ABC1 (ATP-binding cassette transporter A1) mediates the release of cellular cholesterol and phospholipid to form high density lipoprotein. Functions of ABCA1 are highly regulated at the transcriptional and post-transcriptional levels, and the synthesized ABCA1 protein turns over rapidly with a half-life of 1–2 h. To examine whether the functions of ABCA1 are modulated by associated proteins, a yeast two-hybrid library was screened with the C-terminal 120 amino acids of ABCA1. Two PDZ (PSD95-Discs large-ZO1) proteins, 1-syntrophin and Lin7, were found to interact with ABCA1. Immunoprecipitation revealed that a 1-syntrophin interacted with ABCA1 strongly and that the interaction was via the C-terminal 120 amino acids SYV of ABCA1. Co-expression of 1-syntrophin in human embryonic kidney 293 cells retarded degradation of ABCA1 and made the half-life of ABCA1 five times longer than in the cells not expressing 1-syntrophin. This effect is not common among PDZ-containing proteins interacting with ABCA1, because Lin7, which was also found to interact with the C-terminal region of ABCA1, did not have a significant effect on the half-life of ABCA1. Co-expression of 1-syntrophin significantly increased the apoA-I-mediated release of cholesterol. ABCA1 was co-immunoprecipitated with 1-syntrophin from mouse brain. These results suggest that 1-syntrophin is involved in intracellular signaling, which determines the stability of ABCA1 and modulates cellular cholesterol release.

Cholesterol is not catabolized in the peripheral cells and, therefore, is mostly released and transported to the liver for conversion to bile acids to maintain cholesterol homeostasis. The same pathway may also remove cholesterol that has pathologically accumulated in cells, such as at the initial stage of atherosclerosis. The assembly of high density lipoprotein (HDL) particles by lipid-free apolipoproteins with cellular lipid has been recognized as one of the major mechanisms for the cellular cholesterol release (1, 2). ApoA-I-mediated cholesterol efflux is a major event in “reverse cholesterol transport,” a process that generates HDL and transports excess cholesterol from the peripheral tissues, including the arterial wall, to the liver for biliary secretion. The importance of ABCA1 in this active cholesterol-releasing pathway for regulating cholesterol homeostasis became apparent with the finding that it is impaired in the cells from patients with Tangier disease, a genetic deficiency of circulating HDL (3, 4). Tangier disease is caused by mutations in ABCA1. ABCA1 mutations are also a cause of familial HDL deficiency and are associated with premature atherosclerosis (5, 6).

Cholesterol is a prerequisite for cells, but, at the same time, the hyper-accumulation of cholesterol is harmful to cells. Therefore, the expression of ABCA1 is highly regulated at both the transcriptional and post-transcriptional level. The transcription of ABCA1 is regulated by the intracellular oxysterol concentration via the LXR/RXR nuclear receptor (7), and the synthesized ABCA1 protein turns over rapidly with a half-life of 1–2 h (8–10). However, the post-translation regulatory mechanism of ABCA1 is unclear. We analyzed the associated proteins that could be involved in the post-translational regulation of ABCA1. By yeast two-hybrid screening with the C-terminal 120 amino acids of ABCA1, two PDZ (PSD95-Discs large-ZO1)-binding proteins, 1-syntrophin and Lin7, were found to interact with ABCA1. Immunoprecipitation confirmed the association of 1-syntrophin and ABCA1 via its C-terminal amino acids. The importance of this interaction in the regulation of ABCA1 function was examined.

**EXPERIMENTAL PROCEDURES**

Materials—The anti-ABCA1 monoclonal antibody KM3073 was generated against the first extracellular domain of the human ABCA1 protein in rats. Anti-ABCA1 monoclonal antibody KM3110 was generated against the C-terminal 20 amino acids of ABCA1 in mice. Anti-ABCA1 polyclonal antibody, previously described (11), was used for immunostaining. Affinity-purified antibody specific for 1-syntrophin was prepared using recombinant proteins. Human 1-syntrophin (amino acids 169–346) was fused to glutathione S-transferase (GST) in the pGEX vector (Amersham Biosciences) and to the maltose-binding protein (MBP) in the pMAL-c2 vector (New England Biolabs, Inc.). The GST–1-syntrophin protein was used as an antigen. Obtained rabbit antiserum was affinity purified with the column coupled with the MBP–1-syntrophin fusion protein. Anti-FLAG epitope monoclonal antibody M2 was purchased from Sigma. Human apoA-I was a gift from Dr. Shinji Yokoyama, Nagoya City University Graduate School of Medical Sciences.

