Caveolin-3, a muscle-specific caveolin-related protein, is the principal structural protein of caveolae membrane domains in striated muscle cell types (cardiac and skeletal). Autosomal dominant limb girdle muscular dystrophy (LGMD-1C) in humans is due to mutations within the caveolin-3 gene; (i) a 9-base pair microdeletion that removes three amino acids within the caveolin scaffolding domain (ΔTFT) or (ii) a missense mutation within the membrane spanning domain (P → L). The molecular mechanisms by which these two mutations cause muscular dystrophy remain unknown.

Here, we investigate the phenotypic behavior of these caveolin-3 mutations using heterologous expression. Wild type caveolin-3 or caveolin-3 mutants were transiently expressed in NIH 3T3 cells. LGMD-1C mutants of caveolin-3 (ΔTFT or P → L) were primarily retained at the level of a perinuclear compartment that we identified as the Golgi complex in double-labeling experiments, while wild type caveolin-3 was efficiently targeted to the plasma membrane. In accordance with these observations, caveolin-3 mutants formed oligomers of a much larger size than wild type caveolin-3 and were excluded from caveolae-enriched membrane fractions as seen by sucrose density gradient centrifugation. In addition, these caveolin-3 mutants were expressed at significantly lower levels and had a dramatically shortened half-life of ~45–60 min. However, caveolin-3 mutants were palmitoylated to the same extent as wild type caveolin-3, indicating that targeting to the plasma membrane is not required for palmitoylation of caveolin-3. In conclusion, we show that LGMD-1C mutations lead to formation of unstable high molecular mass aggregates of caveolin-3 that are retained within the Golgi complex and are not targeted to the plasma membrane. Consistent with its autosomal dominant form of genetic transmission, we demonstrate that LGMD-1C mutants of caveolin-3 behave in a dominant-negative fashion, causing the retention of wild type caveolin-3 at the level of the Golgi. These data provide a molecular explanation for why caveolin-3 levels are down-regulated in patients with this form of limb girdle muscular dystrophy (LGMD-1C).
9-base pair microdeletion that removes the sequence TFT from the caveolin scaffolding domain; and (ii) a missense mutation that changes a proline to a leucine (P → L) in the transmembrane domain (34). Both mutations lead to a loss of ~85–90% of caveolin-3 protein expression.

The identity of the CAV3 lesions in these LGMD families is instructive. Of the 12 amino acid residues that are invariant in all three human caveolins as well as the two Caenorhabditis elegans homologs, two of these invariant residues are affected by the mutations identified in these two Italian families. One of the invariant prolines is changed to leucine in family A, while one of the invariant phenylalanines is deleted in family B (34). In addition, alanine scanning mutagenesis of a peptide encoding the caveolin scaffolding domain reveals that the FTV(35) sequence in caveolins 1 and 3 is important for the correct recognition of certain caveolin-binding signaling molecules in vitro (35, 36), and the FT residues in this sequence are deleted in family B. This finding provides genetic evidence that this region of the caveolin scaffolding domain is critical in vivo.

One hypothesis is that down-regulation of the caveolin-3 protein in patients with LGMD-1C may reflect the targeting of misfolded caveolin-3 oligomers to a degradative pathway. Here, we evaluate the phenotypic behavior of these caveolin-3 mutants using heterologous expression in NIH 3T3 cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—Antibodies and their sources were as follows: anti-caveolin-3 IgG (mouse mAb 26; Ref. 14), gift of Dr. Roberto Campos-Gonzalez, Transduction Laboratories; anti-Cab45 IgG (rabbit pAb; Ref. 37), gift of Dr. Philip E. Scherer, Albert Einstein College of Medicine. [9,10-3H]palmitic acid was from American Radiolabeled Chemicals, Inc. EXPRE35S protein-labeling mix (containing L-[35S]methionine) was from NEN Life Science Products. All other biochemicals used were of the highest purity available and were obtained from regular commercial sources.

