The cytoplasmically oriented monotopic integral membrane protein stomatin forms high-order oligomers and associates with lipid rafts. To characterize the domains that are involved in oligomerization and detergent-resistant membrane (DRM) association, we expressed truncation and point mutants of stomatin and analyzed their size and buoyancy by ultracentrifugation methods. A small C-terminal region of stomatin that is largely hydrophobic, Ser-Thr-Ile-Val-Ph-Pro-Leu-Pro-Ille (residues 264–272), proved to be crucial for oligomerization, whereas the N-terminal domain (residues 1–20) and the last 12 C-terminal amino acids (residues 276–287) were not essential. The introduction of alanine substitutions in the region 264–272 resulted in the appearance of monomers. Remarkably, only three of these residues, Ile-Val-Ph (residues 266–268), were found to be indispensable for the DRM association. Interestingly, the exchange of Pro–269 and to some extent the residues 270–272, which are essential for oligomerization, did not affect the DRM association of stomatin. This suggests that the formation of oligomers is not necessary for the association of stomatin with DRMs. Internal deletions near the membrane anchoring domain resulted in the formation of intermediate size oligomers suggesting a conformational interdependence of large parts of the C-terminal region. Fluorescence recovery after photobleaching analysis of the tagged, monomeric, non-DRM mutant ST-(1–262)-green fluorescent protein and wild type stomatin StomGFP showed a significantly higher lateral mobility of the truncation mutant in the plasma membrane suggesting a membrane interaction of the respective C-terminal region also in vivo.

The 31-kDa integral protein stomata was first identified as an abundant component of the human erythrocyte membrane (1–3); however, it is also widely expressed in various tissues and cell lines (1, 3–5). The primary structure of 287 amino acids (3, 6) is characterized by a highly charged 24-residue N terminus followed by a 29-residue hydrophobic sequence that is most probably associated with the membrane and the large C-terminal region containing 234 residues. Stomatin has an unusual topology (7), similar to caveolin (8), with the hydrophobic domain forming a putative “hairpin loop” in the lipid bilayer and the N and C termini facing the cytoplasm. Palmitoylation of the cysteine residues Cys-29 and Cys-86 further increases the affinity of stomatin for the membrane (9). The association of stomatin with the lipid droplet phospholipid monolayer (10) suggests that the hydrophobic domain is embedded only in the cytoplasmic leaflet of lipid bilayer membranes.

In the human epithelial cell line of amniotic origin (UAC), stomatin concentrates in plasma membrane protrusions and juxtanuclear vesicles that are positive for the late endosomal/lysosomal marker lysosome-associated membrane protein 2 (LAMP-2) (11). At the plasma membrane, the protein is directly or indirectly linked to the cortical actin microfilaments, and this connection remains intact even after treatment with the actin filament-disrupting drug cytochalasin D (12).

A structural characteristic of stomatin is its organization in high-order homo-oligomeric complexes of about 300 kDa, comprising 9- to 12-mers, within the epithelial plasma membrane (13). In the erythrocyte membrane, these oligomers are even larger, in the range of 200 to 600 kDa (14). A C-terminal truncation mutant of stomatin, ST-(1–262)myc5, is not incorporated into oligomers indicating that the last 25 C-terminal amino acids are required for the oligomeric complex formation (11).

Another hallmark of stomatin is its presence in detergent-resistant membranes (DRMs)2 that are enriched in sphingolipids and cholesterol (11, 14, 15). These DRMs are thought to originate from specialized membrane domains, known as lipid rafts, that are suggested to play a role in cellular events such as receptor signaling, membrane trafficking, and sorting to the apical membrane of polarized cells (16–19). The truncation mutant ST-(1–262)myc5 does not associate with DRMs. Because this mutant lost both the ability to oligomerize and associate with DRMs, we suggested that the raft association of stomatin may depend on its oligomeric structure (11).

In this report, we show that the C-terminal region between residues 264 and 272 is essential for oligomerization and that only three hydrophobic residues (266–268) within this region are necessary for DRM association. The residues 269–272, which are indispensable for oligomerization, are not essential

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2 The abbreviations used are: DRM, detergent-resistant membrane; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; PHB, prohibitin; ROI, region of interest; SPFH, stomatin, prohibitin, flotillin, HRC/K; WT, wild type.
for DRM association, suggesting that stomatin oligomerization is not necessary for raft association. FRAP analyses independently showed that the C terminus (residues 263–287) contained a membrane-interacting domain regulating the lateral mobility.

