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Recruitment of TIP47 to lipid droplets is controlled by the putative hydrophobic cleft

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Abstract

Adipose differentiation-related protein (ADRP) and TIP47 show sequence similarity, particularly in their N-terminal PAT-1 domain. Under standard culture conditions, ADRP existed in most lipid droplets (LDs), whereas TIP47 was observed only in some LDs and recruited to LDs on treatment with fatty acids. By analyzing deletion mutants, we found that the C-terminal half of TIP47, or more specifically the putative hydrophobic cleft [S.J. Hickenbottom, A.R. Kimmel, C. Londos, J.H. Hurley, Structure of a lipid droplet protein; the PAT family member TIP47, Structure (Camb) 12 (2004) 1199–1207.], was involved in LD targeting and responsiveness to fatty acids. The result contrasted with that observed for ADRP and implied a distinct LD-targeting mechanism for TIP47. Consistent with this, overexpression of Rab18 decreased ADRP, but not TIP47, from LDs, and TIP47 did not displace pre-existing ADRP from LDs. But ADRP may be a factor to control the TIP47 behavior, because TIP47 in LDs increased upon down-regulation of ADRP. The results suggested that the putative hydrophobic cleft is critical for the unique characteristics of TIP47.

Keywords: ADRP; TIP47; Rab18; Lipid droplets; Fatty acids

Lipid droplets (LDs) are found in most cell types. They used to be considered only as a passive reservoir of excess lipids, but recent studies have indicated that they may be actively involved in intracellular lipid trafficking, lipid metabolism, signal transduction, and other cellular functions [1–3]. LDs comprise a core of lipid esters and a phospholipid monolayer that forms the outer boundary [4]. A number of proteins have been reported to localize in LDs. These proteins are generally considered to bind to the LD surface [5,6], but some of them may also penetrate deep into the LD core by an unknown mechanism [7].

PAT proteins, which have derived their name from perilipin, adipose differentiation-related protein (ADRP; also known as adipophilin), and TIP47 constitute a family of LD proteins [5]. They exhibit sequence similarity and are believed to have similar putative three-dimensional structures [8]. Perilipin is expressed only in adipocytes and steroidogenic cells, whereas ADRP and TIP47 are expressed in many cell types. The localization of ADRP to LDs has been shown repeatedly [9,10], and the molecular properties and functions of ADRP have been reported [11–16]. In contrast, TIP47 has been well characterized as a cytoplasmic protein that facilitates the transport of mannose-6-phosphate receptors (MPRs) from the endosomes to the trans-Golgi network (TGN) [17,18], but the localization of TIP47 to LDs was under controversy [19,20]. Using specific antibodies and different methodologies, several studies have demonstrated that TIP47 is distributed in LDs [21–24], but the mechanism via which it localizes to LDs has not been elucidated yet.

In this study, we comparatively examined the distributions of ADRP and TIP47 in Huh7 cells. These cells constitutively harbor a large number of LDs under standard culture conditions. Despite their sequence similarity, ADRP and TIP47 showed different behaviors on Rab18 overexpression or the addition of fatty acids. Furthermore,

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we examined the behavior of various TIP47 mutants. We observed that the N-terminal domain of TIP47 that exhibits a high similarity to ADRP did not mediate efficient LD localization, and the C-terminal domain, which was shown to form a hydrophobic cleft [8], was necessary for the recruitment of TIP47 to LDs. Additionally, our results showed that LDs harbor ADRP, TIP47, and Rab18 in various ratios, thus suggesting the co-existence of heterogeneous LDs in a cell.

Materials and methods

Cells and antibodies. Human Huh7, HepG2, mouse Balb/c 3T3, and hamster BHK-21 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank. The cells were cultured in Dulbecco's minimum essential medium supplemented with 10% fetal calf serum, 50 U/ml penicillin, and 0.05 mg/ml streptomycin at 37 °C under 5% CO2:95% air. When required, the cells were treated with 200–400 μM fatty acids that were complexed with fatty acid–free bovine serum albumin (Wako Pure Chem.). Oleic acid, linoleic acid, and docosahexanoic acid (Sigma) were used.

A rabbit anti-TIP47 antibody was raised against a peptide of human TIP47 segment (amino acids (aa) 305–318) as described [21], and it was affinity-purified by using a peptide column. Anti-human ADRP antibody was obtained from Progen. The specificity of the anti-TIP47 and anti-ADRP antibodies and the lack of crossreaction between these two antibodies were confirmed by immunofluorescence microscopy and Western blotting using BHK-21 cells that were transfected with either human TIP47 or human ADRP cDNA (data not shown); this was possible because the antibodies did not react with hamster proteins. Guinea pig anti-TIP47 antibody (Progen), mouse anti-V5 antibody (Invitrogen), biotinylated horse anti-goat IgG antibody (Vector), and anti-IgG conjugated with fluorochromes (Molecular Probes) were purchased commercially.