**Construction of Untagged LGMD-1C Caveolin-3 Mutants**—The cDNA encoding wild type caveolin-3 (Cav-3/WT; Ref. 12) was cloned into the pCAGGS expression vector (gift of Dr. Armin Rehm, Ploegh Laboratory, Harvard Medical School). The following two caveolin-3 mutants were generated: (i) replacement of proline at amino acid position 104 with leucine (Cav-3/P-L) and (ii) deletion of amino acids threonine-phenylalanine-threonine (amino acids 63–65) (Cav-3/ent). Both caveolin-3 mutants were generated by polymerase chain reaction amplification using appropriate internal primers and subcloned into the pCAGGS expression vector. The correctness of intended base substitutions and the absence of unwanted mutations was verified by DNA sequencing.

**Cell Culture and Transient Transfection**—NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with glutamine, antibiotics (penicillin and streptomycin), and 10% donor bovine calf serum. Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% dialyzed fetal bovine serum, 5 mM sodium pyruvate, antibiotics, and L-glutamine. Cells were washed twice with PBS and lysed 30 min at 4 °C in a buffer containing 10 mM Tris, pH 8.0, 0.15 M NaCl, 5 mM EDTA, 1% Triton X-100, 60 mM octyl glucoside. Samples were preheated for 1 h at 4 °C using protein A-Sepharose (20 µl, 50% slurry) and subjected to immunoprecipitation using anti-caveolin-3 antibody (10 µl, mAb 26) and protein A-Sepharose (30 µl, 50% slurry). After three washes with the immunoprecipitation buffer, samples were separated by SDS-PAGE (12.5% acrylamide), and the gel was treated with 1M sodium salicylate for 30 min to maximize substrate sensitivity. The gel was dried at 70 °C for 1 h and exposed on Kodak X-omat NT film at ~70 °C.

**Measurement of the Turnover Rate of Caveolin-3 Mutants**—In order to determine the half-life of transiently expressed caveolin-3/WT, caveolin-3/P → L, and caveolin-3/ent/TTFT, COS-7 cells were labeled 24 h post-transfection for 1 h with 50 µCi/ml EXPRES35S protein-labeling mix (L-[35S]methionine) in methionine-free culture medium (46). Cells were collected at the end of the labeling period (time 0) and 1, 7, 24, and 48 h after replacement of the culture medium with fresh normal growth medium and processed for immunoprecipitation as described above.

**Co-expression of Tagged Wild Type and Mutant Forms of Caveolin-3**—To examine whether LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, we co-transfected NIH 3T3 cells with C-termially Myc-tagged WT caveolin-3 and three different N-terminally GFP-tagged forms of caveolin-3 (WT, P → L, or DTFT). Briefly, the GFP-tagged caveolin-3 fusions were created using the pEGFP-C1 vector (CLONTECH, Inc.). The C-termially Myc-tagged WT caveolin-3 cDNA was as we described previously (12), except it was subcloned into the pCAGGS expression vector. Myc-tagged caveolin-3 was visualized using pAb A-14 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) that is directed against the Myc epitope (EQKLISEEDLN). GFP-tagged caveolin-3 was detected as we previously described for GFP-tagged caveolin-1 (47).

**RESULTS**

**Expression of Wild Type and LGMD-1C Mutants of Caveolin-3**

In order to begin to understand the pathogenesis of limb girdle muscular dystrophy 1C (LGMD-1C), we generated the
same two mutations in wild type caveolin-3 that are seen in patients with the symptomatic disease. These two mutations, as well as wild type caveolin-3, are illustrated schematically in Fig. 1A. We next transiently transfected these caveolin-3 mutants (ΔTFT or P → L) into COS-7 cells and assessed their expression relative to wild type by Western blot analysis with a specific caveolin-3 monoclonal antibody probe (mAb 26). Note that both mutant forms are expressed at significantly lower levels than achieved with wild type caveolin-3. Quantitation revealed that these LGMD-1C mutants were expressed at ~15–20% the level of expression observed for wild type caveolin-3.

