CONTRIBUTIONS to SCIENCE, **1** (3): 267-298 (2000)
Institut d'Estudis Catalans, Barcelona

Knock, knock, knocking on muscle doors. Visions of the transport of substrates across the plasma membrane in muscle

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Abstract

Muscle is a major player in metabolism. It uses large amounts of glucose in the absorptive state and changes in muscle insulin-stimulated glucose uptake alter whole-body glucose disposal. Lipid substrates such as fatty acids or ketone bodies are preferentially used by muscle in certain physiological conditions. Muscle is also the main reservoir of amino acids and protein. The activity of many different plasma membrane transporters such as glucose carriers, carnitine, creatine or amino acid transporters maintain muscle metabolism by taking up or releasing substrates or metabolites across the cell surface. The goal of this review is the molecular characterization of muscle membrane transporter proteins and the analysis of their regulatory roles.

Resum

El múscul té un paper central en el metabolisme. Així, el múscul utilitza quantitats substancials de glucosa durant l'estat absortiu, i els canvis en la captació muscular de la glucosa provoquen alteracions en la utilització global de la glucosa per l'organisme sencer. El múscul constitueix també el principal reservori corporal d'aminoàcids i de proteïnes. A més, el metabolisme muscular és mantingut mitjantçant l'activitat de molts diferents transportadors localitzats a la membrana plasmàtica, com són els transportadors de glucosa, carnitina, creatina o aminoàcids; aquests transportadors capten o alliberen, a través de la membrana plasmàtica de la cèl·lula muscular, diferents substrats o metabòlits. L'objectiu d'aquesta revisió consisteix en la caracterització molecular de les principals proteïnes transportadores presents a la membrana plasmàtica de les cèl·lules musculars, així com l'anàlisi de les seves propietats reguladores.

Keywords: Glucose, amino acids, fatty acids, lactate, creatine, carnitine

Substrates of muscle metabolism

Muscle is subjected to major modifications of energy requirements and as a result regulates the rate of utilization of different substrates. There is substantial evidence that muscle metabolism relies on the activity of membrane proteins which catalyze the uptake of critical substrates for energy production, anabolic processes, or the release of glycolytic metabolites or amino acids. There is an internal «energy store» in the form of phosphocreatine, which is maintained in equilibrium with ATP through the catalysis of creatine kinase; therefore, any fall in ATP concentration leads to the formation of further ATP from ADP, using the energy of phosphocreatine. Creatine is the substrate for the synthesis of phosphocreatine, and is taken up into the muscle cell by creatine carriers. A major substrate under absorptive resting conditions or during exercise is glucose, which is taken up via different glucose transporters that are exquisitely regulated by insulin or exercise. As a result of glucose metabolism, lactate is formed, especially under conditions in which there is an oxygen deficit, and lactate is released via activity of monocarboxylate transporters. In fact, monocarboxylate transporters are also responsible for the uptake into the muscle cell of ketone bodies (β-hydroxybutyrate or acetoacetate), which are efficient substrates of muscle metabolism. The major lipid substrates in muscle are fatty acids, which are especially important in fasting conditions or during aerobic exercise. Although the mechanism of fatty acid uptake is controversial, there is evidence of the participation of membrane proteins. L-Carnitine is essential for the synthesis of acyl-carnitine derivatives, which reach the mitochondrial matrix to undergo β-oxidation; in this regard, carnitine is mainly synthesized in liv-

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er and kidney and must be taken up by muscle via carnitine transporters found in the plasma membrane.

Muscle is not only an important site for work performance and high energy demand, but is also the main reservoir of amino acids and protein. This is due to the activity of many different amino acid transporter agencies that take up and release amino acids. Since a great deal has been learnt recently about these proteins, this review will focus on the membrane proteins that participate in the metabolism of creatine, glucose, amino acids and lipid substrates such as fatty acids and ketone bodies in muscle.

Muscle creatine is taken up from the extracellular milieu

Intracellular phosphocreatine is an essential component of energy metabolism in muscles and brain since it acts as a store of high-energy phosphate which can be converted into ATP by catalysis of creatine kinase. In turn, phosphocreatine is formed from creatine by creatine kinase. Phosphocreatine is spontaneously converted into creatinine, which is excreted by kidney; the daily amount of creatinine excreted in urine is around 15 mmol (2 g). This loss of creatine has to be replenished either from food or by endogenous synthesis. Creatine is synthesized from arginine, glycine and methionine in the liver, pancreas and kidney. Muscles are unable to synthesize creatine, which must be taken up from the bloodstream.

Creatine is increasingly used by athletes as a dietary supplement to improve physical performance when creatine phosphate is needed. Indeed, dietary creatine increases muscle phosphocreatine and creatine concentrations in humans [1-3]. Several studies indicate that a dietary creatine supplement enhances performance in high-intensity, short-term exercise or in intermittent work performance [4, 5], although it does not improve sprint performance in swimmers or runners [6, 7].

Cardiac and skeletal muscle cells take up creatine by a mechanism that depends on sodium, shows an apparent Km for creatine in the micromolar range and is inhibited by the structural analogues 3-guanidinopropionate or 4-guanidinobutyrate [8-10]. Creatine uptake in G8 muscle cells is stimulated by long-term exposure to insulin, IGF-I, isoproterenol or 3,3',5-triiodothyronine (T_3) [9], probably as a consequence of stimulation of Na^+-K^+-ATPase activity. However, there is some controversy as to the effects of insulin on muscle creatine uptake: whereas some authors reported stimulation of creatine uptake [11, 12], others have found that acute treatment with insulin does not alter creatine uptake by soleus muscle [10]. Creatine uptake by L6 muscle cells is down-regulated in the presence of extracellular creatine [13] and carbohydrate ingestion increases skeletal muscle creatine accumulation during creatine supplementation in humans [14]. All these observations indicate that carnitine uptake is regulated and that changes in creatine uptake influence the accumulation of creatine by muscle. In consequence, one way to enhance total intracellular creatine in muscle is to stimulate creatine transport.

Creatine transport is catalyzed in humans by two creatine transporters, named CRT1 and CRT2, which are encoded

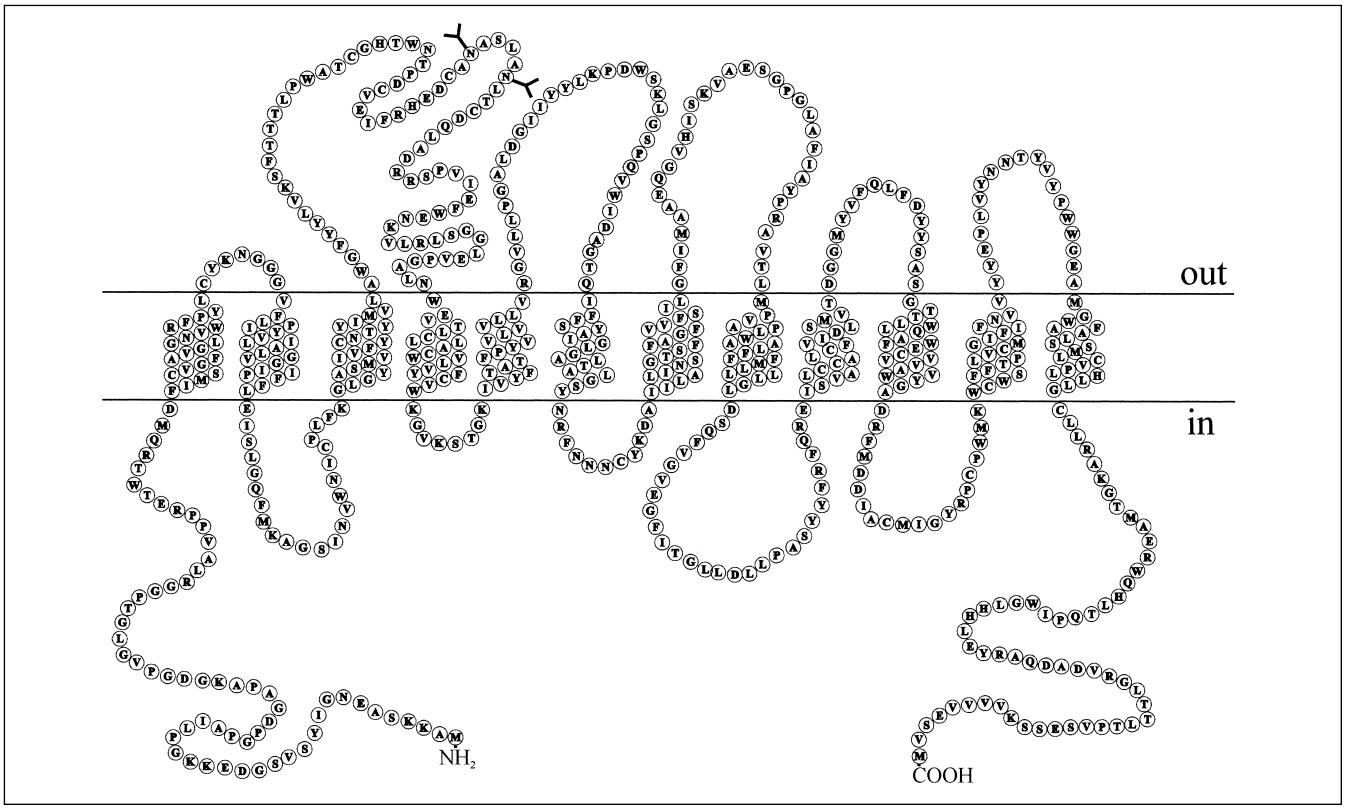

Figure 1. Proposed 12 transmembrane-spanning domain topology model for human creatine transporter (CRT-1). Putative glycosylation sites are indicated by branches.

by different genes. Creatine transporters belong to the superfamily of Na^+- and Cl—dependent neurotransmitter transporters [15] and, in keeping with this, expression of CRTs in *Xenopus* oocytes or in COS-7 cells induces the uptake of creatine, which is dependent on Na^+ and Cl^- ions [16, 17]. The stoichiometry of creatine, Na^+ and Cl^- seems to be 1:2:1 [17]. The creatine transporters have twelve transmembrane domains and human CRT1 shows two potential N-glycosylation sites (Figure 1) [18]. Although the specific structure/function relationship of the creatine transporters has not been studied, they are very close to GABA and taurine transporters. Cardiac and skeletal muscles together with brain, kidney and placenta express CRT1 in humans [18]. At present, the cDNAs for human, rat, rabbit and Torpedo CRT1 creatine transporters have been identified [16, 18-21]; functional analysis indicates that they encode a creatine transporter which shows a Km for creatine in the micromolar range, i.e. very similar to the kinetic data obtained in muscle cells [14]. Human CRT1 gene is located in Xq28, contains 13 exons and spans approximately 8.5 kb of genomic DNA [21, 22]. Unlike the broad expression of CRT1, human CRT2 is only expressed in testis and is located on chromosome 16 (16p11.2) [23, 24]. There are many unanswered questions on the biology of muscle creatine transporter such as the precise mechanism of transport, structure-function relationships, and regulation of subcellular distribution and activity of the carrier. It has been reported that creatine supplementation to rats down-regulates the expression of CRT1 in skeletal muscle [25], which confirms the observation mentioned above that extracellular creatine down-regulates creatine uptake [13].

Glucose transport is exquisitely regulated in muscle

Skeletal muscle accounts for nearly 40% of body mass and is the main tissue involved in the insulin-induced stimulation of glucose uptake. Several studies using the euglycemic-hyperinsulinemic clamp have shown that at circulating levels of insulin in the upper physiological range most of the infused glucose is taken up by skeletal muscle and converted mainly into glycogen [26]. Evidence for the role of muscle glucose uptake in overall glucose homeostasis also comes from studies of transgenic mice over-expressing GLUT1 in skeletal muscle. Thus, over-expression of GLUT1 in transgenic mice increases glucose uptake in muscle, which leads to low plasma glucose concentrations and an increased glucose disappearance rate after a glucose tolerance test [27]. Because of its quantitative role in glucose uptake, alterations in muscle insulin sensitivity have a profound impact on whole body glucose disposal. In this regard, non-insulin-dependent diabetic patients show deficient insulin-induced glucose transport in skeletal muscle [28, 29]. Insulin treatment, exercise or electrical stimulation rapidly increase the rate at which glucose is taken up. Kinetic analysis of the effects of insulin or contraction on muscle glucose transport indicates an enhancement in Vmax values [30]. The stimulation of muscle glucose transport is critical since this process is thought to be a rate-limiting step in the pathway of glucose utilization in skeletal muscle. Several observations support this view: a) intracellular free glucose does not accumulate, regardless of glucose or insulin concentration, in skeletal muscle from control or streptozotocin-induced diabetic rats [31, 32], b) in normoglycemic conditions, both in the absence of insulin and at sub-maximal insulin concentrations, glucose clearance is constant in the perfused rat hindlimb [33], and c) over-expression of GLUT1 in skeletal muscle from transgenic mice leads to a 10-fold increase in muscle glycogen content and a 2-fold increase in muscle lactate, with no increase in muscle glucose-6-phosphate concentrations [34].

Different glucose transporters are expressed in muscle

Skeletal muscle expresses GLUT1 and GLUT4 glucose transporters [35]. Besides GLUT4 and GLUT1, there is some evidence that skeletal muscle of humans, though not that of rats, expresses GLUT5 [36, 37]. Based on the induction of fructose transport activity obtained in *Xenopus* oocytes after injection of human GLUT5 cRNA [38], it is thought that GLUT5 accounts for fructose uptake by muscle. It has been reported recently that skeletal muscle also expresses low mRNA levels of GLUTX1, a novel glucose transporter with unknown physiological properties [39].

