycolysis that may reflect the tendency of rapidly growing tumours to become hypoxic. It is of interest that CD147 is up-regulated in aggressive tumours [5, 46]

whilst the transition from normality to malignancy is accompanied by increased MCT1 expression in the brain [17], although the opposite has been reported in colon carcinomas [36].

MCTs as a site of pharmacological intervention.

There is some evidence that MCTs may play a role in the transport of some drugs across the plasma membrane, such as salicylate and valproic acid [26, 65], atorvastatin [68] and nateglinide [47]. At present, the best MCT inhibitors exert powerful effects on other cellular targets [24, 55]. Furthermore, as outlined above, it is likely that global inhibition of MCT function would have disastrous consequences for the well being of an organism. Thus MCT inhibitors are unlikely to be of pharmacological use, unless isoform specific compounds become available.

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Note added in proof A recent publication has shown that MCT3, like MCT1 and MCT4, probably also requires CD147 for its correct expression at the plasma membrane [Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T, Linser PJ (2003) Loss of MCT1, MCT3, and MCT4 expression in the retinal pigment epithelium and neural retina of the 5A11/basigin-null mouse. Invest Ophthalmol Vis Sci 44:1305–1311]