Detergent Insolubility, Oligomeric State, and Caveolar Targeting of LGMD-1C Mutants of Caveolin-3

Triton Insolubility—Endogenous caveolins 1, 2, and 3 are insoluble in nonionic detergents such as Triton X-100 at low temperatures (10, 12, 25, 26); however, they can be efficiently solubilized by the mild detergent, octyl glucoside (25, 26). It is thought that octyl glucoside solubilization occurs through the displacement of endogenous lipid components (such as glycosphingolipids and cholesterol) that are concentrated within caveolae membranes and interact directly with the caveolins (25–31).

Fig. 2 shows that in transiently transfected NIH 3T3 cells, wild type caveolin-3 is >95% Triton-insoluble, while both LGMD-1C mutants of caveolin-3 are significantly more Triton-soluble. More specifically, Cav-3/ΔTFT was ~25–30% Triton-soluble, while Cav-3/P → L was ~40–50% Triton-soluble. These results clearly indicate that these mutations interfere with the ability of caveolin-3 to achieve optimal Triton insolubility. These results are perhaps surprising, since the mutation within the caveolin scaffolding domain (ΔTFT) more dramatically affects Triton insolubility than the mutation within the membrane-spanning domain (P → L).

Oligomerization—Caveolin-1 forms ~350-kDa homo-oligomers containing ~14–16 caveolin monomers per oligomer (15, 48). These homo-oligomers are thought to function as building blocks in the construction of caveolae membranes. Similarly, caveolin-3 forms homo-oligomers of the same size as caveolin-1 (12). In contrast, caveolin-2 exists as a homodimeric complex or as a high molecular mass hetero-oligomer with caveolin-1 (10, 13).

Thus, we next investigated the oligomeric state of LGMD-1C caveolin-3 mutants. For this purpose, we employed an established velocity gradient system developed previously to study the oligomeric state of caveolins 1, 2, and 3 (10, 12, 15). Fig. 3 shows that wild type caveolin-3 behaved as a high molecular mass complex, migrating between the 200- and 443-kDa molecular mass standards (peak fractions 7 and 8). In contrast, both LGMD-1C caveolin-3 mutants migrated predominantly as high molecular mass oligomers of >443 kDa, forming high molecular mass aggregates. More specifically, virtually all of the P → L mutant migrated in fractions 11 and 12, while ~60% of the ΔTFT mutant was confined to fractions 11 and 12 and ~40% migrated to fractions 7 and 8. Our results dramatically show that these LGMD-1C mutations adversely affect the oligomerization process.

Incorporation into Low Density Triton-insoluble Membrane Fractions That Are Enriched in Caveolae Membranes—To separate membranes enriched in caveolae from the bulk of cellular membranes and cytosolic proteins, an established equilibrium sucrose density gradient system was utilized (19, 25, 27, 28, 31, 35–40).
In this fractionation scheme, immunoblotting with anti-caveolin IgG can be used to track the position of caveolae-derived membranes within these bottom-loaded sucrose gradients. Using this procedure, caveolin-1 is purified 2000-fold relative to total cell lysates as 4–6 mg of caveolin-rich domains (containing 90–95% of total cellular caveolin-1) are obtained from 10 mg of total cellular proteins (19, 43). We and others have shown that these caveolae-enriched fractions exclude >99.95% of total cellular proteins and also markers for noncaveolar plasma membrane, Golgi, lysosomes, mitochondria, and endoplasmic reticulum (25, 27, 28).

Fig. 4 illustrates that in this fractionation scheme wild type caveolin-3 is correctly targeted to these low density Triton-insoluble membranes (fractions 4 and 5) that are enriched in caveolae. In contrast, both LGMD-1C caveolin-3 mutants are quantitatively excluded from these caveolae-enriched fractions. These results indicate that the LGMD-1C mutations clearly prevent the incorporation of caveolin-3 into caveolae membranes.