GLUT4 is the main glucose carrier expressed in skeletal muscle from adult rats, whereas GLUT1 accounts for only 5-10% of total glucose carriers [40], which is similar to data reported in isolated rat adipocytes [41]. The structure/function relationships of these 12 transmembrane domain proteins, which show both the N and the C-terminal domains oriented to the cytosol, have recently been reviewed elsewhere [42] and will not be analyzed here.

Cardiac muscle also expresses both GLUT4 and GLUT1 glucose transporters and, in rat cardiomyocytes, accounts for about 60% and 40%, respectively, of total glucose carriers [43].

In addition to the differences in their expression levels, GLUT1 and GLUT4 show a differential localization in the muscle fibre. Results obtained from sub-cellular division studies or by immunoelectron microscopy indicate that GLUT-1 carriers are located mainly in the plasma membrane, whereas GLUT-4 carriers are more abundant in intracellular membranes (see review 35).

The expression of muscle GLUT4 is regulated

The relative abundance of GLUT4 and GLUT1 in skeletal and cardiac muscles depends on the developmental stage [44]. Thus, during fetal life in the rat, GLUT1 is the predominant glucose carrier and its expression is markedly repressed

perinatally [44-46], as a consequence of alterations lying at a pre-transferral step [44, 45]. In contrast, muscle expression of GLUT4 is low in the fetal rat and a continuous induction of GLUT4 mRNA and protein takes place in the perinatal phase [44-46]. The expression of glucose transporter isoforms during perinatal life is consistent with the observation of a high rate of glucose uptake by fetal rat heart [47].

Thyroid hormones are an important mechanism responsible for the transition of muscle GLUT1 and GLUT4 expression during the perinatal phase. This view is based on the observation that congenital hypothyroidism markedly impairs GLUT4 protein induction when GLUT4 mRNA levels are low. The effect of the deficit in thyroid hormones on GLUT4 expression is reversible and a single injection of T3 causes a marked and rapid increase in the levels of GLUT4 mRNA and protein in heart [46]. Similarly, congenital hypothyroidism leads to a substantial enhancement of GLUT1 protein and mRNA levels in heart [46]. Furthermore, T3 injection in hypothyroid neonates causes a decrease in cardiac levels of GLUT1 mRNA [46]. Thyroid hormones are also involved in the control of glucose transport in skeletal muscle in adulthood. Thus, chronic T3 administration stimulates GLUT4 expression and glucose transport in skeletal muscle from rats [48, 49].

The role of thyroid hormones in controlling the transition of glucose transporter carriers from fetal to neonatal levels in heart is also supported by additional data. Thus, there is analogous behaviour between circulating concentrations of thyroid hormone and the induction of cardiac GLUT4 protein during perinatal development: T4 and T3 levels increase progressively during postnatal life and reach a plateau 2 weeks after birth [50] and, in parallel, cardiac GLUT4 protein expression increases after birth, becomes progressively more abundant, and attains adult levels after day 15 of postnatal life [44, 46].

The contractile activity dependent on innervation regulates the expression of GLUT1 and GLUT4 in skeletal muscle in an inverse manner. This is supported by two distinct types of evidence: a) the induction of GLUT4 and the repression of GLUT1 take place at the end of fetal life and coincide with the timing of skeletal muscle innervation [51], and b) the expression of GLUT1 is enhanced and GLUT4 is repressed in response to muscle denervation during adult life [51-53].

In addition, chronic low-frequency stimulation of muscle *in vivo* or *in vitro* causes the induction of GLUT4 glucose transporter expression above basal levels [54-56]. Physical training increases insulin-mediated whole-body glucose utilization in human subjects, which reflects an adaptation in muscle elicited by local contraction-dependent mechanisms. Physical training in human beings does not alter muscle insulin receptor function, but does enhance the muscle content of GLUT-4 [57, 58]. Similarly, chronic exercise enhances GLUT-4 expression in rat muscle [59]. In fact, it seems that a single bout of exercise enhances GLUT4 expression in muscle [60]. In addition, chronic exercise increases GLUT-4 content in skeletal muscle of obese insulin-resistant Zucker (fa/fa) rats [61].

In spite of the potential therapeutic importance of GLUT4 gene transcription in muscle, there is relatively little information on the specific mechanisms that regulate it. Studies performed in transgenic mice have shown that 2.4 kb of the 5'-flanking region of the human GLUT4 gene fused to a chloramphenicol acetyltransferase reporter gene is specifically expressed in adipose tissue, skeletal muscle and heart [62], which indicates that the 2.4 kb of the GLUT4 gene contains all the sequence elements needed to confer tissue-specific expression.

Work using transgenic mice has also shown skeletal muscle-specific DNA elements located within 730 bp of the GLUT4 5'-flanking DNA [63]. These results are consistent with other studies in C2C12 muscle cells in culture, which have shown that GLUT4 muscle-specific expression is conferred by a 103-bp DNA sequence located between -522 and -420 of rat GLUT4 gene [64]. A myocyte enhancer factor 2 (MEF2) binding site in the GLUT4 promoter located between -466/-457 from the transcription start site has also been proposed as essential for the specific expression of GLUT4 in muscle cells [64].

Studies performed in C2C12 muscle cells have shown that thyroid hormones stimulate the transcription of the rat GLUT4 gene through a 281 bp region in the GLUT-4 promoter located between -517 and -237 from the transcription start site [65]. Recently, Torrance et al. [66] proposed a new, low-affinity binding site for thyroid hormone receptors in GLUT-4 promoter located between bases -457/-426, next to the MEF2 site, although the function of this site has not been demonstrated. These results are consistent with reports that thyroid hormones stimulate GLUT4 gene expression in muscle [46].

It has also been reported that 2400 bp of 5'-flanking DNA is sufficient for regulation of the human GLUT4 gene in transgenic mice during fasting and refeeding [62]. Furthermore, a 1154-bp fragment is necessary to direct the insulin-dependent regulation of the human GLUT4 gene in muscle and adipose tissue from transgenic mice [63].

At present, there are several indications that the regulation of GLUT4 expression in muscle *in vivo* is due to modifications not only at a transcriptional level but also post-transcriptionally. Thus, GLUT4 protein diminishes in the presence of slight or even no changes in GLUT4 mRNA levels in red skeletal muscle from 2-day fasted rats or in white muscle from streptozotocin-induced diabetes [67]; similarly, GLUT4 protein decreases in the absence of changes in mRNA in skeletal muscle from benfluorex-treated rats [68].

GLUT4 protein has also been reported to increase in the absence of changes in mRNA in rat heart during perinatal development [44] and in skeletal muscle from the mdx dystrophic mouse [69].

However, alterations in tissue levels of GLUT4 mRNA have been detected in the absence of changes, or in the presence of inverse changes, in the content of GLUT4 protein. Thus, in hearts from propylthiouracil- or methylmazole-induced hypothyroid rats, GLUT4 protein does not change when GLUT4 mRNA diminishes markedly [46, 70]. In skeletal muscle from glucose-induced hyperglycemic rats [71],

GLUT4 mRNA increases without affecting GLUT4 protein levels. Similarly, streptozotocin-induced diabetes causes a large decrease in cardiac GLUT4 mRNA levels, which is, however, only accompanied by a small decrease in cardiac GLUT4 protein [67]. The precise mechanisms that regulate this adaptation - changes in transfer efficiency or rate of GLUT4 protein degradation - are unknown. However, it has been reported that treatment of streptozotocin-induced diabetic rats with ICI D8731 - an antagonist of the angiotensin type-1 receptor - for 4 months prevents the reduction of cardiac GLUT4 protein under conditions in which mRNA levels stay low [72]. This suggests that angiotensin II influences either directly or indirectly the post-transcriptional regulation of GLUT4 expression in heart.

All these observations suggest that the regulation of muscle GLUT4 expression in transfer or post-transfer steps is relatively frequent and can involve either the preservation of GLUT4 carrier levels in the presence of variable changes in GLUT4 gene expression, or the modification of GLUT4 carriers in the absence of changes in GLUT4 mRNA. These two inverse patterns can be explained by changes in the transfer efficiency of GLUT4 transcripts or in the general machinery of protein synthesis, or by modifications in the rate of GLUT4 protein degradation. In this regard, we have no knowledge of the specific regulatory mechanisms at transfer and/or post-transfer. Considerable effort will be required to unravel the molecular basis for the control of GLUT4 expression in post-transcriptional steps.

Insulin and muscle contraction translocate GLUT4 to distinct cell surfaces in muscle

As mentioned above, glucose transport in skeletal and cardiac muscle is thought to be maintained by the catalytic activity of two glucose transporter isoforms, i.e. GLUT4 and GLUT1. In non-stimulated conditions, GLUT1 is found mainly in the sarcolemma of the muscle fibre, but not in transverse tubules [40, 73]. In contrast, GLUT4 is mostly associated with intracellular membranes [35]. Intracellular GLUT4 is found in large elements, including multivesicular endosomes located in the trans-Golgi network region, and in small tubulovesicular structures [74]. Analysis by immunogold labeling of the subcellular distribution in transgenic mice over-expressing GLUT4 has revealed that most GLUT4 is located in and around the muscle triad [75]. A variety of experimental approaches have shown that in non-stimulated conditions GLUT4 is mainly intracellular in cardiac myocytes [43, 76]. Immunoelectron microscopy of rat heart has shown that GLUT4 labeling is mostly associated with intracellular membranous vesicular structures [77]. These tubulo-vesicular elements are found in different locations: a) perinuclear elements near the Golgi apparatus, b) near the sarcolemma, c) near the transverse tubule membranes and d) near the intercalated disks [78].

Immunocytochemical studies, subcellular division and photolabeling assays (reviewed in 35) indicate that GLUT4 translocates from an intracellular locus to the cell surface of the muscle fibre in response to insulin or exercise. GLUT4 is recruited to selective domains of the sarcolemma [73] and T-tubules of the muscle fiber [74, 79, 80]. There is controversy as to whether insulin unmasks a COOH-terminal GLUT4 epitope in T-tubules in skeletal muscle [74, 75].

There is also evidence that insulin promotes a marked recruitment of GLUT4 carriers to the cell surface in cardiac myocytes [77]. Indeed, GLUT4 is recruited in response to the combination of insulin and exercise both to the sarcolemma and to the T-tubule of cardiac myocytes [77]. Insulin also redistributes GLUT1 from an intracellular site to the cell surface in isolated rat cardiomyocytes or in perfused rat heart [43, 76, 81]. Nevertheless, the effect of insulin on GLUT1 is clearly weaker than its effect on GLUT4 [43, 76]. The insulin-dependent recruitment of GLUT1 in cardiomyocytes contrasts with the lack of effect in skeletal muscle, but is similar to the effect observed in the adipose cell, in which both GLUT4 and GLUT1 translocate in response to insulin [41].

Exercise or muscle contraction also stimulates glucose transport and causes GLUT4 translocation to the cell surface in skeletal muscle [82, 83]; the recruitment of GLUT4 triggered by exercise in rat skeletal muscle affects both sarcolemma and T-tubules [74]. GLUT4 is also translocated to the cell surface in response to contraction, hypoxia or ischemia in cardiac myocytes [84, 85].

In summary, a distinctive feature of muscle cells is that two distinct cell surfaces, sarcolemma and T-tubules, are involved in GLUT4 recruitment in response to insulin or exercise. As such, there are probably important differences in GLUT4 traffic between adipose and muscle cells [86].

GLUT4 trafficking pathway in muscle

GLUT4 trafficking involves several steps both in cardiac and in skeletal muscle. Thus, an intracellular exercise-sensitive GLUT4 pool has been identified by subcellular division from rat skeletal muscle; this GLUT4 pool shows no sensitivity to insulin [87], which indicates that distinct pools may be responsible for exercise- or insulin-dependent GLUT4 translocation.

Additional evidence has come from the purification of two intracellular GLUT4 membrane pools from rat skeletal muscle that differ in their response to insulin *in vivo*: one shows a marked decrease in GLUT4 levels while the other is unaltered [80, 88-91]. These two GLUT4 membrane populations also differ in their polypeptide composition. Vesicle immunoisolation analysis performed in insulin-sensitive or insulin-insensitive intracellular membrane fractions has revealed that SCAMP proteins (secretory carrier-associated membrane proteins), transferrin receptors and cellubrevin are only present in the insulin-insensitive GLUT4 pool. In contrast, VAMP2 protein and IRAP (insulin-regulated aminopeptidase) are detected in GLUT4 vesicles isolated from both intracellular membrane fractions [90, 91].

There is also evidence that there are at least two intracellular GLUT4 vesicle populations in rat cardiomyocytes [43]. Thus, GLUT4 vesicles were immuno-isolated by use of varying amounts of anti-GLUT4 antibody in intracellular membranes from non-stimulated cardiac myocytes [43]. About 40-50% of the GLUT4-vesicles present in intracellular membranes from cardiomyocytes were immuno-isolated by use of small amounts of anti-GLUT4 antibody: in these conditions, very little GLUT1 or SCAMP protein was detected in the immunoprecipitates. Larger amounts of antibody caused maximal immunoadsorption of GLUT4-containing vesicles: in these conditions most of the GLUT1 and SCAMP was detected in the immunoprecipitates [43].

