C-terminal Mutations That Alter the Turnover Number for 3-O-Methylglucose Transport by GLUT1 and GLUT4*

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Robin Dauterive, Stephen Laroux, Robert C. Bunn, Audra Chaisson, Trudy Sanson, and Brent C. Reed‡

From the Department of Biochemistry and Molecular Biology, Louisiana State University School of Medicine,
Shreveport, Louisiana 71130

Turnover numbers for 3-O-methylglucose transport by the homologous glucose transporters GLUT1 and GLUT4 were compared to those for truncated and chimeric transporters expressed in Xenopus oocytes to assess potential regulatory properties of the C-terminal domain. The ability of high intracellular sugar concentrations to increase the turnover number for sugar entry (“accelerated exchange”) by GLUT1 and not by GLUT4 was maintained in oocytes. Replacing the GLUT1 C terminus with that of GLUT4 stimulated turnover 1.6-fold, but abolished accelerated exchange. Thus, the GLUT1 C terminus permits accelerated exchange by GLUT1, but in doing so must interact with other GLUT1 specific sequences since the GLUT4ctrm1 chimera did not exhibit this kinetic property. Removal of 38 C-terminal amino acids from GLUT4 reduced its turnover number by 40% whereas removing only 20 residues or replacing its C terminus with that of GLUT1 increased its turnover number 3.5–3.9 fold. Therefore, using mechanisms independent of those which alter transporter targeting to the plasma membrane, C-terminal mutations in either GLUT1 or GLUT4 can activate transport normally restricted by the native C-terminal domain. These results implicate the C termini as targets of physiological factors, which through covalent modification or direct binding might alter C-terminal interactions to regulate intrinsic GLUT1 and GLUT4 transporter activity.

GLUT1 and GLUT4 are two members of a larger family of facilitative glucose transporters. GLUT1 is expressed in most cell culture lines and in many tissues, whereas GLUT4 is restricted primarily to muscle and adipose tissue (for review, see Ref. 1). Two factors determine the relative effectiveness by which GLUT1 or GLUT4 catalyze sugar transport: (a) the extent to which each transporter is targeted to the cell surface, and (b) their kinetic properties once resident in the plasma membrane. The amino acid sequences of the two transporters share 66% identity and 13% similarity, while non-conservative differences are localized to five domains, which include the N terminus, the large extracellular loop, a portion of transmembrane domains II and III, the large cytosolic loop, and the C terminus (see Fig. 1). An understanding of which of these domains account for the unique targeting and kinetic properties that distinguish GLUT1 and GLUT4 behavior is evolving rapidly.

Cellular control of GLUT4 transporter targeting to internal or plasma membrane compartments is a major mechanism for regulating glucose transport in insulin-responsive tissues. GLUT4, unlike GLUT1, does not undergo an N-terminal internalization sequence, which increases the efficiency of retrieval of GLUT4 relative to GLUT1 from the plasma membrane (2), and a C-terminal sequence, which restricts GLUT4 to intracellular sites if insulin is absent (3–6). When insulin is present, a higher proportion of intracellular GLUT4 than GLUT1 is re-distributed to the plasma membrane to facilitate glucose transport across the plasma membrane (7–11).

The turnover numbers for sugar transport by GLUT1 and GLUT4 are roughly comparable (12, 13), while the Km for GLUT1 is typically higher than that of GLUT4 (12–17). GLUT1 demonstrates a unique kinetic property of “accelerated exchange” in which the Vmax for transport of sugar into a cell or vesicle is much higher when measured under equilibrium exchange than under “zero-trans” conditions when little or no intracellular sugar is present (15, 18–22). The stimulation of sugar influx by a high intracellular concentration of glucose does not require translocation of additional transporters to the plasma membrane. Rather, it occurs through an increase in the rate constant for conversion of the transporter from an inward to outward facing conformation when the inward facing binding site becomes occupied by sugar. This property is not observed with GLUT4 (16, 17).

Both transporters, once resident in the plasma membrane, can exhibit different levels of activity, which are regulated by translocation-independent mechanisms (23–29). The structural features of GLUT1 and GLUT4 that are required for these forms of regulation are not known, although several reports emphasize the importance of the C-terminal domain (26, 29–31). To characterize transport regulatory features that might reside within the C termini, we have compared, using Xenopus oocytes, the kinetic parameters for sugar transport by cell-surface native and mutated GLUT1 and GLUT4 transporters in which the C-terminal domains were interchanged or truncated.

