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Modulatory roles of NHERF1 and NHERF2 in cell surface expression of the glutamate transporter GLAST

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ABSTRACT

The PDZ (PSD-95/Drosophila discs-large protein/zonula occludens protein) domain-containing proteins Na^+/H^+ exchanger regulatory factor 1 (NHERF1) and NHERF2 interact with the glutamate transporter GLAST. To characterize the roles of these NHERF proteins in the plasma membrane targeting of GLAST, we examined the interaction of green fluorescent protein (EGFP)-tagged GLAST with epitope-tagged NHERF proteins in human embryonic kidney (HEK) 293T cells. Co-expression of either NHERF protein increased the cell surface expression of EGFP-GLAST. Deletion of the C-terminal PDZ domain-binding motif caused an increase in EGFP-GLAST with immature endoglycosidase H-sensitive *N*-linked oligosaccharides, suggesting impaired exit of EGFP-GLAST from the endoplasmic reticulum (ER). Immunoprecipitation experiments revealed that NHERF1 predominantly bound EGFP-GLAST containing immature *N*-glycans, whereas NHERF2 co-precipitated EGFP-GLAST with mature *N*-glycans. Expression of a dominant-negative mutant of the GTPase Sar1 increased the interaction of EGFP-GLAST with NHERF1 in the ER. By contrast, immunofluorescence microscopy showed that NHERF2 co-localized with EGFP-GLAST in ER–Golgi intermediate compartments (ERGICs), at the plasma membrane and in early endosomes, but not in the ER. These results suggest that NHERF1 interacts with GLAST during ER export, while NHERF2 interacts with GLAST in the secretory pathway from the ERGIC to the plasma membrane, thereby modulating the cell surface expression of GLAST.

1. Introduction

Glutamate transporters play important physiological roles in various tissues and cells. In the central nervous system, they protect neurons from excitotoxicity by transporting excessive amounts of glutamate into glial cells [1]. They are also expressed in epithelial cells, where they take up glutamate as either a nutrient or a source for glutathione synthesis [2]. To accomplish these functions, glutamate transporters must be localized to the cell surface. The cell surface expression of glutamate transporters is partly regulated by interaction of the transporters with adaptor proteins, including PDZ domain-containing proteins [3]. Glutamate transporters, such as GLAST [4], possess a type I PDZ domain-binding motif (S/T–*X*–*Φ*, where *X* is any residue, and *Φ* is a hydrophobic amino acid) at their C-terminus and bind to specific PDZ domain-containing proteins. GLAST is known to bind to NHERF1 and NHERF2 [5,6], and interaction with these proteins has been shown to stabilize the surface expression of GLAST by linking it to the plasma membrane [5–8].

NHERF1 and NHERF2 possess PDZ domains that scaffold membrane proteins to the cytoskeleton [9]. The proteins share similar domain structures composed of two tandem PDZ domains and protein 4.1-ezrin-radixin-moesin (FERM) binding-domains that recognize FERM family membrane anchor proteins and link them to the actin cytoskeleton [10]. Although NHERF1 and NHERF2 both function as scaffolding proteins for GLAST [5,6], PDZ domain-containing proteins in general are known to play multiple roles in the ER export, surface delivery, and endocytosis [11–13] of membrane proteins. Moreover, previous study has shown that NHERF1 and NHERF2 have different effects on trafficking of the cystic fibrosis transmembrane conductance regulator (CFTR) [14]. The precise roles of NHERF proteins in regulating the cell surface expression of GLAST remain unknown.

The purpose of the present study was to explore the roles of NHERF1 and NHERF2 in the intracellular trafficking of GLAST. To accomplish this, we investigated the interaction between GLAST and NHERF proteins by immunoprecipitation and immunofluorescent microscopy in HEK293T cells transfected with EGFP-tagged GLAST and V5 or FLAG-tagged NHERF1 or NHERF2.

Abbreviations: PDZ, PSD-95/Drosophila discs-large protein/zonula occludens protein; NHERF, Na^+/H^+ exchanger regulatory factor; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediated compartment; EGFP, enhanced green fluorescent protein; COPII, coatomer protein complex II.

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2. Materials and methods

2.1. Construction of plasmids

Canine GLAST cDNA [15] was subcloned into pEGFP-C1 (Clontech, Palo Alto, CA) to generate pEGFPcGLAST for the expression of EGFP-tagged GLAST (EGFP-GLAST). Canine NHERF1 (GenBank Accession Number AB689732) and NHERF2 (GenBank Accession Number AB689733) cDNAs were amplified by PCR using bone marrow cDNA as a template and cloned into the pCRbluntTOPO vector (Invitrogen, Carlsbad, CA) to obtain pCRcNHERF1 and pCRcNHERF2, respectively. A linker encoding the V5 epitope was ligated into pCRcNHERF1 to insert the V5 tag sequence (IPNPLLGLD) next to the initiation codon of NHERF1, and the sequence was then subcloned into a pcDNA3.1(−) vector for the expression of N-terminally V5-tagged NHERF1 (V5-NEHRF1). NHERF1 cDNA was also ligated into the pBICEP–CMV1 vector (Sigma, St Louis, MO) for the expression of N-terminally FLAG-tagged NHERF1 (FLAG-NHERF1). The plasmid encoding N-terminally V5-tagged NHERF2 (V5-NEHRF2) was prepared following the same procedure.