Plasmids. Human TIP47 cDNA was amplified using polymerase chain reaction (PCR) and was inserted into the pcDNA3.1/V5-His-TOPO vector (Invitrogen). Various mutants were obtained by PCR using appropriate primer sets and were cloned to the same vector. The mutants were designed such that they had a V5 tag at the C terminus, or they were expressed as non-tagged molecules. The critical residues that formed the putative hydrophobic cleft were replaced with alanine as follows: the sequence of aa 254–261 was changed from SLGKLRAT to ALGAARAA, aa 343–349 from SLGSSIQ to AAGSAIA, and aa 416–421 from WLVGPF to AAAGAA. In order to synthesize a TIP47-ADRP fusion protein that comprised the N-terminal half of TIP47 and the C-terminal half of ADRP, an *Nhe*I restriction enzyme site was created between the two segments such that the final protein would have two extra amino acids (Ala-Ser) at the center of the molecule. All the plasmids were controlled by DNA sequencing.

cDNA transfection and RNA interference. For cDNA transfection, the plasmid vectors were introduced into the cells by using Lipofectamine 2000 (Invitrogen), and the cells were observed one or two days after transfection. The expression of proteins of the expected sizes was confirmed by Western blotting. For RNA interference (RNAi), SMARTpool siGENOME duplexes (Dharmacon) were used to knockdown the expression of TIP47 and ADRP. A control RNA duplex, namely, siCONTROL Non-Targeting siRNA, was also obtained from Dharmacon. The cells were transfected with siRNA by using RNAiFect (Qiagen). After three days, the cells were harvested for analyses.

Immunofluorescence microscopy. The cells that were cultured on coverslips were observed by immunofluorescence microscopy as described previously [23]. In most of the experiments, the cells were fixed with 3% formaldehyde alone or with 3% formaldehyde containing 0.01–0.025% glutaraldehyde and were permeabilized using 0.01% digitonin for 30 min. In order to analyze the non-tagged TIP47 mutant proteins, mouse Balb/c 3T3 cells were transfected with cDNAs and labeled using the guinea pig anti-TIP47 antibody that does not crossreact with endogenous mouse molecules. LDs were visualized using BODIPY 493/503 (Molecular Probes). The images were captured by a Zeiss Pascal confocal laser scanning microscope or a Zeiss Axiovert fluorescence microscope equipped with the Apotome system.

For the quantification of the image intensity, more than 12 areas of each sample were randomly pictured under identical microscopic settings. The areas in which the labeling intensity was above a certain threshold per unit cell area were assessed by ImageJ (http://rsb.info.nih.gov/ij/). In order to quantify the targeting of the non-tagged TIP47 mutants in LDs, Balb/c 3T3 cells were cotransfected with a mutant cDNA and 1/10th amount of the pEGFP-N1 vector (Clontech) and labeled for human TIP47. The results of a preliminary experiment revealed that the EGFP-positive cells invariably expressed the other protein.

Western blotting. Total cell lysates were solubilized in a sodium dodecyl sulfate sample buffer, and an equal amount of proteins (20 μg) was electrophoresed in 15% acrylamide gels and transferred to a nitrocellulose membrane. The blot was incubated with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies. The reaction was detected by using SuperSignal West Dura Extended Duration Substrate (Pierce).

Results

Intracellular distribution of ADRP and TIP47

Huh7 cells were derived from human hepatoma and harbored many LDs when cultured in a standard medium. TIP47 in Huh7 cells was labeled around LDs by the current protocol (Fig. 1A) [23]. Despite the constant presence of LDs, only 10–20% of the cells showed TIP47-positive LDs, while the remaining cells lacked TIP47 in LDs. Therefore, although LDs were positive for ADRP in all cells, the colocalization of TIP47 and ADRP in LDs occurred only infrequently (Fig. 1B). Even in cells harboring TIP47-positive LDs, many other LDs were devoid of TIP47 and were labeled for ADRP alone (Fig. 1A and B). A small number of LDs, in particular, were labeled for TIP47 alone and not for ADRP (Fig. 1B); LDs of this type were relatively small and were observed not only in Huh7 cells but also in human fibroblasts and other cell types (Fig. 1C). As shown below, in this type of LDs, TIP47 persisted even when its total expression drastically reduced due to RNAi.

Recently, we observed that Rab18 specifically localizes in LDs. Rab18 and ADRP may coexist in the same LDs, but the labeling intensity for these proteins was often reciprocal [25]. LDs that were labeled positively for both Rab18 and TIP47 were also observed; however, such LDs were limited, and in most cases, they were labeled for only one of these proteins (Fig. 1D). These results indicate that LDs in Huh7 cells comprise heterogeneous populations that harbor ADRP, TIP47, and Rab18 in different ratios.

Localization of TIP47 in LDs is increased by fatty acids

On the addition of fatty acids to the medium, TIP47 was rapidly recruited to LDs in Huh7 cells (Fig. 2A). A similar redistribution was observed when other fatty acids such as linoleic acid or docosahexanoic acid were added. The

