

REVIEW ARTICLE

The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation

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Monocarboxylates such as lactate and pyruvate play a central role in cellular metabolism and metabolic communication between tissues. Essential to these roles is their rapid transport across the plasma membrane, which is catalysed by a recently identified family of proton-linked monocarboxylate transporters (MCTs). Nine MCT-related sequences have so far been identified in mammals, each having a different tissue distribution, whereas six related proteins can be recognized in *Caenorhabditis elegans* and 4 in *Saccharomyces cerevisiae*. Direct demonstration of proton-linked lactate and pyruvate transport has been demonstrated for mammalian MCT1–MCT4, but only for MCT1 and MCT2 have detailed analyses of substrate and inhibitor kinetics been described following heterologous expression in *Xenopus* oocytes. MCT1 is ubiquitously expressed, but is especially prominent in heart and red muscle, where it is up-regulated in response to increased work, suggesting a special role in lactic acid oxidation. By contrast, MCT4 is most evident in white muscle and other cells with a high glycolytic rate, such as tumour cells and white blood cells, suggesting it is expressed where lactic acid efflux predominates. MCT2 has a ten-fold higher affinity for

substrates than MCT1 and MCT4 and is found in cells where rapid uptake at low substrate concentrations may be required, including the proximal kidney tubules, neurons and sperm tails. MCT3 is uniquely expressed in the retinal pigment epithelium. The mechanisms involved in regulating the expression of different MCT isoforms remain to be established. However, there is evidence for alternative splicing of the 5′- and 3′-untranslated regions and the use of alternative promoters for some isoforms. In addition, MCT1 and MCT4 have been shown to interact specifically with OX-47 (CD147), a member of the immunoglobulin superfamily with a single transmembrane helix. This interaction appears to assist MCT expression at the cell surface. There is still much work to be done to characterize the properties of the different isoforms and their regulation, which may have wide-ranging implications for health and disease. In the future it will be interesting to explore the linkage of genetic diseases to particular MCTs through their chromosomal location.

Key words: CD-147, lactate, metabolism, pH, pyruvate.

INTRODUCTION

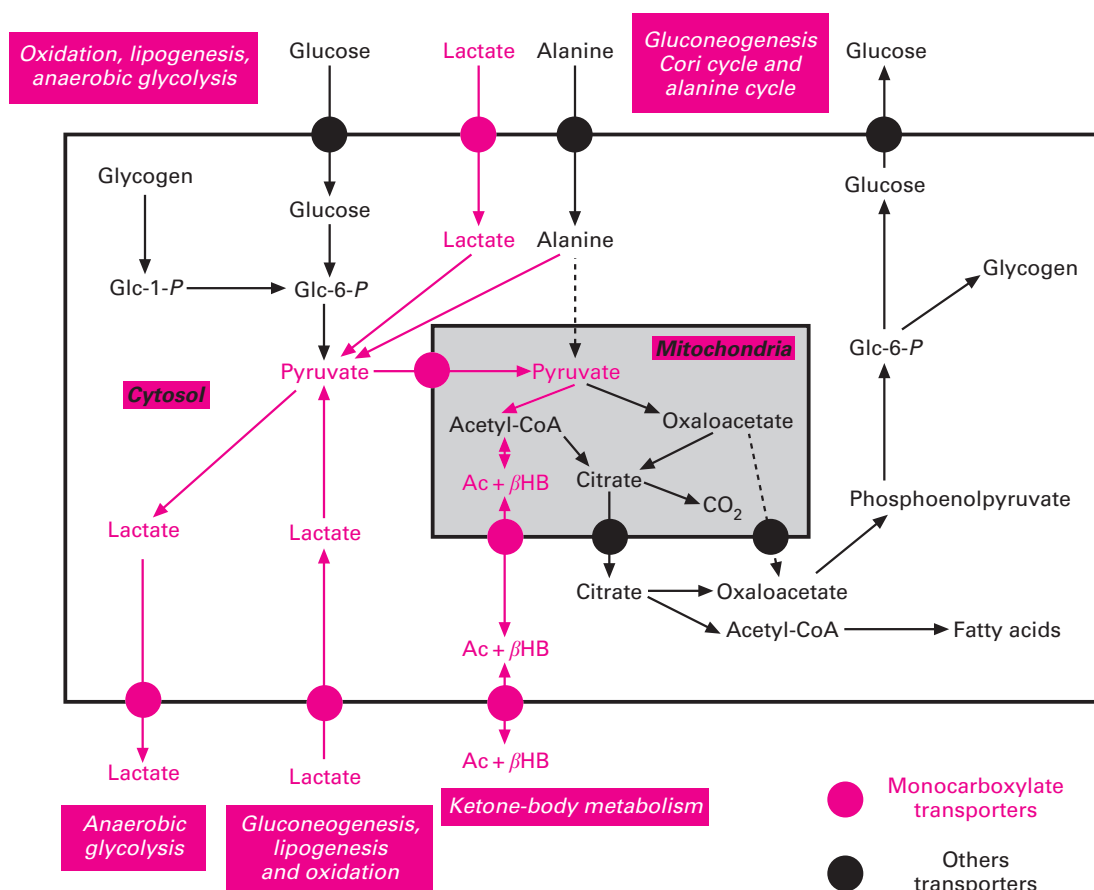
Monocarboxylic acids play a major role in the metabolism of all cells, with lactic acid, the end product of glycolysis, being especially important. Some tissues, such as white skeletal muscle, red blood cells and many tumour cells, rely on this pathway to produce most of their ATP under normal physiological conditions, while all tissues become dependent on this pathway during hypoxia or ischaemia. Glycolysis produces two molecules of lactic acid for every glucose molecule consumed, and these must be transported out of the cell if high rates of glycolysis are to be maintained. If efflux of lactic acid from the cell does not keep pace with production, intracellular concentrations increase and cause the pH of the cytosol to decrease. This leads to inhibition of phosphofructokinase and hence glycolysis. Other tissues, such as brain, heart and red skeletal muscle, readily oxidize lactic acid, which may become a major respiratory fuel under some conditions. In these tissues lactic acid must be rapidly transported into the cell. The same is true for tissues such as the liver, which, through the operation of the Cori cycle, utilize lactate as their dominant gluconeogenic substrate [1–3]. Although it is lactic acid that is both produced and utilized by

metabolism, the p*K* of lactic acid is 3.86, which ensures that it dissociates almost entirely to the lactate anion at physiological pH. This charged species cannot cross the plasma membrane by free diffusion, but requires a specific transport mechanism, provided by proton-linked monocarboxylate transporters (MCTs). These transporters catalyse the facilitated diffusion of lactate with a proton. There is no energy input other than that provided by the concentration gradients of lactate and protons, although the latter, in the form of a pH gradient, can drive the accumulation or exclusion of the lactate anion [2,3].

Although lactate is the monocarboxylate whose transport across the plasma membrane is quantitatively the greatest, the MCTs are also essential for the transport of many other metabolically important monocarboxylates such as pyruvate, the branched-chain oxo acids derived from leucine, valine and isoleucine, and the ketone bodies acetoacetate, β -hydroxybutyrate and acetate. As such, MCTs have a central role in mammalian metabolism and are critical for metabolic communication between cells [2]. This is illustrated in Scheme 1, which also highlights the important role of the mitochondrial monocarboxylate (pyruvate) carrier in carbohydrate and fat metabolism [1,4]. The mitochondrial pyruvate carrier is believed

Abbreviations used: BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; CHC, α -cyano-4-hydroxycinnamate; dbEST, database of expression sequence tags; DBDS, 4,4′-dibenzoylaminostilbene-2,2′-disulphonate; DIDS, 4,4′-di-isothiocyanatostilbene-2,2′-disulphonate; ESTs, expressed sequence tags; MCT, monocarboxylate transporter; pCMBS, *p*-chloromercuribenzenesulphonate; RPE, retinal pigment epithelium; TM, transmembrane (domain); uORF, upstream open reading frame; UTR, untranslated region; VDC, vestibular dark cells; pH_i, intracellular pH.

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Scheme 1 Metabolic pathways involving monocarboxylate transport across the mitochondrial and plasma membranes

Abbreviations: Glc-1-P and Glc-6-P, glucose 1-phosphate and glucose 6-phosphate; Ac + β HB, acetoacetate plus β -hydroxybutyrate.

to be a member of the six-transmembrane-helix mitochondrial carrier family, but has not yet been cloned and sequenced [5]. It is thought to be unrelated to the plasma membrane MCTs, which are the subject of this review, and will not be considered further. The emphasis of this review will be to examine the substantial progress in our understanding of plasma-membrane monocarboxylate transport that has arisen out of the recent cloning and sequencing of several MCTs that are now known to be members of a new transporter family [6]. For further information on their role in the integration of metabolism and a review of earlier studies on the characterization of lactate transport in a variety of cells and tissues, see [2,3,7].

IDENTIFICATION, CLONING AND SEQUENCING OF MCT1

Most early functional studies on plasma-membrane lactate transport were performed using red blood cells, since a homogeneous population of these cells could readily be obtained. Furthermore, in the case of rat and rabbit red blood cells, rates of lactate transport were found to be very rapid and to display conventional Michaelis–Menten kinetics indicative of the presence of a single MCT [8,9]. As such, these cells were an ideal starting material for identifying the protein responsible for transport, and several groups used a variety of covalent labelling techniques in their attempts to accomplish this [10–13]. Unequivocal identification was ultimately achieved in our laboratory using covalent labelling of the protein with 4,4'-di-isothio-

cyanatostilbene-2,2'-disulphonate (DIDS); labelling was prevented by specific inhibitors of the transporter such as α -cyano-4-hydroxycinnamate (CHC). Separation of the labelled protein by SDS/PAGE showed it to have a molecular mass of about 45 kDa [14]. The unlabelled protein was partially purified following detergent solubilization, and transport activity was measured after reconstitution into proteoliposomes [14,15]. Active fractions were enriched in a 45 kDa protein, and when membrane proteins from DIDS-treated red blood cells were taken through the same purification procedure, this protein was found to be DIDS-labelled [16]. N-terminal sequencing showed it to be identical with a putative transporter of unknown function that had been previously cloned by Goldstein et al. [17]. These workers had identified a mutation of the wild-type protein which enhanced mevalonate uptake into Chinese-hamster ovary cells. In subsequent expression studies they demonstrated that the protein catalysed inhibitor-sensitive monocarboxylate transport and named it 'MCT1' [18]. Expression of MCT1 in *Xenopus laevis* oocytes has subsequently allowed more detailed characterization of its properties [19,20]. MCT1s from human, rat and mouse have now been cloned and share about 95% sequence identity with the Chinese-hamster ovary MCT1 (see Table 1) [21–25]. Human MCT1 has been mapped to chromosome band 1p13.2-p12 [21]. Comparison of the 5' untranslated regions (5'-UTRs) from all available MCT1 sequences provides no evidence for alternative splicing in this region, suggesting that MCT1 is transcribed from a universal promoter. This is consistent with

the tissue distribution of MCT1, which is very broad, as will be discussed further below. There is no evidence for alternative splicing in the 3'-UTR region.