MATERIALS AND METHODS

Plasmid Constructions—Each sequence encoding the native, chimeric, or truncated transporters was inserted into the BglII site of pSP64T (32) and retains the 5′- and 3′-untranslated region of Xenopus hemoglobin in the final transcript (Fig. 1). The transcripts of plasmids pSP64T/GLUT1 (33) and pSP64T/GLUT4M, which contains a silent Ile-479(ATT) to Ile-479(ATA) mutation, yield, respectively, native GLUT1 and GLUT4 after translation. The transcript from pSP64T/GLUT1ctrm4 encodes a transporter with amino acids 1–444 of GLUT1 and 461–509 of GLUT4, while that from pSP64T/GLUT4ctrm1 encodes
a transporter with amino acids 1–460 of GLUT4 and 445–492 of GLUT1 containing amino acids 1–455. Glut4 contains amino acids 1–471. Plasmid pSP64T/GLUT4M2 with Leu-490(TTA) of GLUT4 converted to STOP-472(TAA) produces truncated GLUT4 containing amino acids 1–460 of GLUT4 and 445–492 of GLUT1. The non-conservative amino acid substitutions effected by the switch occur in the cytosolic C-terminal domain except the interchange of GLUT4 leucine 466 with GLUT1 phenylalanine 450 at the junction of the transmembrane domain XII and the cytosolic C-terminal domain (Fig. 1).

To verify correct translation of the mutant constructs, GLUT1, GLUT4, GLUT1ctrm4, and GLUT4ctrm1 messages were translated in vitro and the transporter products analyzed by SDS-PAGE. As anticipated, the mobility of each of the smaller GLUT1 and GLUT1ctrm4 translation products was faster than that for GLUT4 or GLUT4ctrm1 (Fig. 2A). Antibody RE01 recognized the C terminus of GLUT4 and GLUT1ctrm4, but not GLUT1 or GLUT4ctrm1, while antibody RE09 recognized the large extracellular loop of GLUT4 and GLUT4ctrm1 but not GLUT1 or GLUT1ctrm4. Antibody RE15 recognized the N terminus of GLUT4, Peptide (amino-C)LINRNEENRAKS-amide for antibody RE18 against the large cytosolic loop of GLUT1 was coupled to keyhole limpet hemocyanin using glutaraldehyde (40, 41). Residues in parentheses are in the native mouse transporter sequence.

**RESULTS**

Effects of Interchanging the C Termini of GLUT1 and GLUT4 on Transport Activity—The C-terminal domain and part of the transmembrane domain XII of GLUT1 (amino acids 1–449) and of GLUT4 (amino acids 460–509) were interchanged at phenylalanine 450 of GLUT4 and phenylalanine 444 of GLUT1 to generate the two chimeras, GLUT1ctrm4 and GLUT4ctrm1. The non-conservative amino acid substitutions effected by the switch occur in the cytosolic C-terminal domain except the interchange of GLUT4 leucine 466 with GLUT1 phenylalanine 450 at the junction of the transmembrane domain XII and the cytosolic C-terminal domain (Fig. 1).

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Transport properties determined for the native and chimeric transporters expressed in Xenopus oocytes are presented in Table I. Under zero-trans conditions, the \( V_{\text{max}}(zt) \) for uptake of sugar by native GLUT1 was 7.6 times that of native GLUT4 and 2-fold that of GLUT1ctrm4. The \( V_{\text{max}}(zt) \) for GLUT4ctrm1 was 6.3 times that of native GLUT4. No statistical difference (\( p > 0.05 \)) was observed between the \( K_{\text{m}}(zt) \) values for zero-trans uptake of 3-O-methylglucose, which averaged 9.3 mM for both the native and chimeric transporters.

In erythrocytes the \( V_{\text{max}}(ee) \) for sugar transport by GLUT1 under equilibrium exchange conditions is typically higher than the corresponding \( V_{\text{max}}(zt) \) for influx measured when the intracellular concentration of sugar is low (20–22, 42). This property is qualitatively retained by the mouse GLUT1 transporter when expressed in Xenopus oocytes (Table I). At 22°C the \( V_{\text{max}}(ee) \) for GLUT1 was 2.5 times the \( V_{\text{max}}(zt) \) (\( p < 0.016 \)), while the \( K_{\text{m}}(ee) \) was 4.1 times the \( K_{\text{m}}(zt) \) (\( p < 0.001 \)). In contrast, the \( V_{\text{max}}(ee) \) and \( V_{\text{max}}(zt) \) (influx) for transport by GLUT4 expressed in Xenopus oocytes were not statistically different (\( p > 0.05 \)). The \( K_{\text{m}}(ee) \) for transport by GLUT4, however, was significantly higher than the \( K_{\text{m}}(zt) \) (\( p < 0.020 \)).