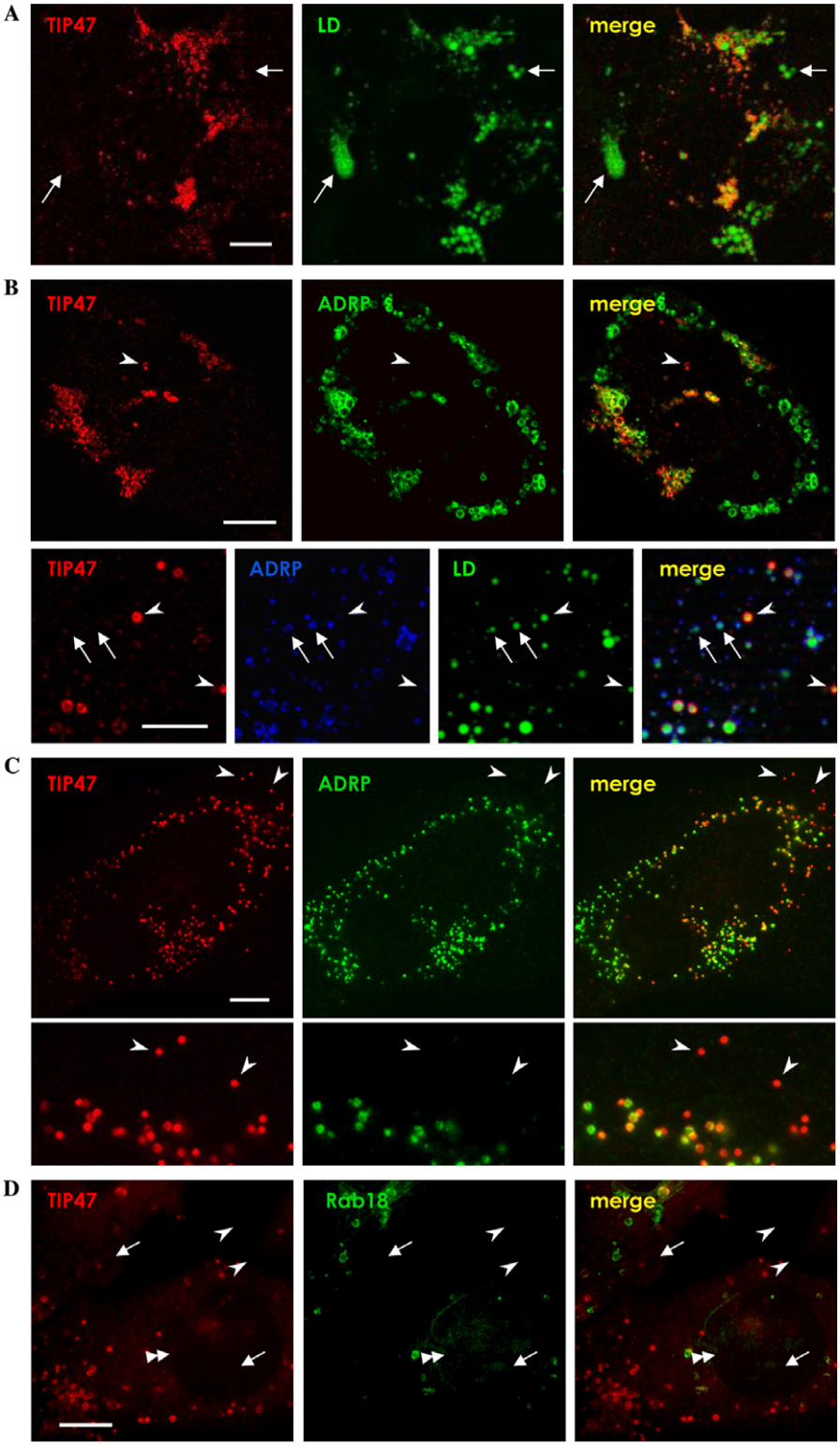

Fig. 1. Distribution of TIP47, ADRP, and Rab18 in LDs. (A) Huh7 cells. TIP47 labeling was observed as round rings of various sizes (red) that surrounded LDs; they were identified by staining with BODIPY 493/503 (green). In Huh7 cells that were cultured in a standard medium, TIP47-positive LDs and TIP47-negative LDs (arrows) coexisted. (B) Huh7 cells. Upper panel: TIP47 (red) and ADRP (green) colocalized in many cases. However, ADRP-positive, TIP47-negative LDs and a relatively small number of ADRP-negative, TIP47-positive LDs (arrowheads) were also observed. Lower panel: triple labeling of TIP47 (red), ADRP (blue), and BODIPY 493/503 (green) revealed that ADRP-positive, TIP47-negative LDs (arrows) and ADRP-negative, TIP47-positive LDs (arrowheads) were present. (C) Human fibroblasts cultured with 200 μM oleic acid for 2 days. Under this condition, all the cells contained TIP47-positive LDs. A small number of TIP47-positive, ADRP-negative LDs (arrowheads) were observed consistently. (D) Huh7 cells. TIP47-positive, Rab18-negative LDs (arrows); TIP47-negative, Rab18-positive LDs (arrowheads); and TIP47-positive, Rab18-positive LDs (double arrowheads) were observed. Bars, 10 μm.

