Regulation and Mechanisms of ATP-Binding Cassette Transporter A1-Mediated Cellular Cholesterol Efflux

Nan Wang, Alan R. Tall

Abstract—ATP-binding cassette transporter A1 (ABCA1) plays a major role in cholesterol homeostasis and HDL metabolism. ABCA1 mediates cellular cholesterol and phospholipid efflux to lipid-poor apolipoproteins, and upregulation of ABCA1 activity is antiatherogenic. ApoA-I, the major apolipoprotein component of HDL, promotes ABCA1-mediated cholesterol and phospholipid efflux, probably by directly binding to ABCA1. ABCA1 gene expression is markedly increased in cholesterol-loaded cells as a result of activation of LXR/RXR. ABCA1 protein turnover is rapid. ABCA1 contains a PEST—proline (P), glutamate (E), serine (S), and threonine (T)—sequence in the intracellular segment that mediates ABCA1 degradation by a thiol protease, calpain. ApoA-I and apoE stabilize ABCA1 in a novel mode of regulation by decreasing PEST sequence-mediated calpain proteolysis. ABCA1-mediated cholesterol and phospholipid efflux are distinctly regulated and affected by the activity of other gene products. Stearyl CoA desaturase decreases ABCA1-mediated cholesterol efflux but not phospholipid efflux, likely by decreasing the cholesterol pool available to ABCA1. This and other evidence suggest that ABCA1 promotes cholesterol and phospholipid efflux, probably by directly transporting both lipids as substrates. (Arterioscler Thromb Vasc Biol. 2003;23:1178-1184.)

Key Words: lipoprotein metabolism ■ risk factors ■ cell biology ■ gene regulation

Atherosclerosis, the major cause of death in industrialized societies, is initiated by the retention in arteries of cholesterol-enriched, apoB-containing lipoproteins and their subsequent uptake by arterial wall macrophages, giving rise to macrophage foam cells.1 HDL and HDL apolipoproteins protect against the development of atherosclerosis, probably primarily by stimulating the efflux of cholesterol from macrophage foam cells.2,3 A breakthrough in this area of research has been the elucidation of mutations in the ATP-binding cassette transporter A1 (ABCA1)4–6 as the genetic defect in Tangier disease, a disorder characterized by HDL deficiency, defective apolipoprotein-mediated phospholipid and cholesterol efflux from cells, and the accumulation of macrophage foam cells in various tissues, including arteries. The pheno-
ligands in vivo.12

induced in cholesterol-loaded cells as a result of activation of
ABCA1 gene expression have revealed that ABCA1 is
mediated phospholipids and cholesterol efflux from cells,18
different laboratories had demonstrated apolipoprotein-
and function of ABCA1, emphasizing recent work on the
initial step in HDL formation.7,8

The finding that ABCA1 mutations cause Tangier disease
has stimulated studies of ABCA1 functions in vivo and in
vitro. As reviewed elsewhere in this series, these studies
confirm the pivotal role of ABCA1 in HDL formation and
demonstrate an antiatherogenic role of ABCA1, especially
in Tangier disease fibroblasts. Subsequent to the discovery
of apoA-I-mediated cellular cholesterol efflux to apoA-I.
24,25 Using a floppase activity of ABCA1 because PS levels on the exofacial leaflet
mediated cholesterol and phospholipid efflux from cells.
26 These studies also showed that apoA-I could be
cosmically cross-linked to ABCA1, suggesting close proximity
of apoA-I to ABCA1 (ie, within about 11 Å).17 These
findings led us to suggest that apoA-I directly binds ABCA1
to form a complex that is required for ABCA1-facilitated
cellular cholesterol and phospholipid efflux.17 In contrast to
this idea, Chambenoit et al23 proposed that apoA-I binding
might not involve a direct molecular interaction between
ABCA1 and apoA-I but rather a modified distribution of plasma membrane lipids induced by ABCA1 expression,
leading to “docking” of apoA-I molecules at the cell surface,
perhaps in close proximity to ABCA1. The authors provided
3 lines of evidence to support this hypothesis. First, apoA-I
binding requires functional ABCA1 because an ABCA1
mutant with defective ATPase activity fails to bind apoA-I.
Second, ABCA1 expression at the cell surface is not linearly
correlated with apoA-I binding, as determined by fluorescence quantification of apoA-I binding and cell surface
ABCA1 protein levels. Third, the lateral mobility of ABCA1
green fluorescent protein in membranes appears to be differ-
ent from that of membrane-bound apoA-I.23 The authors
suggested that apoA-I is likely bound to phosphatidylyserine
(PS) that is presented to the exofacial leaflet by the “floppase”
activity of ABCA1 because PS levels on the exofacial leaflet
are increased by ABCA1 expression.23 However, Smith et al24 reported that although ABCA1 expression increased PS
levels on the exofacial leaflet, the increased PS was insuffi-
cient to mediate cellular apoA-I binding and lipid efflux
because annexin V, a PS-binding protein, did not compete
with ABCA1-induced apoA-I binding, nor did it affect
ABCA1-mediated lipid efflux to apoA-I.24,25 Using a fluores-
cence photobleaching technique, this study also suggested
that apoA-I bound to an integral membrane protein in
ABCA1-expressing cells.24