Although the chimeric transporters GLUT1ctrm4 and GLUT4ctrm1 retained transport activity, replacement of the C terminus of GLUT1 with that of GLUT4 abolished accelerated exchange since the \( V_{\text{max}}(zt) \) and \( V_{\text{max}}(ee) \) for GLUT1ctrm4 were indistinguishable (Table I). Replacement of the C terminus of GLUT4 with that of GLUT1, however, did not confer accelerated exchange upon the GLUT4ctrm1 chimera, since its values of \( V_{\text{max}}(zt) \) and \( V_{\text{max}}(ee) \) for transport (like those for GLUT4 and GLUT1ctrm4) were statistically indistinguishable. While the \( K_{\text{m}}(ee) \) for GLUT1 was significantly higher (\( p < 0.003 \)), no significant differences (\( p > 0.05 \)) between the \( K_{\text{m}}(ee) \)'s for GLUT1ctrm4, GLUT4ctrm1, and GLUT4 were observed. The GLUT1ctrm4 and GLUT4ctrm1 chimeras generated by the interexchange, therefore, retained high transport activity but did not retain or gain, respectively, GLUT1's ability to exhibit accelerated exchange.

Effect of C-terminal Truncation of GLUT4 on Transport Activity—Two truncated forms of GLUT4 were constructed, GLUT4M1 and GLUT4M2, which are missing 38 and 20 C-terminal amino acids, respectively (Fig. 1). To confirm translation of the proper mutant transporters, [35S]methionine-labeled transporters from oocytes were analyzed by SDS-PAGE (Fig. 2B). The two sizes of transporter present in each immunoprecipitation represent different states of glycosylation of the transporter (43). Antibodies against the N terminus (Fig. 2B, left panel) and against the large extracellular loop of GLUT4 (data not shown) recognized each of the three transporters. As predicted, the mobility of GLUT4M1 was faster than that of GLUT4M2, and GLUT4M2 migrated faster than native
GLUT4. Only native GLUT4, but neither GLUT4M1 nor GLUT4M2, was immunoprecipitated by antibody against the GLUT4 C terminus (Fig. 2B, right panel). These results confirm the absence of C-terminal epitope in the truncated mutants.

At 0.5 mM 3-O-methylglucose, the zero-trans rates of sugar uptake by either native GLUT4 or truncated GLUT4M1 expressed in oocytes were indistinguishable (p > 0.05), but both were significantly higher than that of water injected oocytes (Table I). Interestingly, the rate of 3-O-methylglucose uptake by oocytes expressing GLUT4M2 was 3.6 times that of oocytes expressing native GLUT4 (p < 0.04). The V_{max(zt)} for GLUT4M1 was 30% lower than that of GLUT4 (p < 0.038), while the V_{max(zt)} for GLUT4M2 was 4.5 times that of native GLUT4 (p < 0.001). The values of K_{m(zt)} obtained for GLUT4, GLUT4M1, and GLUT4M2 ranged between 7.6 and 9.3 mM, but were not statistically different (p > 0.05). Thus, without altering K_{m(zt)}, removal of 38 C-terminal amino acids of GLUT4 only minimally reduces transport activity, while removal of 20 amino acids dramatically increases transport rates.

Comparison of the Plasma Membrane Distribution and Concentration of Native and C-terminal Mutants of GLUT1 and GLUT4 Expressed in Xenopus Oocytes—The relative surface concentrations of the native and mutant transporters were determined to assess the extent to which altered transport rates associated with each mutation reflected an alteration of the transporter’s intrinsic catalytic properties (turnover number), rather than simply a change in its cell-surface concentration. The antibodies required for this assay reacted poorly with Western blots; therefore, the amounts of [35S]methionine-labeled transporters present in total or isolated plasma membranes were quantitated by immunoprecipitation and SDS-PAGE (Fig. 3A). The percentage of total transporter recovered in the plasma membranes is presented in Fig. 3B. These distribution data provided the first internal control for quantifying the relative transporter concentrations in the plasma membrane. The measured transporter distributions should be (and are) consistent with those of previous studies which established that transporters containing the GLUT4 C terminus, i.e. GLUT4 and GLUT1ctrm4, display lower percentages in the plasma membrane than transporters such as GLUT1, GLUT4M1, GLUT4M2, or GLUT4ctrm1 missing all or a portion of this domain (Fig. 3B). The truncation mutant GLUT4M2 should (and does) exhibit a plasma membrane distribution higher (2-fold) than that of native GLUT4 after disruption of the dileucine motif in the C terminus known to be critical for efficient sequestration of GLUT4 (4–6). Although lacking an intact GLUT4 C terminus, neither GLUT4M1, GLUT4M2, nor GLUT4ctrm1 transporter attains as high a distribution in the plasma membrane as exhibited by native GLUT1. This also is consistent with their retention of N-terminal sequences present in GLUT4 and lacking in GLUT1, which partially contribute to the internalization and/or sequestration of GLUT4 (4–6).