Analysis of GLUT4 distribution in skeletal muscle fibers has also identified distinct intracellular GLUT4 compartments. Thus, in non-stimulated conditions, around 23% of intracellular GLUT4 is associated with large structures located in the TGN region, and 77% with small tubulovesicular structures [74]: both compartments are recruited by insulin and contraction [74]. In turn, the GLUT4 detected in small tubulovesicular structures can be further subdivided into transferrin receptor-positive and transferrin receptor-negative elements [74].

The observations concerning skeletal muscle and cardiac myocytes are consistent with the presence of at least two intracellular GLUT4 membrane populations in non-stimulated muscle cells (see Figure 2):

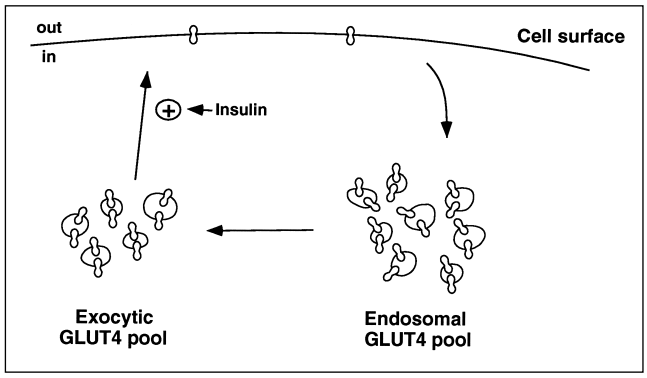

Figure 2. Scheme of proposed GLUT4 traffic pathway in skeletal muscle and cardiac myocytes.
Results obtained in skeletal muscle and cardiac myocytes are consistent with GLUT4 present in an endosomal compartment (which selectively contains cellubrevin and SCAMPs in skeletal muscle and SCAMPs and GLUT1 in cardiac myocytes) and in an exocytic/storage compartment, which contains a more limited number of proteins. This latter compartment is recruited to the cell surface in response to insulin. The endosomal compartment comes from internalization of GLUT4. There is evidence, although not shown in the scheme, that the endosomal GLUT4 compartment, in isolated rat cardiac myocytes, undergoes recruitment to the cell surface in response to insulin (reference 43).

a) an endosomal GLUT4 compartment with a high content of GLUT1 and SCAMPs (in cardiac myocytes) and high abundance of SCAMPs, transferrin receptors and cellubrevin (in skeletal muscle),

b) storage/exocytic GLUT4 vesicles with low GLUT1 and SCAMPs content (in cardiomyocytes) and high VAMP2 and IRAP content (in skeletal muscle).

The pattern of protein composition of these two GLUT4 pools suggests that the storage pool derives from the endosomal pool. According to this proposal, GLUT4 would be sorted from the endosomal membrane population to the storage/exocytic pool together with VAMP2 or IRAP (at least in skeletal muscle) and the storage compartment would be subject to recruitment in response to insulin (Figure 2). In cardiac myocytes, there is also evidence that insulin recruits the endosomal compartment to the cell surface [43]. However, direct evidence that GLUT4 moves from the cell surface to the endosomal population, that GLUT4 is sorted from the endosomal to the storage pool or that exocytic GLUT4-vesicles are recruited directly to the cell surface is lacking.

Putative proteins involved in glucose transporter trafficking in muscle

As reviewed above, there is evidence that GLUT4 moves through a series of steps in muscle cells and, therefore, different proteins control the various trafficking processes. Current knowledge on this issue is scarce: most of the work has been done in adipose cells or in heterologous cell systems.

Since non-metabolizable analogues of GTP stimulate GLUT4 recruitment to the cell surface of permeabilized adipocytes and cardiac myocytes [92, 93], several authors have searched for the presence of a GTP-binding protein. Rab4 (a small GTP-binding protein) was detected in GLUT4 vesicles derived from adipocyte and from skeletal muscle [94, 95]. Interestingly, rab4 is found in early endosomes in CHO cells, and is excluded from the recycling compartment [96, 97]. Its over-expression causes a redistribution of transferrin receptors from endosomes to the plasma membrane [98]. The over-expression of rab4 in adipocytes also increases intracellular retention of GLUT4 [99]. Similarly, co-expression of rab4 and GLUT4 in *Xenopus* oocytes reduces the abundance of GLUT4 at the cell surface and diminishes glucose transport [100]. Furthermore, transfer of a synthetic peptide corresponding to the carboxyl-terminal domain of rab4 to adipose cells inhibits insulin-induced GLUT4 translocation [101]. Insulin, but not exercise, caused a decrease in rab4 in intracellular membranes from rat skeletal muscle [95]. All these results support the view that rab4 indeed regulates GLUT4 trafficking in adipose and muscle cells. All these observations suggest that rab4 promotes enhanced sorting of GLUT4 from the endosomal to the storage compartment.

Rab4 is not the only small GTP-binding protein involved in GLUT4 trafficking in muscle cells. Thus, a small molecular mass GTP-binding protein, p24, has been detected in GLUT4 vesicles obtained from rat heart [102]: insulin causes a marked decrease in the abundance of p24 associated with intracellular GLUT4-vesicles [102]. This protein and its role in GLUT4 traffic has not yet been identified.

Vesicle fusion in eukaryotic cells is regulated by vSNARE and tSNARE proteins. Proteins originally identified as t-SNAREs involved in synaptic vesicle exocytosis, such as syntaxin 1A/1B or syntaxin 4, have also been found in cell

surface membranes from isolated rat adipocytes, cardiomyocytes or skeletal muscle [91, 103]. Furthermore, the v-SNARE proteins, VAMP2 and cellubrevin, have been detected in GLUT4 vesicles derived from rat skeletal muscle [91] and cellubrevin has also been found in GLUT4 vesicles derived from isolated rat cardiomyocytes [91]. These results are consistent with previous reports indicating that cellubrevin and VAMP2 colocalize with GLUT4 in intracellular membranes obtained from adipocytes [104, 105]. As to the role of these proteins in GLUT4 traffic, it has been reported that the cleavage of VAMP2 and cellubrevin by botulinum neurotoxins B or D inhibits the translocation of GLUT4 in 3T3-L1 adipocytes [106, 107]. In addition, introduction of the cytoplasmic domains of syntaxin 4, VAMP2 or cellubrevin results in an inhibition of insulin-stimulated GLUT4 translocation to the plasma membrane in 3T3-L1 adipocytes [108]. In summary, the presence of VAMP2 in the exocytic GLUT4 compartment suggests that it plays a role in the docking and fusion of GLUT4 vesicles to the cell surface in the muscle fiber. Cellubrevin interacts with syntaxin 1 and SNAP-25 [109] and seems to be involved in exocytosis of transferrin receptor-containing vesicles in CHO cells [110], but it does not affect fusion of early endosomes *in vitro* [111]. As cellubrevin is located in the endosomal GLUT4 compartment, it probably has a role in the fusion events involved in the sorting of GLUT4 from the endosomal to the exocytic pools.

There may also be differences in the specific v- and t-SNAREs responsible for the traffic of GLUT4 in insulin-sensitive cells. Thus, cardiac myocytes express syntaxin 1A and 1B as well as SNAP-25 and are mainly located at the plasma membrane [91]. In contrast, adipocytes do not express either syntaxin 1 or SNAP-25, although they do express SNAP23 [112, 113]. In addition, cardiac myocytes do not express VAMP2, which rules out its role in GLUT4 exocytosis, unlike what occurs in adipose cells and skeletal muscle, in which VAMP2 co-localizes with GLUT4 in the storage compartment [91, 105]. Whether these differences are attributable to the operation of distinct isoforms or to the acquisition of v-SNARE proteins during exocytosis is unknown.

Semicarbazide-sensitive amine oxidase has recently been found to co-localize with GLUT4 in the endosomal compartment from adipose or muscle cells [114, 115]. Interestingly, substrates of semicarbazide-sensitive amine oxidase in combination with very low concentrations of vanadate stimulate glucose transport and GLUT4 translocation to the cell surface in isolated rat adipocytes [115, 116]. Whether these proteins have a regulatory role in GLUT4 traffic remains unanswered.

Another protein that participates in insulin-induced GLUT4 translocation in cells is protein kinase B, also named akt, which lies downstream from phosphatidylinositol 3-kinase. In fact, insulin, but not contraction, activates akt in rat skeletal muscle [117]. Akt-2 has been found in GLUT4 vesicles obtained from isolated rat adipocytes previously treated with insulin [118]. Furthermore, akt-2 phosphorylates proteins that are components of GLUT4 vesicles in response to insulin [119]. This strongly supports the concept that akt-2 phosphorylates a component responsible for triggering the exocytosis of GLUT4 to the cell surface, and might link insulin signaling to activation of GLUT4 exocytosis. No data are yet available concerning the association of akt-2 and GLUT4 vesicles in muscle.

Muscles release lactate and take up lactate and ketone bodies

Metabolism of monocarboxylic acids in muscle

Skeletal muscle produces and removes lactate at the same time. Lactate is formed during periods of high energy demand or during rapid fluctuations in energy requirements. The formation of lactate during exercise is a result of an excess of pyruvate formation and is not necessarily the result of anaerobic conditions, since lactate can be produced in oxidative fibres of fully oxygenated muscles. The lactate taken up by the muscle can be used for glycogen synthesis, especially in fast-twitch fibers [120] or for oxidation in slow-twitch fibers [121, 122]. Thus, at any given moment, lactate can be produced in glycolytic fibres and oxidized in more oxidative fibres. Due to its large mass, skeletal muscle is the main producer and consumer of lactate in the body. Other monocarboxylate compounds are ketone bodies and α-ketoacids derived from amino acids. Ketone bodies are actively consumed by muscle during fasting or during recovery after exercise, when β-hydroxybutyrate or acetoacetate concentrations in plasma are high. In contrast, it has been reported that a substantial portion of the branched-chain amino acids deaminated by muscle are released to the circulation as α-ketoacids [123, 124].

Lactate uptake and release are stereospecific, inhibitable by cinnamate and pH-dependent [125]. Lactate uptake by soleus muscle shows a Km near 13 mM [126]. Lactate flux across the membrane regulates intracellular muscle pH. Thus, measurements of intracellular pH after muscle contraction have demonstrated that pH recovery is inhibited by the lactate transport inhibitor cinnamate [127]. Studies using sarcolemmal vesicles have revealed that lactate transport is transactivated by lactate and that high H^+ concentration at the trans-side inhibits the transport [128]. It is also clear that lactate transport is stimulated by protons at the cis-side, which has been attributed to fast H^+ binding to the carrier [125]. The transport kinetic parameters and the substrate selectivity have led to the concept of lactate-proton co-transport in skeletal muscle [129] in which the transport is stereospecific and electroneutral, probably 1:1, and H^+ binds first to the carrier.

Obviously, lactate and H^+ efflux from muscle is important during exercise since it reduces the intracellular concentration of both species and prevents cellular acidification. Some indirect observations support the view that lactate transport controls lactate utilization in skeletal muscle. As such, under many different experimental conditions there is a considerable inward-directed lactate gradient [130-132], which suggests that the capacity for lactate transport across

the membrane is not much higher than the internal utilization. This remains to be established.

Lactate transport is not subject to acute regulation in muscle. However, there are several examples indicating that lactate transport is regulated in a long-term manner. Thus, lactate uptake is faster in soleus than in extensor digitorum longus muscle in rats [133] and, similarly, lactate transport is greater in sarcolemmal vesicles derived from slow-twitch fibers than in vesicles from fast-twitch fibres [134]. These data suggest a higher lactate-proton transport rate in red fibres than in white. Furthermore, situations characterized by up-regulation of lactate transport in skeletal muscle include exercise training [135, 136], chronic electrical stimulation [137] or administration of thyroid hormones [138]. In contrast, other conditions such as muscle denervation [139, 140], unweighting in rats [141] or aging [134] down-regulate lactate transport.

Monocarboxylate transporters

Different membrane transporter proteins catalyze the proton-linked transport of lactate, pyruvate, branched-chain α-ketoacids derived from leucine, valine and isoleucine, β-hydroxypyruvate, acetoacetate and acetate, which have been named monocarboxylate transporters. At present, eight different monocarboxylate transporter isoforms have been identified in human tissues (MCT1-8, the latter also known as XPCT). An extensive review of monocarboxylate transporters has recently been written by Halestrap and Price [142].

The first monocarboxylate transporter identified, MCT1, was isolated from Chinese-hamster ovary cells [143]. MCT1s from human, rat and mouse have now been cloned and share about 95% sequence identity with Chinese-hamster MCT1 [144-148]. Human MCT1 shares high identity with human MCT2, MCT3 and MCT 4 (ranging from 45 to 57%) and lower identity with the rest of the members so far identified (25 to 30%) [143, 149].

Hydrophobicity analysis of the monocarboxylate transporters suggests a structure with 12 transmembrane segments, presumed to be α-helical. It seems probable that there are 12 transmembrane domains with the N- and C-termini oriented to the cytosol. Furthermore, the MCT family members show the highest sequence conservation in the putative transmembrane region. Results obtained in studies of proteolytic digestion [150] are in keeping with a model of MCT1 like the one shown in Figure 3. In addition, MCT1 does not show N-glycosylation [146], which raises the question of the nature of the mechanisms involved in intracellular traffic. In this connection, there is a possible association of MCT proteins and an ancillary protein OX-47, which may be instrumental in directing MCTs to the cell surface (see review 142). An association between a 12 transmembrane-spanning domain protein transporter and an ancillary protein has been well substantiated for some amino acid transporters.