CLONING AND SEQUENCING OF OTHER MAMMALIAN MCT ISOFORMS

MCT2

Extensive studies on the kinetics and substrate and inhibitor specificity of monocarboxylate transport into red blood cells, heart cells, tumour cells and hepatocytes led us to propose the existence of a family of MCTs (reviewed in [2,7]). This was confirmed by Goldstein and Brown's group, who cloned and sequenced a second isoform of MCT (MCT2) from a hamster liver cDNA library [26]. MCT2 shares 60% identity with MCT1 and, with the use of a baculovirus expression system, was shown to catalyse CHC-sensitive pyruvate transport. Very recently more detailed characterization of MCT2 was achieved by expression in *Xenopus laevis* oocytes [27,28]. MCT2 has now been cloned and sequenced from rat, mouse and human, with the human MCT2 being mapped to chromosome band 12q13 [25,27,29,30]. MCT2 is not as widely distributed as MCT1, and there is also evidence for alternatively spliced mRNA species. Thus, for both rat and human MCT2, two separate groups have sequenced cDNA clones independently (see Table 1), and comparison of these sequences reveals the presence of two different 5'-UTRs, even in the case of human MCT2, where both cDNAs were from liver. In both species the sequences diverge 30 nt upstream of the AUG codon, suggesting the existence of different leader exons due to different promoter usage (as is seen for chicken MCT3, to be discussed below). In the case of mouse MCT2 there appears to be an additional sequence present upstream of the first coding exon, indicating the presence of a longer or additional exon. Thus it seems probable that mammalian MCT2 is regulated by the use of several promoters and/or alternative splicing within the 5'-UTR, a phenomenon that has also been observed for other transporters [31–34]. Alternative splicing of the 3'-UTR also seems likely, since there are differences in the 3'-UTR sequences in the cDNA clones from different laboratories as revealed by searches of the database of expressed sequence tags (dbESTs). Furthermore, Northern-blot analysis reveals the presence of a range of transcript sizes from about 2 kb to 14 kb whose relative abundance is tissue-dependent [25,27,29]. Sequencing of the MCT2 gene, and further analysis will be required to reveal the full complexity of MCT2 transcriptional regulation.

MCT3 (REMP) and MCT4

The next member of the MCT family to be cloned and sequenced was from a chicken retinal pigment epithelium (RPE) cDNA library [35,36]. Philp and colleagues originally named this protein 'REMP', and later, when its identity was confirmed as an MCT isoform, they renamed it MCT3. It shares 43% and 45% sequence identity with MCT1 and MCT2 respectively and was confirmed to transport lactate by expression in a thyroid epithelial cell line, although detailed characterization has not been reported [36]. Unlike MCT1 and MCT2, which are widely distributed, MCT3 is found only in the RPE, where its location is restricted to the basolateral membrane in the adult [35,37]. The chicken MCT3 gene has been sequenced [38] and shown to contain two alternative exons (1a and 1b) for the 5'-UTR, giving rise to mature mRNA transcripts of 2.45 kb and 2.2 kb respectively. The 2.2 kb form (MCT3b) is found early in embryonic development, but is replaced by the 2.45 kb form later in de-

velopment and in the adult chicken RPE [38]. These data imply regulation of MCT3 expression during development through the control of different promoters.

Most recently, by searching the dbEST for fragments of MCT-like sequences, we identified four new potential members of the MCT family which were subsequently cloned and sequenced [6]. The derived protein sequences exhibited 25–50% identity with MCT1 and included one human protein sequence that was more closely related to chicken MCT3 (67% identity) than MCT1 or MCT2 (43% and 45% identity respectively). As none of the many other ESTs found at the time more closely matched chicken MCT3, and as some of the constituent human ESTs were from retinal cDNA libraries, this sequence was named mammalian MCT3. We also cloned the rat orthologue of MCT3 [39]. At both the protein and mRNA level this mammalian MCT3 revealed a much broader tissue distribution than chicken MCT3 (confined to the RPE), with a particularly strong signal in muscle [6,39]. However, Philp and co-workers subsequently cloned cDNAs from mouse and rat RPE cells that were also related to chicken MCT3, but clearly distinct from the mammalian MCT3 cloned in this laboratory (see Table 1). The tissue distribution of this new mammalian MCT3 was determined with specific antibodies, and expression was shown to be confined to the RPE, as is the case for chicken MCT3 [37]. These data suggest that this second mammalian MCT3, and not the first one cloned, is the true mammalian equivalent of chicken MCT3. Thus the first cloned mammalian MCT3 was renamed MCT4, with the isoform cloned from mammalian RPE retaining the name MCT3 (see [39]). This nomenclature is used throughout the rest of this review. MCT4 has recently been expressed in *Xenopus* oocytes by Bröer and colleagues and confirmed to catalyse the proton-linked transport of L-lactate [40], but no detailed characterization has yet been performed.

The sequences of MCT3 and MCT4 are highly related, as would be expected, but surprisingly, at both the nucleotide and protein sequence level, human MCT4 is actually more closely related to chicken MCT3 than is human MCT3. The same is also true when rat MCT3 and MCT4 sequences are compared with chicken MCT3, and it will be important to establish whether a chicken MCT isoform exists that is more similar in distribution and function to mammalian MCT4. In the light of what we currently know, it seems probable that the rather confusing relationship between MCT3 and MCT4 represents an evolutionary quirk, with both isoforms diverging from a common ancestor in a different manner in birds and mammals. Comparison of the kinetics and substrate specificities of mammalian MCT3 and MCT4 may be revealing in this context, since similarities might explain the ease of divergence of distribution without compromising transport properties. It may also be significant that there is an EST sequence from *Xenopus neurula* embryo (accession number AI031360) which is more closely related to mammalian MCT4 than either chicken or mammalian MCT3.

Recent sequencing of a region of human chromosome 22 has uncovered a gene that almost certainly corresponds to human MCT3 (see Table 1). The coding sequence is interrupted by three introns, and these are in exactly the same position as in the chicken gene. The human MCT1 gene also has four coding exons [21], although only the position of the last intron has been published. This exon boundary is in exactly the same position as in chicken and human MCT3, suggesting a common evolutionary origin of the two genes. However, somewhat confusingly, when comparing the human and chicken MCT3 genome structure it is likely that, in terms of DNA sequence alone, two different transporter isoforms are actually being compared (see discussion

Table 1 Known and putative monocarboxylate transporter-related sequences from eukaryotes and prokaryotes

Unigene symbol and Unigene number refer to entries in the Unigene database of unique human, mouse and rat genes. SP: XXNNNN indicates a SwissProt database entry. Only example prokaryotic transporters are given; for a more complete list see [61]. Several EST sequences also exist for mouse MCT4, MCT5, MCT6, MCT7 and MCT9 (not shown in the Table). Several DNA sequence database entries exist for the yeast sequences – only one is given in the Table below. The additional entries can be found within the annotations of the relevant SwissProt entry. The *Trypanosoma cruzi* sequences listed under MCT1 are virtually identical with those of rat MCT 1 at the nucleotide level, questioning the identity of the *Trypanosoma* entries. References for unpublished sequences: (a) Orsenigo, M. N., Tosco, M., Bazzini, C., Laforenza, U. and Faelli, A. (1999) Accession number AJ.236865; (b) Koehler-Stec, E. M., Simpson, I. A., Vannucci, S. J., Landschulz, K. T. and Landschulz, W. H. (1998) accession numbers AF058054 and AF058055; (c) Tanaka, M., Tanaka, T. and Mitsui, Y. (1997) accession numbers U40854 and U40855; (d) Enerson, B. E., Zhdankin, O. Y. and Drewes, L. R. (1996) accession number U62316; (e) Dao, L., Landschulz, W. H. and Landschulz, K. T. (1998) accession number AF058056; (f) Philp, N. J. and Yoon, H. (1998) accession number AF059258; (g) Philp, N. J. and Yoon, H. (1997) accession number AF019111; (h) Phillimore, B. (1999) accession number AL031587; (i) Murphy, L., Harris, D. and Barrell, B. (1999) accession number AL009193; (j) Kovalenko, T. A. and Alatorsev, V. E. (1999) accession number AJ.238706; (k) Arino, J., Casamayor, A., Gamio, F. J., Gancedo, C., Lafuente, M. J., Aldea, M., Casas, C. and Herrero, E. (1996) accession number Z74861; (l) Cziepluch, C., Jauniaux, J. C., Kordes, E., Poirey, R., Pujol, A. and Tobiasch, E. (1996) accession number Z75214.

(a) Sequences related to MCT 1

| MCT (Unigene symbol) | Alternative (former) name | Organism (source) | Accession numbers | | Unigene number | Notes | Reference | |
|----------------------------|---------------------------|--------------------------------|------------------------------|-----------------------------|---------------------|------------------------------|------------------|-------|
| | | | DNA | Protein | | | | |
| MCT1 (SLC16A1) | MEV | CHO (met-18b-2 cells) | M97382 | SP:Q03064, AAB59630, A44458 | | Mutant form of MCT1 | [17] | |
| | | MOT1 | CHO | L25842 | SP:Q03064, AAB59731 | | | [17] |
| | Rabbit (red blood cells) | | – | AAB32442 | | N-terminal protein sequence | [16] | |
| | Human (heart) | | L31801 | SP:P53985, AAC41707, A55568 | Hs.75321 | Gene structure also reported | [21] | |
| | MCT2 (SLC16A7) | MOT2 | Rat (skeletal muscle) | X86216 | SP:P53987, CAA60116 | Rn.6085 | | [22] |
| | | | Rat (intestine) | D63834 | BAA09894, JC4399 | | | [24] |
| | | | Rat (intestine–jejunum) | AJ.236865 | CAB37948 | | Partial sequence | (a) |
| | | | Mouse (Ehrlich Lettré cells) | X82438 | SP:P53986, CAA57819 | Mm.9086 | | [23] |
| | | | Mouse (kidney) | AF058055 | AAC13720 | | | (b) |
| | | | Mongolian gerbil (brain) | AF029766 | AAB84218 | | Partial sequence | [105] |
| <i>Trypanosoma cruzi</i> ? | | | U40854, U40855 | AAB65515, AAB65516 | | See note | (c) | |
| MCT3 | REMP | Syrian hamster (liver) | A55626, L31957 | SP:P53988, AAC42046 | | | [26] | |
| | | Rat (testis) | X97445 | SP:Q63344, CAA66074 | Rn.10524 | | [29] | |
| | | Rat (brain) | U62316 | AAB04023 | | | (d) | |
| | | Human (liver) | AF049608 | AAC70919 | Hs.132183 | | [27] | |
| | | Human (liver) | AF058056 | AAC13721 | | | (e) | |
| | | Mouse (kidney) | AF058054 | AAC13719 | Mm.29161 | | (b) | |
| | | Mongolian gerbil (brain) | AF029767 | AAB84219 | | Partial sequence | [105] | |
| | | Chicken (RPE cells) | U15685 (mRNA) | AAB52367 | | | [35,36] | |
| MCT4 (SLC16A3) | (MCT3) | | AF000240 (gene) | AAB61338 | | | [38] | |
| | | Rat (RPE) | AF059258 | AAC18120 | Rn.14526 | | (f) | |
| | | Mouse (RPE) | AF019111 | AAB70582 | Mm.6212 | Partial sequence | (g) | |
| | | Human | AL031587 (gene) | CAB37479 | * | From genomic sequence | (h) | |
| MCT5 (SLC16A4) | (MCT4) | Human (circulating blood) | U81800 | AAC52015 | Hs.85838 | | [6] | |
| | | Rat (skeletal muscle) | U87627 | AAC53591 | Rn.10826 | | [6,39] | |
| | | <i>Xenopus laevis</i> (embryo) | AI031360 | – | | Single EST sequence | [159] | |
| MCT6 (SLC16A5) | (MCT5) | Human (placenta) | U59185 | AAB72035 | Hs.23590 | | [6] | |
| MCT7 (SLC16A6) | (MCT6) | Human (placenta) | U59299 | AAC52013 | Hs.90911 | | [6] | |
| MCT8 (SLC16A2) | XPCT (MCT7) | Human (circulating blood) | U79745 | AAC52014 | Hs.114924 | | [6] | |
| | | Human (foetal brain) | U05315 (mRNA) | SP:P36021, AAB60374 | Hs.75317 | | [41] | |
| | | | U05316-21 (gene) | AAB60375 | | | | |
| | | Mouse (liver) | AF045692 | AAC40078 | Mm.5045 | | [43] | |

Table 1 (cont.)