The controversy about apoA-I/ABCA1 interaction has also
been looked into from a different angle using apoA-I mut-
ants.26 The C-terminal lipid-binding helix 10 of apoA-I was
identified to be critical for apoA-I-mediated cholesterol
efflux from ABCA1-expressing cells,26 and a positive correla-
tion between cholesterol efflux and the lipid-binding char-
acteristics of apoA-I was observed.26 These findings led to a
composite model to explain the interaction between apoA-I
and ABCA1, that is, helix 10 of apoA-I tethers the lipid-free
apolipoprotein to the ABCA1-generated lipid domain that
then diffuses within the plane of the membrane until it comes
in contact with ABCA1, where a protein–protein interaction
could lead to the lipidation of apoA-I.26 However, the data do
not distinguish whether apoA-I/lipid binding precedes the
interaction of apoA-I with ABCA1 or the other way around.

The ABCA1 molecule has 2 large extracellular loops,
probably linked by a disulfide bond, located on each of the 2
transmembrane domains (Figure 1 and27,28). Importantly,
many ABCA1 missense mutations causing Tangier disease
have been identified in these loops.29 A functional test of
these mutations in transfected cells revealed defects in apoA-I
binding and cellular lipid efflux.27,28 Interestingly, one of the
mutants, W590S, showed lipid efflux deficiency but moder-

Figure 1. ApoA-I–mediated stabilization of ABCA1 by inhibiting
PEST-dependent calpain proteolysis. ApoA-I binds to ABCA1
and causes inhibition of calpain-catalyzed proteolysis of ABCA1
by promoting cellular phospholipid efflux or by inducing a con-
formational change of ABCA1 that inhibits access of calpain to
the PEST sequence. Calpeptin is a specific inhibitor of calpain
and increases ABCA1 protein by reducing calpain proteolysis.

Role of Apolipoprotein Binding to ABCA1 in
the Regulation of Lipid Efflux
ABCA1 is a large membrane protein with 2 transmembrane
domains and 2 nucleotide-binding folds linked by an intra-
cellular peptide segment (Figure 1). After translation,
ABCA1 is further processed by glycosylation and presented
at the cell surface.15 The primary function of ABCA1 is to
promote cellular cholesterol and phospholipid efflux, which
requires the presence of extracellular lipid-poor apolipopro-
tins.16,17 However, the detailed molecular events linking
apolipoproteins to ABCA1-facilitated cellular lipid efflux are
still not clear. Before the discovery of ABCA1, a number of
different laboratories had demonstrated apolipoprotein-
mediated phospholipids and cholesterol efflux from cells,18–20
and Francis et al21 discovered that this process was defective
in Tangier disease fibroblasts. Subsequent to the discovery
that ABCA1 mutations cause Tangier disease, we and others
found that transfection of the full-length cDNA of ABCA1 in
cells resulted in increased apoA-I binding to the cell sur-
face.17,22 These studies also showed that apoA-I could be
chemically cross-linked to ABCA1, suggesting close proximity
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mutants, W590S, showed lipid efflux deficiency but moder-
ately increased apoA-I binding. These findings have been confirmed independently and strongly suggested that apoA-I directly binds ABCA1 at the cell surface without requiring the ability of ABCA1 to mediate lipid efflux. Together, these results also suggest that apoA-I binding requires an ABCA1 molecule assuming an optimal structural conformation that is maintained by a functional ATPase because cell-surface ABCA1 mutants with defective ATPase fail to bind apoA-I. The moderate increase in apoA-I binding to W590S may imply that dissociation of apoA-I from ABCA1 could be facilitated by lipid efflux to apolipoproteins bound to ABCA1 and is consistent with the earlier finding that ABCA1 binds apoA-I but not HDL.

Extrahepatic ABCA1 functions to promote cholesterol efflux from peripheral tissues to lipid-poor apolipoproteins that in turn deliver the lipid load back to liver for disposal, a process likely involving scavenger receptor B1, which has high affinity for lipid-rich HDLs but low affinity for lipid-poor apolipoproteins. Hepatic ABCA1 is likely involved in pre-β HDL formation in liver, demonstrated by increased pre-β HDL as well as more mature HDL levels in mice with adenovirus-mediated expression of ABCA1. Like scavenger receptor B1, ABCA1 binds not only apoA-I but also other apolipoproteins, including apoE. Thus, the antiatherogenic effect of apoE in vivo could be partially explained by lipid efflux from macrophage foam cells coordinated with ABCA1.