Human Sar1 cDNA (GenBank Accession Number NM020150) was amplified by PCR using HEK293 cDNA as the template, and then cloned into pCMV-myc (Clontech) to obtain the expression vector encoding N-terminally myc-tagged Sar1 (myc-Sar1). The myc-tagged dominant-negative mutant of H79G Sar1 [16] was generated by site directed mutagenesis.

The sequences of the oligonucleotides used in this study are listed in Supplementary Material Table S1

2.2. Cell culture and transfection

HEK293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin G and 100 μg/ml streptomycin at 37 °C and 5% CO_2. Transfection was performed using linear polyethylenimines (Polysciences, Inc., Warrington, PA).

2.3. Analysis of proteins

Protein samples were separated by SDS–PAGE and analyzed by immunoblotting, as previously described [17]. Proteins were detected using anti-GFP (MBL, Nagoya, Japan), anti-V5 (Funakoshi, Tokyo, Japan), or anti-myc (MBL) antibodies.

Cell surface biotinylation, immunoprecipitation, and deglycosylation analyses were performed as previously described [17].

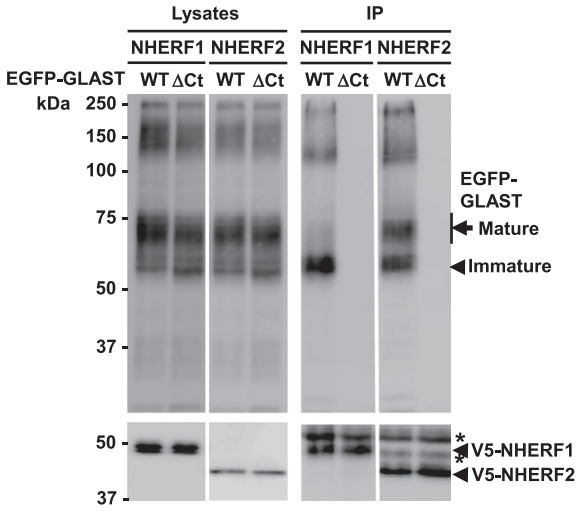

Fig. 2. Interaction of the C-terminal PDZ-domain binding motif of GLAST with PDZ-domain proteins in HEK293T cells. EGFP-GLAST (*WT*) or EGFP-GLASTΔCt (*ΔCt*), and V5-NHERF1 or V5-NHERF2, were co-expressed in HEK293T cells. Cells were lysed and V5-tagged NHERF proteins were immunoprecipitated using an anti-V5 antibody. GLAST (upper panel) and NHERF (lower panel) polypeptides in total cell lysates (*Lysates*) and immunoprecipitates (*IP*) were detected by immunoblotting with anti-GFP or anti-V5 antibodies. GLAST proteins possessing mature or immature *N*-glycans are indicated by an arrow or an arrowhead, respectively. Asterisks on the immunoblots for V5-NHERF proteins indicate IgG heavy chains.

2.4. Immunofluorescent staining and confocal laser microscopy

Immunofluorescent staining was performed as previously described [17]. Briefly, transfected cells were fixed in methanol and then stained with anti-V5 and anti-calnexin (1:200; Clontech), anti-ERGIC53 (1:200; Sigma), anti-Sec23 (1:200; Sigma), or anti-Rab5 (1:200; Cell Signaling Technology, Danvers, MA), followed by detection with AlexaFluor568-labeled anti-murine

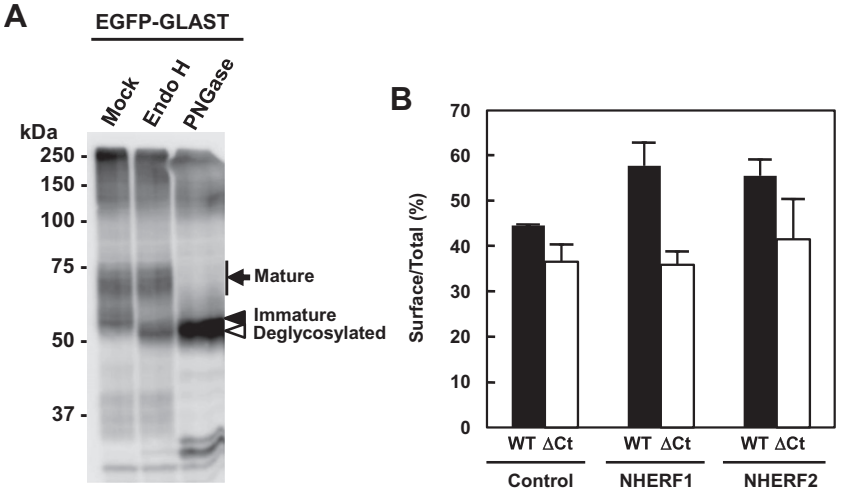

Fig. 1. Effects of NHERF1 and NHERF2 on the cell surface expression of GLAST in HEK293T cells. (A) The lysates from HEK293T cells expressing EGFP-GLAST were treated with endoglycosidase H (*Endo H*) or peptide *N*-glycosidase F (*PNGase*). EGFP signals were detected by immunoblotting with an anti-GFP antibody (*IB*). GLAST with mature or immature *N*-glycans were indicated by an arrow or an arrowhead, respectively. Deglycosylated GLAST signals are indicated by an empty arrowhead. The positions of marker proteins are shown in kDa. (B) HEK293T cells were transfected with EGFP-GLAST (*WT*) or EGFP-GLASTΔCt (*ΔCt*), and with V5-tagged NHERF1 or NHERF2. Cell surface proteins were biotinylated with sulfo-NHS-SS-biotin and solubilized, then separated from the total cell lysates using streptavidin beads. EGFP-GLAST was detected by immunoblotting and the signal intensities were measured by densitometry. Data are expressed as the mean ± SD ($n = 3$) of the abundance of the *Surface* relative to the *Total*.