**Immunolocalization of LGMD-1C Mutants of Caveolin-3 to a Perinuclear Intracellular Compartment**

As the LGMD-1C mutants of caveolin-3 were excluded from caveolae membranes, we next determined their subcellular localization by immunofluorescence using confocal microscopy. Fig. 5 shows the localization of these mutants. The distribution of wild type caveolin-3 is shown for comparison. Both LGMD-1C mutants were primarily retained at the level of a perinuclear compartment and did not reach the plasma membrane. In contrast, wild type caveolin-3 was efficiently targeted to the plasma membrane under these conditions. We identified this perinuclear compartment as the Golgi complex (Fig. 6) by performing double-labeling experiments with antibodies directed against the resident Golgi marker protein, Cab45, that is endogenously expressed (37).

**LGMD-1C Mutants of Caveolin-3 Have a Much Shorter Half-life but Still Undergo Palmitoylation to Normal Levels**

Given that the LGMD-1C mutants of caveolin-3 are not expressed to the same level as the wild type protein (Fig. 1B) and they are retained in an intracellular compartment (Figs. 5 and 6), these results indirectly suggest that such mutations may affect the stability of the caveolin-3 protein product. Thus, we performed a series of pulse-chase experiments designed to evaluate the turnover rate of caveolin-3 in transiently trans-
fected cells. Fig. 7 shows that wild type caveolin-3 has a half-life of \( \sim 5.25 \) h. In striking contrast, both LGMD-1C mutants have a half-life of \( \sim 45–60 \) min. This dramatic reduction in half-life is predicted to reduce the steady state levels of these LGMD-1C mutants to \( \sim 15–20\% \) of wild type levels (1/5th–1/7th), as we observe experimentally by Western blot analysis (Fig. 1). These results may explain why both mutations lead to a loss of \( \sim 85–90\% \) of caveolin-3 protein expression in patients with LGMD-1C (34).

Since caveolin-1 is palmitoylated on three cysteine residues within its C-terminal region (49) and these cysteines are absolutely conserved between caveolins 1 and 3 (12), we next evaluated the palmitoylation state of caveolin-3. As predicted, wild type caveolin-3 was heavily palmitoylated (Fig. 8A). Similarly, both LGMD-1C mutants of caveolin-3 were also palmitoylated. As a control for expression levels, we also assessed the relative amount of wild type and mutant caveolin-3 in lysates derived from these experiments (Fig. 8B). If we normalize for the fact that these caveolin-3 mutants are not expressed as well as the wild type (Fig. 8C), we find that both mutants undergo palmitoylation to a similar extent as wild type caveolin-3. Since the LGMD-1C mutants of caveolin-3 are retained at the level of the Golgi, these results indicate that palmitoylation of caveolin-3 can take place prior to its transport to the cell surface.

**LGMD-1C Mutants of Caveolin-3 Have a Dominant Negative Phenotype, Causing the Retention of Wild Type Caveolin-3 at the Level of the Golgi**