| MCT (Unigene symbol) | Alternative (former) name | Organism (source) | Accession numbers | | Unigene number | Notes | Reference |
|----------------------|---------------------------|----------------------------------|---------------------|----------------------|----------------|------------------------------|-----------------------------|
| | | | DNA | Protein | | | |
| MCT9 ? | | Human | | | | Hs.126805 | EST sequences; see the text |
| | YKW1 | <i>Drosophila melanogaster</i> | AL009193, AJ.238706 | CAA15693, CAB42050 | | From genomic sequence | (i),(j) |
| | | <i>S. cerevisiae</i> | Z28221 | SP: P36032, CAA82066 | | ORF YKL221w | [160] |
| | | <i>S. cerevisiae</i> | Z74861 | CAA99138 | | ORF YOL119c | (k) |
| | | <i>S. cerevisiae</i> | Z75214 | CAA99626 | | ORF YOR306c | (l) |
| | | <i>C. elegans</i> | AAB71245 | AF026202 | | Locus C10E2.6 | [162] |
| | | <i>C. elegans</i> | AAD14701 | AF125952 | | Locus C01B4.9 | [162] |
| | | <i>C. elegans</i> | Z78545 | CAB01766 | | Locus M03B6.2 | [162] |
| | | <i>C. elegans</i> | U29379 | AAA68732 | | Locus K05B2.5 | [162] |
| | | <i>C. elegans</i> | U41105 | AAA82406 | | Locus T02G5.12 | [162] |
| | | <i>C. elegans</i> | Z70206 | CAA94126 | | Locus C49F8.2 | [162] |
| | oxIT-2 | <i>Sulfolobus solfataricus</i> | Y08256 | CAA69453 | | ORF c01003 | [163] |
| | | <i>Archaeoglobus fulgidus</i> | AE001079 | AAB90866 | | Oxalate/formate antiporter | [164] |
| | | <i>Bacillus subtilis</i> | Z99105 | CAB12011 | | ybfB | [165] |
| | | <i>Escherichia coli</i> | M64787 | SP: P23910 | | Role in arabinose metabolism | [166] |
| | | <i>Pseudomonas abietaniphila</i> | AF119621 | AAD21074 | | | [167] |

(b) Monocarboxylate transporters whose sequences are not related to MCT1

| MCT (Unigene symbol) | Alternative (former) name | Organism (source) | Accession numbers | | Notes | Reference |
|----------------------|---------------------------|--------------------------------|-------------------|----------------------|-------------|-----------|
| | | | DNA | Protein | | |
| | JEN1 | <i>S. cerevisiae</i> | U24155 | SP: P36035, AAB60291 | ORF YKL217w | [50] |
| | Lactate permease | <i>Escherichia coli</i> | | SP: P33231 | Gene lctP | [168] |
| | Lactate permease | <i>Haemophilus influenzae</i> | | SP: Q57251 | | [169] |
| | Lactate permease | <i>Bacillus subtilis</i> | | SP: P71067 | | [170] |
| | Lactate permease | <i>Sulfolobus solfataricus</i> | Y08256 | CAA69452 | ORF c01002 | [163] |

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          S V S F F F V P L A E R W K      N S L T *
Human TCAGTTTCCTTTTTTTTGTACCATTGGCCGAAAGATGGAAA----AACAGTCTGACCTGAAAGAAAGAAGACTGCAATCAAGTGAGAGCTAAACAAAAGAAAACCTAA
          S V S L S F V P L A E R W K R K Q S D L L R T T I K *
Mouse TCAGTTTCCTGTCTTTTGTACCCTGGCTGAAAGATGGAAACGCAACAGTCTGACCTTCTGAGGACTACAATCAAGTAAGAGCCTAACAAACAAAAATCTAAT
          S V S L F F V P L A E R W K L K Q S D L L R T T I K *
Rat   TCAGTTTCCTTTTTTTTGTACCCTGGCTGAAAGATGGAAACTCAAGCAGTCTGACCTTCTAAGGACTACAATCAAGTAAGAGCCTAACAAAAAATAAAAAA

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Figure 1 Comparison of the region around the stop codon in mammalian MCT5

Sequences for the 3' ESTs of mouse (accession number AI390702) and rat (accession number AI547803) were from dbEST, whereas the full human sequence is available (U59185).

above). This highlights the dangers of comparing apparently related transporter isoforms across distant species, although the complete conservation of intron positions within the coding sequences might be expected if the genes diverged from a common ancestor.

MCT5–MCT7

In addition to the identification of MCT4, our search of dbEST for fragments of MCT-like sequences enabled us to identify, clone and sequence three more novel members of the MCT family, now renamed MCT5, MCT6 and MCT7 with 25–30% amino acid sequence identity with MCT1 [6]. These members of the MCT family have not been functionally expressed and characterized, although their tissue distribution in humans has been determined by Northern-blot analysis [6].

MCT5 is of interest because it has an Alu insertion event in the 3'-UTR and a truncated C-terminus [6] (an Alu region is a region cut by the restriction endonuclease *AluI*). Since our initial searching of the dbEST [6], continued generation of EST sequences has revealed several for MCT5 from mouse and rat, and comparison of these with the human MCT5 sequence gives interesting insight into the Alu insertion. Previously [6] it was observed that human MCT5 (then named MCT4) had a much shorter C-terminal tail than other MCT family members (see Figure 2 below), and that an insertion sequence was present within the 3'-UTR of the cDNA. It is now clear from comparison with the 3'-UTR of human MCT5 that the mouse MCT5 sequence (example accession number AI390702) contains a relative deletion corresponding to the Alu insertion sequence in human MCT5 (not shown). The available 3'-end of the rat sequence (accession number AI547803), which is probably too short to be the true 3'-end, despite the presence of a short poly(A) tail, does not extend to the Alu insertion point in the human sequence. In comparing the human with the rat and mouse MCT5 sequences near their stop codons, the human sequences can be seen to contain a 4 bp deletion which causes a frameshift and introduces a stop codon in frame some eight amino acids before that present in rat and human MCT5 (see Figure 1). Consequently, the Alu insertion event is unlikely to have interrupted the original coding sequence of human MCT5, being downstream of the termination codon in rat and mouse MCT5. The frameshift causing deletion in the human sequence thus appears to be a separate event. Even with eight more amino acids than human MCT5, rat and mouse MCT5 still have much shorter 3'-UTRs than the other MCT family members. Human MCT5 contains five short overlapping upstream open reading frames (uORFs) within its 182 nt 5'-UTR [6]. A mouse EST sequence was found (accession number AI527817) corresponding to the 5' end of mouse MCT5 cDNA. The 142 nt 5'-UTR contains two short uORFs (12 and 2 codons long), suggesting a conserved, perhaps regulatory, role for these uORFs.

XPCT (MCT8)

During investigation of X-chromosome inactivation, gene sequencing revealed another MCT-related sequence [41], although no attempt was made to express and characterize the gene product. It is not clear whether the translation start site of this sequence is at the first AUG codon (giving a predicted 67 kDa protein) or second AUG codon (74 amino acids shorter, with a predicted 60 kDa protein). In either case the protein possesses a long N-terminal extension relative to MCT1, containing a PEST sequence motif indicative of rapid degradation [42]. Thus it was originally named 'XPCT' for 'X-linked PEST-containing transporter', but has since been renamed MCT8 [6,39] (see Table 1). The XPCT mRNA was found to be highly expressed in human liver and heart [6,41]. A mouse homologue of this protein has recently been identified whose mRNA is expressed highly in kidney in addition to liver [43]. The mouse XPCT protein sequence begins at the position equivalent to AUG-2 (amino acid 75) in the human XPCT and contains a duplicated 20-amino-acid peptide within its PEST domain, but is otherwise highly conserved. The mouse and human genes both have a six-exon structure, with conservation of intron positions [41,43]. Only the final intron is conserved in position relative to any of the three introns in MCT3 (which is also conserved in MCT1), as might be expected from the lower degree of conservation between MCT8 and other human MCT family members. There are no published reports of heterologous expression of this member of the MCT family, and thus no confirmation that it catalyses proton-linked monocarboxylate transport. However, recently it has been reported that purification of a lysosomal proton-linked sialic acid transporter yields a protein that runs at 57 kDa on SDS/PAGE and can transport L-lactate [44]. It was suggested that this might be a member of the MCT family, and MCT8 (XPCT) would be a possible candidate. Not only is the predicted size of MCT8 greater than the other MCTs because of the PEST extension, but in yeast this sequence is able to target membrane proteins to the lysosome [45]. However, the substrate specificity of the transporter is very broad, sharing similarities with the organic-anion-transporter family, including the ability to transport the dicarboxylate 2-oxoglutarate [46].

MCT9 and other mammalian EST sequences

Since our earlier dbEST searches [6], we have now identified another set of human ESTs which may represent a further MCT isoform. Full cDNA sequences are not currently available, but the partial protein sequence is shown in the sequence alignment in Figure 2. These ESTs map to chromosome 10 (see UNIGENE entry; Table 1). Two mouse MCT9 EST sequences also exist (accession numbers AI316907 and AI316929). Many new mouse and rat ESTs can be identified for MCT4 (which has yet to be

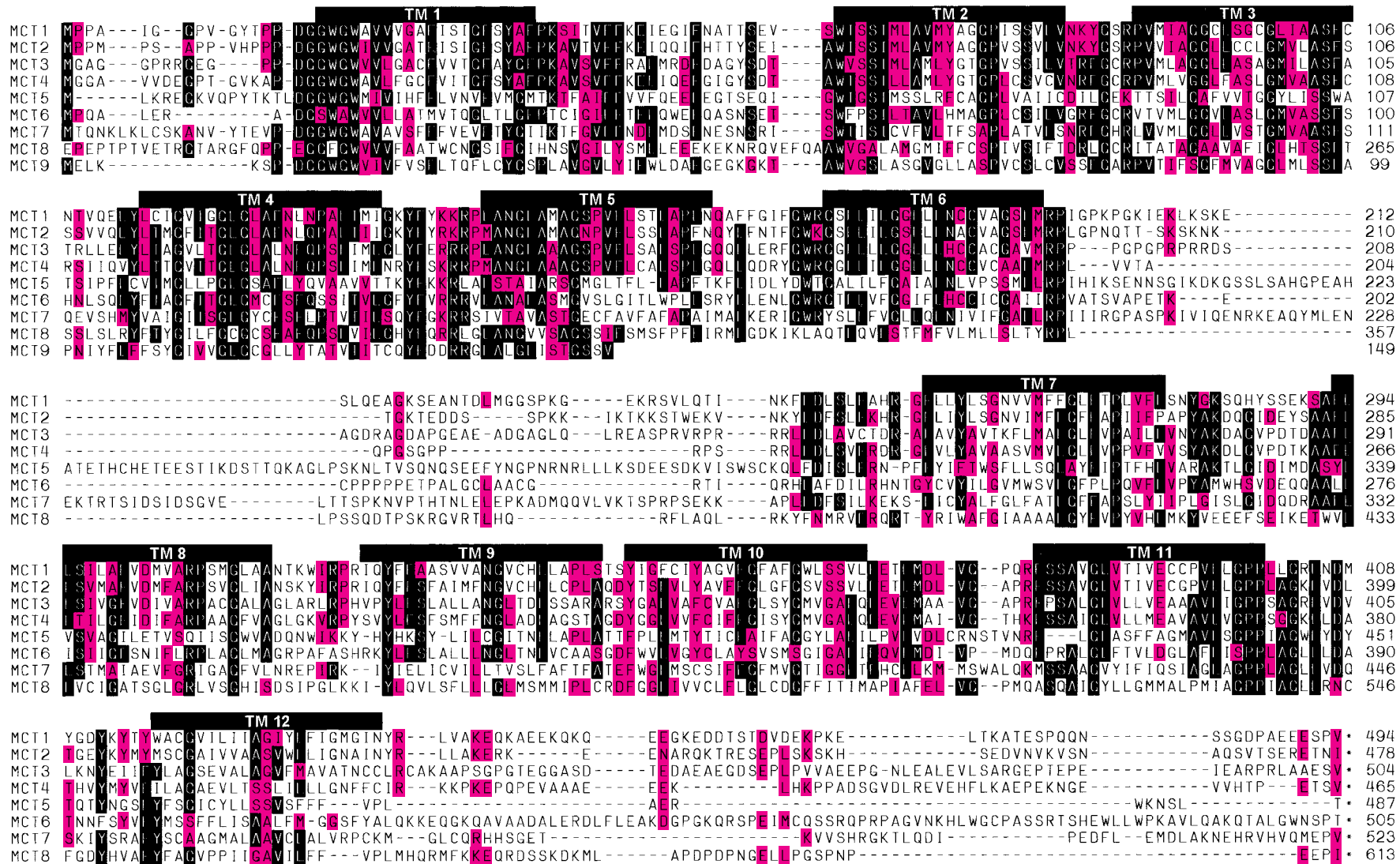


Figure 2 Alignment of human MCT family protein sequences

The sequences were aligned using the clustal V algorithm using DNASTar Lasergene software with default parameters. Positions which are identical in at least five sequences are shown as white text on a black background. Positions conserved in four or more sequences are coloured in black text on a red background. Note that, for MCT8, the first 150 amino acids (PEST sequence) are not included, whereas for MCT9 the full sequence is not yet available.