EXPERIMENTAL PROCEDURES

Reagents—The mouse monoclonal antibodies against stomatin, GARP-50 and GARP-61, have been described (1) and were used directly from the hybridoma supernatants. Protease inhibitors were from Sigma, laboratory chemicals were from Merck, and reinforced nitrocellulose Optitran BA-S 83 was from Schleicher & Schuell.

Cell Culture—Human epithelial cells of amniotic origin, squamous epithelial carcinoma cells (A431), and HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Invitrogen) and antibiotics (100 units/ml penicillin and streptomycin) under standard conditions.

DNA Mutagenesis and Cell Transfection—The vector pEF-Puro.PL3, used to stably express stomatin truncation mutants or alanine substitutions in full-length stomatin, has been described (13, 20). The cDNAs of truncated stomatin variants were generated by polymerase chain reaction and were directionally cloned into the unique restriction sites SpeI or EcoRI, respectively. Subsequently, the cDNAs encoding full-length stomatin point mutants and internal deletion mutants were generated by overlap extension using complementary oligonucleotides carrying the desired mutation/deletion and peripheral primers carrying the 5’- or 3’-end of stomatin cDNA flanked by the restriction sites SpeI or EcoRI, respectively. Subsequently, the cDNA fragments were subcloned into pEFBOs-Puro.PL3. The cDNAs encoding full-length stomatin were generated by polymerase chain reaction and were directionally cloned into the unique restriction sites SpeI/EcoRV of pEFBOS-Puro.PL3. The cDNAs encoding full-length stomatin point mutants and internal deletion mutants were generated by overlap extension using complementary oligonucleotides carrying the desired mutation/deletion and peripheral primers carrying the 5’- or 3’-end of stomatin cDNA flanked by the restriction sites SpeI or EcoRI, respectively. Subsequently, the cDNA fragments were subcloned into pEF-Puro.PL3. A431 or UAC cells were stably transfected using the Protactin mammary transfection system (Promega).

Membrane Preparation—Membrane preparations of confluent A431 or UAC clones (one 15-cm dish each) were performed using two centrifugation steps as described previously (13). Briefly, the membranes of confluent A431 or UAC cells stably expressing stomatin mutants or alanine substitutions in full-length stomatin, has been described (13). The cDNAs of truncated stomatin variants were generated by polymerase chain reaction and were directionally cloned into the unique restriction sites SpeI or EcoRI, respectively. Subsequently, the cDNAs encoding full-length stomatin was adjusted to 50% sucrose by the addition of 490 μl of ice-cold TNE buffer containing 0.1% Triton X-100 at 4 °C for 10 min. The lysate was adjusted to 50% sucrose by the addition of 490 μl of 80% sucrose in TNE and overlaid with 3 ml of 35% sucrose followed by 1.2 ml of 5% sucrose in the same buffer. After ultracentrifugation at 48,000 rpm in an SW 55 rotor (Beckman) for 18 h, 8 fractions were collected from the top and analyzed by Western blotting. DRM components were identified in the low-density fractions 2 and 3, whereas Triton X-100-soluble proteins were found in the dense fractions 6, 7, and 8.

Western Blotting—Immunoblotting was performed as described (10) using the SuperSignal system (Pierce). For the detection of stomatin mutants, the mouse monoclonal antibody against the N terminus of stomatin, GARP-50 (diluted 1/15), was used except for the N-terminal truncation mutant ST-(21–287) that was detected by GARP-61 (diluted 1/15), an antibody against a C-terminal epitope of stomatin.