To examine whether LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, we next co-transfected NIH 3T3 cells with C-terminally Myc-tagged WT caveolin-3 and three different N-terminally GFP-tagged forms of caveolin-3 (WT, P→L, or ΔTFT). Fig. 9A shows that in cells co-expressing wild type caveolin-3 (Myc) and LGMD-1C mutant forms of caveolin-3 (GFP), both are co-localized to the Golgi complex (middle and lower panels). In contrast, in cells co-expressing wild type tagged forms of caveolin-3 (Myc and GFP), both tagged forms are co-localized to the plasma membrane (upper panel). These results clearly indicate that both the LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, causing the retention of wild type caveolin-3 at the level of the Golgi.
FIG. 8. Wild type and mutant forms of caveolin-3 both undergo palmitoylation to the same extent. A, metabolic labeling and immunoprecipitation. COS-7 cells were transiently transfected with wild type and mutant forms of caveolin-3. Forty-eight hours post-transfection, cells were labeled for 4 h with [9,10-3H]palmitic acid (150 µCi/ml). After immunoprecipitation with caveolin-3-specific antibodies, samples were separated by SDS-PAGE and visualized by autoradiography. B, Western blot analysis. As a control for relative expression levels, lysates were also subjected to immunoblotting with antibodies directed against caveolin-3. Note that both caveolin-3 mutants undergo palmitoylation to the same extent as wild type caveolin-3 if we normalize for the fact that these caveolin-3 mutants are not expressed as well as the wild type. C, graphical representation of A and B (derived from several independent experiments). After quantitation, a relative ratio (palmitate incorporation/protein) was calculated and normalized to 1 for wild type caveolin-3. Note that the P→L mutant showed a ratio of ~1.4, while the ΔTFT mutant yielded a ratio of ~0.8. These results indicate that both LGMD-1C mutants of caveolin-3 are palmitoylated to a similar level as wild type caveolin-3.

FIG. 9. Co-expression of wild type and LGMD-1C mutant forms of caveolin-3 in a single cell. To examine whether LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, we co-transfected NIH 3T3 cells with C-terminally Myc-tagged WT caveolin-3 and three different N-terminally GFP-tagged forms of caveolin-3 (WT, P→L, or ΔTFT). A, immunolocalization. Note that in cells co-expressing wild type tagged forms of caveolin-3 (Myc and GFP), both tagged forms are co-localized to the plasma membrane (upper panel). However, in cells co-expressing wild type caveolin-3 (Myc) and LGMD-1C mutant forms of caveolin-3 (GFP), both are co-localized to the Golgi complex (middle and lower panels). B, Western blot analysis. The expression of wild type caveolin-3 and caveolin-3 mutants (ΔTFT or P→L) was assessed by immunoblotting with antibodies directed against the Myc epitope and GFP. Note that in cells co-expressing wild type tagged forms of caveolin-3 (Myc and GFP), both wild type tagged forms are well expressed (lane 1). However, in cells co-expressing wild type caveolin-3 (Myc) and LGMD-1C mutant forms of caveolin-3 (GFP) (lanes 2 and 3), both wild type and LGMD-1C mutants are expressed at dramatically lower levels (~15–20% the level of expression seen in lane 1). Taken together, these results indicate that the LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, causing the retention of wild type caveolin-3 at the level of the Golgi.
mutants are expressed at dramatically lower levels (~15–20% the level of expression seen in lane 1). These results directly demonstrate that co-expression of wild type caveolin-3 with LGMD-1C mutant forms can dramatically reduce the steady-state expression levels of wild type caveolin-3 by ~80–85%.

These observations are consistent with the previous genetic observation that the transmission of LGMD-1C occurs in an autosomal dominant fashion; i.e. only one mutant copy of caveolin-3 is sufficient to cause the symptomatic disease and reduce caveolin-3 levels by ~85–90% in patients (34).

**LGMD-1C Mutants of Caveolin-3 Do Not Affect the Distribution or the Expression of Endogenous Caveolins 1 and 2**

LGMD-1C mutants of caveolin-3 may also affect the expression, properties, or distribution of caveolins 1 and 2 that are endogenously expressed in NIH 3T3 cells. For example, LGMD-1C mutants of caveolin-3 may form mixed hetero-oligomers with caveolins 1 and 2, leading to their retention in the Golgi and degradation. However, this is unlikely, since we have recently shown that wild type caveolin-3 does not form mixed oligomers with caveolin-1 or -2 (50).

To examine whether LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion with regard to the localization of caveolins 1 and 2, we next transiently transfected NIH 3T3 cells with wild type caveolin-3 or the LGMD-1C mutant forms of caveolin-3. To monitor the effects of LGMD-1C mutants on endogenous caveolins 1 and 2, we employed specific antibody probes that recognize only caveolin-1 or caveolin-2, as we have generated and described previously (13, 40).