Animals—16-week-old 1-syntrophin (+/−) (12) and wild-type C57BL/6 mice were used in this study. The animals were allowed ad libitum access to food and drinking water. Mice carrying mutations were identified by Southern blot analysis as described (12).
Stabilization of ABCA1 by α1-Syntrophin

ABCA1 Interacts with Two PDZ-binding Proteins—To search for proteins that are associated with the C-terminal region of ABCA1, a fusion construct of the Ga4 DNA-binding domain with the C-terminal 120 amino acids of human ABCA1 was used as bait for two-hybrid screening. The identified genes contained two PDZ-containing proteins, α1-syntrophin (10 clones) and Lin7 (two clones). To determine whether the interaction between ABCA1 and α1-syntrophin or Lin7 occurs in vivo, we transfected FLAG-tagged α1-syntrophin, FLAG-tagged Lin7, or FLAG-tagged vinexin β (15) (as a negative control) together with ABCA1 into HEK293 cells. Lysates prepared from transfected cells were immunoprecipitated with anti-FLAG antibody, and precipitates were evaluated by immunoblotting with anti-ABCA1 antibody. As shown in Fig. 1, ABCA1 was co-immunoprecipitated with FLAG-tagged α1-syntrophin or FLAG-tagged Lin7, but not with FLAG-tagged vinexin β. ABCA1 was not precipitated with mouse IgG control from any of the lysates in which the expression of ABCA1 (Fig. 1) and FLAG-tagged proteins (data not shown) were detected by immunoblotting. More than 25% of the ABCA1 expressed in HER293 was roughly estimated to be co-immunoprecipitated with FLAG-tagged α1-syntrophin, suggesting strong interaction between ABCA1 and α1-syntrophin (Fig. 2). The interaction between ABCA1 and Lin7 seemed to be weak, because the amount of precipitated ABCA1 with Lin7 was much less than that with α1-syntrophin. The amount of ABCA1 in lysates was consistently higher when co-expressed with α1-syntrophin than with other proteins.

ABCA1 Interacts with α1-Syntrophin via the C-terminal Three Amino Acids—ABCA1 contains the amino acid sequence ESYV at the C terminus, which has been described as a binding target for syntrophin PDZ domains (16). To determine whether the C-terminal three amino acids SYV are important for the interaction, ABCA1ΔSYV, in which these amino acids were trimmed, was co-expressed with FLAG-tagged α1-syntrophin in HER293 cells. Although the expression of ABCA1ΔSYV was detected in the lysates, no ABCA1ΔSYV was co-precipitated with FLAG-tagged α1-syntrophin. These results suggest that the interaction is mediated with the C-terminal three amino acids SYV of ABCA1 and α1-syntrophin PDZ domains.

Co-localization of the ABCA1 and PDZ-containing Proteins α1-Syntrophin and Lin7—ABCA1 is mainly localized to plasma membrane but is also substantially expressed in intracellular compartments (11, 17–20). To determine whether ABCA1 and α1-syntrophin or Lin7 are co-localized in cells, ABCA1 was co-transfected with FLAG-tagged α1-syntrophin or FLAG-tagged Lin7 into HEK293 cells. The subcellular localization of these proteins was examined under a confocal laser scanning microscope. α1-Syntrophin resided mainly on plasma mem-

![Image](http://www.jbc.org/)

FIG. 1. In vivo association of ABCA1 with α1-syntrophin. HER293 cells were co-transfected with human ABCA1 and FLAG-tagged α1-syntrophin, FLAG-tagged Lin7, or FLAG-tagged vinexin β. Cell lysates were immunoprecipitated (IP) with anti-FLAG antibody. Immunocomplexes and cell lysates (5%) were subjected to immunoblotting using anti-ABCA1 monoclonal antibody KM3110, generated against the C-terminal 20 amino acids of ABCA1. Mouse IgG was used as a negative control. The data are representative of three independent experiments.