The data in Fig. 3B do not provide an accurate comparison of the relative plasma membrane concentrations for each transporter, since the total amounts of each expressed transporter protein were comparable, but not identical. Rather, the transporter concentrations in the plasma membrane were calculated as described under “Materials and Methods” using the integrated intensities of the transporter bands from isolated plasma membranes (Fig. 3A, right panel) and the concentration expressed relative to GLUT4 (Fig. 3C). These data provide the second internal control to validate proper quantitation of each surface transporter. The surface ratio (GLUT1/GLUT4) was established by Nishimura and colleagues (13) both by isolating plasma membrane/vitelline membrane complexes from [35S]methionine-labeled oocytes (as in this study) and by labeling GLUT1 and GLUT4 on the surface of oocyte plasma membranes with ATB-BMPA. The ratio GLUT1/GLUT4 in the isolated plasma membranes determined in this study (Fig. 3C) was 3.5 and agrees well with the value of 3.8 determined by
The observed difference in ratio can be accounted for by at most an 8% contamination of the GLUT4 in the plasma membrane by GLUT4 from internal membrane sources. Thus, the selected techniques effectively separate the plasma membrane from internal vesicles containing GLUT1 or GLUT4, and reveal that the concentrations of GLUT4M1, GLUT4M2, and GLUT1ctrm4 transporters in the plasma membrane of Xenopus oocytes were not statistically different than that of GLUT4 (p > 0.05), while those of GLUT1 (GLUT1/GLUT4 ratio = 3.5) (p < 0.001) and GLUT4ctrm1 (GLUT4ctrm1/GLUT4 ratio = 1.8) (p < 0.019) were significantly higher (Fig. 3C).

Relative Turnover Numbers for Sugar Transport by Native and C-terminal Mutant GLUT1 and GLUT4 Transporters—To compare the turnover numbers of each transporter, the measured value of $V_{\text{max(ee)}}$ (Table I) for each transporter was corrected appropriately for a higher or lower level of cell-surface protein expression relative to GLUT4 by dividing its $V_{\text{max(ee)}}$ by the ratio (Fig. 3C) of its cell-surface concentration to that of GLUT4. Such normalized values reflect the projected value of...
Our demonstration that mouse GLUT1M1 was calculated to be 23% that of mouse GLUT1. Unlike the mutated rabbit transporter, significant transport capacity is retained by the truncated mouse GLUT1.

The 4-fold higher turnover number for the GLUT4ctrm1 chimera could have arisen from a transport activating property of the GLUT1 C terminus, since GLUT1 in our study is more active than GLUT4. However, since the turnover number for the truncated transporter GLUT4M2 was also nearly 4 times that of the native transporter (Fig. 3D), a more reasonable interpretation for the turnover numbers for both GLUT4ctrm1 and GLUT4M2 being higher than GLUT4 is that in both instances an inhibitory domain has been removed, which includes all or a subset of amino acid residues 490–509 of GLUT4. Likewise, the higher turnover number observed for GLUT4ctrm4 arises from the removal of the inhibitory region present in the GLUT1C terminus and not through activation by the GLUT4 C terminus, since the latter was derived from the less active GLUT4 transporter.

Both GLUT1 and GLUT4 C-terminal domains, therefore, suppress native transport capacity and for GLUT4 this inhibitory region has been localized to the C-terminal 20 amino acids. The physiological role of the inhibitory domain in GLUT4 is unclear. The C-terminal 29 amino acid residues of rat GLUT4 must be present for analogs of cAMP and AMP to inhibit GLUT4 transport in CHO cells, and transfer of this domain to GLUT1 generated a GLUT1ctrm4 chimera, which acquired nucleotide analog-induced inhibition of sugar transport (29). It appears unlikely that C-terminal modifications in GLUT4 are responsible for the inhibitory activity of the C-terminal domain, since the latter was derived from the less active GLUT4 transporter.