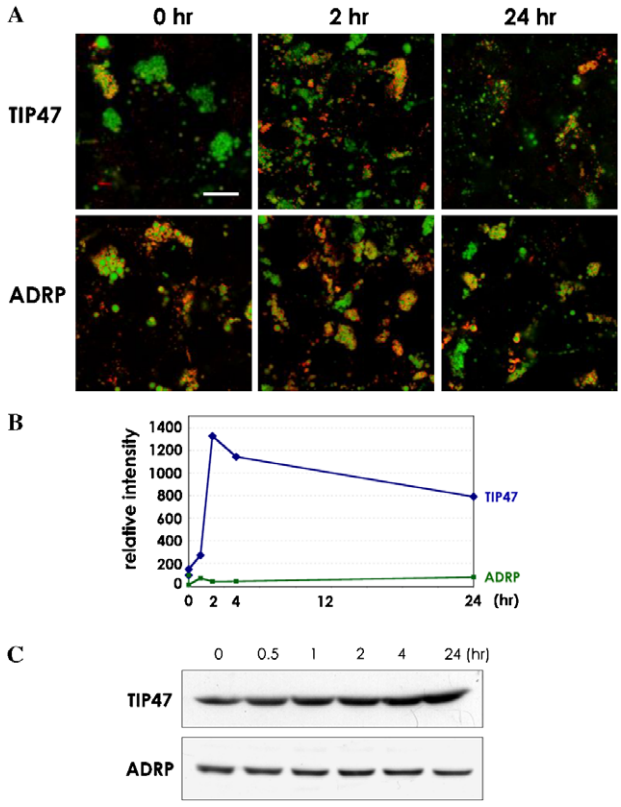

Fig. 2. TIP47 in Huh7 cells treated with oleic acid. (A) The frequency of TIP47-positive LDs increased significantly on treatment with 200 μM oleic acid for 2 h; however, the frequency of TIP47-positive LDs did not increase further when incubated for a longer period of time (upper panel). In contrast, the ADRP-positive LDs did not show any change in their frequency on treatment with oleic acid (lower panel). Bar, 10 μm. (B) Semiquantitation of the total intensity of the TIP47 and ADRP labeling per unit cell area. The labeling intensity of TIP47 in LDs increased rapidly after the oleic acid treatment, became the strongest at 2 h, and then decreased gradually. In contrast, ADRP labeling did not change significantly up to 24 h. The graph shows the representative results of three independent experiments. (C) Western blotting. Each lane was loaded with the same amount of proteins (20 μg). The expression of TIP47 per protein weight increased steadily after oleic acid treatment; however, the time course did not correlate with that of the immunofluorescence labeling intensity. ADRP expression did not show a significant change during the same period.

redistribution of TIP47 to LDs during de novo formation in preadipocytes was reported when this study was being performed [26]. But a brief fatty acid treatment of Huh7 cells did not visibly increase the number or volume of LDs (data not shown). Thus, it is likely that the recruitment of TIP47 in Huh7 cells occurred to the preexisting LDs. The ratio of LDs labeled for TIP47 was the highest at 2 h after the addition of oleic acid, and it decreased gradually later (Fig. 2B). In contrast, the intensity of ADRP labeling did not show a significant change on the addition of oleic acid. The total expression of TIP47 in Huh7 cells also increased by the oleic acid treatment (Fig. 2C), but its increase was considerably slower than that of the LD labeling. These results showed that the increase of TIP47 localization in LDs was not related to the TIP47 expression level.

Overexpression of Rab18 did not affect TIP47

Rab18 overexpression displaced ADRP from LDs [25]. Considering the sequence similarity between ADRP and TIP47, we assumed that Rab18 could exert an identical effect on TIP47 and examined this possibility in HepG2 cells. We used this cell line because in these cells, the displacement of ADRP by Rab18 was observed more clearly as compared to that in Huh7 cells; in HepG2 cells also, ADRP was observed in almost all LDs, whereas TIP47 was found in a small percentage of LDs (data not shown). The effect of Rab18 on TIP47 was examined by comparing the labeling intensity of TIP47 between EGFP-Rab18-positive and EGFP-Rab18-negative LDs in individual cells. We observed that while EGFP-Rab18-positive LDs always showed a weaker labeling intensity for ADRP than other LDs (Fig. 3A), TIP47 labeling did not produce constant results (Fig. 3B). There was the innate variability of TIP47 labeling in LDs, but obviously Rab18 overexpression did not affect TIP47 in LDs in the same manner as it did ADRP.

Knockdown of TIP47 and ADRP

The mutual relationship between TIP47 and ADRP was further explored by knocking down either one of these proteins by RNAi. In comparison to the control cells that were treated with control random siRNA, both TIP47 and ADRP were reduced significantly when treated with their respective siRNA (Fig. 4A). The knockdown of either protein did not significantly affect the total expression of the other protein.

Immunofluorescence microscopy revealed that TIP47 knockdown did not affect ADRP labeling (Fig. 4B). Interestingly, however, residual TIP47 labeling was often observed in the ADRP-negative LDs after the knockdown, while the ADRP-positive LDs in the same cell were devoid of TIP47. This result suggested a unique property of TIP47-positive, ADRP-negative LDs. Contrastingly, after the ADRP knockdown, intense TIP47 labeling was observed in LDs in the majority of cells without any further treatment (Fig. 4B and C). The increase in level of TIP47 was most conspicuous in the cells showing a significant reduction in the level of ADRP. Because the total expression of TIP47 did not increase by the ADRP knockdown, this result suggested that the presence of ADRP is a factor that restricts the occurrence of TIP47 in LDs.