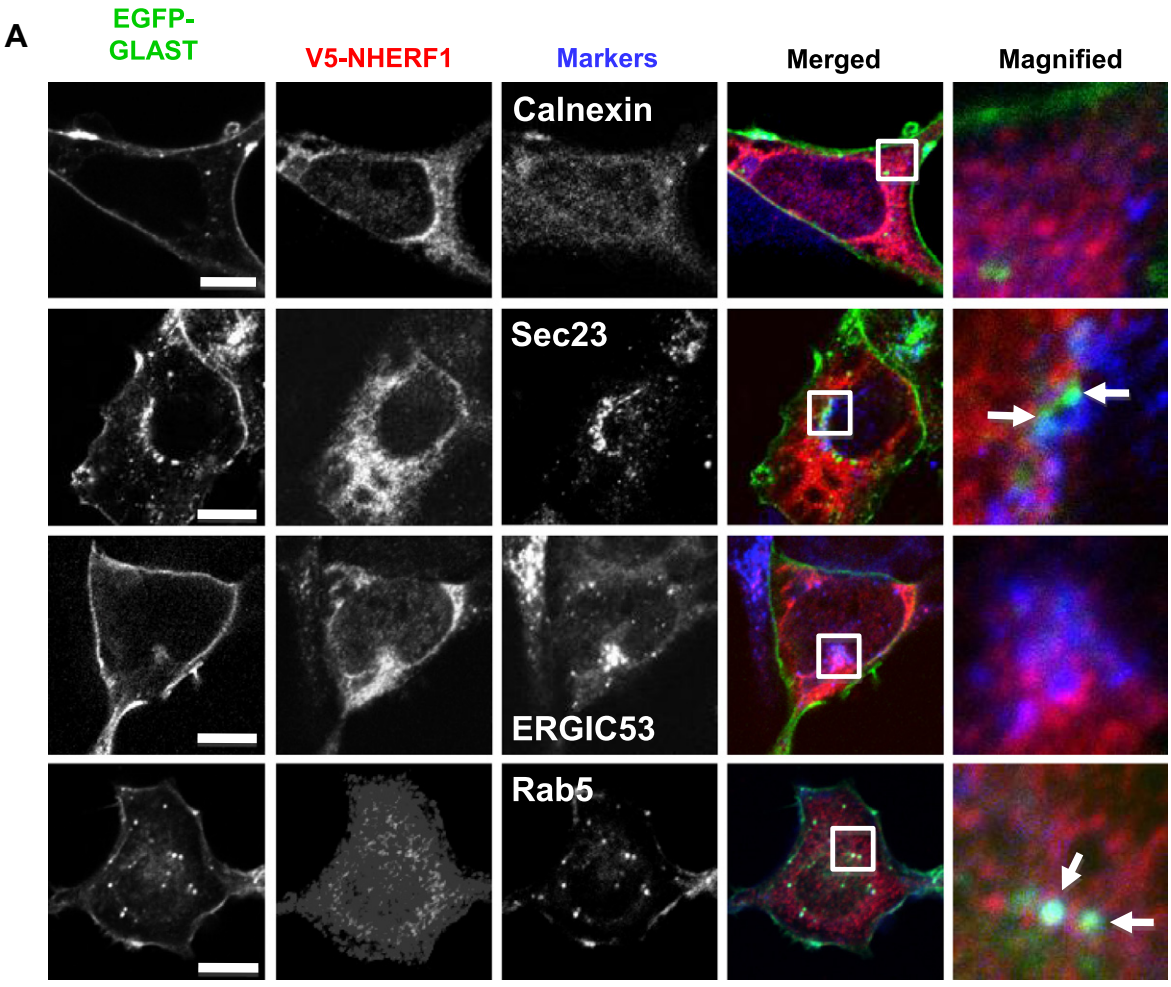

Fig. 3. Localization of GLAST with NHERF proteins in HEK293T cells. (A) EGFP-GLAST (*EGFP-GLAST*) and V5-NHERF1 (*V5-NHERF*) in transfected cells were detected with the organelle markers calnexin (*Calnexin*), Sec23 (*Sec23*), ERGIC53 (*ERGIC53*), or Rab5 (*Rab5*). Merged images are shown with magnified areas shown in the boxes. Arrows indicate co-localization of EGFP-GLAST with Sec23 or Rab5. (B) Cells expressing EGFP-GLAST and V5-NHERF2 were examined as described above. Arrowheads indicate signals at the plasma membrane. Arrows indicate co-localization of EGFP-GLAST with Sec23 or Rab5. Thin arrows and empty arrowheads indicate co-localization of V5-HNERF2 with EGFP-GLAST and co-localization of V5-NHERF2 with ERGIC, respectively. In both A and B, signals for EGFP-GLAST, V5-NHERF proteins and markers are shown in green, red, and blue, respectively. Scale bars indicate 10 μm and boxes show 5 μm squares. (C) Cells expressing FLAG-NHERF1 (*NHERF1*) and V5-NHERF2 (*NHERF2*) were analyzed for calnexin (*Calnexin*) or ERGIC53 (*ERGIC53*) expression and localization. The boxed 5 μm squares shown in most left panels show magnified areas (*Magnified*) and their merged images (*Merged*). Small squares or circles indicate signals positive for NHERF1 and NHERF2 but negative for ERGIC53, or signals positive for NHERF2 and ERGIC53 but negative for NHERF1, respectively. In the merged images, signals for FLAG-NHERF1, V5-NHERF2 and either calnexin or ERGIC53 are shown in green, red, and blue, respectively. Scale bars indicate 10 μm.