Effect of Truncation at Lys-456 on the Turnover of 3-O-Methylglucose by Mouse GLUT1—Our demonstration that mouse GLUT4M1 retained 60% of the turnover capacity of native mouse GLUT4 was unexpected, since truncation of rabbit GLUT1 at the same relative position in the C terminus generated a transporter that was totally inactive in CHO cells (30) and in Xenopus oocytes (44). The transport capacity of truncated GLUT1, therefore, was re-evaluated using mouse GLUT1M1, which contains the same Lys-456 STOP mutation used to truncate rabbit GLUT1 (Fig. 1). Uptake of 0.5 mM 3-O-methylglucose by oocytes expressing GLUT1M1 was 10-fold higher than that of water injected oocytes (Fig. 4A), while the V\text{max}_{(zt)} for GLUT1M1 was 14% that of GLUT1. When corrected for the differences in plasma membrane content of the two transporters (Fig. 4B), the relative turnover number for mouse GLUT1M1 was calculated to be 23% that of mouse GLUT1. Unlike the mutated rabbit transporter, significant transport capacity is retained by the truncated mouse GLUT1.

**DISCUSSION**

The C-terminal domains of GLUT1 and GLUT4 indirectly regulate cellular sugar uptake because they serve as recognition sequences for the cellular machinery that controls sorting to intracellular or plasma membrane sites. This study was initiated to determine whether these domains, apart from their sorting roles, might also function to regulate transport activity (turnover number) of transporters resident in the plasma membrane. The first 11 amino acids adjacent to transmembrane domain XII in the two C termini contain 1 similar and 10 identical amino acid residues (Fig. 1). Of the remaining C-terminal amino acids, 50% of the differences represent non-conservative changes. Thus, the C-terminal domains of GLUT1 and GLUT4 contain both conserved and divergent regions, which could confer either shared or unique regulatory properties to each of the transporters.

To examine the properties of the divergent sequences, the C-terminal domains were interchanged to alter, in effect, all dissimilar amino acids distal to Phe-444 (GLUT1) and Phe-460 (GLUT4). The resultant chimeric transporters GLUT1ctrm4 and GLUT4ctrm1 exhibited turnover numbers higher than those of the respective native GLUT1 and GLUT4 (Fig. 3D). The 4-fold higher turnover number for the GLUT4ctrm1 chimera has a turnover number higher than those of the respective native GLUT1 and GLUT4 (Fig. 3D). The resultant chimeric transporters GLUT1ctrm4 and GLUT4ctrm1 exhibited turnover numbers higher than those of the respective native GLUT1 and GLUT4 (Fig. 3D). The general conclusion from these experiments is that the inhibitory region has been removed, which includes all or a subset of amino acid residues 490–509 of GLUT4. Likewise, the higher turnover number observed for GLUT4ctrm4 arises from the removal of the inhibitory region present in the GLUT1 C terminus and not through activation by the GLUT4 C terminus, since the latter was derived from the less active GLUT4 transporter.

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from an alteration of the C-terminal 22 amino acids of GLUT1. We have demonstrated the respective ability and inability of GLUT1 (15) and GLUT4 (16, 17) to exhibit accelerated exchange in their native cell environments is retained when the two transporters are expressed in Xenopus oocytes. Therefore, the turnover number for sugar entry catalyzed by GLUT1 can be elevated 2.5-fold ($V_{\text{max}}/V_{\text{max}}^\text{ee} = 2.5$; Table I) or higher (46) in the oocyte environment simply by raising the intracellular sugar concentration. No previous reports to our knowledge have identified regions of amino acid diversity that account for such “accelerated exchange” kinetics exhibited by GLUT1 and not by GLUT4. This study demonstrates that replacing the C-terminal domain of GLUT1 with that of GLUT4 abolishes accelerated exchange. More importantly, this kinetic property is not transferred with the C-terminal domain of GLUT1 when it replaces that of GLUT4. Apparently, a prerequisite for accelerated exchange kinetics is the ability of unique sequences within the C-terminal domain of GLUT1 to interact specifically with one or more unique sequences in another domain of GLUT1. This might occur directly or in combination with other regulatory factors to establish a low rate of conversion of the transporter from the inward facing to the outward facing conformation by forming an energy barrier to changes in transporter conformation when the inward facing site of the transporter is unoccupied by sugar. These restrictive interactions, which presumably are disrupted to activate transport when internal sugar concentrations are raised, might be the same interactions that are destroyed when the GLUT1 C terminus is replaced by that of GLUT4 to simultaneously activate transport and abolish accelerated exchange.