Molecular segments necessary for the localization of TIP47 to LDs

The above results imply that ADRP and TIP47 are localized to LDs using different cues. But these two proteins show the highest similarity with regard to their N-terminal PAT-1 domain, which we as well as others showed sufficient for the LD localization of ADRP [13,14,16]. In order to analyze whether the corresponding

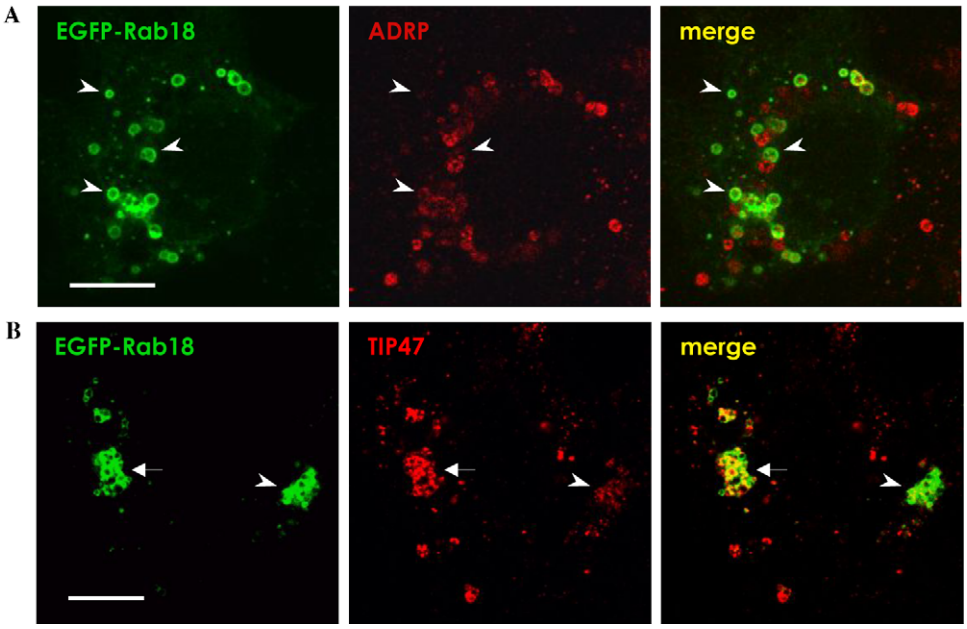

Fig. 3. Effect of Rab18 overexpression in HepG2 cells. (A) The labeling intensity of EGFP-Rab18 and ADRP in each LD was observed in a reciprocal manner. The level of ADRP labeling was weak in EGFP-Rab18-positive LDs (arrowheads). (B) The intensity of EGFP-Rab18 and TIP47 in LDs did not show a constant correlation. EGFP-Rab18-positive LDs showed variable levels of TIP47 labeling (compare the arrow and the arrowhead). Bars, 10 μm.

domain of TIP47 supports LD localization, we constructed deletion mutants of TIP47 and observed their distribution in Huh7 cells by immunofluorescence microscopy. For this purpose, the TIP47 mutants that were tagged with a V5 epitope at the C terminus were first expressed in Huh7 cells and then labeled by the anti-V5 antibody. The overall result was similar to that obtained in the case of non-tagged proteins described below; however, full-length TIP47 tagged with V5 often showed an unusual cytosolic distribution (Fig. 5A), and its LD localization hardly increased on the addition of oleic acid (Fig. 5B). This behavior was considerably different from that of the endogenous protein, thus suggesting that the tag affected the behavior of TIP47. A deleterious effect of tags on TIP47 functions has also been reported [27].

Thus, to precisely analyze the effect of mutation, we constructed deletion mutants without any tags. The mutant proteins were expressed in mouse Balb/c 3T3 cells and labeled using the guinea pig anti-human TIP47 antibody; it was confirmed that this antibody did not recognize endogenous mouse TIP47 (data not shown). The antibody recognized the N-terminal portion of TIP47, and thus did not label 183–434 or endogenous TIP47 variant pp17a, but this protein was already reported not to localize in LDs [28]. The localization of the non-tagged full-length human TIP47 to LDs markedly increased after oleic acid addition, thus indicating that the protein behaved in a manner similar to the endogenous protein (Fig. 5C). In comparison to the V5-tagged mutants, the non-tagged mutants showed a general increase in the ratio of LD localization.

The N-terminal segments alone, i.e., 1–140, 1–183, and 1–246, were not sufficient for efficient LD localization, thus indicating the importance of the C-terminal portion. A fusion protein comprising the TIP47 N-terminal half (1–195) and the ADRP C-terminal half (185–437) was targeted to LDs in the absence of oleic acid, thus suggesting that both LD localization and the responsiveness to fatty acids of TIP47 were controlled by the C-terminal half. Consistent with this, deletion of a segment similar to a tandem repeat of S3-12, i.e., Δ(73–145), did not affect the LD localization.

On the other hand, small deletions in the C terminus, i.e., 1–316 and 1–384, induced highly efficient LD localization even in the absence of oleic acid. We speculated that the C-terminal deletions caused the abovementioned effect by disrupting the putative hydrophobic cleft [8]. We tested this possibility by replacing the critical amino acids that formed the cleft wall with alanine residues. The above assumption was supported because two of three such mutants were largely localized to LDs even in the absence of oleic acid (Fig. 5A and C). Another notable result was that the deletion of the putative Rab9-binding domain, i.e., Δ(159–173), caused a marked reduction in LD localization. This result suggested that the binding of Rab9 may be necessary for the localization of TIP47 to LDs.