IgG (Molecular Probes, Eugene, OR), AlexaFluor405-labeled anti-rabbit IgG (Molecular Probes), or Cy3-conjugated anti-FLAGM2 antibody (1:500; Sigma). The cells were examined using a Zeiss confocal laser LSM5 PASCAL microscope.

2.5. Statistical analysis

The statistical significance of differences was assessed with the paired Student's *t*-test.

3. Results

EGFP-GLAST appeared as broad bands of 60–75 kDa monomers and 150 kDa dimers in immunoblot analysis (Fig. 1A). Deglycosylation of GLAST proteins, which possess two *N*-glycosylation sites [4,15], revealed that the *N*-glycans of GLAST polypeptides with apparent molecular masses of 65–75 kDa were resistant to endo H digestion, whereas *N*-glycans of the minor 60 kDa polypeptides were digested by endo H to yield an apparent molecular mass of 55 kDa, comparable to that of GLAST deglycosylated with PNGase F (Fig. 1A). This indicates that 65–75 kDa GLAST proteins possess mature complex-type *N*-glycans, which are processed in the Golgi apparatus, and that the minor 60 kDa molecular species have endo H-sensitive immature high mannose-type *N*-glycans, acquired in the ER.

Cell surface biotinylation assay showed that in control cells, the cell surface fraction of EGFP-GLAST comprised 44.6% ± 0.9% ($n = 3$) of the total amount. This fraction was reduced to 36.6% ± 3.8% ($n = 3$, $p < 0.05$) by the deletion of the three C-terminal amino acid residues with the sequence TKM, corresponding to the type I PDZ domain-binding motif (EGFP-GLASTΔCt, Fig. 1B and Supplementary Material Fig. S1). Moreover, co-expression of V5-NHERF1, and V5-NHERF2, increased the relative abundance of cell surface EGFP-GLAST to 57.8% ± 5.2% ($n = 3$), and 55.6% ± 3.6% ($n = 3$), respectively. Again, deletion of the C-terminal TKM motif abolished the effects of NHERF overexpression, returning the cell surface levels of EGFP-GLAST to that of control cells (Fig. 1B). These data demonstrate that cell surface expression of EGFP-GLAST is increased by interaction with endogenous PDZ proteins and markedly enhanced by overexpression of NHERF1