FRAP Analysis—HeLa cells were transiently transfected with pEGFP-N3-stomatin or pEGFP-N3-ST-(1–262) as described (10) using Lipofectamine 2000 (Invitrogen). 12 h after transfection, cells were seeded on 35-mm glass-bottom dishes (MatTek Corp., Ashland, MA), and FRAP analysis was performed after another 24 h, essentially as described (21, 22). Cells were washed with Hanks’ balanced salt solution and mounted in pre-warmed Hanks’ balanced salt solution (supplemented with 25 mM Heps, pH 7.4) on a Zeiss LSM 510 Meta confocal microscope equipped with a stage heater (37 °C). Cells were imaged with a ×63 NA1.4 Plan-Apochromat oil immersion lens at 4-fold magnification. For quantitative FRAP, pre- and postbleach images were monitored with the 488 nm laser line of a 30-milliwatt argon laser set to 60% laser power at 1% transmission and a confocal pinhole of 1.5 airy units. Photobleaching of a circular region of interest (ROI) with a diameter of 1.8 μm was performed by 12 bleach iterations with all argon laser lines set to 100% transmission. Images were recorded with a 2-s time lapse at a scan time of about 0.8 s. Fluorescence intensity data of the whole cell ROI, the bleached ROI, and a background ROI were quantified using the physiology evaluation software package (Carl Zeiss Microscopy, Jena, Germany). Recovery curves were bleach- and background-corrected, and mobile fractions were calculated as described (22). For the calculation of the recovery half-time (t½), the normalized and bleach-corrected recovery data were fitted to the following formula to estimate recovery kinetics, l(t) = a(1 - exp(-kt)). The recovery half-time was then calculated as t½ = ln 2/k as described (23). For qualitative FRAP, the diameter of the bleach ROI was increased to 3.4 μm, the pinhole was set to 1 airy unit, and laser transmission was increased to 2–3% for pre- and postbleach images. Postbleach images were acquired with a 30-s time lapse, and 4-line averaging was used to get high quality pictures. The other settings were identical to those used in the quantitative experiments.

RESULTS

Amino Acid Residues between Positions 262 and 275 Are Essential for Oligomerization—We have previously shown that the last 25 C-terminal amino acid residues of stomatin are essential for homo-oligomerization (13). To determine whether the
rather basic C-terminal end, \textsuperscript{270}QGIIGAKHSHLG\textsuperscript{287}, or the more proximal, mainly hydrophobic region, \textsuperscript{262}KNSTIVFPLPIDML\textsuperscript{275} (Fig. 1), was important for oligomerization, we generated several C-terminal truncation mutants and stably expressed them in A431 or UAC cells. A431 cells were chosen because they lacked endogenous stomatin that might interfere with mutant-mutant interactions. The cell membranes were isolated and solubilized in 1% Triton X-100 at room temperature, and the protein complexes were separated according to size by linear density gradient centrifugation. SDS-PAGE of the collected fractions, subsequent Western blotting, and densitometric evaluation showed that about 90% of the C-terminal truncation mutant, ST-(1–275), is oligomeric (Figs. 2 and 6), similar to WT stomatin, ST-(1–287). This result shows that the outermost 12 residues of the C terminus are not required for oligomeric complex formation. Likewise, the short hydrophilic N terminus is not involved in oligomerization because the N-terminal truncation mutant, ST-(21–287), shows a similar distribution within the gradient. In contrast, further truncation at the C terminus, as in ST-(1–270), ST-(1–266), and ST-(1–262), resulted in complete loss of the oligomeric complexes (Figs. 2 and 6). The upper bands (marked \textit{end}) that are visible in the analyses of ST-(1–262) and ST-(21–287) represent the endogenous stomatin of UAC cells and show that the gradi-
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Amino Acid Residues 264–272 Are Indispensable for Oligomerization—To determine in detail which amino acids within the region 262–275 are essential for oligomerization, we performed an alanine scan of this region in full-length stomatin (Fig. 1). The alanine-substituted point mutants were stably expressed in A431 cells and subjected to density gradient centrifugation, and the collected fractions were analyzed by SDS-PAGE and Western blotting. As shown in Figs. 3 and 6, the mutation of each of the amino acid residues in the region 265TIVFPL270 and Ile-272 resulted in the complete loss of oligomeric complexes. The residues Ser-264 and Pro-271 are also important because the oligomers of these mutants only account for about 18% (Figs. 3 and 6). Therefore, we conclude that the oligomerization domain is located between residues 264 and 272. The residues flanking this region, Lys-262, Asn-263, Asp-273, Met-274, and Leu-275, apparently play a minor role in the formation of stomatin oligomers.