Discussion

Targeting of TIP47 to LDs

In comparison with ADRP and perilipin, TIP47 was unique in that it was recruited to LDs on treatment with fatty acids. The N-terminal half of TIP47 contains the 11-mer repeats that are found in many lipid-binding proteins [29]. Furthermore, as compared to the C-terminal half, the N-terminal half, or more specifically the PAT-1

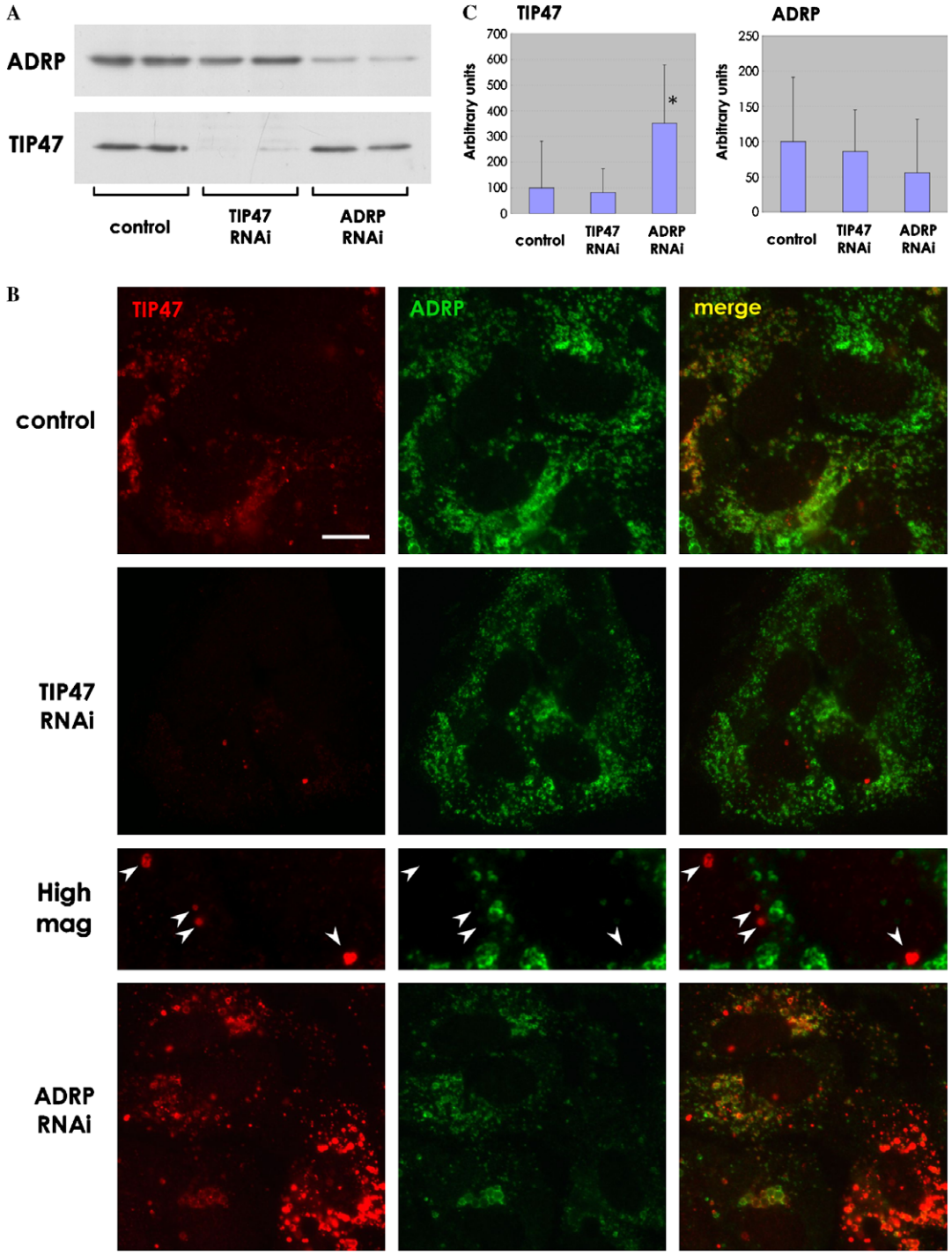

Fig. 4. Knockdown of TIP47 and ADRP in Huh7 cells by RNAi. (A) Western blotting. Each experiment was performed in duplicate. The RNAi procedure caused an effective reduction of the target proteins, while the other protein was not affected significantly. (B) In cells transfected with control siRNA, LDs were labeled in various ratios for both ADRP and TIP47. When treated with TIP47 siRNA, the expression of TIP47 was effectively suppressed, but the labeling of ADRP remained unaffected. Interestingly, residual TIP47 labeling was localized to ADRP-negative LDs (arrowheads in high magnification figures). When treated with ADRP RNAi, variable degrees of reduction occurred in the ADRP expression in different cells; however, in the cells wherein ADRP was weakly labeled, TIP47 labeling was generally enhanced. Bar, 10 μm. (C) Cells that were treated with the control, TIP47, and ADRP siRNAs were labeled for TIP47 and ADRP, and the area showing the labeling above the threshold intensity per unit cell area was quantified. Knockdown of ADRP increased the area positively labeled for TIP47 significantly (*$p < 0.01$, Student's t test).