Table 2 Known chromosomal locations for MCT genes

The chromosomal locations for *S. cerevisiae* and *C. elegans* genes are not presented here, but can be obtained from the relevant database entries (see Table 1).

| MCT | Unigene symbol | Unigene number | Chromosomal location | Reference |
|-----------------------|----------------|----------------|----------------------|-----------|
| MCT1 Human | SLC16A1 | Hs.75231 | 1p13.2-p12 | [65] |
| MCT2 Human | SLC16A7 | Hs.132183 | 12q13 | [27] |
| MCT3 Human | — | Hs.126783 | 22q12.3-13.2 | Table 1 |
| MCT4 Human | SLC16A3 | Hs.85838 | 17 | Unigene |
| MCT5 Human | SLC16A4 | Hs.23590 | 1 | Unigene |
| MCT6 Human | SLC16A5 | Hs.90911 | ? | — |
| MCT7 Human | SLC16A6 | Hs.114924 | ? | — |
| MCT8 Human (XPCT) | SLC16A2 | Hs.75317 | Xq13.2 | [41] |
| MCT9 Human | — | Hs.126805 | 10 | Unigene |
| MCT8 Mouse (XPCT) | SLC16A2 | Mm.5045 | X | [43] |
| MCT <i>Drosophila</i> | — | — | X 2E | Table 1 |

cloned from mouse), and MCT5–MCT7 (which have only been cloned from humans). These sequences will not be discussed further here, since they add few insights to what is already known.

CHROMOSOMAL LOCATIONS OF MAMMALIAN MCTS

The known chromosomal locations of human MCT genes are given in Table 2. Some of these entries are taken from mapping data present in the Unigene database (<http://www.ncbi.nlm.nih.gov/Schuler/UniGene>). In future, such mapping data may be useful in relating genetic diseases to particular transporter isoforms. For further details the reader is referred to the On-Line Mendelian Inheritance in Man (OMIM) database (<http://www.ncbi.nlm.nih.gov/omim>). To date, no specific genetic diseases have been linked to a particular MCT isoform by this means, although an MCT deficiency has been described at the functional level [47], and there is a preliminary report of polymorphisms in MCT1 that may correlate with impaired lactate transport [48].

MCT FAMILY MEMBERS IN NON-MAMMALIAN SPECIES

Since our previous sequence survey [6], several MCT sequences have appeared in publications or sequence databases, including human and mouse MCT2, rat MCT3 and mouse MCT8 (XPCT). These are collected together in Table 1. Recently database entries for *Trypanosoma cruzi* MCT1 have appeared (accession numbers U40854 and U40855). However, their nucleotide sequences almost exactly match those of rat MCT1 and thus it seems likely that the true source of these sequences is the host rather than the parasite.

Drosophila MCTS

Sequencing of the *Drosophila melanogaster* X chromosome has revealed an MCT family member (see Table 1). Of the other MCT isoforms, this *Drosophila* sequence most closely resembles a number of potential MCTS from *C. elegans* (see below) followed by chicken MCT3 and human MCT5 and MCT4. Recently a large number of *Drosophila* EST sequences have been generated. Amongst them sequences representing at least six additional *Drosophila* MCT family members can be identified (N. T. Price and A. P. Halestrap, unpublished work). Interestingly, some of the *Drosophila* ESTs were derived from Schneider 2 cells, which

do not possess any detectable proton-linked lactate transport activity (N. T. Price, M. C. Wilson and A. P. Halestrap, unpublished work). These data suggest that such MCT family members may not be proton-linked; they might also transport different monocarboxylates or even unrelated substrates.

MCTS identified in *C. elegans* and *S. cerevisiae*

Our previous survey also [6] identified four MCT family members in both the nematode worm *C. elegans* and the yeast *S. cerevisiae*. Subsequently two additional sequences from *C. elegans* have appeared (Table 1). Thus the MCT family members thus far identified include nine in mammals, possibly seven in *Drosophila*, six in *C. elegans*, with only four in unicellular *S. cerevisiae*.

EVOLUTIONARY RELATIONSHIPS BETWEEN MCT FAMILY MEMBERS

A cladogram is presented in Figure 3 to show the relatedness and hence possible evolutionary history of members of the MCT family, including non-mammalian ones. This confirms that chicken MCT3 is more closely related in sequence to mammalian MCT4 than mammalian MCT3, as discussed above. In the case of the mammalian MCTS, conservation of sequence between the isoforms is greatest for MCT1–MCT4 (> 50%), and for all of these isoforms their ability to transport lactate and pyruvate has been experimentally verified as described above. This suggests that these isoforms may form a subgrouping of transporters whose main function is the transport of short-chain monocarboxylates such as lactate, pyruvate and the ketone bodies. For the other isoforms conservation is significantly less (< 30%), perhaps indicating the evolution of distinct substrate specificities or the replacement of the proton linkage with co-transport of another cation such as Na⁺. The cladogram suggests that the six *C. elegans* MCT family members and the four from *S. cerevisiae* form separate groups which themselves evolved independently from an earlier ancestor. Characterization of transport-protein families by genome analysis, particularly for prokaryotic transporters, has recently been reviewed elsewhere [49].

As yet, none of the non-mammalian sequences have been heterologously expressed to confirm their identity as monocarboxylate transporters. In a unicellular organism such as *S. cerevisiae*, whose transporter families have been reviewed recently by Horák [50], different MCT isoforms cannot reflect kinetic adaptation for tissue-specific requirements and are therefore

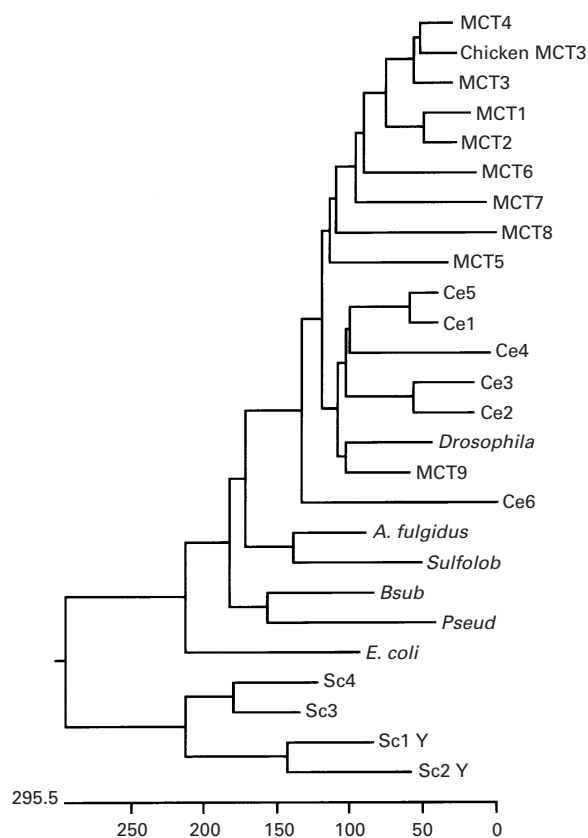


Figure 3 Possible evolutionary relationship of MCT related sequences

Protein sequences were aligned as in Figure 2 and displayed as a cladogram using DNASTar Lasergene software. The x-axis represents sequence divergence in arbitrary units calculated by the software. Sc1–Sc4 and Ce1–Ce6 refer to the sequences from *S. cerevisiae* and *C. elegans* respectively in the order shown in Table 1. It should be noted that MCT9 is only a partial sequence and thus its position in the cladogram is only provisional. Abbreviations: *A. fulgidus*, *Archaeoglobus fulgidus*; *Sulfolob*, *Sulfolobus*; *Bsub*, *Bacillus subtilis*; *Pseud*, *Pseudomonas*; *E. coli*, *Escherichia coli*.

more likely to possess different substrate specificities. It is well established that yeasts contain a proton-linked lactate and acetate transporter on their plasma membrane [51–54], but it is disruption of the *JEN1* gene that abolishes uptake of lactate [55]. Jen1 is predicted to be a transporter from its sequence, but it does not belong to the MCT family, nor is it more closely related to the prokaryotic lactate permeases than other members of the transporter superfamily. This would suggest that none of the four yeast MCT family members are able to transport lactate, and the identity of their true substrates must await further experimentation. Thus the available data suggest that the evolution of transporters for lactate must have proceeded differently in *S. cerevisiae* and higher eukaryotes, although confirmation of this must await a direct demonstration that Jen1 is a lactate transporter.

COMMON FEATURES OF MAMMALIAN MCT SEQUENCES

The existence of a mammalian monocarboxylate transporter family is now firmly established and contains at least nine members in humans. An alignment of the transmembrane domains of all the currently identified members of the human MCT family (nine) is shown Figure 2.

Predicted topology

Hydropathy plots using the Kyte–Doolittle algorithm [56] and the TMpred program [57] predict the number of transmembrane α -helical (TM) domains to be 12 for MCT1, MCT2, MCT3, MCT7 and MCT8 and between 10 and 12 for the other MCTs. Thus it seems probable that there are 12 TM domains with the N- and C-termini located within the cytoplasm, as illustrated in Figure 4. For MCT1 in the red-blood-cell plasma membrane we have tested the topological predictions experimentally using proteolytic digestion, and the data fully support the prediction [58]. Thus both the N- and C-terminal sequence and the loop between TMs 6 and 7 could not be cleaved by proteases in intact red blood cells, but could be cleaved in leaky ghosts. The only external loop that could be cleaved by proteases added to intact red blood cells was that between TMs 11 and 12.

The 12-TM helix topology is shared by many other plasma-membrane transporters such as the GLUT family [59,60]. Furthermore, the MCT family members, like members of other transporter families, exhibit the greatest sequence conservation in the putative TM regions, and the shorter loop regions between them. In contrast, the hydrophilic regions of the sequences (the N-terminal residues preceding TM1, the loop region between TMs 6 and 7, and the C-terminal residues succeeding TM12) show little conservation. Indeed the size of the loop region between TM6 and TM7 varies substantially from 105 and 93 residues in MCT5 and MCT7, through 67, 66, 49, 47 and 40 residues in MCT1, MCT3, MCT2, MCT6 and MCT8, to only 29 residues in MCT4. Such divergent hydrophilic regions are a common feature of 12-TM transporter family sequences [61], and it is unlikely that these regions are directly involved in transport. Rather they may be critical for other aspects of function, such as substrate specificity or regulation of transport activity, as will be discussed further below.

Glycosylation

Analysis of the sequences for potential glycosylation sites shows that the only suitable asparagine residues predicted to be in an extracellular loop (see below) and thus potential N-glycosylation sites are Asn³⁵³ in MCT3, Asn⁴⁵⁶ in MCT5, Asn¹⁰² and Asn³⁹³ in MCT6 and Asn⁵⁷ in MCT7. However, in all cases the predicted size of these loop regions suggest that they are too small for N-linked glycosylation to take place [62]. Thus, as is the case for MCT1 and MCT2, it is unlikely that the new MCT isoforms are glycosylated. Indeed, for MCT1 and MCT3 this has been confirmed experimentally [23,36].