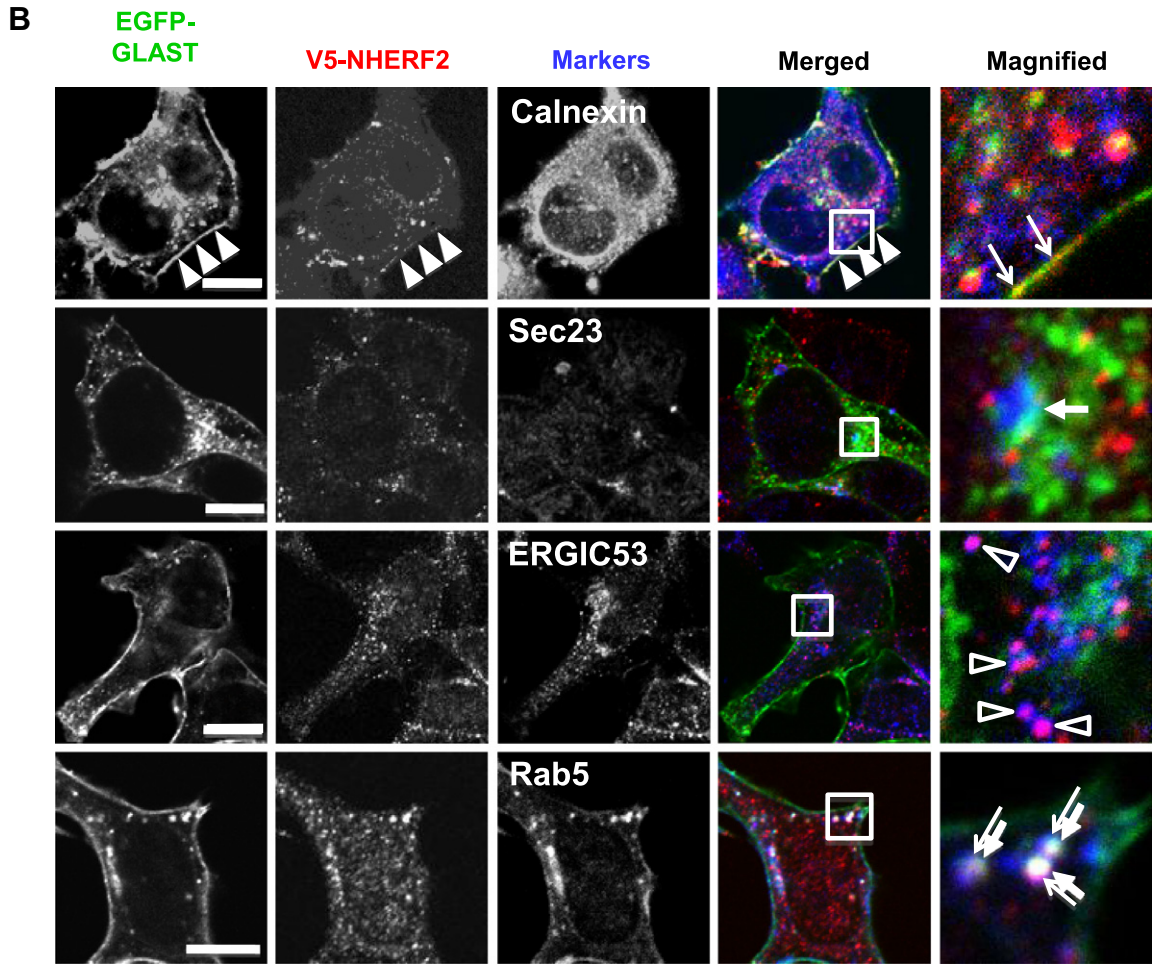

Fig. 3. (*continued*)

and NHERF2, presumably through interaction at the C-terminal PDZ domain-binding motif.

Next, the interaction of GLAST with NHERF1 or NHERF2 was analyzed by immunoprecipitation (Fig. 2). In total lysates from cells co-transfected with V5-NHERF1, the immature form of GLAST was more abundant in cells expressing EGFP-GLASTΔCt than in cells expressing the EGFP-GLAST (23.3% ± 1.0% *vs.* 15.8% ± 1.4%, $n = 3$, $p < 0.001$; Fig. 2, *Lysate*). Likewise, the percentage of immature GLAST was significantly higher in the cells expressing EGFP-GLASTΔCt than in cells expressing wild-type GLAST (20.5% ± 0.5% *vs.* 16.0% ± 0.6%, $n = 3$, $p < 0.05$) in the presence of NHERF2. Immunoprecipitation of NHERF proteins showed that approximately 5% of the total EGFP-GLAST (mature + immature) co-precipitated with V5-NHERF1 or V5-NHERF2 (Fig. 2, *IP*). By contrast, no EGFP-GLAST ΔCt was detected in the immunoprecipitates of NHERF1 or NHERF2, confirming that GLAST interacts with NHERF1 and NHERF2 at the C-terminus. Interestingly, EGFP-GLAST that co-precipitated with NHERF1 was comprised exclusively of molecules possessing immature *N*-glycans, whereas NHERF2 co-precipitated equal amounts of both the mature and immature forms of EGFP-GLAST.

Together, these results suggest that NHERF1 interacts transiently with GLAST bearing immature *N*-glycans primarily in the ER, while NHERF2 binds to GLAST possessing either immature or mature *N*-glycans in a post-ER compartment. The data also suggest that association with NHERF1 facilitates the exit of GLAST from the ER and subsequent *N*-glycan maturation.

Based on these findings, the subcellular localization of EGFP-GLAST and NHERF proteins in transfected cells was examined by confocal laser microscopy using antibodies to organelle markers: calnexin as a marker of the ER; Sec23 as a marker of the ER exit site; ERGIC53 as a marker of the ER–Golgi intermediate compartment (ERGIC); and Rab5 as a marker of early endosomes. The results revealed that EGFP-GLAST was principally localized to the periphery of the cells with some granular distribution in the cytoplasm, which coincided with Sec23 and Rab5 staining. No differences were observed between cells co-transfected with V5-NHERF1 (Fig. 3A) and those expressing V5-NHERF2 (Fig. 3B). Signals from V5-NHERF1 produced a reticular or granular appearance in the cytoplasm. Most of those signals were separate from the signals generated by calnexin, Sec23, and ERGIC53, although V5-NHERF1 was localized in close proximity to these organelle markers (Fig. 3A). This close but inconsistent distribution of NHERF1 and the ER markers, calnexin and Sec23, may indicate that association of NHERF1 with GLAST on the ER membrane is a rapid and transient process.

On the other hand, V5-NHERF2 exhibited a dispersed and punctate appearance and showed marked differences from V5-NHERF1 in its subcellular localization (Fig. 3B). V5-NHERF2 signals were partially coincident with ERGIC53, but differed from those of calnexin and Sec23, although they were closely distributed. Moreover, some of the punctate structures positive for EGFP-GLAST at the plasma membrane or in vesicles positive for both EGFP-GLAST and Rab5 in the cytoplasm were also coincident with V5-NHERF2, but not with V5-NHERF1.