region showed higher similarity to ADRP. Therefore, we suspected that the N-terminal and C-terminal halves of TIP47 are related to LD binding and fatty acid responsiveness, respectively. However, the present result showed that the N-terminal half of TIP47, e.g., 1–183 and 1–246, is not effective for targeting to LDs. Moreover, the region with

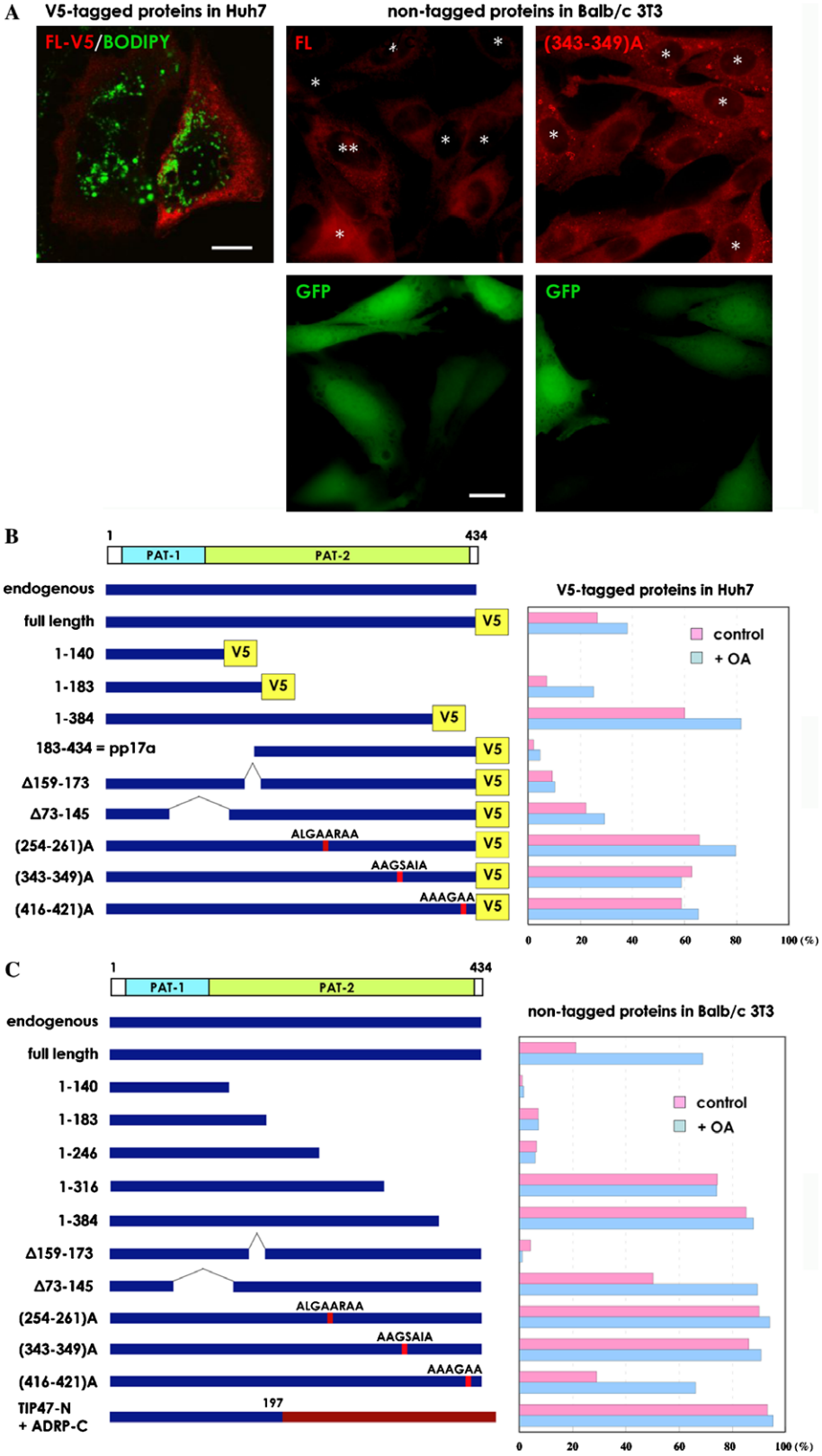

Fig. 5. Analysis of the TIP47 mutants. (A) Left panel, Huh7 cells. Full-length TIP47 tagged with V5 at the C terminus showed peculiar cytosolic labeling that was never observed for endogenous TIP47. Right panel, non-tagged TIP47 cDNAs and pEGFP were cotransfected to Balb/c 3T3 cells and labeled with the human TIP47-specific antibody. In the transfected cells (*), full-length TIP47 showed punctuate labeling only in a few cells, whereas the TIP47 mutant (343–349)A showed distinct punctuate labeling in most cells. In a separate experiment, punctuate labeling was confirmed to be derived from LDs. Bars, 10 μm. (B,C) The percentage of cells showing positive LD labeling in the control cells (pink bars) and in cells treated with oleic acid for 2 h (blue bars). (B) V5-tagged TIP47 mutants in the Huh7 cells; (C) non-tagged TIP47 mutants in Balb/c 3T3 cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

sequence similarity to a tandem 11-mer repeat of S3-12, i.e., 73–145, was not necessary for the LD localization; this result was consistent with that of Targett-Adams et al. [16] on ADRP. The C-terminal half or pp17a was also not localized to LDs [28]. When considered together with the results of other mutants, it was suggested that a large portion of TIP47 that spanned the N- and C-terminal segments is required for LD binding as well as responsiveness to fatty acids.