STRUCTURE–FUNCTION RELATIONSHIPS IN THE MCT FAMILY

The possible roles of the conserved sequence motifs of the MCT family have been discussed in detail previously [6]. A full understanding of the significance of these motifs and the role of other conserved residues must await the detailed characterization of their distinct kinetics and substrate and inhibitor specificities, coupled with the results of site-directed mutagenesis. However, the sequence comparisons do highlight some important points. In TMs 1–6 there are 15 residues which are absolutely conserved across all nine mammalian sequences (Figure 2), and many more positions where only conservative substitutions occur. Two highly conserved regions stand out in particular: the sequence with consensus **[D/E]G[G/S][W/F][G/A]W**, which traverses the lead into TM1, and the consensus **YFXK[R/K][R/L]-XLAX[G/A]XAXAG**, which leads into TM5 (residues shown in **bold** are conserved in all of the sequences, residues in square brackets indicate alternative amino acids, residues that are in

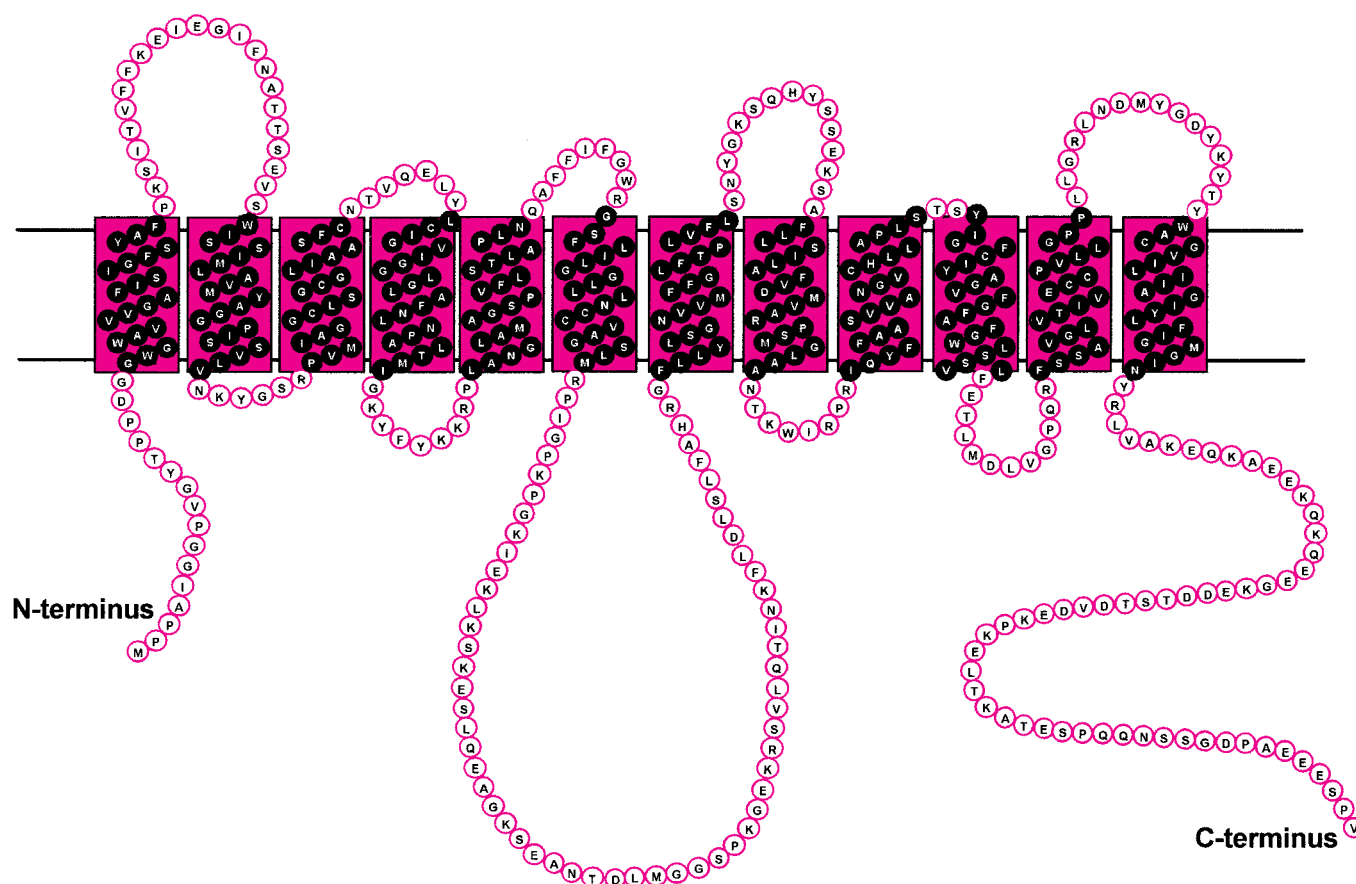


Figure 4 Proposed membrane topology of the MCT family

The sequence shown is that of human MCT1.

normal type are the consensus amino acid at that position and 'X' represents any amino acid). When the non-mammalian MCT family members are included, there is still conservation of these sequence motifs [6]. It has been proposed that the N-terminal domains are more important for energy (e.g. H^+ or Na^+) coupling, membrane insertion and/or correct structure maintenance, whereas the C-terminal domains may be more important for the determination of substrate specificity [61]. Inhibitor and photoaffinity-labelling studies on sugar transporters [59,60] and the characteristics of chimaeras constructed with regions from different glucose-transporter isoforms [63] are consistent with this hypothesis. Since MCTs are monocarboxylate/proton co-transporters, it might be predicted that the amino acid residues responsible for proton binding and translocation are in TMs 1–6. A possible candidate residue for this role would be the only conserved aspartate/glutamate residue in the N-terminal half of the protein, which is at the start of TM1 (Asp¹⁵ of human MCT1). However, site-directed mutagenesis to change this residue to an asparagine residue was without effect on transport activity. By contrast, even the conservative change of Asp³⁰² to Glu in TM8 of rat MCT1 led to total loss of lactate transport activity [64].

There is also evidence for the C-terminal half of MCT1 being involved in substrate specificity. Thus conversion of Phe³⁶⁰ in TM10 of Chinese-hamster MCT1 into Cys enables MCT1 to transport mevalonate, while decreasing its ability to transport lactate and pyruvate [17,65]. Furthermore, we have shown that

the binding site of MCT1 for DIDS, which acts at or near the substrate-binding site [14], is in the C-terminal half of the transporter [58]. Indeed, conversion of either of two exofacial lysine residues into glutamine residues (Lys²⁹⁰ in the loop between TMs 7 and 8 and Lys⁴¹³ in the loop between TMs 11 and 12), prevents irreversible inhibition of MCT1 by DIDS [66]. A positively charged group that binds the carboxylate anion is a feature that is likely to be present in all MCTs. In red-blood-cell MCT1 arginine probably fulfils this role, since the arginine-specific reagent phenylglyoxal inhibits MCT1-mediated lactate transport [2,12,67]. This is also the case in lactate dehydrogenase [68]. An arginine residue in TM8 (Arg³¹³ of human MCT1) is conserved in all the putative MCTs from higher eukaryotes, except MCT5 (see Figure 2) and also in all but one of the *S. cerevisiae* and *C. elegans* sequences. Recent studies in Stefan Bröer's laboratory have demonstrated that site-directed mutagenesis of this residue greatly reduces the affinity of MCT1 for lactate, with transport of L-lactate being less than 3% of control values at 0.1 mM and not saturating even at 50 mM [64]. If this arginine residue is involved in binding the carboxylate moiety, its replacement by a glutamine in MCT5 suggests that this MCT isoform may not transport a monocarboxylate. Another arginine/lysine residue that is totally conserved in all sequences is located within the relatively highly conserved region YFXK[R/K][R/L]XLAX[G/A]XAXAG between TMs 4 and 5 mentioned above. However, since the N-terminal half of the molecule is not thought to be involved in substrate binding, this

residue is unlikely to be involved in binding the carboxylate group.

UNIQUE PROPERTIES OF THE DIFFERENT MCT ISOFORMS

MCT1

Kinetics

The kinetics of pyruvate and lactate transport into red blood cells have been thoroughly characterized using radiotracer techniques, and these can be taken as representing the kinetics of MCT1, since this is the only endogenous MCT present in the red-blood-cell plasma membrane (see [2,9]). By determining the effects of pH on the kinetics of net flux of lactic acid and lactate exchange, kinetics, were shown to follow an ordered, sequential mechanism [69,70]. Transport involves a proton binding to the transporter initially, followed by a lactate anion. Translocation of lactate and proton across the membrane occurs next, followed by their sequential release from the transporter on the other side of the membrane. The process is freely reversible, with equilibrium being reached when $[\text{lactate}]_{\text{in}}/[\text{lactate}]_{\text{out}} = [\text{H}^+]_{\text{out}}/[\text{H}^+]_{\text{in}}$. The kinetic parameters for lactic acid influx and efflux

are thermodynamically constrained according to the Haldane equation:

$$(V_{\text{max}}/K_m)_{\text{influx}} = (V_{\text{max}}/K_m)_{\text{efflux}}$$

The rate-limiting step for net lactic acid flux is the return of the free carrier across the membrane that is required for the completion of the translocation cycle, as illustrated by the observation that rates of monocarboxylate exchange are substantially higher than those of net transport. As the pH is decreased between 6 and 8 on the side that lactate is added, transport is stimulated primarily through a decrease in the K_m for lactate. Transport can also be stimulated by raising the pH on the opposite side of the membrane. This increases the V_{max} of transport by stimulating the rate at which the unloaded carrier reorients in the membrane.

Substrate and inhibitor specificity

The substrate and inhibitor specificity of MCT1 were also studied extensively in red blood cells by measurement of the inhibition of the rate of [^{14}C]lactate uptake (see [2,9]). Subsequently these studies were confirmed and extended using Ehrlich Lettré tumour

Table 3 Comparison of K_m (a) and K_i (b) values for various substrates and inhibitors of different MCT isoforms, and inhibition by different inhibitors at 0.1 mM (c)

Data for tumour cells and muscle vesicles are taken from [67] and [3] respectively. Data for MCT1 and MCT2 expressed in *Xenopus* oocytes are from [20] and [28] respectively.

(a)

| Substrate | K_m (mM) | | | |
|-------------------------------|-------------|--------|------|-----------------|
| | Tumour cell | Oocyte | | Muscle vesicles |
| | | MCT1 | MCT2 | |
| Lactate | 4.5 | 3.5 | 0.5 | 13–40 |
| D,L- β -Hydroxybutyrate | 12.5 | 12.5 | 1.2 | High |
| Pyruvate | 0.72 | 1.0 | 0.08 | > 50 |
| 2-Oxoisopentanoate | — | 1.3 | 0.3 | > 50 |
| 2-Oxoisohexanoate | — | 0.7 | 0.1 | — |
| Acetoacetate (3-oxobutyrate) | 5.5 | 5.5 | 0.8 | High |

(b)

| Inhibitor | K_i (mM) | | | |
|-----------|-------------|--------|------|-----------------|
| | Tumour cell | Oocyte | | Muscle vesicles |
| | | MCT1 | MCT2 | |
| CHC | 166 | 425 | 24 | 4000 |
| Phloretin | 5.1 | 28 | 14 | > 1500 |

(c)

| Inhibitor | Inhibition at 0.1 mM (%) | | | |
|-----------|--------------------------|--------|------|-----------------|
| | Tumour cell | Oocyte | | Muscle vesicles |
| | | MCT1 | MCT2 | |
| DIDS | 434 | — | — | > 1000 |
| DBDS | 30 | 0 | 56 | — |
| pCMBS | 50 | 90 | 0 | 25 |

cells which, like red blood cells, contain exclusively MCT1. In this case continuous measurements of monocarboxylate transport were made by monitoring the substrate-induced decrease in intracellular pH (pH_i) using the pH-sensitive fluorescent dye 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). This technique has shown itself to be superior to the discontinuous radiotracer technique, and has the advantage that it can be used at more physiological temperatures and is not dependent on the availability of radioactively labelled substrates [67]. Most recently, additional confirmatory data have been obtained following expression of the MCT1 in *Xenopus* oocytes [20]. Taken together, these studies demonstrate that MCT1 can transport a wide range of short-chain monocarboxylates, the K_m values decreasing as the chain length increases from two to four carbon atoms. Monocarboxylates substituted in the C-2 and C-3 positions are good substrates, with the carbonyl group being especially favoured and C-2 substitution being preferred over C-3. Indeed, many naturally occurring monocarboxylates, such as pyruvate, lactate, acetoacetate and β -hydroxybutyrate, fall into this category, and K_m values for some of the most important of these are given in Table 3. For lactate, MCT1 is stereospecific, with the K_m for the L-isomer (5–10 mM) being an order of magnitude lower than for the D-isomer. However, this is not true for other monocarboxylates such as 2-chloropropionate or β -hydroxybutyrate. Monocarboxylates with longer branched aliphatic or aromatic side chains also bind to the transporter, but are not readily released following translocation and may act as potent inhibitors. One of these is the now classical inhibitor of lactate transport, CHC [71].