Transport characteristics of MCT1 have been extensively studied in human erythrocytes (which only express MCT1) or after heterologous expression in *Xenopus* oocytes [151-154]. Kinetics studies indicate that MCT1 is a proton/lactate co-transporter that follows an ordered, sequential mechanism [151, 152]. Transport begins with proton binding to the transporter, which is followed by binding of lactate anion. Translocation of lactate and proton across the membrane occurs next, followed by their sequential release on the other side of the membrane. MCT1 can transport a wide variety of

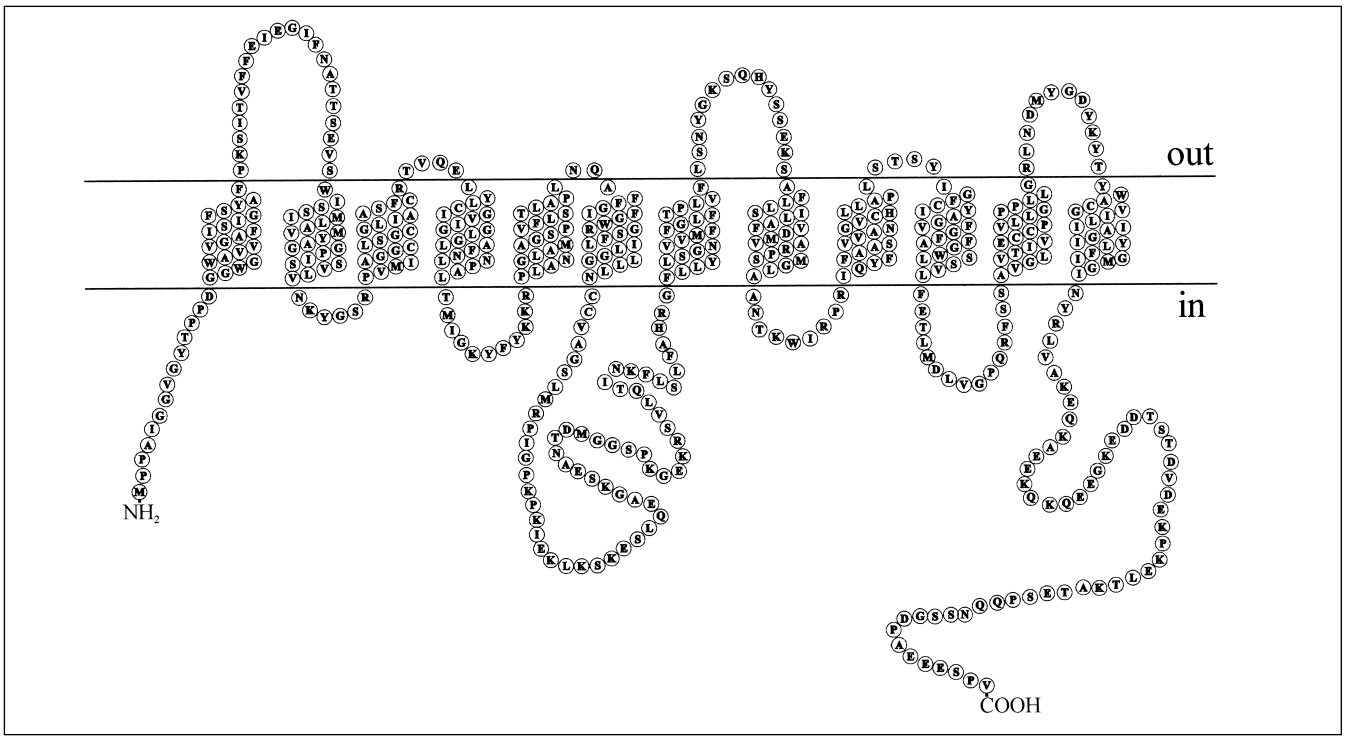

Figure 3. Proposed topology of MCT-1.
The sequence shown is that of *Cricetulus longicaudatus* (Chinese hamster) MCT-1.

short-chain monocarboxylates, the Km values decreasing as their 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 favored and C-2 substitution preferred over C-3. Transport of lactate is stereospecific, but not that of other substrates such as β-hydroxybutyrate or 2-chloropropionate. Monocarboxylates with longer-branched aliphatic or aromatic side chains also bind to the transporter but are not quickly released following translocation and may act as potent inhibitors. MCT4 has a lower affinity for lactate (Km higher than 20 mM) than MCT1 (Km of 0.5 mM) after heterologous expression in *Xenopus* oocytes [155]. No functional data are available for MCT5, MCT6, MCT 7 or XPCT.

Skeletal muscle expresses MCT1 and MCT4 monocarboxylate transporters [143, 155-157]. MCT1 expression correlates with its mitochondrial content. Thus, rat muscles enriched in slow oxidative fibers express large amounts of MCT1, whereas muscles with a high content of fast-twitch glycolytic fibers contain hardly any [155, 156]. Similarly, there is a positive correlation between MCT1 expression and the occurrence of type I fibers in human muscles [158]. In contrast, MCT4 is present in all rat muscles but less in predominantly oxidative muscle [156] and MCT4 density is independent of fiber type in human muscle [158]. Heart expresses high levels of MCT1 both in human and rat [142, 143]; MCT1 is found at the cell surface of cardiomyocytes and is particularly concentrated in the intercalated discs [142, 143]. MCT1 is not the only monocarboxylate transporter that is expressed in heart although there is some variability among species; thus, human but not rat heart expresses MCT4 [143], and Syrian hamster heart expresses MCT2 [159]. Furthermore, Northern blot data suggest that both human skeletal muscle and heart express other monocarboxylate transporters with as yet unknown functions: skeletal muscle expresses MCT6 and XPCT and heart expresses MCT5, MCT6, MCT7 and XPCT [149]. In all, data so far available indicate the expression of different MCT isoforms in skeletal and cardiac muscle; further studies will be required to characterize the specific cellular distribution. The distribution of MCT1 and MCT4 in different muscle fibers has led to the suggestion that MCT1 expression in skeletal muscle may reflect the need to take up lactate for oxidative purposes, whereas MCT4 may be important for lactate efflux. A lot of research is still required before the function of these MCT isoforms in muscle can be demonstrated.

In keeping with the observations that lactate uptake is regulated in muscle, it is becoming clear that the expression of MCTs is subject to regulation in muscle. Endurance exercise and high-intensity chronic electrical stimulation enhance MCT1 expression in rat skeletal muscle [156, 160] and exercise training also increases MCT1 in rat heart [160]. In humans, exercise training causes MCT1 up-regulation in muscle [161]. In contrast, muscle denervation reduces the expression of both MCT1 and MCT4 in muscle [156]. The mechanisms involved in the regulation of MCT1 and MCT4 in muscle remain to be determined.

How do fatty acids cross the plasma membrane in muscle?

Muscle utilizes large amounts of fatty acids: for example, a large percentage of the energy required to run a marathon race is derived from muscular oxidation of fatty acids, most of which come from the bloodstream. The mechanisms by which fatty acids cross the membrane are controversial. On the one hand, there is evidence from unilamellar protein-free phospholipid vesicles that fatty acids cross membranes efficiently by simple diffusion [162]. On the other hand, some researchers have provided evidence for carrier-mediated uptake [163].

Fatty acid diffusion across membranes may be divided into several steps: a) desorption of the fatty acids from albumin to which they are bound, b) absorption of an unbound monomeric form to the outer leaflet of the plasma membrane, c) passage of fatty acids across the membrane (flip-flop), and d) desorption of fatty acids from the cytosolic leaflet of the plasma membrane. The utilization of unilamellar protein-free phospholipid vesicles has led to reports that the rate constant for adsorption of fatty acid monomers or for adsorption of fatty acids bound to albumin (at high fatty acid/albumin ratio) to phospholipid bilayer vesicles is extremely high [164-166]. A more controversial aspect is the transmembrane movement of fatty acids or flip-flop across the membrane. Many studies use the pH-sensitive fluorophore pyranin to measure rates of flip-flop of fatty acids into phospholipid vesicles; some studies have shown that rates of flip-flop are high enough to account for fatty acid uptake into cells (rate constant for flip-flop > 10 s^{-1}) [166, 167]. In contrast, other studies in which flip-flop of long chain anthroyloxy fatty acids has been measured show that the rate is low (rate constant < 0.003 s^{-1}) [168]. The rate of fatty acid desorption from membranes is slower than the rates of adsorption and depends on fatty acid length and unsaturation [162]. However, there is controversy as to whether the rates of fatty acid desorption are lower or higher than flip-flop [162, 168]. Thus, these studies performed in model systems suggest that proteins are not essential for the movement of fatty acids across membranes, although there is discussion on the rates and rate-limiting steps.

There is also growing evidence suggesting a carrier-mediated uptake of fatty acids into cells which is based on: a) the saturation kinetics of fatty acid uptake [169, 170], which in rat cardiomyocytes accounts for 80% of palmitate uptake [169], b) the alteration of fatty acid uptake by covalent modification of proteins [171, 172], and c) the identification of several proteins which enhance fatty acid uptake [163]. In this regard, several membrane-associated proteins have been identified and are expressed in cardiac and skeletal muscles, namely, a plasma membrane fatty acid-binding protein (FABPpm), a fatty acid translocase (FAT) and a fatty acid transport protein (FATP).

The plasmalemmal FABP, or FABPpm, is a peripheral membrane protein which is identical to the mitochondrial isoform of aspartate aminotransferase [173, 174]. The cellu-

lar mechanisms by which the mitochondrial isoform of aspartate aminotransferase reaches the inner side of the plasma membrane and the domain/s that permit binding to the plasma membrane are not understood. There are several indications that FABPpm participates in the uptake of fatty acids by cells. Thus, antibodies directed against the FABPpm diminished oleate uptake in isolated rat cardiomyocytes [175] and in 3T3-L1 adipocytes [176]. They also reduced palmitate uptake in preparations of giant sarcolemmal vesicles isolated from heart or skeletal muscle [177]. Inhibition with antibodies suggests that FABPpm may account for up to 50% of fatty acid uptake. Furthermore, 3T3 fibroblasts transfected with FABPpm/aspartate aminotransferase show enhanced saturable oleate uptake in parallel to an increase in FABPpm bound to the plasma membrane [178]. The mechanisms by which FABPpm mediates fatty acid uptake remain unknown. In this regard, the precise role of FABPpm in fatty acid transport across phospholipid vesicles must be determined.

Recent reports indicate that FABPpm expression is regulated in muscles. Thus, the expression of FABPpm protein and mRNA is very high in heart, intermediate in red skeletal muscle and lower in white muscle, which parallels the capacity of fat oxidation [177, 179, 180]. Furthermore, endurance training, which enhances the capacity for fatty acid oxidation in muscle, also increases FABPpm [181].

Fatty acid translocase (FAT) is an integral membrane protein which is considered the rat homologue of human CD36 [182] and which binds fatty acids. FAT has two predicted transmembrane domains and is oriented so that it has two short intracellular segments (Figure 4). The remaining part of FAT is extracellular, containing 10 potential N-linked glycosylation sites, which may explain why the molecular mass of the isolated protein (88 kDa) is much greater than the mass deduced from the cDNA sequence (53 kDa). Studies on the purified bovine isoform of CD36 indicate that the six centrally clustered cysteines are linked by disulfide bonds, resulting in 1-3, 2-6 and 4-5 arrangements of the disulfide bridges [183]. Based on the homology with the fatty acid binding site of heart-type fatty acid-binding protein (H-FABP), a potential binding site for fatty acids may lie in its extracellular segment between residues 127 and 279 [184]. Several observations support a role of FAT in fatty acid uptake by cells, namely that the expression of FAT in Ob17PY fibroblasts (which do not express endogenous FAT) enhances fatty acid uptake [185] and that transgenic mice showing muscle-specific over-expression of FAT are characterized by an enhanced muscle capacity to oxidize fatty acids in response to muscle contraction and show low plasma concentrations of triglycerides and fatty acids [186]. As for FABPpm, the mechanism by which FAT contributes to fatty acid uptake and the extent of the participation of FAT in the rate of fatty acid uptake by tissues is unknown. However, FAT has a predicted structure which does not fit with a membrane transporter, so its role remains enigmatic. Recently, and perhaps this is germane to this issue, it was reported that the expression of FAT in cardiac myocytes in culture does not enhance fatty acid uptake, which suggests that it requires additional protein components for its operation as a «fatty acid translocase» [187]. In any case, as mentioned for FABPpm, studies in liposomes are required in order to analyze the precise role of FAT.