The above result was quite different from that of ADRP mutants [13,14,16], implying that TIP47 and ADRP do not bind to LDs via a common mechanism. This inference was supported by the following results. First, the overexpression of Rab18 removed ADRP, but not TIP47, from LDs. Second, TIP47 recruitment on oleic acid treatment occurred in preexisting LDs that harbored ADRP; we also observed that TIP47 overexpression did not affect ADRP (data not shown). Third, in contrast to Rab18, endogenous TIP47 did not show reciprocal labeling intensities with ADRP in LDs. Based on these findings, we speculated that TIP47 and ADRP do not compete directly with each other and may bind to LDs by different modalities. However, it is still reasonable to consider that the presence of ADRP is a factor that influences LD localization of TIP47 because TIP47 increases in LDs on ADRP knockdown.

The responsiveness of TIP47 to fatty acids was lost when short segments were truncated from the C terminus or when the amino acids that were believed to form the hydrophobic cleft [8] were replaced with alanine residues. The C-terminal half is predicted to form the hydrophobic cleft, and therefore, its truncation may have had the same effect as that produced by alanine replacement. The cleft is assumed to incorporate a hydrophobic molecule, but it is too small to bind to membranes or LDs. Based on these suppositions, we speculate that the binding of an appropriate molecule to the cleft induces a conformational change and modulates the affinity of TIP47 to LDs. The disruption of the cleft by mutations may have mimicked this conformational change, thus abolishing the responsiveness to fatty acids.

Molecules that bind the hydrophobic cleft are not known, but they may be fatty acids, or alternatively, they may be other molecules that are mobilized to react with fatty acids to generate lipid esters. In fact, we observed that the application of all-*trans*-retinol to retinal pigment epithelial cells caused a similar translocation of TIP47 to LDs (Tsuiki et al., manuscript in preparation). In contrast, although a similar cleft was predicted to be present in ADRP [8], the protein was constitutively localized to LDs and did not show any responsiveness to fatty acids. The reason for this difference between TIP47 and ADRP is unclear; however, it may be explained by the presence of redundant LD-targeting sequences in ADRP [13,14,16]. Alternatively, the hydrophobic cleft of ADRP may exhibit a higher affinity to binding molecules, and the concentration of these molecules, including fatty acids, in the normal cell cytosol may be sufficient to induce an appropriate conformation.

Presence of heterogeneous LDs

The results of this study showed the coexistence of heterogeneous LDs that contain ADRP, TIP47, and Rab18 in different ratios. The labeling intensities of ADRP and Rab18 in LDs are reciprocal to each other [25], but the labeling intensity of TIP47 did not show any apparent correlation with either ADRP or Rab18. Therefore LDs in a cell may be classified into at least four different groups, depending upon the ADRP/Rab18 ratio and the presence of TIP47. In particular, LDs with TIP47, but no or little ADRP, were observed in many cell types. A similar result was reported in HeLa cells that were cultured with fatty acids [19], but the specificity of the antibody used in that study was questioned [20]. TIP47 in those ADRP-negative, TIP47-positive LDs persisted even when the total expression of TIP47 was significantly suppressed. This result indicates the uniqueness of these LDs; their significance is of interest for future studies.

LDs that are not coated by ADRP have been observed under several experimental conditions. First, when cells were treated by brefeldin A, LDs persisted for several hours after losing ADRP [30]. Second, when Rab18 was overexpressed due to cDNA transfection, LDs harboring Rab18 were devoid or severely depleted of ADRP [25]. Finally, even when the ADRP expression was considerably suppressed by RNAi, many LDs persisted [25]. Very recently, an analysis of ADRP-null mice was reported; it showed that although the hepatic cells of the mice harbored fewer LDs than their normal counterparts, LDs could still be observed [31]. These results suggest that ADRP is not essential in maintaining the structural integrity of LDs.

Relationship with trafficking functions of TIP47

The physiological role of TIP47 in LDs is a debatable issue. We carried out the knockdown of TIP47 by RNAi and measured the amount of lipid esters in the cells, but the effect was not significant (Tauchi-Sato and Fujimoto, unpublished data). This result would imply that the absence of TIP47 did not influence the integrity of LDs or the storage of lipid esters drastically. The function of TIP47 as an adaptor molecule of MPR and its involvement in the endosome-TGN retrieval pathway have been characterized in detail [17,18]. In this context, it is interesting that the mutant lacking the Rab9-binding portion or Δ(159–173) was barely targeted to LDs. Previous studies have shown that an impairment in the Rab9 binding induced morphological changes in late endosomes and abnormal trafficking of MPRs [16,27]. The present result raised an intriguing possibility that the binding of Rab9 is required for TIP47 to be recruited to LDs, and this recruitment may be functionally related to MPR trafficking. In other words, LDs and the intracellular vesicular trafficking may be correlated by TIP47. We are now testing this possibility.

Acknowledgments

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