Inhibitors of MCT1 fall into four broad categories: first, substituted aromatic monocarboxylates such as CHC and phenylpyruvate; secondly, inhibitors of anion transport such as the stilbenedisulphonates, niflumate and 5-nitro-2-(3-phenylpropylamino)benzoate; thirdly, bioflavonoids such as phloretin and quercetin; fourth miscellaneous inhibitors including thiol reagents such as *p*-chloromercuribenzenesulphonate (pCMBS) and amino reagents (e.g. pyridoxal phosphate and phenylglyoxal). It is important to note that none of these inhibitors is specific for inhibition of MCT1, and caution must be exercised when using them to investigate the role of MCT in cellular function. Such caution is particularly important when using CHC, since this compound is two orders of magnitude more potent at inhibiting pyruvate transport into mitochondria than it is at inhibiting lactate transport across the plasma membrane [72]. Thus, when used with cells in which some oxidation of glucose to CO_2 and water is occurring, CHC prevents oxidation of glycolytically derived pyruvate and leads to a large increase in lactic acid production and decrease in pH_i independent of any effect on plasma-membrane MCT activity [73].

MCT2

In comparison with MCT1, the properties of MCT2 are poorly understood. Although the kinetics of monocarboxylate transport into hepatocytes have been studied extensively [74], these cells are now known to contain both MCT1 and MCT2 in similar amounts and thus cannot be used to characterize the properties of MCT2 [29]. Recently Lin et al. [27] expressed human MCT2 in *Xenopus* oocytes and showed it to have a very high affinity for pyruvate (K_m 25 μM). These observations have been confirmed and extended for rat MCT2 by Bröer et al. [28]. Measured K_m and K_i values for most substrates and inhibitors are 6–10 times lower than for MCT1, as shown in Table 3, K_m values for pyruvate, L-lactate, acetoacetate and D,L- β -hydroxybutyrate be-

ing 0.08, 0.5, 0.8 and 1.2 mM respectively. Unlike MCT1, MCT2 is insensitive to inhibition by pCMBS [26,28].

Other MCT isoforms

No detailed analyses of the properties of either MCT3 or MCT4 have been described. In COS and NBL1 cells, both of which express large amounts of MCT4, transport kinetics for L- and D-lactate and pyruvate are similar to those for MCT1 [39]. Furthermore, in frog sartorius muscle, which as a white muscle might be expected to express predominantly MCT4 [39], microelectrode studies gave a K_m for L-lactate of about 10 mM [75]. However, when MCT4 was expressed in *Xenopus* oocytes, a K_m value for L-lactate of 22 mM was determined [40]. This is similar to the values derived for L-lactate transport into giant sarcolemmal vesicles from glycolytic muscle fibres (containing primarily MCT4) [3].

TISSUE DISTRIBUTION AND PHYSIOLOGICAL ROLE OF THE DIFFERENT MAMMALIAN MCT ISOFORMS

It would be predicted that the existence of several different MCT isoforms has a physiological significance that is related either to their unique properties or to their regulation. In either case, their tissue distribution should give some clues as to their role. However, care should be exercised when extrapolating between species, as illustrated by the relationship between MCT3 and MCT4 in chickens and mammals discussed above. We have determined the expression of mRNA for all the MCT isoforms in a wide range of human tissues using Northern-blot analysis [6], and similar studies for MCT1 and MCT2 have been reported by Lin et al. [27]. In addition, for MCT1 and MCT2, less extensive analysis of rat, mouse and hamster tissues have been reported [17,25,29,76], whereas for MCT3 such data are only available for chicken tissues [35]. These studies indicate that the expression of mRNA for each of the eight MCT isoforms has a distinct tissue-dependency. For example, MCT1 and MCT4 are expressed to different extents in most tissues, whereas MCT2 has a more restrictive distribution, and MCT3 is found exclusively in the RPE. However, for most isoforms, one or two tissues showed especially high levels of mRNA, including MCT2 in testis, MCT4 in skeletal muscle, MCT5 in placenta, MCT6 in kidney and placenta, MCT7 in pancreas and brain and MCT8 in liver, kidney and heart. Although these data may provide some clues as to the tissue distribution of the expressed protein, it is more important to determine this directly and to establish the localization of the protein within the tissue. For MCT1–MCT4 this has been achieved by using Western blotting and both immunofluorescence microscopy and immunogold electron microscopy with specific antipeptide antibodies. There are currently no published data for MCT5–MCT8, and these members of the MCT family will not be considered further.

MCT1 was found to be present in almost all tissues, in many cases with specific locations within each tissue [18,26,29,30,39,77], as will be discussed further below. In contrast MCT2 is expressed in fewer tissues, and where it is expressed together with MCT1 its exact location within the tissue is different, suggesting a unique functional role. MCT2 is unusual in that there appear to be substantial species differences in both its amino acid sequence and tissue distribution [29]. The latter may be related to splice variants in the 5'-UTR that are indicative of alternative promoter usage, as discussed above. MCT3 is exclusively located in the basal membrane of the RPE in both chicken and rat, in contrast

with MCT1, which was found on the apical surface [37,78]. The use of alternative 5'-UTRs is often associated with spatial distribution of mRNA forms, but in the case of chicken MCT3 the two different mRNAs, resulting from different promoter usage, are involved in temporal rather than spatial regulation [38] (see above). MCT4 is expressed particularly strongly in skeletal muscle, as will be discussed further below, and is also the major MCT isoform in white blood cells and some mammalian cell lines. 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, and hence require rapid lactic acid efflux [39]. In this context it is noteworthy that among the ESTs that correspond to human MCT4 mRNA, the cDNA libraries employed were frequently derived from tumour cell lines (which are often highly glycolytic) and placenta (which rapidly exports lactic acid from the foetal to the maternal circulation).

Skeletal muscle

Skeletal muscle is the main producer of lactic acid in the body, since many fibres, especially in 'white muscle' have few mitochondria and depend on high rates of glycolysis for most of their ATP production. However, lactic acid can also be taken up by some skeletal-muscle fibres and used as a respiratory fuel. This is especially the case in 'red muscle', which has a high mitochondrial content. The net production of lactic acid by muscle is most pronounced in the transition from rest to heavy exercise, when there is a rapid increase in energy demand, but this is not normally due to a lack of oxygen. Rather the maximal glycolytic capacity of muscle exceeds the maximal oxidative capacity, and the acceleration of glycolysis at the onset of muscle activity is faster than that of the oxidative pathway (see [79]). Thus lactic acid is an important metabolic intermediate for skeletal muscles, requiring its rapid transport across the sarcolemma in either direction, depending on the fibre type and workload (see [3,40,80]). Although it is not possible to determine accurate transport kinetics of lactic acid influx and efflux in intact skeletal muscles, measurements are possible using small and giant sarcolemmal membrane vesicles. Such studies (reviewed in [3,40,80]) confirmed that lactate and other monocarboxylates cross the skeletal-muscle sarcolemma by means of a saturable, stereospecific transport system which shows an obligatory 1:1 coupling between lactate and H⁺. K_m values of between 13 and 40 mM have been reported for L-lactate in most studies with rat and human sarcolemmal vesicles, and transport was shown to be inhibited reversibly by CHC and irreversibly by organomercurials such as pCMBs. In these ways the characteristics of skeletal-muscle lactic acid transport resemble those of many other cells (see [2,3]). Subsequent studies using Western blotting and immunofluorescence microscopy have confirmed the presence of both MCT1 and MCT4 in skeletal muscle, with little if any MCT2 [18,26,39,81].

MCT1 expression in individual muscles correlates with their mitochondrial content. Muscles such as soleus that contain predominately slow oxidative fibres express large amounts of MCT1, whereas muscles with a high content of fast-twitch glycolytic fibres, such as the white gastrocnemius and white tibialis anterior, contain almost none [39,40,81]. In human skeletal muscle a positive relationship was found between MCT1 density and the occurrence of type I fibres (defined according to myosin-heavy-chain isoforms) [82]. These data suggest that MCT1 expression in muscle fibres may reflect the need to transport lactic acid into the cell for oxidation as a respiratory fuel. In contrast, MCT4 is present in all rat muscles, but less in predominantly oxidative muscle such as soleus [39]. In human

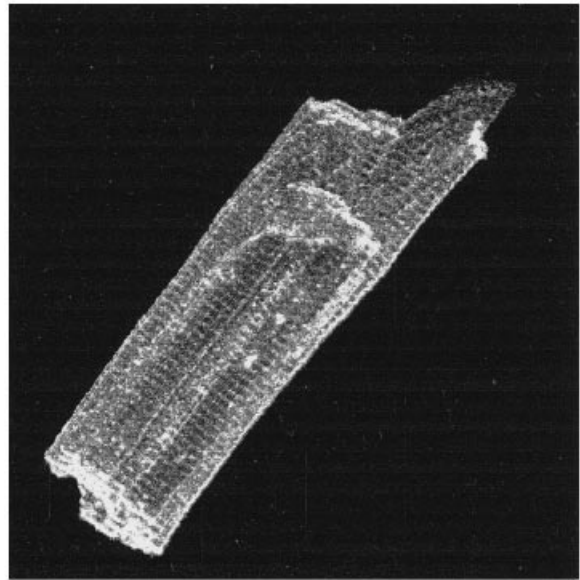


Figure 5 MCT1 distribution in an isolated rat heart cell

MCT1 was revealed with immunofluorescence laser scanning confocal microscopy using a C-terminal antipeptide antibody specific to rat MCT1 (C. Heddle and A. P. Halestrap, previously unpublished results). The white areas indicate MCT1.

muscles, MCT4 density was independent of fibre type and the inter-individual variation was large [82]. These data imply that MCT4 is important for lactic acid efflux from muscles that rely more on glycolytic metabolism for their ATP production.

Heart

Lactate, pyruvate and the ketone bodies acetoacetate, β -hydroxybutyrate and acetate are important fuels for the heart under aerobic conditions and must be transported into the cell. In contrast, under hypoxic conditions, the heart uses glycolysis in its attempt to maintain the production of ATP required for ionic homeostasis and contraction. The resulting lactic acid must be transported out of the cell, to prevent its intracellular accumulation and consequent decrease in pH_i [7]. Were this to occur, glycolytic ATP production would be diminished and contractile function impaired [83–85]. Indeed, using 4,4'-dibenzoylamino-stilbene-2,2'-disulphonate (DBDS), an inhibitor of lactate transport discovered in this laboratory, it has been confirmed that lactic acid transport out of the heart is important in re-establishing intracellular pH following periods of ischaemia [86]. We have performed extensive kinetic studies of monocarboxylate transport into isolated heart cells from the rat and guinea-pig using both radiotracer and BCECF-fluorescence techniques. Our data strongly imply the existence of two MCTs within rat and guinea-pig heart cells, namely one isoform with a high affinity for substrates and strongly inhibited by stilbenedisulphonates such as DBDS, and another isoform with lower affinity for substrates and only weakly inhibited by DBDS [7,87–91].