The divergent sequences of GLUT1 and GLUT4, therefore, express differences in the degree to which they suppress transport and their involvement in accelerated exchange. Do the regions of amino acid identity in the C termini impart a common trait to GLUT1 and GLUT4? The amino acids in a “core” region common to both GLUT1 and GLUT4 (Fig. 1B, shaded area) have been considered essential to GLUT1 function. While the loss of 12 or 22 C-terminal amino acids from GLUT1 appears inconsequential, removal of the entire C-terminal 42 amino acids destroys >95% of the activity (46), and loss of the C-terminal 37 amino acids of rabbit GLUT1 totally inactivates the transporter by locking it into an inward facing conformation (30, 44). Our observations indicate that one or more of the identical amino acids and not the dissimilar amino acids within residues 457–468 of GLUT1 or 473–484 of GLUT4 are critical for normal transporter function since interchange of the C termini of mouse GLUT1 and GLUT4 only enhances sugar turnover. Although the high amino acid identity within the “core” region would predict that truncation of GLUT4 at the same site as rabbit GLUT1 should totally inactivate mouse GLUT4, this was not observed. The turnover number for GLUT4M1 remained 60% that of native GLUT4. Since this result was unexpected, we reevaluated the effect of truncation of GLUT1 at Lys-456 on transport activity using the mutant mouse GLUT1M1 and determined the turnover number for mouse GLUT1M1 to be >20% that of native mouse GLUT1. It is unclear why such subtle species differences should render the truncated rabbit mutant totally inactive yet allow the truncated mouse mutant to retain significant activity. Perhaps one or more of the 17 amino acid residues dispersed throughout the rabbit transporter which differ from those of the mouse transporter render the rabbit isoform more sensitive to C-terminal truncation within this region. Is this “core” region of amino acid identity therefore less important to GLUT4 transporter function since GLUT4M1 retains 60% of the transport capacity of native GLUT4? Apparently it is not. A better appreciation of the common role of this region in GLUT1 and GLUT4 arises when comparing the turnover number of GLUT4M1 to that of GLUT4M2. When the activated mutant of GLUT4 (GLUT4M2) is truncated further to form GLUT4M1, 84% of GLUT4M2 turnover capacity is lost. This is comparable to the 77% loss of GLUT1 activity observed after truncation to form the mutant GLUT1M1. Thus the common amino acids distal to Lys-456 of GLUT1 and Arg-472 of GLUT4 are not absolutely essential for function in the respective transporters, but do permit each transporter to express a 4-5-fold higher turnover capacity. Truncated GLUT4M1 appears nearly as active as native GLUT4 only because of the presence in GLUT4 of the C-terminal inhibitory domain, which restricts the full transport potential of native GLUT4.

In summary, the C-terminal domains of mouse GLUT1 and especially mouse GLUT4 limit the maximal transport capacity of the respective transporters in which they reside. Stimulation of GLUT4 activity can occur either by removing 20 amino acids from the C terminus or by replacing the GLUT4 C terminus with that of GLUT1. Higher transport rates by GLUT1 can be induced simply by raising the internal sugar concentration or by replacing the C-terminal domain with that of GLUT4. Each of these methods of activation have relatively little physiological basis, but share the common property that they may serve to alter interactions of the native C-terminal domain with other domains of the transporter and/or with other cytosolic proteins or factors which serve to regulate transporter activity. This is especially relevant in view of the recent identification of proteins capable of binding directly to fusion proteins containing either GLUT1 or GLUT4 C termini (47). Several physiologically relevant examples of transport regulation which occur in cells by mechanisms independent of GLUT1 or GLUT4 redistribution were cited in the introduction. Perhaps these forms of activation target and alter C-terminal domain interactions to shift the transporters from a less to more active state as were accomplished by the mutations. Cited studies and our results emphasize the importance of investigating further the potential role of the C-terminal domain as a target for physiological mechanisms that regulate sugar turnover through activation of the transporter resident in the plasma membrane, an important form of regulation separate from the well documented activation of cellular sugar uptake via transporter redistribution.

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