Fig. 10 shows that in NIH 3T3 cells transiently expressing either wild type or mutant forms of caveolin-3, the distribution of caveolins 1 and 2 was not affected; i.e. they were efficiently targeted to the plasma membrane. However, in these same cells, the LGMD-1C mutants of caveolin-3 were retained at the level of the Golgi complex. These results clearly indicate that the LGMD-1C mutants do not affect the distribution of caveolins 1 and 2.

To assess whether LGMD-1C mutants of caveolin-3 can affect the steady state expression levels of caveolins 1 and 2, we next derived NIH 3T3 cells stably expressing either wild type caveolin-3 or the LGMD-1C mutants of caveolin-3. Interestingly, the expression levels of endogenous caveolins 1 and 2 were not affected by the expression of LGMD-1C mutants of caveolin-3 (Fig. 11A). Consistent with these observations, LGMD-1C mutants of caveolin-3 also did not affect the localization of caveolin-1 (Fig. 11B).

Taken together, these results clearly demonstrate that LGMD-1C mutants of caveolin-3 selectively cause the retention of wild type caveolin-3, but not caveolins 1 and 2, at the level of the Golgi complex, dramatically reducing its steady-state expression levels.

**DISCUSSION**

LGMD-1C is an autosomal dominant form of limb girdle muscular dystrophy that is genetically caused by mutations
within the coding regions of the caveolin-3 gene. In collaboration with Minetti and colleagues (34), we have recently identified two different families in Italy with this autosomal dominant form of limb girdle muscular dystrophy that is due to a deficiency in caveolin-3 expression. In these patients, by quantitative immunofluorescence and Western blot analysis, the levels of the caveolin-3 protein are reduced by ~85–90%.

Analysis of their genomic DNA reveals two distinct mutations: (i) a 9-base pair microdeletion that removes the sequence TFT from the caveolin scaffolding domain and (ii) a missense mutation that changes a proline to a leucine (P → L) in the transmembrane domain (34). Since these mutations are heterozygous and show an autosomal dominant form of transmission, we postulated that these mutations must cause the formation of a dominant-negative form of caveolin-3 (34). One hypothesis is that this may lead to the degradation of wild type caveolin-3, since caveolins 1, 2, and 3 are known to form both hetero- and homo-oligomers (10, 12, 13, 15, 51). However, the mechanisms that underlie the pathogenesis of this form of muscular dystrophy remain unknown.

Here, we show that these LGMD-1C mutations (i) decrease the steady-state expression levels of caveolin-3, (ii) increase the Triton solubility and oligomeric state of caveolin-3, (iii) cause caveolin-3 to be excluded from caveolae-enriched membrane domains, (iv) lead to the intracellular retention of caveolin-3 in a perinuclear compartment that we identify as the Golgi complex, and (v) dramatically shorten the half-life of caveolin-3 by ~5–7-fold. However, these mutations do not prevent the palmitoylation of the caveolin-3 protein product. These results are summarized in Table I. Taken together, these data indicate that LGMD-1C mutations cause the formation of unstable high molecular mass aggregates of caveolin-3 that are retained within the Golgi complex and are not targeted to the plasma membrane. In addition, these results provide a molecular explanation for why caveolin-3 levels are dramatically downregulated in patients with LGMD-1C.