Western and Northern blotting has confirmed the presence of large amounts of MCT1 in both human and rat hearts. Immunofluorescence microscopy suggests that this isoform is particularly concentrated in the intercalated-disc region at the end of the myocytes [7,18]. More detailed analysis with confocal microscopy confirms this location, but also shows some MCT1 to be

associated with the t-tubules (Figure 5). Greater resolution with immunogold electron microscopy has demonstrated that MCT1 avoids the desmosomes and gap junctions of the intercalated discs [92]. These studies confirmed that MCT1 is found along the length of the t-tubules and may also be associated with plasmalemmal invaginations characteristic of caveolae. No evidence was found for internal membrane compartments containing MCT1, implying that expression of the protein is constitutive. It is likely that MCT1 reflects the DBDS-insensitive MCT activity measured in the kinetic studies. Indeed when lactate transport was measured across the intercalated-disc region of isolated rat heart cells (MCT1-rich) it was found to be insensitive to DBDS, whereas transport in the middle of the cell (MCT1-poor) was DBDS-sensitive [7]. Yet the actual transport rates in the centre of the cell were faster than at the ends, implying a high concentration of the DBDS-sensitive carrier in this region of the cell. This is unlikely to be MCT4, which, although present in human hearts, could not be detected in rat heart cells [39] and in any case has a lower affinity for lactate and stilbenedisulphonates (see above). MCT2 would appear to be a likely candidate for the second MCT isoform in heart, since it is a high-affinity, stilbenedisulphonate-sensitive, carrier [28], although unlike MCT2 it appears to be insensitive to 5-nitro-2-(3-phenylpropylamino)-benzoate [28,91]. However, our C-terminal antibody, which readily detected MCT2 as a 40 kDa protein in Western blots of membrane proteins from brain, liver, kidney and testis, failed to detect MCT2 in rat heart plasma membranes [13]; nor did immunofluorescence microscopy reveal the presence of any MCT2 in isolated rat heart cells [7,29]. Furthermore, Northern blots showed that MCT2 mRNA was barely detectable in rat or mouse heart [6,25,29], although in human heart a range of MCT2 mRNA transcript sizes was detected [27]. Hamster heart also expressed significant quantities of MCT2 mRNA [29], and MCT2 protein could be detected by immunofluorescence microscopy [26]. However, the location of the expressed protein was similar to that of MCT1 [26], and would not seem able to account for the observed rates of lactate transport in the middle of the heart cell. Thus the identity of the second MCT isoform in heart cells remains to be elucidated, although its properties suggest that it is likely to be similar to MCT2, perhaps with some C-terminal modification that renders it undetectable by our MCT2 antibody.

Brain

Glucose is usually the major fuel for the brain, and the blood/brain barrier is relatively impermeable to lactate and the ketone bodies [93]. However, during conditions such as starvation and diabetes, the permeability increases and these monocarboxylates become more important substrates for the provision of energy [94]. Their use as fuels is even more pronounced in the neonatal animals, and this is accompanied by a much greater increase in the permeability of the blood/brain barrier to these monocarboxylates [76,95,96]. Little or no MCT2 was found to be expressed in the endothelial cells under any conditions, but MCT1 expression was detected and was much greater in the neonatal brain [30,77,96]. Using immunogold electron microscopy it was shown that the cerebral blood vessels of 17-day-old suckling pups have 25 times as much MCT1 associated with both the luminal and abluminal membranes of the capillary endothelium than do adults [77]. The increase in MCT1 protein expression was associated with a parallel increase in MCT1 mRNA as detected by both Northern-blot analysis and *in situ* hybridization [76]. Thus it seems probable that MCT1 is responsible for the high rates of lactate and ketone-body transport across the endothelium of the cerebral microvasculature.

Surrounding the brain capillaries are astrocytes, whose foot processes are in close contact with plasma membrane of the endothelial cells. MCT1 is the major MCT isoform present in astroglial cells [30,77], and this is confirmed by monocarboxylate transport kinetics of cultured astroglial cells [19,97]. However, within the foot processes MCT1 expression is low, whereas that of MCT2 is high [30,77]. This may reflect the high affinity of MCT2 for lactate and the ketone bodies, which would make it well suited to transport these monocarboxylates rapidly into the astrocytes once they have crossed the endothelium. Paradoxically, however, MCT2 expression in these foot processes is somewhat decreased in neonatal animals [30], although MCT1 expression is greatly increased [96]. Astrocytes are also thought to play an important role in producing lactic acid derived from glycolysis, which can then be used as a respiratory fuel for neurons. This process is stimulated in response to increased brain activity, because active uptake of glutamate into the astrocytes increases ATP turnover [98,99]. In hypoxia or ischaemia, glycolysis becomes the only means of ATP production and lactic acid concentration in the brain rise enormously [99]. This is exacerbated by the low permeability of the blood/brain barrier to lactate, and it is interesting to speculate whether the enhanced monocarboxylate transport activity in the neonatal animal may be of benefit in combating a period of hypoxia that might be experienced at birth. If ischaemia or hypoxia are allowed to continue too long, the accumulation of lactic acid and the decrease in pH cause an inhibition of glycolysis, a decrease in cellular ATP concentrations, ionic imbalance and, ultimately, cell death [100]. However, if the period of ischaemia is short, the lactic acid acts as a preferred respiratory fuel for the neurons on reperfusion [99].

The ability of neurons to oxidize lactic acid under both normal physiological conditions and pathological conditions is similar to that of the heart and red skeletal muscle, and, like these tissues, neurons express the heart isoform of lactate dehydrogenase (H-4) [101]. The identity of the MCT isoform(s) in neurons is presently unclear. Immunogold electron microscopy demonstrated that they express MCT1, although not to the same extent as do astrocytes and endothelial cells, but no MCT2 expression was detected using a C-terminal antipeptide antibody [77,96]. However, Western blotting has shown that a membrane fraction derived from the brain contains MCT2, but little MCT1 [29]. Furthermore, Northern-blot analysis has shown that, unlike cultured astroglial cells, cultured neurons contain mRNA for MCT2, but not for MCT1. In addition, these cells exhibit a high-affinity, pCMBS-insensitive lactate transporter with kinetic characteristics similar to those of MCT2 [19]. In contrast, peripheral nerves exhibit monocarboxylate transport kinetics more reminiscent of MCT1 than MCT2 [100]. Northern-blot analysis and *in situ* hybridization confirm that MCT2 mRNA is present in brain, but at lower levels than MCT1 mRNA [25,29,76]. The distribution of the two mRNA species in the cortex, cerebellum and hippocampus is similar, but not identical. It would seem desirable for neurons to express MCT2, since this would provide them with a greater ability to take up lactate and ketone bodies into the cells when their concentrations are low. The inability of the immunogold electron microscopy to detect MCT2 protein in neurons is reminiscent of the situation in the heart, where kinetic evidence and Northern-blot analysis suggests MCT2 should be present, but the antibody does not detect it. Once again, an alternative spliced form of MCT2 that is not detected by the antibody could provide an explanation. Thus it is interesting that the predominant form of MCT2 mRNA present in brain is much larger (9 kb) than that found in liver, kidney and testis (2.7 kb) – tissues in which the antibody detects protein [25,29,76]. There

are no data on the expression of the other MCT isoforms in brain, although Northern-blot analysis of human brain suggests little or no MCT4, MCT5 or MCT6, whereas MCT7 and MCT8 are present at quite high levels [6].

Retina and inner ear

The transport of lactate across the plasma membrane of the various cell layers of the retina is fundamental for its proper function, which is dependent on high rates of glycolysis. The RPE forms the outer blood/retinal barrier and is responsible for the transport of metabolites between the choroidal blood supply adjacent to the basolateral surface of the RPE and the neural retina on its apical surface. The latter extends processes that protrude between the photoreceptor cell outer segments, although there is no direct contact between the two. Rather they are separated by the sub-retinal space, whose composition and volume are controlled by the RPE. In addition to photoreceptor cells, the neural retina contains other neurons and glial cells (Müller cells). The latter two cell types are thought to be primarily glycolytic, whereas the photoreceptors are thought to rely more on oxidative metabolism for their energy production. In mammalian retina it has been shown that some of the lactate released by Müller glial cells is used for oxidation as a respiratory fuel by the photoreceptors in the outer retina, whereas the rest is removed via the blood supply [102]. The distribution of MCT1, MCT2, MCT3 and MCT4 in the retina have been determined by immunofluorescence microscopy and immunogold electron microscopy [37,78,103]. MCT3 is exclusively located on the basolateral surface of the RPE, whereas MCT1 is highly expressed on its apical surface and absent on the basolateral surface. As in the brain, both the luminal and abluminal plasma membranes of the retinal microvessel endothelium express high levels of MCT1, as do Müller-cell microvilli, the plasma membranes of the rod inner segments and all retinal layers between the inner and external limiting membranes. In one study, MCT2 was found to be abundantly expressed on the inner (basal) plasma membrane of Müller cells and by glial-cell processes surrounding the microvessels, as well as in the synaptic and nuclear layers of the neural retina [103]. However, in another study with a different MCT2 antibody, no significant expression of MCT2 was detected [78]. MCT4 is expressed in the neural retina and Müller-cell microvilli, but not to any significant extent in the RPE of adult rats. However, in neonatal rats both basolateral and apical membranes of the RPE express some MCT4 [78]. Taken together, these data suggest that MCT3 is responsible for lactate efflux from the RPE into the choroidal blood supply, whereas lactate transport between most other cells in the retina probably involves MCT1, with a minor contribution from MCT4. MCT2, with its higher affinity for lactate, may play a specific role in the exchange of lactate between glia and neurons, similar to that proposed for the brain. It has been proposed that MCT1 on the apical surface of the RPE may play an additional role in regulating the volume of the subretinal space, since lactate/H⁺ transport by MCT1 is accompanied by water transport [104]. An accumulation of lactate within the sub-retinal space would cause osmotic swelling, that could cause the retina to become detached from the RPE. However, the ability of MCT1 (in association with MCT3) to transport rapidly both lactic acid and water across the RPE and into the blood will prevent this.

In the inner ear, the vestibular dark cells (VDC) form the epithelial layer that separates the endolymph (apical side) and perilymph (basolateral side). Glycolytically produced lactate must cross this epithelium, and studies of lactate and pyruvate transport into gerbil VDC using BCECF measurements of pH_i

have shown the existence of a proton-linked transport mechanism with characteristics similar to MCT1. Transport could be demonstrated across both the apical and basolateral plasma membranes, and reverse-transcription PCR confirmed the presence of MCT1 mRNA. However, MCT2 mRNA was also detected, but whether the two isoforms have a unique distributions on the apical and basolateral surfaces has not been reported [105]. Proton-linked monocarboxylate transport into the marginal cells of the stria vascularis has also been reported, and here, too, reverse-transcription PCR has suggested the presence of both MCT1 and MCT2 [106].

Other tissues

Characteristics of monocarboxylate transport into a wide range of other tissues have been determined, and the results have been reviewed elsewhere [2]. However, only in a few tissues have functional data been correlated with demonstration of the presence of different MCT isoforms. In spermatozoa, MCT1 is found exclusively in the head region and MCT2 in the tail. The relevance of this to lactate metabolism in spermatozoa is unclear, but may relate to their possession of a unique mitochondrial lactate dehydrogenase isoenzyme [26]. In kidney cortex, MCT1 is prevalent on the basolateral surface of epithelial cells in the proximal tubules, in contrast with the medulla, where MCT2 is found on the basolateral surface of epithelial cells in the collecting ducts. There are also reports of a sodium-dependent active lactate-transport system in kidney which might work in concert with MCT1 and MCT2 to scavenge lactate and other monocarboxylates from the glomerular filtrate (see [2]). The very high levels of MCT6 mRNA found in the human kidney would make MCT6 a possible candidate for such a sodium-dependent transporter [6]. In the stomach, MCT1 is abundant on the basolateral surface of epithelial cells, whereas MCT2 is plentiful in parietal cells of oxyntic glands [26]. The distribution of MCT isoforms in liver is species-dependent. In hamster liver, MCT2 is the major isoform, and little or no MCT1 is found [26]. However, this is not the case in rat liver, where MCT1 is present at higher levels than MCT2 [29], especially in the periportal region (M. C. Wilson and A. P. Halestrap, unpublished work). Northern-blot analysis suggests that MCT1 is also the predominant isoform in human and mouse liver [6,25,27,29]. Furthermore, the kinetics of monocarboxylate transport into isolated liver cells resemble those of MCT1 more than those of MCT2, although pCMBS maximally inhibits transport by about 80%, suggesting that about 20% of transport activity may be mediated by MCT2 and 80% by MCT1 [74].