![Image](http://www.jbc.org/)

FIG. 2. ABCA1 interacts with α1-syntrophin via the C-terminal three amino acids. HER293 cells were co-transfected with ABCA1 or ABCA1ΔSYV, in which the C-terminal three amino acids were trimmed, and with FLAG-tagged α1-syntrophin. Cell lysates were immunoprecipitated (IP) with anti-FLAG antibody. Immunocomplexes and cell lysates (5%) were subjected to immunoblotting using the anti-ABCA1 monoclonal antibody KM3073, generated against the first extracellular domain of the human ABCA1, and an anti-FLAG antibody. The data are representative of two independent experiments.

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bran, where it co-localized with ABCA1 (Fig. 3A). Lin7 also localized mainly on plasma membrane and appeared not to be uniformly distributed but rather clustered in a specific region of plasma membrane in some cells. In those regions, high expression of ABCA1 and the formation of filopodia were observed (Fig. 3B).

**Interaction of α1-Syntrophin with ABCA1 in Mouse Brain—** Among syntrophin isoforms, α1-syntrophin is mainly expressed in brain, skeletal muscle, and heart in mouse (21). To examine whether ABCA1 and α1-syntrophin interact physiologically, we tried co-immunoprecipitation of these two proteins from mouse brain. Lysates prepared from mouse brain were immunoprecipitated with anti-α1-syntrophin antibody, and precipitates were evaluated by immunoblotting with anti-ABCA1 antibody. As shown in Fig. 4, mouse ABCA1 was co-immunoprecipitated with α1-syntrophin, but not with control IgG. This interaction was confirmed to be specific, because ABCA1 was not precipitated from brain of α1-Syn−/− mice (Fig. 4).

**α1-Syntrophin Modulates Turnover of ABCA1—** PDZ-containing proteins have been reported to be involved in protein stability. For example, interaction with β2-syntrophin controls the degradation of ICA512, which connects insulin secretory granules to the utrophin complex and the actin cytoskeleton, by calpain (22), and the stability of AQP4 (23) and neuronal nitric-oxide synthase (12) is suggested as being controlled by α1-syntrophin. The amount of ABCA1 in lysates was consistently higher on co-expression with α1-syntrophin than with other proteins as shown in Fig. 1. Therefore, we examined the effect of α1-syntrophin on the stability of ABCA1. FLAG-tagged α1-syntrophin or FLAG-tagged Lin7 was transiently co-expressed with ABCA1 in HEK293 cells. At 48 h after transfection, the medium was replaced with 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium containing 100 μg/ml cycloheximide, and cellular protein synthesis was inhibited to block synthesis of newly synthesized ABCA1. After the indicated times, the amount of ABCA1 was measured by immunoblotting (Fig. 5A). After the inhibition of cellular protein synthesis, 80% of ABCA1 was degraded in 7 h, and the half-life was about 2 h as reported previously (10). Thus, ABCA1 protein turns over rapidly in HEK293 cells. When ABCA1 was co-expressed with α1-syntrophin, only ~30% of ABCA1 was degraded in a 7-h treatment with cycloheximide, and the half-life was estimated to be 10 h (Fig. 5B). Lin7, which is a PDZ protein and also binds to the C terminus region of ABCA1, did not show a significant effect on the half-life of ABCA1. The half-life of ABCA1ΔSYV was scarcely affected by co-expression of α1-syntrophin (data not shown). These results suggest that α1-syntrophin decreases ABCA1 protein degradation by interacting with the C terminus three amino acids of ABCA1.

**α1-Syntrophin Increases apoA-I-mediated Cholesterol Efflux by ABCA1—** To analyze the functional consequences of decreased ABCA1 protein degradation in the presence of α1-syntrophin, the apoA-I-mediated release of cholesterol was examined from HEK293 cells transiently cotransfected with ABCA1 and α1-syntrophin (Fig. 6). Human ABCA1 transiently expressed in HEK293 cells supported the apoA-I-mediated release of cholesterol as previously reported with ABCA1-green fluorescent protein (13). Co-expression of α1-syntrophin significantly increased the apoA-I-mediated release of cholesterol, although expression of α1-syntrophin alone did not affect it.