In addition, we analyzed several stomatin variants carrying point mutations that were located more proximal to the membrane attachment site. The residues Thr-181 and Trp-184 were part of a conserved region within the stomatin family, TDAWG185 (bold letters indicate more than 60% identity)
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FIGURE 6. Summary of the oligomerization and DRM association data of stomatin mutants. For the WT stomatin and the indicated mutants, the intensity of Western blot bands was determined by densitometry and quantified by ImageQuant 5.1 software. The position numbers of the amino acid residues are indicated. Below the amino acid sequence, the values of oligomerization (oligomers) and DRM (raft) association (percent relative to WT stomatin) for the respective point mutants are given. In the lower part of the figure, the values for the truncation mutants ST-(1–266), ST-(1–270), and ST-(1–275) are shown. For the calculation of percent oligomerization, the sum of the densitometric values obtained for fractions 2 and 3 (monomers) plus 10 and 11 (oligomers) was set to 100%. For DRM association, the sum of fractions 2 and 3 (DMRs) and 6 – 8 (non-DMRs) was set to 100%. Using these calculations, 96% of WT stomatin was oligomeric, and 80% was associated with DRMs.

—We have already shown that endogenous stomatin associates with lipid rafts in UAC cells, whereas the monomeric C-terminal truncation mutant, ST-(1–262), is not detected in DRMs (11). To analyze the effect of the outermost C terminus on the DRM association in more detail, we performed flotation experiments using A431 cells expressing three additional stomatin truncation mutants. As mentioned above, we used A431 cells to rule out the possible interference of endogenous stomatin with lipid raft association of the mutants via oligomerization. However, lipid rafts in these cells appeared to be less stable and/or less abundant than in UAC cells because under the conditions used for the UAC cells, even the WT stomatin was excluded from DRMs and apparently dissolved (data not shown). We therefore reduced the Triton X-100 concentration during solubilization at 4 °C to final 0.1%. This resulted in the association of 80% of WT stomatin with the floating fractions and the exclusion of the monomeric, C-terminal truncation mutant ST-(1–266) (Figs. 4 and 6), as observed with UAC cells (not shown). To demonstrate the applicability of this modified technique, the presence of two other raft markers, placentaly alkaline phosphatase and flotillin-2, was tested. The distribution of A431-derived DRMs proved to be similar to the UAC-derived DRMs, which were isolated with 1% Triton X-100. Throughout the following experiments, these two raft markers served as positive controls for the proper isolation of DRMs (not shown).

To test whether the reduced detergent concentration affects the oligomerization behavior of stomatin and mutants, we solubilized the cell membranes in 0.1% Triton X-100 at room temperature and performed linear gradient centrifugation in 0.1% Triton X-100. For all tested constructs, we obtained equal results compared with the experiments with 1% Triton X-100 (data not shown).

ST-(1–275), lacking only the outermost 12 amino acid residues, shows a distribution similar to WT stomatin regarding both oligomerization and DRM association (Figs. 4 and 6). Interestingly, the completely monomeric C-terminal truncation mutant ST-(1–270) shows some association with DRMs. The analysis of the oligomeric N-terminal truncation mutant ST-(21–287) revealed, as expected, that the hydrophilic N terminus was not necessary for DRM association (Figs. 4 and 6).

Ile-266, Val-267, and Phe-268 Are Crucial for DRM Association but Not for Oligomerization—To study the significance of the amino acid residues in the region 262–275 for DRM association, we determined the buoyancy of the stomatin point mutants that already had been analyzed for oligomerization. Interestingly, only three amino acids, Ile-266, Val-267, and Phe-268, are indispensable for DRM association (Figs. 5 and 6). Surprisingly, the monomeric mutant ST-P269A associated with DRMs equally well as WT stomatin (Figs. 5 and 6) challenging the hypothesis that oligomerization is necessary for raft association. Several other monomeric point mutants, namely ST-S264A, ST-T265A, ST-L270A, ST-P271A, and ST-D272A, are also associated with DRMs (Fig. 5) albeit to a reduced extent when compared with WT stomatin (Fig. 6). The remaining stomatin mutants, ST-K262A, ST-N263A, ST-D273A, ST-M274A, and ST-L275A, are partially oligomeric and also show reduced DRM association.