FAT/CD36 gene was recently identified as a causal gene for insulin resistance in spontaneously hypertensive rats (SHR), based on quantitative trait loci mapping in the FAT

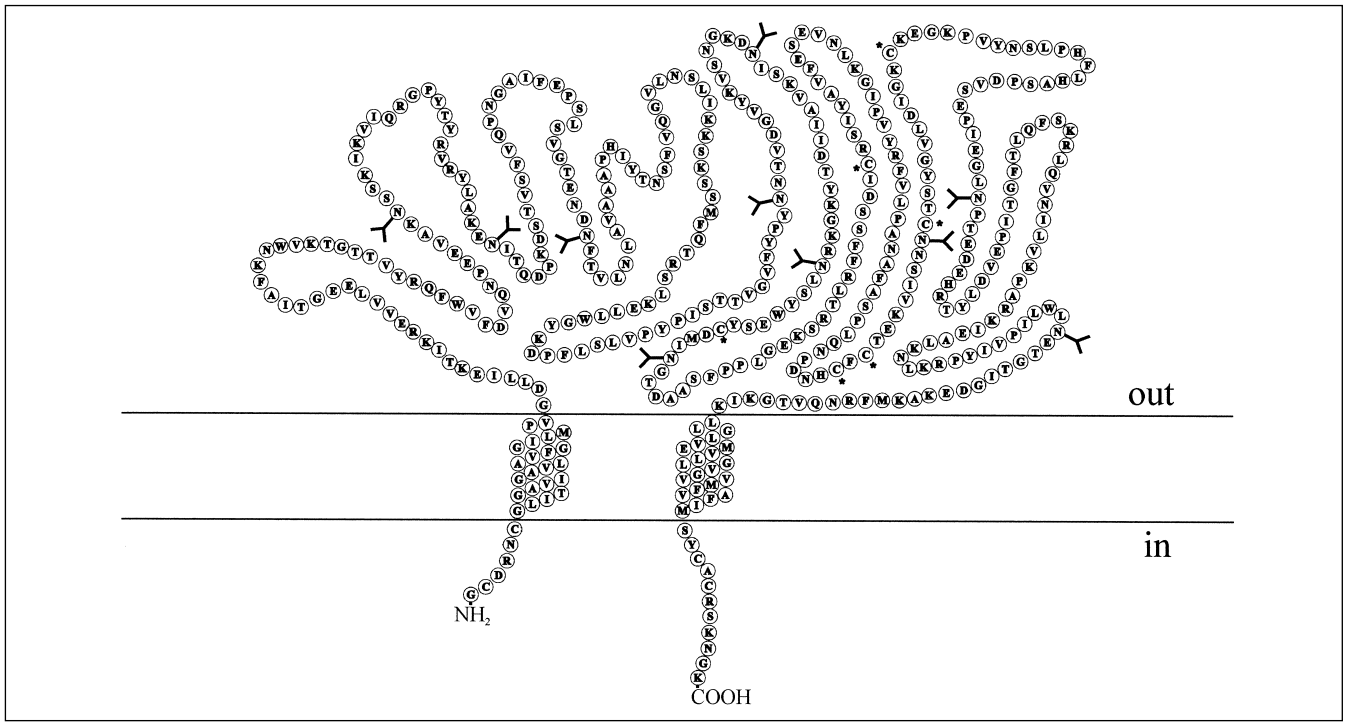

Figure 4. Proposed topology of rat fatty acid translocase (FAT)/CD36.
The asterisks indicate the six centrally clustered cysteines that are linked by disulfide bonds in 1-3, 2-6 and 4-5 arrangements.

gene, defective FAT gene expression in adipose tissue and a deletional mutation in FAT [188]. However, a further study demonstrated that FAT gene defect was unlikely to be a major cause of insulin-resistance phenotype in SHR since no mutations in FAT were detected in the original SHR rats [189].

FAT is expressed in muscles and is subject to regulation. During development, mRNA and protein levels are up-regulated in rat heart, which is parallel to an enhancement of its capacity to utilize fatty acids [190, 191]. Chronic stimulation increases muscle fatty acid uptake by giant muscle vesicles, and enhances muscle FAT expression [192]. Furthermore, the expression of FAT protein and mRNA is very high in heart, intermediate in red skeletal muscle and lower in white muscle, which parallels their capacity of fat oxidation [177, 179, 180].

The mouse fatty acid transport protein (FATP) was initially identified in an expression cloning strategy by its ability to enhance fatty acid uptake [193]. Mouse FATP (FATP-1) encodes an integral membrane protein with six predicted membrane-spanning regions, and three potential N-linked glycosylation sites; both the N-and C-terminal ends are oriented to the cytosolic side (Figure 5). The human, rat and *Saccharomyces cerevisiae* orthologues of FATP have been isolated [194-196]. In fact, a total of five members of the family have been identified in murine tissues (FATP1 to 5) and, at least, FATP-1, FATP-2 and FATP-5 enhance fatty acid uptake when expressed in COS cells [197]. In addition, FATP shows homology with rat very long-chain fatty acid CoA synthetases [196].

Stable over-expression of FATP confers enhanced ability to take up fatty acids [193] and facilitates the uptake of saturated and mono-enoic long-chain fatty acids with 14-22 carbons suggesting that it has broad specificity with respect to fatty acid chain length and degree of saturation [193]. The mechanism by which FATP facilitates fatty acid uptake is unknown. In this connection, it has been reported that murine FATP binds ATP [194], and studies with the yeast orthologue of FATP indicate that it displays very long-chain fatty acid synthetase activity, which might be essential for normal very long-chain fatty acid homeostasis [196]. Comparisons of the sequences of murine, human and yeast orthologues have identified a conserved motif IYTSGTTGXPK, also found in a number of proteins that form adenylated intermediates [194]. Substitution of alanine for serine 250 in this motif from murine FATP inhibits long-chain fatty acid uptake and ATP binding [194]. Taking all these data together, it is reasonable to propose that FATP facilitates fatty acid uptake via membrane-associated fatty acid CoA synthetase activity rather than by transport catalysis. However, as for FABPpm and FAT/CD36, it is crucial to analyze the role of FATP in vesicle models as well as the effect of *in vivo* ablation of FATP on fatty acid homeostasis.

FATP is expressed in cardiac and skeletal muscles [182]. Data exist which indicate that its expression is regulated in adipocytes [198, 199] and muscles [200]. Thus, FATP mRNA levels are down-regulated in heart, skeletal muscle, adipose tissue, brain and other tissues in response to lipopolysaccharide (endotoxin) treatment *in vivo* under conditions in which fatty acid uptake is reduced [200].

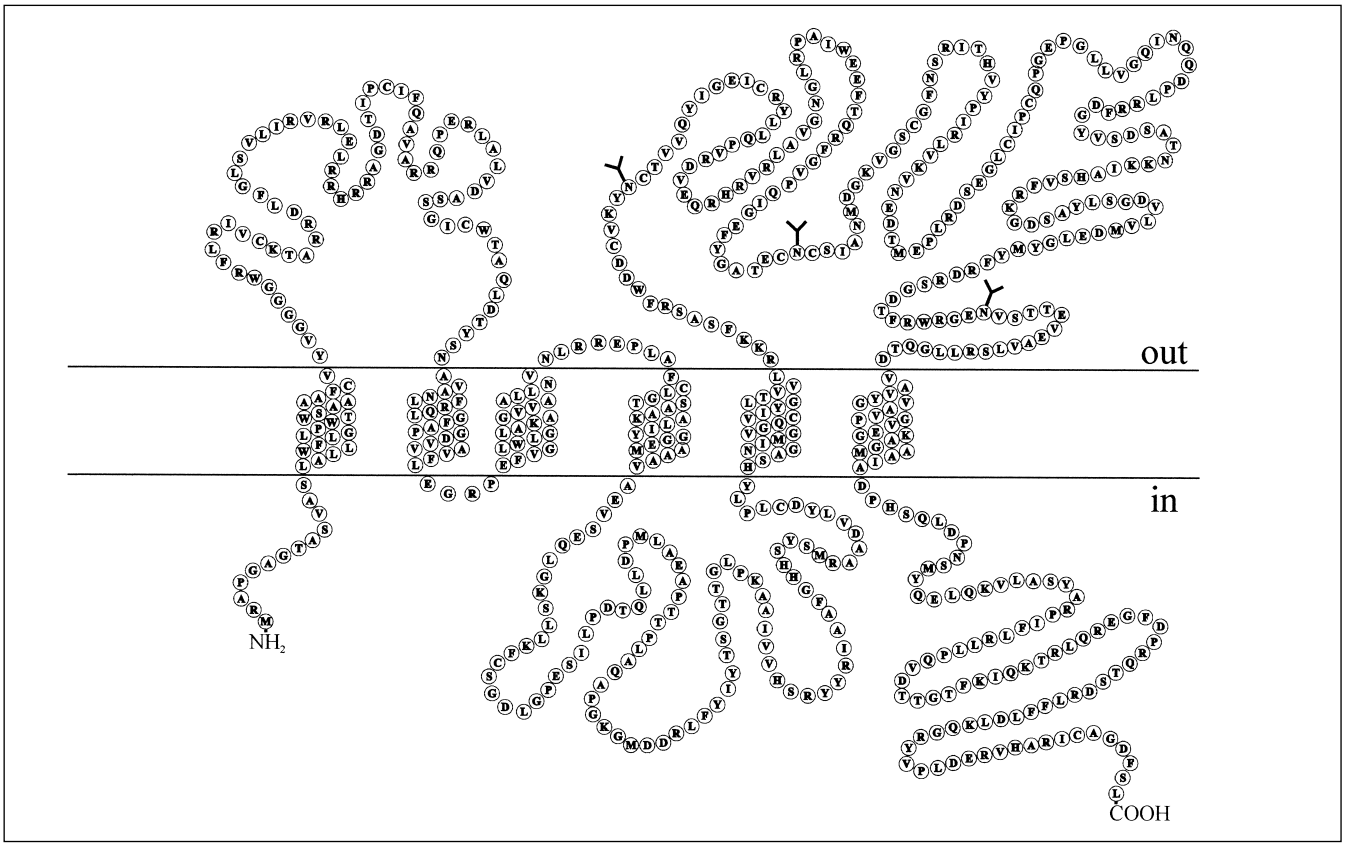

Figure 5. Proposed topology of mouse fatty acid transport protein FATP-1.

Carnitine uptake is essential for muscle metabolism

L-carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential component in the transport of long-chain fatty acids into mitochondria, where they undergo β-oxidation. The enzyme carnitine palmitoyl transferase (CPT-1) catalyzes the conversion of carnitine and long-chain fatty acids to long-chain acyl carnitine esters, which are then transported to the mitochondrial matrix. L-carnitine is synthesized endogenously from the essential amino acid lysine, with terminal methyl groups donated by S-adenosylmethionine. Methylation of the lysine side chain occurs when lysine is present in proteins. In humans the final reaction in the synthesis of carnitine is catalyzed by a cytosolic hydroxylase, which operates mainly in liver, brain and kidney, and is not present in cardiac or skeletal muscles [201, 202]. In addition to endogenous synthesis, a notable amount of carnitine is provided by the diet, especially meat and dairy products. As such, the requirements for carnitine in cardiac and skeletal muscles are met by uptake from the extracellular milieu and carnitine is normally maintained at a steady level in blood at near 50 μmol/l. It is eliminated as free carnitine or acylcarnitine almost exclusively by the kidneys, where it is filtered and reabsorbed in the proximal tubule. The concentrations of carnitine in human heart or muscle are 20-50-fold greater than in serum [201, 202]. Failure of muscles to obtain sufficient carnitine results in cardiomyopathy or muscular weakness.

Carnitine uptake has been studied in different tissues and cells in culture, and kinetic data suggest the operation of several transporters. Liver and brain show a low-affinity (Km in the range of 2-10 mM) high-capacity carnitine uptake [203-205], while fibroblasts and heart cells have a high-affinity (Km of 5-10 μM) low-capacity system [206-209]. In addition, kidney brush-border membrane vesicles show both high-affinity and low-affinity components for carnitine uptake (Km values of 17 μM and 15 mM, respectively) [210]. Characterization of carnitine transport by renal brush-border vesicles shows Na^+-dependency, electrogenicity and inhibition by structural analogues such as butyrobetaine, L-acetylcarnitine, trimethyl-lysine or D-carnitine [210].

Carnitine uptake by muscle cells varies with the differentiation state; thus, human myoblasts in culture are characterized by a high-affinity uptake (Km of 5 μM) [206, 211], and muscle cell differentiation causes the appearance of a low-affinity component for carnitine uptake (Km of approximately 0.2 mM) [212, 213]. Carnitine uptake by rat skeletal muscle is characterized by high Km values, suggesting the operation of a low-affinity carnitine carrier [214, 215].

Whether the low and high carnitine transport components identified in different tissues or cells are the result of the expression of different transporters or correspond to differential activity states of a single transporter is unknown.

A human gene, named OCTN2, and a rat gene, CT1, which are very similar (85%) and encode carnitine transporters, have recently been isolated [216, 217]. Human OCTN2 has been mapped to chromosome 5q31.1-32 [216]. OCTN2 and CT1 belong to the family of organic cation transporters that includes OCT1, OCT2, OCT3 and OCTN1; OCTN2 is an integral membrane protein showing 12 transmembrane domains, 3 potential glycosylation sites in the first extracellular loops and both the N- and the C-terminal ends oriented intracellularly [218] (Figure 6). OCTN2 is highly expressed in kidney, heart, skeletal muscle and placenta.