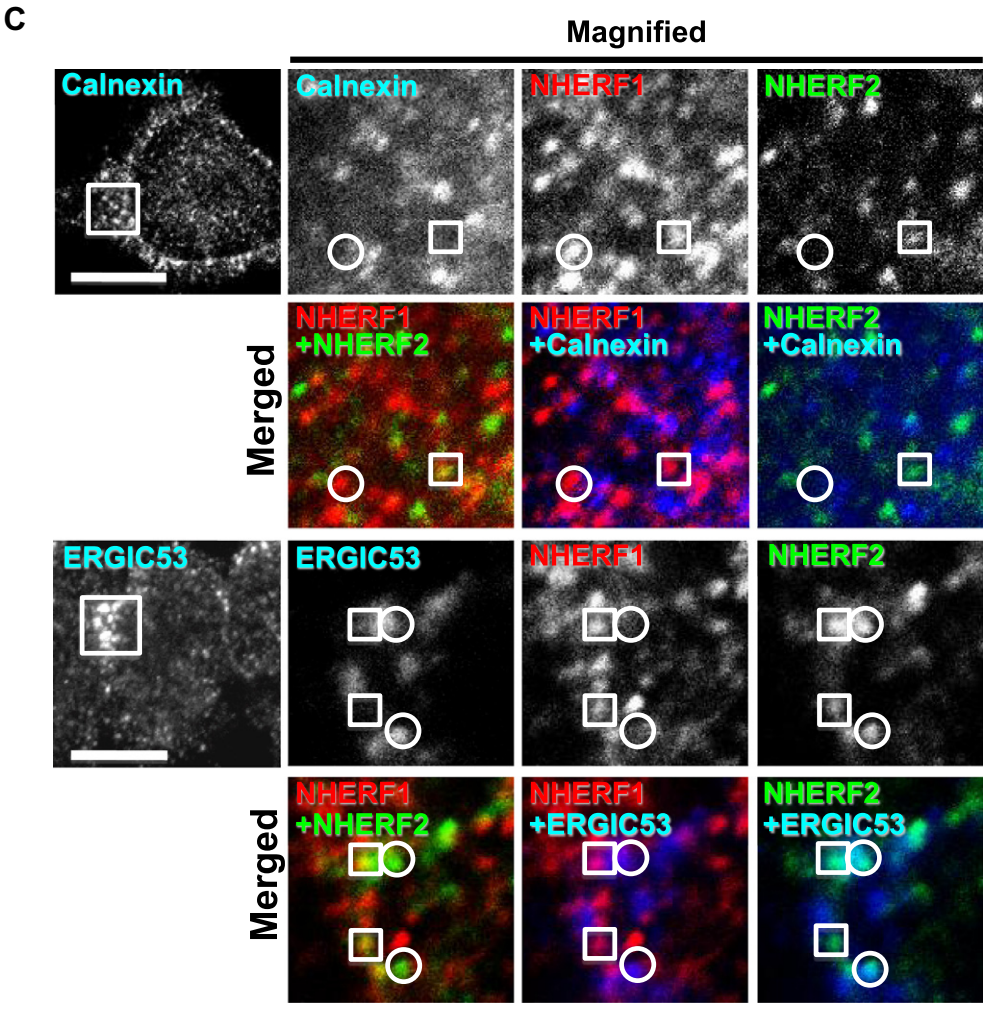

Fig. 3. (*continued*)

Co-expression of FLAG-NHERF1 and V5-NHERF2 showed that the fluorescent signals of these NHERF isoforms coincided in some punctate or reticular structures, demonstrating that they do co-exist in some compartments (Fig. 3C). Structures that were positive for both NHERF1 and NHERF2 were negative for calnexin and ERGIC53, although NHERF1 and NHERF2 were in close proximity to calnexin and Sec23, as described above (Fig. 3A and B). However, it should be noted that a few vesicular compartments that were positive for V5-NHERF2, but not for FLAG-NHERF1, were also positive for ERGIC53, suggesting a selective interaction of NHERF2 with GLAST in the ERGIC. This finding suggested that the change in binding partners of GLAST from NHERF1 to NHERF2 occurs during vesicular transport from the ER to the ERGIC.

To examine this hypothesis, the effect of overexpression of the small GTPase Sar1, a primary component of coatomer protein complex II (COPII) that forms the vesicle to export cargo proteins from the ER, and its dominant-negative mutant Sar1 H79G [16] were analyzed. When wild-type Sar1 was overexpressed, the total cell lysate primarily contained EGFP-GLAST bearing mature *N*-glycans. Conversely, EGFP-GLAST with immature *N*-glycans was the primary form found in cells co-transfected with Sar1 H79G (Fig. 4A, *Lysates*). No differences in this result were observed between cells co-transfected with NHERF1 or NHERF2. Compared with the pattern of *N*-glycan processing shown in Fig. 2, these data demonstrate that overexpressed wild-type Sar1 increased ER exit and subsequent *N*-glycan maturation of EGFP-GLAST, whereas expression of Sar1 H79G resulted in confinement of EGFP-GLAST in the ER.

Under these conditions, EGFP-GLAST bearing mature *N*-glycans co-precipitated with V5-NHERF2, but not with V5-NHERF1 (Fig. 4A, *IP*). Moreover, EGFP-GLAST possessing immature *N*-glycans co-precipitated with V5-NHERF2 as well as with V5-NHERF1, but to a lesser degree. The presence of Sar1 H79G in the immunoprecipitates of NHERF proteins at levels similar to that of EGFP-GLAST also indicated an association between GLAST, Sar1, and NHERF proteins at the site of COPII vesicle formation. These data provide convincing evidence that GLAST interacts with NHERF1 primarily in the ER and with NHERF2 after exit from the ER, and that the interaction with NHERF1 increases the export of GLAST from the ER.