**DISCUSSION**

In this study, we identified α1-syntrophin as a protein interacting strongly with ABCA1 via the C-terminal three amino acids of ABCA1.
acids SVY of ABCA1. Co-expression of α1-syntrophin retarded degradation of ABCA1 and made the half-life of ABCA1 in HEK293 cells five times longer than in the cells not expressing α1-syntrophin. This effect is not common among PDZ-containing proteins interacting with ABCA1, because Lin7, which also binds to the C terminus region of ABCA1, did not show a similar effect. Co-expression of α1-syntrophin significantly increased the apoA-I-mediated release of cholesterol. Because this interaction was observed in mouse brain, α1-syntrophin could be involved in lipid homeostasis in brain.

Mammalian cells have developed sophisticated mechanisms to ensure adequate cellular cholesterol levels, because cholesterol plays a critical role in several important cell functions, including protein trafficking, membrane vesiculation, and signal transduction, and, at the same time, hyper-accumulation of cholesterol is harmful for cells. Plasma membrane cholesterol content, for example, is regulated through a feedback mechanism controlled by sterol regulatory element binding protein-2 (SREBP-2) (24, 25). To eliminate excess cholesterol from the cell, expression of ABCA1, a key molecule for apoA-I-mediated cholesterol efflux, is stimulated by intracellular oxyester via the LXR/RXR nuclear receptor (26). The synthesized ABCA1 protein turns over rapidly with a half-life of 1–2 h (8, 10) to cancel cholesterol efflux by ABCA1. Because co-expression of α1-syntrophin retarded degradation of ABCA1 and made the half-life of ABCA1 in HEK293 cells five times longer than in the cells not expressing α1-syntrophin, α1-syntrophin is expected to be involved in intracellular signaling, which determines the stability of ABCA1.

Recently, it has been proposed that ABCA1 is regulated in two different ABCA1 degradation pathways under various cellular conditions: (a) a basal calpain degradation pathway that is turned off by interaction with apolipoproteins (9, 10); and (ii) a ubiquitin-proteasome pathway that is activated by marked free cholesterol loading (27). A sequence rich in proline, glutamate, serine, and threonine (PEST sequence) just before the second membrane spanning domain of ABCA1 (amino acid residue 1283–1306) is involved in regulating the calpain degradation of ABCA1 (10). Although the nature of the apoA-I-ABCA1 interaction is not fully understood, conformational alteration of ABCA1 through the PEST sequence may be induced by its direct or indirect interaction with apoA-I, which may render ABCA1 resistant to proteolysis by calpain. Because ABCA1 was ubiquitinated as well when co-expressed with α1-syntrophin (data not shown), α1-syntrophin seems not to affect ABCA1 degradation in an ubiquitin-proteasome pathway. Binding of α1-syntrophin to the C terminus of ABCA1 may cause a conformational alteration similar to that caused by apoA-I binding and render ABCA1 resistant to proteolysis by calpain.

Syntrophins are a family of five proteins (α1, β1, β2, γ1, and γ2,) containing two pleckstrin homology domains, a PDZ domain, and a C-terminal syntrophin-unique region (28). Analysis of α-Syn mice has demonstrated that perivascular localization of AQP4 in brain requires α1-syntrophin (23) and that the stability of AQP4 (23) and neuronal nitric-oxide synthase (12) decreases in the absence of α1-syntrophin. β2-syntrophin was also reported to interact with ABCA1 and was proposed to participate in the retaining of ABCA1 in cytoplasmic vesicles by forming a ABCA1-β2-syntrophin-utrophin complex (29). It is possible that α1-syntrophin is also involved in endocytotic recycling of ABCA1. Extracellular lipid-free apoA-I may first interact with ABCA1 on plasma membrane, but it is not clear whether the formation of HDL takes place extracellularly or if intracellular events, such as endocytotic recycling, are involved (30). It is intriguing that apoA-I and α1-syntrophin have a similar effect on ABCA1 turnover. A mutation of ABCA1 that causes Tangier disease (5905) does not affect apoA-I binding or initial ATP binding/hydrolysis but results in a defect in lipid efflux (11, 31, 32). Because apoA-I failed to affect calpain degradation of ABCA1-WS90S in HEK293 cells (10), additional signals following apoA-I binding to ABCA1 are speculated to be necessary for the subsequent inhibition of calpain degradation. It is possible that PDZ-containing proteins such as α1-syntrophin are involved in intracellular signaling, which determines the stability of ABCA1.

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Stabilization of ABCA1 by \(\alpha 1\)-Syntrophin

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