Studies of the intestinal distribution of MCT isoforms has been restricted to the caecum of hamster and the colon of humans and pigs, and in both cases MCT1, but not MCT2, is present [26,107]. MCT1 is believed to play an important role in the uptake of lactate, pyruvate and short-chain fatty acids, such as butyrate and propionate, from the intestinal lumen into the blood [107–109]. In the endocrine pancreas the β -cells of the islets of Langerhans are unusual in that they possess little, if any, MCT activity [110,111]. This is believed to reflect the importance of pyruvate oxidation (rather than its conversion into lactate) in the provision of the ATP used to signal the stimulation of insulin secretion in response to glucose [112]. There is strong experimental evidence for proton-linked monocarboxylate in the placenta (see [2]), and Northern blots show the presence of all MCT isoforms except MCT2 and MCT3 [6]. However, detailed analysis of the location of each isoform within the complex array of membranes in the placenta has not been reported.

REGULATION OF MCT EXPRESSION

With the exception of skeletal muscle, which will be discussed more fully below, relatively little is known about the regulation of MCT expression in different tissues. As discussed above, in the brain there is clear evidence for a large increase in MCT1 expression in the newborn, and this is accompanied by an increase in mRNA, which suggests transcriptional regulation. However, to date there is no published promoter analysis for any of the MCT isoforms. In the retina, changes in MCT4 and MCT1 expression may occur during the neonatal period, but measurements of mRNA have not been made [78]. Developmental regulation of chicken MCT3 expression in the RPE by alternative splicing of the 5'-UTR mRNA has been described above. In the liver, earlier reports of an increase in lactate transport activity in starvation could not be confirmed in this laboratory, and no change in MCT1 or MCT2 expression was detected even after 48 h starvation [29]. For both MCT1 and MCT2 there is little correlation between the mRNA levels and the expression of protein in different tissues [29]. In the case of MCT1 the 3'-UTR of MCT1 is very long (1.6 kb) and may play a role in translational regulation either by looping back and interacting with the UTR or by binding to regulatory factors/binding proteins which make the mRNA unavailable for translation [113]. The importance of such translational regulation is becoming increasingly recognized and more usually involves specific sequences and secondary structure in the 5'-UTR region with which initiation factors and regulatory factors interact to enhance or repress translation [114,115]. For MCT2, the presence of a wide range of different mRNA transcript sizes [25,27,29,76] suggests that tissue-specific post-transcriptional regulation of MCT2 expression may occur through alternative splicing within the 5'- or 3'-UTRs and/or different promoter usage [29]. For human and mouse MCT5, the proposed 5'-UTR contains short overlapping open reading frames (see above). Such minicistrons are also present in the upstream region of human Na⁺/H⁺ exchanger NHE-1 and are known to be inhibitory to translational efficiency [116]. Thus it seems possible that MCT5 may be subject to translational regulation.

As yet no evidence for alternative splicing within the coding regions of MCTs has been found. Since each TM within the transporter probably interacts with at least two other TMs, should any such splicing occur it might be expected to be confined to the N- and C-termini and loop regions, as is the case for other transporters [33,34,117–119]. However, it seems more likely that regulation of the expression, properties and perhaps subcellular location of MCTs is achieved through the use of distinct isoforms transcribed from independent genes, rather than through generation of splice variants from a smaller number of genes.

Regulation of MCT expression in muscle

Both endurance and high-intensity training increase the maximal rate of lactate transport into sarcolemmal vesicles derived from rat skeletal muscle by 30–100% [120,121], and chronic electrical stimulation of the rat hind-limb has a similar effect [122]. These changes in transport activity were accompanied by an increase in MCT1 expression, present predominately in the red fibres, but not in MCT4 expression which is present in all fibres [39,123]. However, other studies on the effects of training, also using sarcolemmal vesicles, showed that the greatest increase in lactate transport capacity took place in the white fibres [124], which would imply an increase in MCT4 expression or activity. MCT1 expression in heart muscle also increased in response to training of rats, and to a greater extent than in skeletal muscle [123]. Thus

it seems clear that an increase in physical activity can improve lactate/H⁺ transport capacity of muscle by up-regulating MCT1 expression. In contrast, when muscle activity is impaired, as in denervated rat muscle [125,126], rates of lactate transport by sarcolemmal vesicles are diminished, and this is associated with a significant decrease in the expression of both MCT1 and MCT4 [39].

Increases in lactate transport activity and MCT expression in response to training have also been measured in human muscle. In one study, 7 days of bicycle training increased MCT1 content of vastus lateralis muscle by 18%, and this was accompanied by an increase in the femoral venous lactate concentration during exercise for a given muscle lactate content [127]. In another study, high-intensity knee-extensor exercise was found to increase the content of MCT1 and MCT4 by 76 and 32%, respectively, and this was associated with a 12% increase in sarcolemmal lactate transport measured in vesicles formed from needle biopsies [128]. Furthermore, after training, the release of lactate and H⁺ were higher at a given cellular-to-interstitial concentration gradient of lactate than before training. In contrast, rates of lactate transport into sarcolemmal vesicles derived from spinal-cord-injured patients are significantly decreased [129]. It may be concluded, therefore, that the lactate/H⁺ transport capacity in humans can be improved by training. However, in a cross-sectional study using vesicles made from needle biopsies it was shown that human subjects can have very different lactate transport capacities, and some extremely well-trained subjects have a very high capacity [130]. This may reflect not only the extent of training, but also inherent athletic ability. There is currently no evidence for any short-term (hormonal) regulation of MCT activity.

Although a considerable body of data confirms that the transport of lactate across rat skeletal-muscle plasma membranes can undergo adaptive changes, the mechanisms involved in this regulation are not known. It might be expected that regulation is transcriptional, but the relatively long (1.6 kb) 3'-UTR of MCT1 might also allow translational control of expression, as outlined above. Alternatively, it may be that pools of MCT1 mRNA are stored in mitochondrial ribonucleoproteins, allowing rapid translation when more MCT1 transporter is needed. The signal(s) that switches on expression, whether it is transcriptional or translational, remains to be identified. β -Adrenergic stimulation of the heart and skeletal muscle is one such signal that is likely to be associated with exercise, and this has been shown to increase the stability of lactate dehydrogenase-M mRNA. The mechanism responsible involves protein kinase A-mediated binding of specific proteins to a U-rich domain in the 3'-UTR [131]. Elevated lactate concentrations and hypoxia would appear to be other appropriate signals. Hypoxia is known to up-regulate the expression of lactate dehydrogenase-M through mechanisms involving several transcription factors and response elements that also regulate the expression of other glycolytic enzymes and GLUT1. These include hypoxia-inducible factor 1, cyclic AMP response element and the erythropoietin hypoxic enhancer [132–134]. Since the distribution of MCT4 within muscle fibres is similar to that of lactate dehydrogenase-M [39,81], it would not be surprising if hypoxia were to increase MCT4 expression by a similar mechanism. Furthermore, endurance training in humans and chronic muscle stimulation of rats have been shown to increase GLUT1 expression [135–142].

MCT REGULATION THROUGH ASSOCIATION WITH THE PROTEIN OX-47

An additional means by which MCT expression or activity might be regulated is through an interaction with an ancillary protein

for which there is strong evidence. When red blood cells are incubated with DIDS, MCT1 becomes specifically cross-linked to a 70 kDa membrane glycoprotein (GP-70 or embigin) [143]. GP-70, a membrane-spanning glycoprotein, is a cell-adhesion molecule of the immunoglobulin superfamily that is expressed most strongly in embryonic tissues, where it is developmentally regulated [144,145]. However, in most adult tissues its expression is weak, whereas that of a closely related protein called OX-47 in the rat (called basigin, CE9, neurothelin, M6, CD147 or EMMPRIN in other species), is strong [146,147]. The transmembrane domain of OX-47 is highly conserved between species and is unusual in that it contains a negatively charged glutamic acid residue which is thought to interact specifically with other membrane proteins [148]. Thus it seems probable that OX-47, like GP-70, can interact specifically with MCT1 and MCT4 in the plasma membrane, and evidence for this has been provided by their co-immunoprecipitation with antibodies against OX-47 [149,150]. Furthermore, using confocal microscopy, we have shown co-localization of OX-47 and MCT1 in isolated heart cells, both being concentrated on the plasma membrane in the intercalated disc and t-tubule regions [149]. This interaction could have significance for the regulation of MCT activity, either through a direct effect on the catalytic activity of the transporter or through regulating its translocation to the membrane. There are precedents for both of these suggestions. Thus glycophorin associates with the anion exchanger AE1, and acts as a chaperone to increase its translocation from the endoplasmic reticulum to the Golgi and plasma membrane [151,152]. It has also been shown that expression of active amino acid transporters requires their interaction with CD98 [153,154], whereas long-chain-fatty-acid transport has been associated with both a 12-transmembrane-helix protein and a single-transmembrane-helix glycoprotein (CD36), which may indicate a similar interaction [155,156]. Indeed, it may be that such interactions with ancillary membrane-spanning glycoproteins are essential for the activity of many plasma-membrane transporters.

We have recently found that, when MCT1 or MCT4 are overexpressed in COS or HeLa cells, most of the protein fails to reach the membrane, but remains associated with the endoplasmic reticulum and Golgi apparatus. However, when co-expressed with OX-47, the proteins are properly directed to the plasma membrane (M. C. Wilson and A. P. Halestrap, unpublished work). Thus it seems most probable that OX-47 is required for the proper translocation of the MCTs to the plasma membrane. Such an interaction would give additional scope for regulation of MCT activity, a possibility for which there is a precedent – the stimulation of neutral-amino-acid transport into cultured cells by system A in response to hypertonic shock, which is known to involve an ancillary protein [157]. The glycosylation state of OX-47 can vary considerable between different tissues. Furthermore, its expression is up-regulated by stimuli that enhance the metabolic activity of cells and lead to stimulation of glycolysis and increased expression of glucose transporters [158]. An attractive possibility is that such changes in OX-47 expression or glycosylation may be involved in a parallel stimulation or modulation of monocarboxylate transport under such conditions.

FUTURE DIRECTIONS

The central position of monocarboxylates, such as lactate and pyruvate, in cellular metabolism and metabolic communication between tissues makes an understanding of the mechanism and regulation of their transport across the plasma membrane of

particular importance. The recent cloning of a family of MCTs has opened up many new opportunities to achieve this goal, but at present such studies are still in their infancy. There is much work to be done to characterize the properties of the different isoforms that have already been cloned, and with the completion of the human (and other) genome projects, more MCT family members may well be recognized. The use of *Xenopus* oocytes is proving the most versatile heterologous expression system for such characterization, and is also suitable for studying structure–function relationships using site-directed mutagenesis. Some family members may well prove not to transport simple monocarboxylates or catalyse proton-linked transport. Identifying their substrates will be difficult, although help may be provided by the linking of genetic diseases to particular transporters through their chromosomal location. Regulation of MCT expression is likely to prove an exciting area of research that has important and wide-ranging implications, including the improvement of athletic performance and the adaptive response to disease states such as ischaemia. It already appears that both transcriptional and perhaps translational regulation of MCT expression may occur, and elucidation of the mechanisms involved will be critical. For the former, determination of the organization of the MCT genes and an analysis of their promoter regions for different regulatory elements will be essential. In the case of translational control, the presence of the spliced variants in the 3'-UTR may well prove significant, perhaps through the involvement of different binding proteins. In addition, it is likely that there are ancillary proteins in addition to OX-47 that have an important role to play in the regulation of MCT expression, and thus the regulation of the expression of these proteins will need to be considered.

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