Overexpression of wild-type Sar1 caused no apparent change in the subcellular localization of EGFP-GLAST and V5-NHERF1 (Fig. 4B). However, in cells expressing Sar1 H79G, fluorescent signals from EGFP-GLAST, V5-NHERF1, and calnexin partially co-localized with each other. This indicated that co-localization of EGFP-GLAST with NHERF1 in the ER was evident when ER export was abrogated by expression of the Sar1 mutant, supporting the hypothesis that the interaction between GLAST and NHERF1 is a transient event in the ER. Furthermore, in cells in which COPII-mediated exit from the ER was suppressed by the expression of Sar1 H79G, V5-NHERF2 co-localized with some EGFP-GLAST, but not with calnexin (Fig. 4B). These findings agree with those

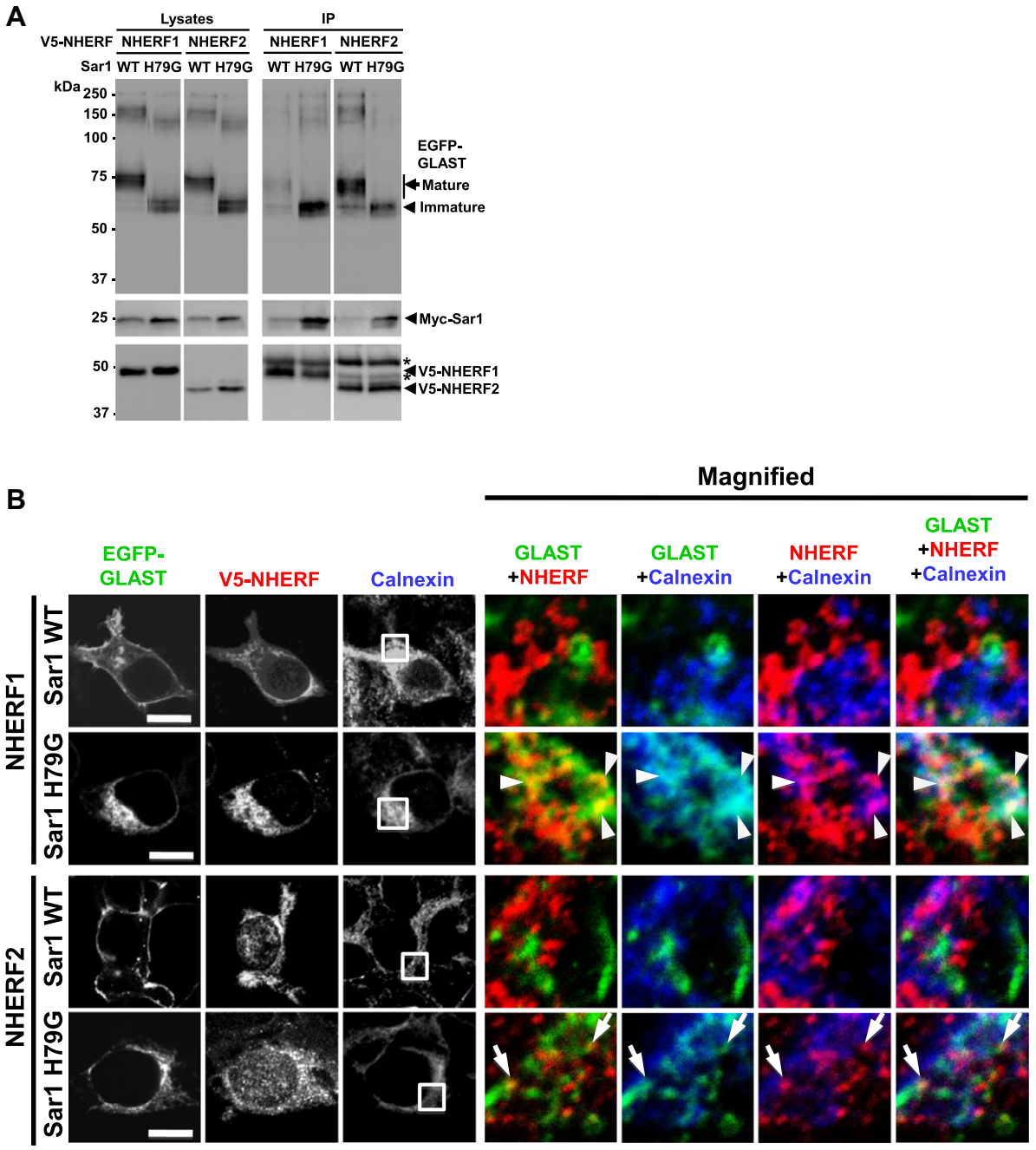

Fig. 4. Effects of Sar1 H79G on the interaction of GLAST with NHERF proteins. (A) EGFP-GLAST and V5-NHERF1 or V5-NHERF2 were co-expressed in HEK293T cells in the presence of wild-type Sar1 (*WT*) or Sar1 H79G (*H79G*) and then immunoprecipitated with a V5 antibody. EGFP-GLAST, NHERF, and Sar1 polypeptides in the total cell lysates (*Lysates*) and the immunoprecipitates (*IP*) were detected by immunoblotting with anti-GFP, anti-V5, and anti-myc antibodies, respectively. Indications are the same as in Fig. 2. (B) Cells expressing EGFP-GLAST with V5-NHERF1 or V5-NHERF2 in the presence of wild-type Sar1 (*Sar1 WT*) or Sar1 H79G (*Sar1 H79G*) were also examined for calnexin (*Calnexin*) expression, as described in the legend for Fig. 3. Boxed areas (5 μm squares) are magnified and merged images. EGFP-GLAST (green), V5-NHERF proteins (red), and calnexin (blue) are shown (*Magnified*). Arrowheads indicate signals positive for EGFP-GLAST, V5-NHERF1, and calnexin. Arrows indicate signals positive for EGFP-GLAST and V5-NHERF2 but not for calnexin. Scale bars indicate 10 μm.

obtained by immunoprecipitation (Fig. 4A) and confirm the serial and differential interaction between GLAST and NHERF1, or NHERF2, in the early stage of the secretory pathway.

4. Discussion

The present study demonstrates that NHERF1 and NHERF2 interact serially with GLAST in the secretory pathway. In this mechanism, GLAST, a vesicular cargo, transfers from NHERF1 to NHERF2 during COPII-mediated vesicular transport from the ER to the ERGIC. The sequential interaction of NHERF proteins with GLAST suggests that NHERF proteins not only stabilize GLAST at the plasma membrane [5,6], but also regulate the vesicular trafficking of the transporter, as evidenced by the finding that the interaction of GLAST with NHERF1 facilitated the export of GLAST from the ER (Fig. 2). Facilitated export from the ER has also been reported for the glutamate transporter EAAC1 via the adaptor protein PDZ-K1 [7], although the molecular mechanism of this process is unknown. Previous studies have demonstrated that the ER-resident protein GTRAP3-18 interacts with and delays

the ER exit of glutamate transporters, including GLAST, through binding to the C-terminal cytoplasmic tail of the transporter [18,19]. NHERF1 may compete with GTRAP3-18 for binding to the C-terminal region of GLAST, thus facilitating its exit from the ER. Another possibility may include interaction of the COPII component, Sec24, and the ER export motif RI/RL/RVX$_8$D, which are found in the C-terminal regions of the SLC6 family of neurotransmitter transporters [20,21]. Because mammalian GLAST also contains a sequence that conforms to the RI/RL/RVX$_8$D-class motif in the C-terminal region (e.g., R477LRTTTNVLGD in canine GLAST), NHERF1 may facilitate the ER exit of GLAST by promoting the binding of Sec24 to GLAST.