Interestingly, comparison of the known protein sequences of mammalian caveolins 1, 2, and 3 with C. elegans caveolins 1 and 2, reveals that only 12 amino acid residues are invariant between worms and humans (51). These include two charged residues (Arg and Asp), five aromatic residues (three Phe, Trp, and Tyr), two prolines, two serines, and a glycine. Of these 12 invariant residues, two are affected by the LGMD-1C mutations identified in caveolin-3. One of the invariant prolines is changed to leucine in family A (P → L), while one of the invariant phenylalanines is deleted in family B (ΔTFT) (34). Our current results with caveolin-3 suggest that mutation of these evolutionarily conserved residues may have dire consequences for the structure or functioning of caveolin-3 and possibly the entire caveolin gene family. Consistent with its autosomal dominant form of genetic transmission, we demonstrate here that LGMD-1C mutants of caveolin-3 behave in a dominant negative fashion, causing the retention of wild type caveolin-3 at the level of the Golgi.

Two other mutations within the coding sequence of the caveolin-3 gene have been described that are associated with a proximal form of muscular dystrophy (52). One of these mutations was homozygous, suggesting a possible autosomal recessive inheritance (52). Interestingly, these two additional mutations map to the caveolin-3 scaffolding domain. Thus, three out of the four mutations identified are clustered within the caveolin scaffolding domain, a 20-amino acid membrane-proximal region of caveolin-3.

Given that these LGMD-1C mutations cause caveolin-3 to form abnormal high molecular mass oligomers that are retained intracellularly and have a much higher turnover rate, it is likely that these mutations cause misfolding of the caveolin-3 protein and lead to its rapid degradation (t_1/2 ~ 1 h). In support of this notion, protein misfolding secondary to mutations or environmental factors is now thought to be a common mechanism that underlies the pathogenesis of a number of human diseases, including cystic fibrosis, Prion disease, and Alzheimer's disease (53–55). This interpretation is consistent with the finding that three out of four thus far identified caveolin-3
mutations cluster within the caveolin scaffolding domain, which is involved both in the self-assembly of caveolins into high molecular mass homo-oligomers and in the hetero-oligomeric interaction of caveolins with signaling molecules (4).

Wild type caveolin-3 has been shown to interact with a variety of signaling molecules and to inhibit their signaling activity both in vitro and in vivo (14, 35, 36, 56). These LGMD-1C mutants of caveolin-3 may cause the retention of certain signaling molecules at the level of the Golgi. One prediction is that retention of signaling molecules at the level of the Golgi by mutant forms of caveolin-3 will occur and, therefore, these mutants will be better inhibitors of signal transduction, although they may be expressed at lower levels. Alternatively, these mutants may no longer recognize signaling molecules and, therefore, will fail to act as inhibitors of signal transduction.

Future studies will address the effects of expressing LGMD-1C mutants of caveolin-3 in skeletal muscle cells in culture. Since C2C12 cells normally up-regulate endogenous caveolin-3 expression during myoblast differentiation and fusion (12, 14), these cells are a good model system to assess the effects of co-expressing wild type and mutant forms of caveolin-3. The added benefit of using C2C12 cells is that this would allow us to examine the behavior of the LGMD-1C mutants in the context of skeletal muscle cell differentiation. However, we feel that it is more prudent to first establish the behavior of LGMD-1C mutants of caveolin-3 in the simpler context of a heterologous expression system, before moving to the slightly more complicated C2C12 differentiation system. We predict that expression of the LGMD-1C mutants of caveolin-3 in C2C12 cells will induce the extensive down-regulation/degradation of wild type caveolin-3, as seen in LGMD-1C patients (34) and NIH 3T3 cells (this report).

TABLE I

| Car-3 | Expression | Triton solubility | Oligomer formation | Caveolar targeting | Cellular distribution | Turnover rate (t 1/2) | Lipid modification |
|-------|------------|------------------|-------------------|-------------------|-----------------------|-----------------------|-------------------|
| WT    | Normal     | <5               | ~200–443          | +                 | Plasma membrane       | ~5.25 h               | +                 |
| P → L | Decreased  | ~25–30           | >443              | –                 | Golgi                 | ~45–60 min           | +                 |
| ΔTFT  | Decreased  | ~40–50           | >443              | –                 | Golgi                 | ~45–60 min           | +                 |

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