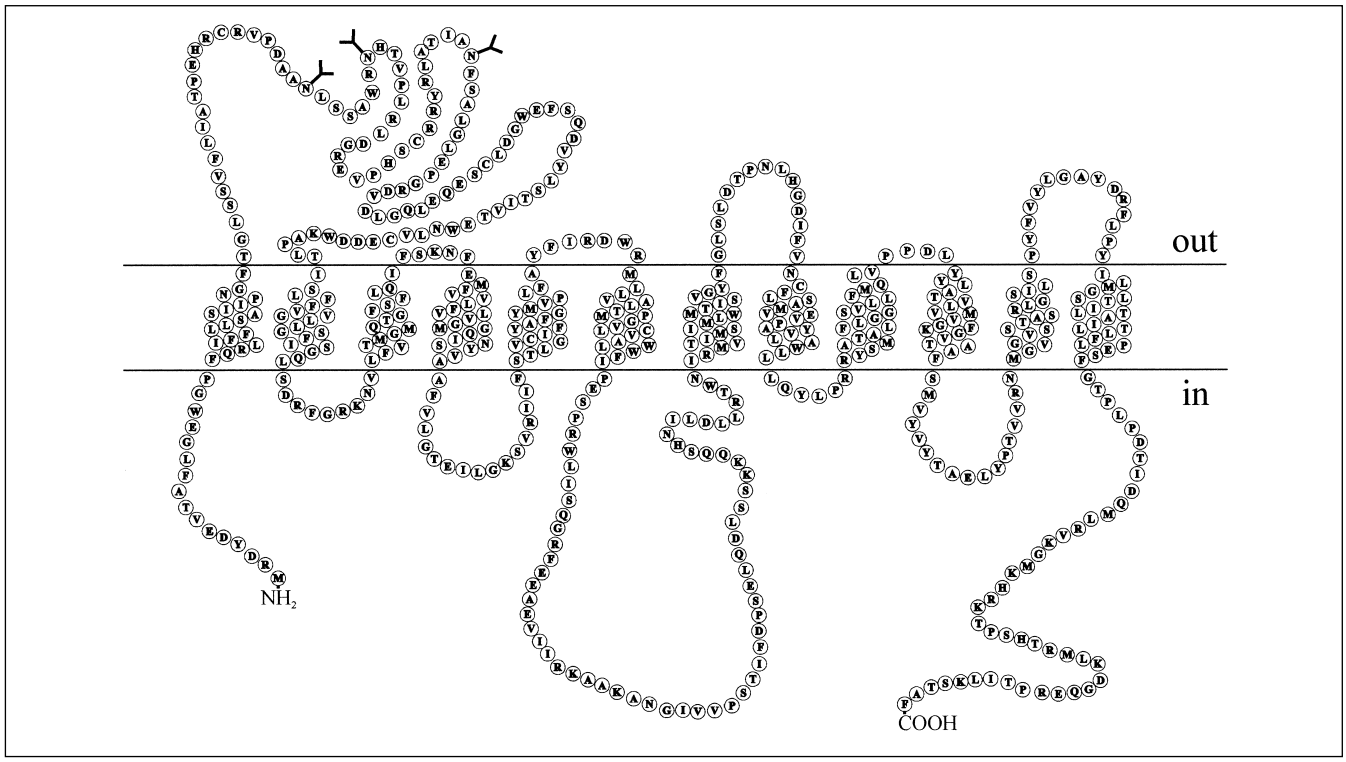

Figure 6. Proposed topology of human carnitine transporter OCTN-2.

Surprisingly enough, CT1 is mainly expressed in kidney, liver, intestine, testis and placenta and is very weakly expressed in skeletal muscle, and no expression is detected in heart [218]. Whether this is due to differences between human and rat tissues or to CT1 and OCTN2 not being orthologous remains unknown.

Expression studies of OCTN2 in human embryonic HEK293 cells indicate that it mediates uptake of carnitine against a concentration gradient with an apparent Michaelis-Menten constant of 5-15 μmol/l [218, 219]. One Na^+ is co-transported with one carnitine molecule [219]. Many zwitterionic drugs such as cephaloridine and many cationic drugs such as verapamil and quinidine inhibit OCTN2 transport activity [219]. In fact, OCTN2 is a Na^+-dependent carnitine transporter as well as a carrier for organic cations which operates in a Na^+-independent manner [220]. This is consistent with the fact that organic cation transporters are Na^+-independent [221]. The role of Na^+ is to enhance the affinity of OCTN2 for carnitine [220].

Primary systemic carnitine deficiency is an autosomal recessive disorder characterized by cardiomyopathy, skeletal myopathy, hypoglycemia and hyperammonemia. Several studies indicate that this disease is a consequence of a defect in carnitine transport [208]. This has also been reported in the mouse model, juvenile visceral steatosis (jvs mice) [222], which results in renal wastage and very low intracellular levels of carnitine. Recently, it has been found that systemic carnitine deficiency is caused by mutations in the OCTN2 gene that lead to deficient cellular carnitine uptake [223-228].

Several observations suggest additional carnitine transporters, apart from OCTN2, in muscle tissues. These argue that, as OCTN2 is a high-affinity carnitine carrier and muscle also expresses a low-affinity carrier, and that myopathic carnitine deficiency is characterized by normal concentrations of carnitine in blood and in liver but low concentrations in muscle, this gives rise to muscle weakness and lipid storage myopathy [229]. This raises the question of whether the low-affinity carnitine carrier is a separate entity and suggests that a defect in this low-affinity carrier may be the cause of the primary myopathic carnitine deficiency.

Amino acid release and uptake by muscle is performed by amino acid transporters

Skeletal muscle constitutes a large proportion of body weight in most mammals, including Humans. As a consequence of its large mass, muscle is the body's main reservoir of amino acids and protein. Under anabolic conditions, muscle takes up amino acids from the extracellular space, in a pattern conforming to its protein composition. In contrast, in catabolic states or when protein synthesis is depressed, the pattern of amino acid release from muscle does not depend on the muscle's protein composition. Thus, alanine and glutamine account for 40-50% of the total amino acids released by human forearm and by the isolated perfused rat hindquarter, whereas they comprise at most 15% of muscle protein [230-233] as a consequence of the *de novo* synthesis of alanine and glutamine in muscle [233-235]. In contrast to alanine and glutamine, several amino acids such as leucine, isoleucine, valine, aspartate and glutamate are released from skeletal muscle in lower amounts than would be expected from their content in muscle protein, probably reflecting their active catabolism in muscle [230, 233]. Furthermore, skeletal muscle is a main site of branched-chain amino acid metabolism; muscle takes up branched-chain amino acids and converts them into the α-ketoacid species which are released into the circulation [123, 124, 233, 236-239] (see section on monocarboxylate transporters).

Other amino acids such as glycine, cysteine, serine, threonine, methionine, proline, lysine, arginine, histidine, phenylalanine, tyrosine, and tryptophan can be either removed from the extracellular space by muscle for incorporation into protein or released upon proteolysis [123].

This complex pattern of amino acid influx and efflux in muscle is catalyzed by many different amino acid carriers. In the early 1960s different substrate specificity transport systems for amino acids were identified in mammalian cells, which revealed the existence of general properties of mammalian amino acid transport such as stereospecificity and broad ssubstrate specificity. Since these initial studies, the main functional criteria used to define amino acid transporters are the type of amino acid carried (zwitterionic, cationic or anionic) and the thermodynamic properties of the transport (active transport, facilitative diffusion). This functional classification is still used (Table 1), since structural information on mammalian amino acid transporters is not complete.

Amino acid transport agencies in muscle

Studies performed on isolated perfused rat hindquarter, on incubated muscle preparation or on muscle cells in culture have revealed a number of amino acid transport systems (Table 1). Most neutral amino acids are transported in muscle via four distinct transport agencies, i.e. systems A, ASC and N^m which show Na^+-dependency and system L, which is Na^+-independent [240-244]. There is also evidence for the operation in muscle of the transport of the β-amino acid, taurine, by a Na^+-dependent carrier [245]. Cationic amino acids are transported in muscle by the Na^+-independent system y^+ [246] and aspartate and glutamate are taken up in muscle cells and in perfused hindquarter via Na^+-dependent and Na^+-independent mechanisms (Table 1) [246, 247]. Because of the limitations imposed in the studies using skeletal muscle, it is likely that there are more amino acid agencies in this tissue than the ones identified by kinetic means.

There is substantial evidence that system A transport activity is regulated in skeletal muscle. Thus, both insulin and exercise stimulate system A activity in muscle acutely [240, 242, 248-253]. The stimulatory effect of insulin has also been

Table 1. Amino acid transport systems present in the plasma membrane of mamalian cells

		Isolated cDNAs	*Transport Activity in Muscle*
Sodium-dependent	*ZWITTERIONIC AMINO ACIDS*		
A	Serves mainly for small amino acids. highly regulated. Tolerates an N-methyl group. Sensitive to pH changes. Trans-inhibition associated. Widespread.		Yes
ASC	Excludes N-methylated amino acids. trans-stimulation associated. Widesread.	ASCT1-2	Yes
N	For gin, Asn and His. Sensitive to pH changes. Restricted to hepatocytes. Variant N^m in muscle.	SN1	Yes
BETA	Transports β-Ala, taurine and GABA. Chloride dependent. Variants known. Widespread.	series GAT-1 to 3 BGT-1, TAUT	Yes
GLY	Transports Gly and sarcosine. Chloride dependent. Bariants known. Present in several tissues	GLYT1-2	
IMINO	Handies proline, hydroxyprolines and N-methylated glycines. Interacts with MeAIB. In intestinal brush-border membranes.		
PHE	Phe and Met. In brush-border membranes.		
B^o	Broad specificity for most zwitterionic amino acids, including branched aromatic ones. Accepts BCH but not Me AIB. In brush-border membranes. Most probably identical to system NBB (renamed B).		
Sodium-independent			
L	Mainly for bulky side chain amino acids. *Trans*-stimulated. Bicyclic amino acids as model substrates. Variants described. Widespread.	4F2hc/LAT-1, -2	Yes
Sodium-dependent	*CATIONIC AMINO ACIDS*		
$B^{o,+}$	Broad specificity for zwitterionic and dibasic amino acids. Accepts BCH but not with MeAIB. In blastocysts, Xenopus oocytes and probably also in brush border membranes.	$B^{o,+}$-AT	
Sodium-independent			
b^+	Cation preferring. Does not interact with homoserine even in the presence of sodium. Variants known.		
y^+	Handles cationic, and zwitterionic amino acids with sodium. Variants known. Sensitive to N-ethyl maleimide. Widespread.	CAT-1 to 4	Yes
y^+L	Handles cationic amino acids, and zwitterionic amino acids with high affinity only with sodium. Insensitive to N-ethyl maleimide. In erythrocytes and placenta.	4F2hc/$y^-{}^+$ LAT-1, -2	
$b^{0,+}$	Like $B^{o,+}$ but limited by positions of branching. Hot inhibited by BCH. In blastocysts, and in brush border membranes.	rBAT/$b^{o,+}$ -AT	
Sodium-dependent	*ANIONIC AMINO ACIDS*		
^{X}-AG	Similarly reactive with L-Glu and D- and L-Asp. K+ -dependent. Widespread	EAAT1 to 5	Yes
Sodium-independent			
x-C	Cystine completes and exchanges with Glu. In heptocytes and fibrloblasts. Electroneutral.	xCT	Yes

MeAIB, methylaminoisobutyric acid; BCH, a-aminoendobicyclo-[2,2,1]-heptane-2-carboxylic acid.

described in human muscle [254]. In addition, amino acid starvation up-regulates system A activity in muscle by a mechanism that requires both protein synthesis and intact microtubular function [255]. The effect of insulin on system A in muscle is characterized by an enhanced Vmax of transport, is independent of protein synthesis, independent of the Na^+-electrochemical gradient and does not require intact microtubular function [253, 255, 256], suggesting a possible direct effect of insulin on the system A transporter. System A activity in skeletal muscle is also acutely regulated by phospholipase C and by vanadate [257, 258]; the latter compound may activate system A by increasing the intracellular pH [258]. System A may also be regulated by long-term mechanisms and, in this regard, it has been reported that streptozotocin-induced diabetes causes up-regulation of system A activity in both red and white muscle [259]. The precise nature of the mechanisms that regulate system A is unknown since identification of the system A gene has not been reported.

The activity of system N^m is responsible for the transport of glutamine and asparagine in a sodium- and pH-dependent manner, with stoichiometry of 1 Na^+:1 glutamine [243, 246]. N^m is responsible for both the influx and the efflux of glutamine in muscle and is subject to regulation. Glutamine transport in muscle is up-regulated in response to incubation in a glutamine-free medium [260], i.e. it undergoes adaptive regulation. Insulin stimulates N^m activity in muscle by a mechanism that is characterized by enhanced Vmax and sensitivity to cycloheximide inhibition [261]. N^m transport activity is also subject to rapid regulation after changes in cell volume. Thus, hypo-osmotic swelling causes stimulation and hyperosmotic shrinkage induces inhibition of Na^+-dependent glutamine uptake in cultured muscle cells, effects which are blocked by the phosphatidylinositol 3-kinase, wortmannin [262, 263]. The cloning of one N system, named SN1, has recently been reported [264], which may accelerate the identification of the N^m carrier and thus the molecular analysis of the regulatory mechanisms that operate in muscle.

Another system that is subject to regulation in muscle is the transport of the β-amino acid taurine. Taurine uptake shows sodium dependency in muscle and cardiomyocytes, and fibre type dictates differences in uptake so that taurine uptake is greater in red muscle than in white [265]. In addition, chronic electrical stimulation of the extensor digitorum longus stimulates taurine transport [266]. In keeping with a role as an organic osmolyte, hypotonicity strongly activates taurine efflux from muscle cells in culture [267].