The C Terminus Modifies the Lateral Mobility of Stomatin in the Plasma Membrane—Our in vitro analyses defined the C-terminal region 262–275 to be important for oligomerization and residues 266–268 for DRM association. To test whether this region is also relevant for interactions in vivo, we studied its effect on the lateral mobility in the plasma membrane. We performed FRAP analyses of the GFP-tagged WT stomatin, StomGFP, and the monomeric, non-DRM truncation mutant, ST-(1–262)GFP, respectively. HeLa cells transiently expressing these fusion proteins were studied at 37 °C. For qualitative analysis, an ROI with a diameter of 3.4 μm in the lateral membrane of these cells was photobleached, and the recovery of the respective fluorescent protein was documented after 30 and 120 s. For each protein, a representative experiment is shown in Fig. 7A. For quantitative analysis, we reduced the diameter of the bleach ROI to 1.8 μm to reduce the recovery time. Recovery was monitored at 2-s intervals for 230 s for StomGFP and for 135 s for ST-(1–262)GFP. The recovery curves shown in Fig. 7B were calculated from seven identical measurements each, as described (22). The recovery curves in Fig. 7B clearly demonstrate that a large proportion of StomGFP is immobile and does not recover (mobile fraction (Mf) = 56%). The monomeric, non-DRM mutant ST-(1–262)GFP shows an increased mobile fraction (Mf) = 82%). To analyze the diffusion speed of the two constructs, we calculated the recovery half-time (t1/2) and found that the ST-(1–262)GFP construct has a lower (t1/2) value and therefore its diffusional velocity is higher than the one for
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DISCUSSION

Previous studies showed that the outermost C terminus of stomatin is involved in oligomerization (13) and DRM association (11). To identify the amino acid residues involved, we performed detailed truncation and alanine scanning analyses in combination with density gradient experiments. We demonstrate here that the small, largely hydrophobic region within the C terminus of stomatin, Ser-Thr-Ile-Val-Phe-Pro-Leu-Pro-Ile (residues 264–272), is absolutely necessary for oligomerization, with Leu-275 marking the C-terminal boundary. On the other hand, DRM association depends on only three of these residues, Ile-Val-Phe (266–268). Remarkably, Pro-269 and the residues 270–272 within the oligomerization domain have no or little influence on DRM association of stomatin suggesting that oligomerization is not necessary for DRM association. Interestingly, the residues Ile-Asp-Met-Leu (residues 272–275) were recently identified as a putative PDZ3 binding motif by an eukaryotic linear motif (ELM) search (24) suggesting diverse interactions with multiple binding partners via multi-PDZ proteins that are known organizers of large protein complexes involved in ion transport and signaling (25).

Our finding that hydrophobic amino acid residues play the major role in oligomerization is in accordance with previous data. First, the oligomers of endogenous stomatin in UAC cells were not affected by high salt concentrations during the isolation procedure.

Second, in COS7 cells, photo-cross-linking of endogenous stomatin via photoactivable derivatives of Leu and Met resulted in the formation of dimers and trimers (26). On the other hand, stomatin can also be cross-linked by the hydrophilic cross-linker disuccinimidyl suberate (13, 26), indicating that hydrophilic parts of stomatin interact. However, our findings show that high-order oligomerization of stomatin depends on the region 264–272 and suggest that this interaction has a predominantly hydrophobic character.

In UAC, Madin-Darby canine kidney cells, and A431 cells, WT stomatin forms complexes comprising 9- to 12-mers but nothing is known as to how they are organized. Topologically and biochemically, stomatin resembles the caveolins, proteins that are integral components of caveolae that play an important role in vesicular transport, intracellular cholesterol homeostasis, and modulation of signaling pathways (27–31). For caveolin-1, two structural models have been proposed; one is a network of interacting oligomers comprising 14–16 monomers (32, 33), and the other model is that of large filaments of heptameric rings that are visible in electron microscopy (34). Our finding of intermediate size mutant stomatin oligomers (trimers to pentamers) suggests that the formation of wild type 9- to 12-mers depends on the interplay of at least two different interactions, mediated by a proximal and a distal region of the C terminus. If one of these interactions gave rise to trimers, these complexes would form a network in analogy to the first model of caveolin oligomers. However, it is more likely that the internal deletions interfere with the conformation of stomatin and thereby indirectly affect the oligomerization domain.

On a wider scale, the structural and functional importance of the C terminus of stomatin is also reflected by the evolutionary conservation within stomatin-related proteins. Computational analyses of these proteins, some only distantly related to stomatin, illustrate this conservation. A comparison of the two parameters $M_f$ and $t_{1/2}$ for StomGFP and ST-(1–262)GFP was calculated from the recovery data as described under “Experimental Procedures.” Mean values for $M_f$ and $t_{1/2}$ (n = 7) are shown; error bars represent ± 1 S.D. The paired Student’s t test was used to assess the statistical significance of the differences between the results for StomGFP and ST-(1–262)GFP.