Moreover, this study shows that NHERF1 and NHERF2 transiently localize to the ER and the ERGIC, and facilitate the exit of GLAST from the ER (Fig. 3). It is noteworthy that mass spectrometry also revealed the presence of non-muscle myosin heavy chain II (NMII) in the immunoprecipitates of HEK293T cells transfected with V5-NHERF proteins (K.S. and M.I., unpublished observations). NMII interacts with NHERF1 and the actin cytoskeleton [22] and promotes the retrograde transport of cargo proteins from the Golgi to the ER [23]. Although NHERF proteins tether membrane transporters by binding to actin cytoskeleton through FERM proteins [10], the association of NHERF proteins with the actin cytoskeleton through NMII may be involved in the intracellular vesicular trafficking of GLAST.

The present study also demonstrates that NHERF2 colocalizes with GLAST at the plasma membrane and in early endosomes (Fig. 3), suggesting that NHERF2 is involved in the endocytosis of GLAST. PDZ domain-containing proteins have been shown to affect the endocytosis of CFTR [13], and differences in the affinities of NHERF1 and NHERF2 for CFTR regulate the balance between endocytic recycling and degradation [14]. Removal of the PDZ-target motif increased the endocytosis of EAAC1 by promoting the binding of clathrin and α-adaptin to EAAC1 [7] through the tyrosine-based internalization motif YXXΦ [24]. Since mammalian GLAST possesses a similar well-conserved motif in the C-terminal region (e.g., Y523QLI in canine GLAST), the balance between endocytosis, recycling and degradation of GLAST would be affected by interaction with NHERF2.

NHERF1 and NHERF2 perform an overlapping function as scaffolding proteins at the plasma membrane [25]. Although NHERF1 and NHERF2 are expressed at higher levels in epithelial cells [10], NHERF1 is enriched in tissues possessing extensively polarized epithelia, such as renal tubular cells, while NHERF2 has a more restricted tissue distribution, with the highest expression in the lung [10]. Moreover, the cellular distribution of NHERF1 and NHERF2 in polarized epithelial cells are mutually exclusive [26]. Conversely, both NHERF1 [5] and NHERF2 [6] are expressed in brain astrocytes, where GLAST is also highly expressed. Therefore, the serial interaction of NHERF proteins with GLAST during COPII-mediated trafficking of GLAST would occur in brain astrocytes, but not in epithelial cells. Thus, NHERF proteins may upregulate the cell surface expression of GLAST in a tissue- and cell-specific manner.

In summary, we demonstrate here that the organelle-specific interactions of GLAST with NHERF1 or NHERF2 play significant roles in regulating the intracellular trafficking of GLAST. These interactions may correspondingly modulate the cell surface expression level of GLAST by accelerating its ER–Golgi trafficking.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.059.

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