The transport of anionic amino acids is also regulated in muscle. Thus, insulin increases glutamate uptake by human forearm muscle [268]. There are differences between the agencies that participate in glutamate transport in the muscle fiber or in cultured muscle cells. Whereas glutamate transport in the perfused rat hindlimb shows stereospecificity and sodium independence [246], glutamate is transported in muscle cells in culture by Na^+-dependent (probably X^-_{AG}) and Na^+-independent (probably x^-_C) agencies [247]. The sodium-dependent component of glutamate transport is up-regulated by incubation in the presence of glutamine by a mechanism that is sensitive to inhibitors of both transcription and protein synthesis [247].

Expression of amino acid transporters in muscle

The primary sequence of most amino acid transport agencies in mammalian cells has been identified. At present, amino acid transporters are classified in 5 different gene families shown in Table 2: 1) family of sodium-independent cationic amino acid transporters, 2) superfamily of sodium- and chloride-dependent neurotransmitter transporters which also includes amino acid transporters, 3) superfamily of sodium-dependent transporters for anionic and zwitteri-

Table 2. Families of amino acid transporters

		Expression in muscle
A) CATIONIC AMINO ACID TRANSPORTERS		
CAT-1		Yes
CAT-2		Yes
CAT-2a		Yes
CAT-3		
CAT-4		
B) SODIUM- AND CHLORIDE-DEPENDENT AMINO ACID TRANSPORTERS		
GAT-1		
GAT-2		
GAT-3		
BGT-1		Yes
TAUT		Yes
GLYT1		
GLYT2		
PROT		
B0,+-AT		
C) SODIUM-DEPENDENT TRANSPORTERS FOR ANIONIC AND ZWITTERIONIC AMINO ACIDS		
EAAT1 (rat GLAST)		Yes
EAAT2 (rat GLT-1)		
EAAT3 (rabbit EAAC1)		Yes
EAAT4		
EAAT5		Yes
ASCT1		Yes
ASCT2		Yes
D) HETEROMULTIMERIC AMINO ACID TRANSPORTERS		
Heavy subunit	*Light subunit*	
4F2hc		Yes
	LAT-1	
	LAT-2	Yes
	y^+LAT-1	
	y^+LAT-2	
	xCT	
rBAT		
	$b^{0,+}$AT	
E) SODIUM- AND PROTON-DEPENDENT VESICULAR NEUROTRANSMITTER TRANSPORTERS		
SN1		

onic amino acids, 4) family of heteromultimeric amino acid transporters, with two distinct subunits (Table 2), and 5) the recently identified SN1 transporter which belongs to the family of sodium- and proton-dependent vesicular neurotransmitter transporters, and which is not expressed in muscle [264]. For a thorough discussion of amino acid transporter families identified up to 1998, see reference 15.

Most of the work performed up to now in this field has involved molecular identification of the transporters, functional studies after over-expression in cells, and a few studies on structure/function relationships and topological properties. Almost nothing is known about the specific function of these transporters in muscle. In fact, since the reported information is basically limited to the expression of mRNA for transporters in muscle, in many instances we do not even know whether the transporter is expressed in the muscle fibre or in cardiac myocytes.

Family of sodium-independent cationic amino acids

These carriers catalyze the sodium-independent transport of cationic amino acids (L-arginine, L-lysine or L-ornitine) with high affinity (Km in the micromolar range), as is the case of CAT-1 or CAT-2, or with low affinity (Km in the millimolar range) by CAT-2a [269-271]. CAT transporters comprise the functionally defined cationic amino acid system y^+ [269-272]. Based on Northern blot assays, cardiac and skeletal muscles are known to express CAT-1, CAT-2 and CAT-2a [269].

CAT-2 and CAT-2a are isoforms derived from mutually exclusive alternative splicing of the primary transcript, which differ only in a 41- to 42-amino acid segment located in the intracellular loop between transmembrane domains VIII and IX of the 14 transmembrane model (Figure 7) [271].

Transport of cationic amino acids via CAT-1 is voltage-dependent: hyperpolarization increases the Vmax and decreases the Km for influx, and the reverse for efflux [273]. For CAT-1, CAT-2 and CAT-2a, the transport is electrogenic (positive charge follows the cationic amino acid flux) and stereospecific [273-275]. In addition, CAT-1, CAT-2 and CAT-2a have variable trans-stimulation of arginine uptake, although trans-stimulation of CAT-2a is minimal [276-278].

Structural information on CAT transporters is scarce and most available data come from the analysis of mouse CAT-1. CAT transporters lack a characteristic signal peptide and in consequence the N-terminus is considered to be cytosolic [270, 272]. Hydrophobicity profiles predict either 12 or 14 transmembrane-spanning domains, although most data support the 14 transmembrane domain model [15, 279, 280] (Figure 7).

The specific role of the different CAT transporters expressed in muscle, CAT-1, CAT-2 and CAT-2a, is unknown. However, the kinetics of the different CAT transporters and the plasma and intracellular concentrations of cationic amino acids lead to the reasonable assumption that CAT-1 and CAT-2, if indeed they are expressed in muscle cells, participate in the uptake of cationic amino acids, whereas the low-affinity transporter CAT-2a might be more active in the efflux of lysine and arginine. In this regard, it is known that there is differential regulation of these carriers. Thus, fasting or stress induced in mice by surgical trauma, situations characterized by muscle protein catabolism and muscle release of cationic amino acids enhance CAT-2a gene expression in skeletal muscle in the absence of alterations in CAT-1 or in CAT-2 [281, 282]. Whether the up-regulation of CAT-2 gene expression contributes to muscle protein catabolism by enhancing the release of cationic amino acids from

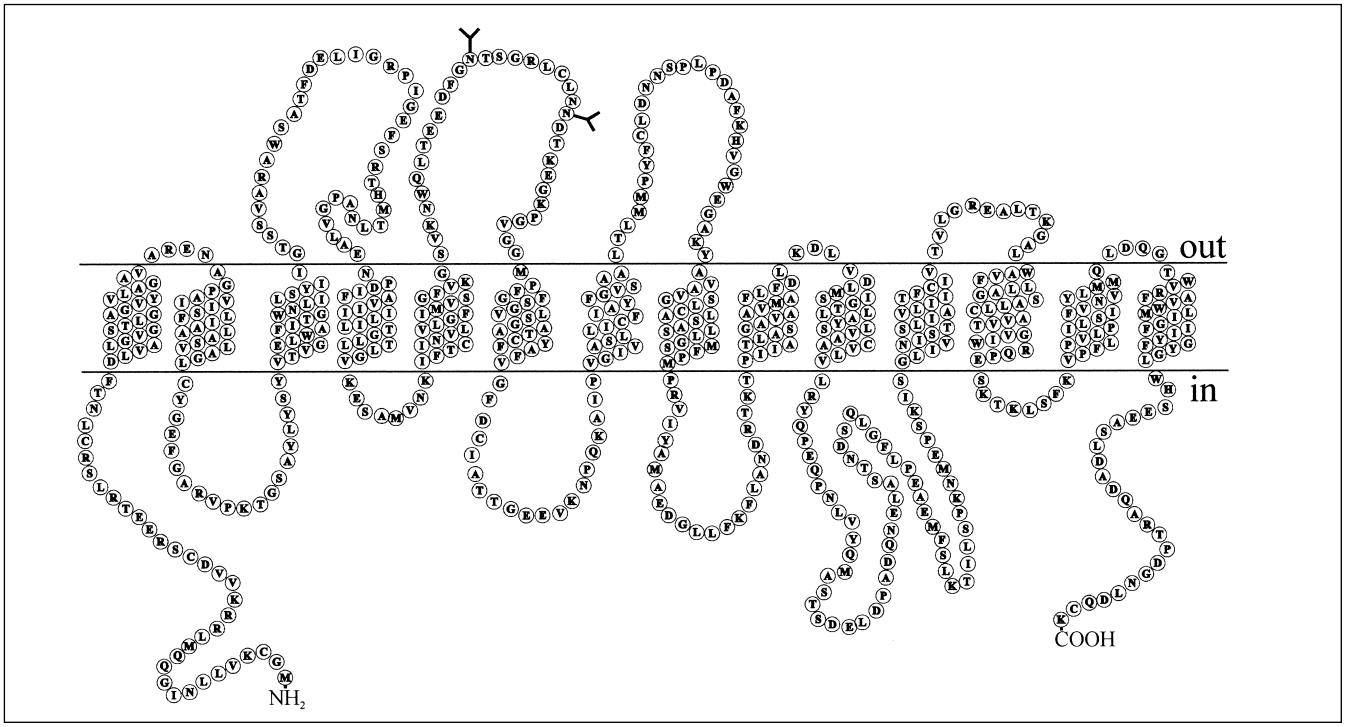

Figure 7. Proposed topology of human CAT-1 transporter.

muscle to the extracellular milieu or, alternatively, whether CAT-2a contributes to the buffering of protein catabolism by enhancing the expression of a low-affinity transporter, remains unknown.

Family of sodium- and chloride-dependent amino acid transporters

Different transporters belonging to this family have been isolated and catalyze the uptake of amino acids such as GABA, betaine, taurine, glycine or proline (Tables 1 and 2) (for review, see 15). The superfamily also includes transporters for neurotransmitters such as dopamine, serotonin or norepinephrine as well as the creatine transporter reviewed in section 3 [283, 284]. Sodium and chloride dependence have been reported for all these transporters. Skeletal muscle expresses substantial levels of mRNA corresponding to BGT-1 and TAUT [285, 286]; however, no further information is available. BGT-1 catalyzes the transport of GABA, betaine and L-proline as assessed in Xenopus oocytes, COS cells or 9HTEo cells [285, 287, 288]. TAUT transporter takes up taurine and β-alanine with high affinity after expression in *Xenopus oocytes*, COS7 cells or HeLa cells [289-292]. The function of these two transporters in muscle remains unknown; however, since taurine and betaine are non-perturbing osmolytes, they may counteract differences in osmolarity between muscle cells and the extracellular medium. In this regard, the activity of TAUT and BGT1 transporters is regulated in MDCK renal cells, RAW 264.7 cells or H4IIE rat hepatoma cells by tonicity [293-295].

The amino acid transporters in this family show high homology (at least 40%) in their primary structure, with nearly 150 well-conserved amino acid residues [15]. As general features, all these transporters lack a signal peptide, show a good prognosis for 12 transmembrane domains, with the N- and C-terminus located intracellularly, and one to four putative N-glycosylation sites between transmembrane domains 3 and 4, as shown for the creatine transporter CRT1 (Figure 1). This prognosis has been validated in different studies for GAT-1 and GLYT1 [296, 297].

Family of sodium-dependent transporters for anionic and zwitterionic amino acids

This family comprises five anionic amino acid transporters (EAAT1 to EAAT5) and two zwitterionic amino acid transporters (ASCT1 and ASCT2) (Table 2) which are sodium-dependent (for review, see 15). For a detailed explanation of the nomenclature used by researchers, see also reference 15. On the basis of sequence homology, this family can be subdivided into two subfamilies: the human EAAT isoforms (the anionic transporters), which show 36-65% amino acid sequence identity between them, and the human zwitterionic amino acid transporters ASCT1 and ASCT2 which show 57% identity between them. The characteristics of their expressed transport activity suggests that EAAT1-5 isoforms are variants of the system X^-_{AG}, and that ASCT1 and ASCT2 are variants of the system ASC.

Northern blot analysis indicates that skeletal muscle expresses EAAT1 and EAAT3 [298, 299]; EAAT5 may also be expressed in muscle, although the bands shown in Northern blot assays for skeletal muscle and heart were near 2.0 kb whereas the EAAT5 cloned from retina was 3.1 kb [300]. The precise distribution of EAATs in muscle tissue is unknown,

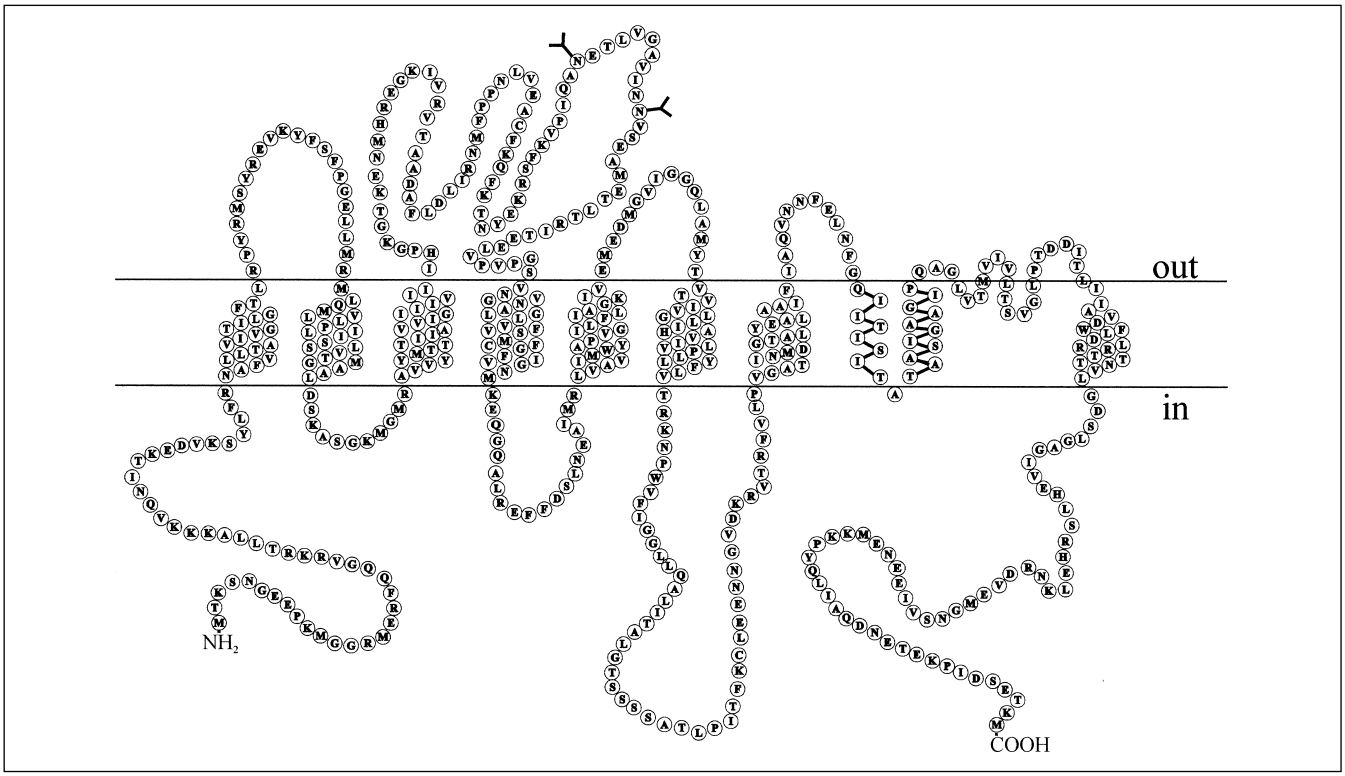

Figure 8. Proposed topology of human anionic amino acid transporter EAAT-1.

although they may participate in the muscle uptake of anionic amino acids. Muscle also expresses ASCT1 and ASCT2, which may contribute to the ASC transport activity reported in this tissue [301, 302].