StomGFP. Fig. 7C shows a comparison of the two parameters $M_f$ and $t_{1/2}$.

FIGURE 7. The lateral mobility of the GFP-tagged truncation mutant ST-(1–262)GFP is elevated. HeLa cells transiently expressing full-length StomGFP and the truncation mutant ST-(1–262)GFP, respectively, were subjected to FRAP analysis. A, qualitative FRAP. An ROI of 3.4 μm diameter was bleached, and the recovery of StomGFP or ST-(1–262)GFP in the plasma membrane after 30 and 120 s is shown. Bars = 10 μm. B, quantitative FRAP. The recovery curves show the mean intensity ± S.D. from seven cells each. C, comparison of the mobile fractions ($M_f$) and the recovery half-times ($t_{1/2}$) for StomGFP and ST-(1–262)GFP was calculated from the recovery data as described under “Experimental Procedures.” Mean values for $M_{f}$ and $t_{1/2}$ (n = 7) are shown; error bars represent ± 1 S.D. The paired Student’s t test was used to assess the statistical significance of the differences between the results for StomGFP and ST-(1–262)GFP.

$\Delta$ E. Umlauf and R. Prohaska, unpublished data.
tin, have compiled various domains, such as the SPFH (35), the prohibitin-like (PHB), and the Band_7 domains (Fig. 1). The solution structure of a shortened SPFH domain of flotillin-2 has been solved recently by NMR (Protein Data Bank accession number 1WIN) (36), with the residues 50–172 aligning with residues 105–196 of stomatin, and revealed a compact, ellipsoid-globular shape formed by β-sheet and α-helical structures. The findings that several members of the SPFH/PHB superfamilies namely, stomatin, flotillin-1 and -2, prohibitin, and podocin, form oligomers (13, 14, 37–40) and associate with DRMs (11, 15, 41–43) have suggested that the SPFH/PHB domain confers structure (oligomerization) and function (lipid raft association) (44, 45) to these proteins. However, we have shown in this study that oligomerization and DRM association of stomatin depend on a sequence outside the SPFH/PHB domain. Likewise, oligomerization of flotillin-2 is conferred by the flotillin domain and not the SPFH domain demonstrating that complex formation does not require insertion into the membrane. Moreover, it was suggested that N-terminal myristoylation and palmitoylation of flotillin-2 regulate its lipid raft association (37). For flotillin-1 it has been shown that the SPFH/PHB domain is partially targeted to plasma membrane DRMs when palmitoylated (46), whereas in a different study the DRM association is dependent on the N-terminal hydrophobic region of the SPFH/PHB domain (47). The role of the stomatin membrane anchoring domain (residues 25–53) has not been investigated thoroughly; however, a deletion mutant, ST-(53–287), lacking this domain leads to the appearance of dot-like structures in the cytoplasm indicat directly or indirectly linked to the cortical actin microfilaments (12), it is also conceivable that stomatin is connected to the cytoskeleton via lipid rafts and thereby exhibits a large immobile fraction. The mobile fraction of the non-raft, non-oligomeric construct ST-(1–262)GFP is clearly increased compared with StomGFP. This could be explained either by a direct interaction of the C terminus with cytoskeletal elements or by an interaction through lipid rafts. Likewise, the cell adhesion molecule NrCAM is thought to be coupled to the cytoskeleton via its lipid raft association (53). Similarly, laser tweezer surface scanning resistance experiments showed that aggregates of the glycosylphosphatidylinositol-anchored protein Qa-2 are immobilized via association with lipid rafts or the cytoskeleton (54). Alternatively, oligomerization of stomatin could be necessary for an interaction with cytoskeletal elements and could also directly influence the lateral diffusion of stomatin. In summary, we characterized the C-terminal domain of stomatin that is necessary for homo-oligomerization and DRM association. This C-terminal region has an influence on the mobile fraction and on the diffusion speed of the protein. The exact relation between diffusional mobility, lipid raft association, and oligomerization of stomatin has yet to be determined and will be the topic of future studies. Our data are in line with the proposed function of stomatin as an oligomeric scaffolding component in specific lipid rafts that may interact with other scaffolding or regulatory proteins to form large protein-lipid complexes possibly involved in signaling and transport.

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