The transporters of this family show potassium dependence in addition to sodium dependence when expressed in different cell systems, with the possible exception of the zwitterionic amino acid transporters [300, 303, 304]. Thus, the glutamate transporters are sodium cotransporters and potassium countertransporters. ASCT1 and ASCT2 are probably electroneutral sodium-dependent amino acid exchangers that do not interact with potassium. Surprisingly enough, some of these transporters (EAATs and ASCTs) function as a substrate (amino acid and sodium)-gated chloride channel in addition to their amino acid transport mode of action [300, 303, 305]. However, the molecular basis for this ligand-gated chloride channel activity remains unknown.

The main common features of the members of this family are: a) the absence of a cleavable signal sequence, suggesting a cytosolic localization of the N-terminus, b) the presence of two canonical sites for N-linked glycosylation on the extracellular loop between transmembrane domains 3 and 4, and c) the presence of six highly conserved putative membrane-spanning domains in the N-terminal half of the proteins (Figure 8). There has been controversy on the topology of these transporters, which has been resolved for the rat EAAT2 (named GLT-1) by using single cysteine mutants and assaying biotinylation [306]. Data indicate that rat EAAT2 has eight transmembrane domains; between the seventh and the eighth membrane-spanning domains, a structure reminiscent of a pore loop and an outward-facing hydrophobic linker are positioned [306]. This topology is presented for the isoform EAAT1, which is expressed in skeletal muscle, in Figure 8.

Family of heteromultimeric amino acid transporters

Very recently, a new family of amino acid transporters has been identified. All the members of this family are consist of light subunits, with structure compatible with a transporter, which must combine with a heavy subunit, either 4F2hc or rBAT, in order to be fully active [307-316]. Several members of this family have been identified and show many different types of sodium-independent amino acid exchange, which permit the uptake of zwitterionic, anionic or cationic amino acids (Table 1). Thus, LAT-1 and LAT-2 are variants of L type transport activity for neutral amino acids [307, 308, 311-313], y^+-LAT-1 and y^+-LAT-2 are variants of the transport system y^+-L for cationic amino acids [309, 310], $b^{0,+}$-AT functions as the transport system $b^{0,+}$ with affinity for both zwitterionic and cationic amino acids [314, 315], and xCT represents the transport activity system x^-_C for anionic amino acids [316]. It is likely that some additional isoforms will be found in mammalian tissues. Molecular genetics has revealed that rBAT, $b^{0,+}$-AT and y^+-LAT-1 are crucial in renal function and mutations in those genes have been identified in cystinuria and in lysinuric protein intolerance [314, 315, 317-319].

The only isoform so far described in skeletal muscle is LAT-2 [311]. Since co-expression of LAT-2 and 4F2hc in *Xenopus* oocytes induces L-type amino acid transport activity characterized by broad specificity for small (glycine, alanine, serine, threonine and cysteine) and large zwitterionic amino acids (leucine, isoleucine, phenylalanine, methionine, tyrosine, histidine, triptophan, valine, asparagine and glutamine) [311], LAT-2 may contribute to the sodium-indepen-

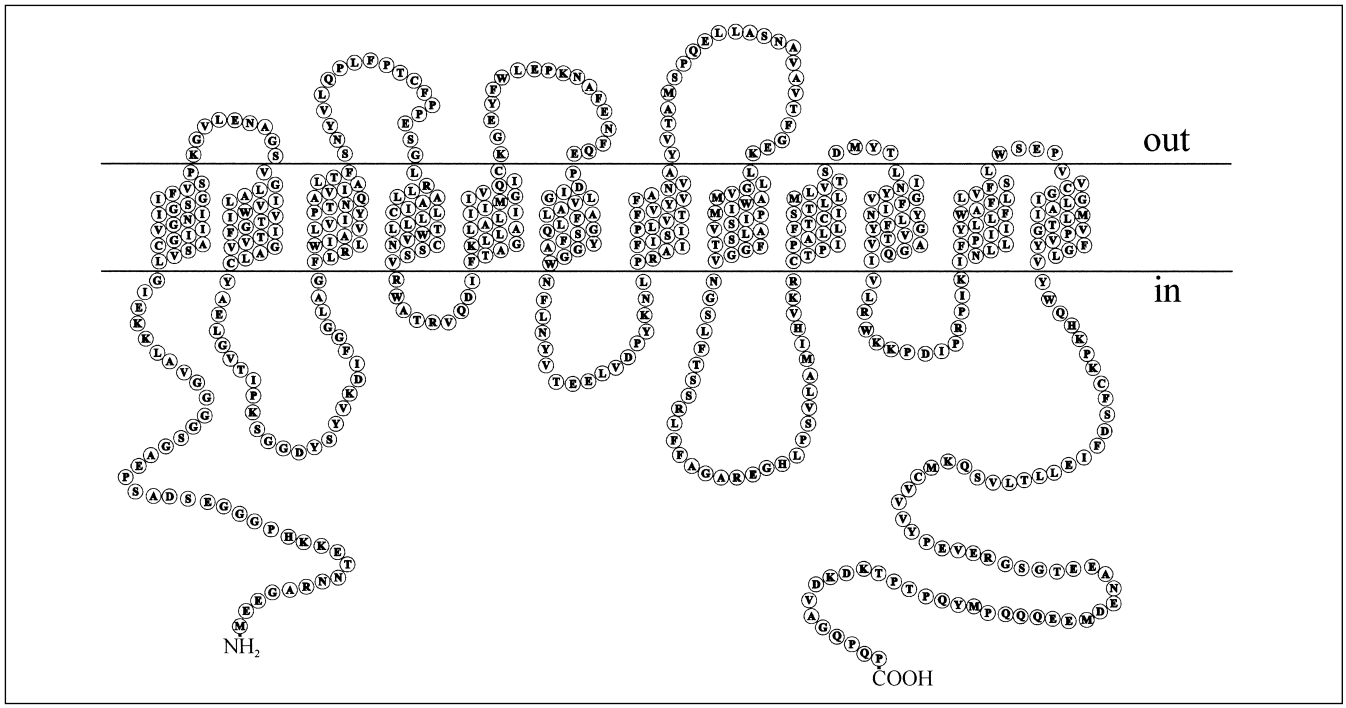

Figure 9. Proposed topology of human L-amino acid transporter-2, LAT-2.

dent uptake or release of these amino acids in muscle. Because of the quantitative importance of alanine and glutamine release by muscle in a variety of physiological conditions, we postulate that LAT-2 may contribute to this function in muscle. In addition, since LAT-2 works as an exchanger, it is likely that alanine or glutamine release is concomitant to the uptake of branched-chain amino acids.

Human LAT-2 shows an amino acid sequence identity of 50, 44 and 45% to human LAT-1, y^+-LAT-1 and y^+-LAT-2, respectively [311]. Hydrophobicity studies suggest 12 transmembrane domains with both the N- and C-terminal segments located intracellularly (Figure 9). There is only one putative N-glycosylation site between the putative transmembrane segments VIII and IX; however, according to the predictions, this segment is intracellular and additionally is not glycosylated in an *in vitro* microsomal system [308], which is in keeping with the observations on the rest of the family. No other experimental data have been produced to support the topological predictions.

LAT-2 interacts with 4F2hc (4F2 cell surface antigen heavy chain), an ubiquitous membrane protein. The formation of this complex (with unknown stoichiometry) permits the arrival of LAT-2 to the cell surface; expression of a tagged version of LAT-2 in *Xenopus* oocytes in the absence of 4F2hc causes its intracellular accumulation and non-arrival at the cell surface [311]. y^+-LAT-1 and 4F2hc are linked by disulfide bridges with residue cysteine 109 of human 4F2hc [311]. Protein 4F2hc has no membrane leader sequence so its N-terminus is located intracellularly and a single transmembrane domain is predicted, and biochemical evidence suggests that 4F2hc is a type-II integral membrane N-glycoprotein [320-322].

Future prospects

A major task in the field of plasma membrane transporters during the last few years has been the identification of their gene coding. In fact, there is still a lot of work to be done on the identification of the molecular biology of membrane transporters, since several important ones such as amino acid transporters A or N^m have not yet been identified, and more carnitine transporters or isoforms for known transporters may be identified in the future. The study of the biological properties of glucose transporters, identified in the middle to late eighties, has received much more attention because of their relationship to insulin action and insulin resistance. However, knowledge of the biology of membrane transporters other than glucose transporters is extremely scarce. In consequence, it is high time that the precise function in cells and thus in muscle metabolism of all the identified membrane transporters with a large metabolism impact was understood. This will be accomplished by analysis of the cell biology of these proteins, by generation of transgenic animals where the transporter is over-expressed in a tissue-dependent manner, and by the *in vivo* ablation of specific genes in a tissue-specific or time-dependent way. This is not an easy task, especially in skeletal muscle, due to the inherent difficulty of this tissue and the lack of good cell models; for example, much more is known about glucose transporters in adipose cells than in skeletal muscle, mostly for the reasons specified above.

These studies seeking to assess cellular function may also identify new target molecules for therapy. There is a need for new drugs to ameliorate insulin resistance in type-2 diabetes mellitus and obesity, to prevent and reverse cachexia induced by many different conditions, and to reverse muscle atrophy caused by immobility or aging. We now know that the GLUT4 glucose transporter is an appropriate target for therapy in insulin-resistant states and considerable efforts are being made to identify molecules that regulate the presence of this transporter at the cell surface. Perhaps some transporters reviewed in this article may regulate the size of the muscle fiber or permit the entry of molecules that are important for the proliferation or the differentiation of muscle cells.

Acknowledgements

We thank all our laboratory colleagues for enlightening daily discussions on plasma membrane transporters, and Robin Rycroft for editorial work. This work was supported in part by Dirección General de la Investigación Científica y Técnica (PB95/0971, PM96/0060 and PM98-0197), Fondo de Investigación Sanitaria (97/2101 and 00/1125), Fundació la Marató de TV3 (981310), Direcció General de la Recerca, Generalitat de Catalunya (1999SGR00039), Spain and by the projects BIOMED 2 CT98-BMH4-3514 and QLG-CT-1999-00295 from European Union.

Abbreviations

IGF-I, insulin-like growth factor-I; MEF-2, myocyte enhancer factor 2; SCAMP, secretory carrier membrane protein; tSNARE, soluble NSF attachment protein receptor at the target membrane; VAMP-2, vesicle-associated membrane protein-2; vSNARE, soluble NSF attachment protein receptor on the vesicle.

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The authors work at the Departament de Bioquímica i Biologia Molecular *in the Universitat de Barcelona.*

Manuel Palacín and Antonio Zorzano are Professors of Biochemistry and Molecular Biology at the Universitat de Barcelona. At present they are co-leading a research team of more than twenty scientists working on the molecular biology of membrane transporters and on the identification of new targets in the treatment of diabetes mellitus and aminoacidurias. So far, Dr. Palacín has identified three distinct genes that are responsible for the development of two types of cystinuria and lysinuric protein intolerance, and Dr. Zorzano has identified several mechanisms by which glucose transporters are regulated in insulin-sensitive tissues. As a result of their joint effort, they have published nearly 100 research articles: the accumulated impact factor of their published work during the last five years is more than 360 with an average impact factor per article of more than 7.

At present, they hold public funding from different institutions such as the Generalitat de Catalunya, Fundació la Marató de TV3, Ministerio de Educación y Cultura, Fondo de Investigación Sanitaria (Spain) and from the European Community. They also maintain active links with different pharmaceutical companies.

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They also have a strong commitment to university teaching. Thus, Dr. Zorzano was the first Director of the Studies of Biochemistry (from 1992 to 1995) at the Universitat de Barcelona. Dr. Palacín was Main Advisor of the Studies of Human Biology at the Universitat Pompeu Fabra from 1996-98. In addition, they have supervised a total of 15 Ph.D. thesis during the last 10 years.

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