**Apolipoprotein Binding in the Regulation of ABCA1 Turnover**

Cellular expression of ABCA1 is highly regulated. As a consequence of increased ABCA1 expression, apolipoprotein-mediated cellular cholesterol efflux is also enhanced, leading to a decreased cellular cholesterol accumulation. Highly regulated ABCA1 expression may reflect the necessity to tightly regulate ABCA1 protein levels by the cell to maintain cellular cholesterol and phospholipid homeostasis. During cholesterol loading of macrophages, cellular cholesterol and phospholipid content are increased, the latter is caused by increased phospholipid synthesis and is a protective response against cytotoxicity caused by cholesterol accumulation. Therefore, induction of ABCA1 expression could help the cell shed excess cholesterol and phospholipids and reach a new steady state of cellular lipid metabolism. However, overexpressed ABCA1 in the absence of cholesterol loading causes an altered membrane structure, which could be detrimental to the cell. Oram et al. showed that the turnover of ABCA1 protein was rapid with a short half-life of less than 1 hour in murine macrophage-like cells. Thus, the induction of ABCA1 expression by cholesterol loading and rapid turnover of ABCA1 protein suggest the existence of cellular mechanisms tightly regulating the protein levels of ABCA1 to maintain lipid homeostasis.

The cellular processes regulating the turnover of ABCA1 protein have only recently received attention. ABCA1 is present at the cell surface and proposed to function primarily at plasma membranes. However, intracellular localizations of ABCA1 have been observed, and ABCA1-mediated lipid efflux may involve intracellular lipid trafficking or apoA-I endocytotic recycling. Using ABCA1 green fluorescent protein fusion protein and confocal and time-lapse fluorescence microscopy, Neufeld et al. found that ABCA1 is on the cell surface and in intracellular vesicles, including early endosomes, late endosomes, and lysosomes. They have suggested that delivery of ABCA1 to lysosomes could be a mechanism to regulate ABCA1 protein turnover and to modulate its cell surface expression and function. A key role of late endosomes/lysosomes in providing cholesterol for ABCA1-mediated efflux is indicated by the severe defect in apolipoprotein-mediated cholesterol efflux in Niemann-Pick C macrophages.

Regulation of protein turnover of membrane receptors by their ligands has been reported. Because apoA-I directly binds ABCA1, we speculated that apoA-I might regulate ABCA1 protein turnover. Indeed, we found that apoA-I binding increased ABCA1 protein levels in mouse primary hepatocytes, peritoneal macrophages, and transfected cells without affecting ABCA1 mRNA levels. We also showed that apoE had a similar positive effect on ABCA1 protein levels. Arakawa and Yokoyama independently reported that apoA-I increased ABCA1 protein in human THP-1 cells. Furthermore, they demonstrated that the positive effect of apoA-I on ABCA1 was likely mediated by inhibiting ABCA1 degradation by an unknown thiol protease. We obtained similar results showing that nonspecific thiol protease inhibitors increased ABCA1 protein levels in transfected 293 cells.

Many short-lived proteins have specific peptide motifs that target proteins for rapid degradation. One of the motifs is PEST, which is defined as a peptide sequence enriched in proline (P), glutamate (E), serine (S), and threonine (T), and usually flanked by lysine (K), arginine (R), or histidine (H) residues. Using the program PESTfind, we identified a conserved potential PEST sequence in ABCA1 (Figure 1). This PEST sequence appeared to be important in regulation of ABCA1 function because PEST deletion resulted in a 4- to 5-fold increase in cell surface ABCA1 protein and significantly increased ABCA1-mediated lipid efflux and apoA-I binding. PEST sequences often increase protein turnover by enhancing protein ubiquitination and proteasomal degradation. In contrast with the lack of effect of lactacystin, a specific inhibitor of proteasome, on ABCA1 protein levels in THP-1 cells as reported by Arakawa and Yokoyama, ABCA1 was ubiquitinated in 293 cells, and lactacystin moderately increased ABCA1 protein levels in both 293 cells and mouse peritoneal macrophages, suggesting that the proteasomal degradation pathway is involved in ABCA1 turnover. Additional evidence supporting the involvement of the proteasomal pathway in ABCA1 turnover came from another study showing that free cholesterol accumulation in mouse peritoneal macrophages resulted in reduced ABCA1 protein levels that could be reversed by lactacystin treatment. However, we found that the PEST deletion ABCA1 mutant was still ubiquitinated and the protein level of the mutant was also increased by lactacystin treatment, suggesting that ubiquitination and proteasomal degradation of ABCA1 is not controlled by the PEST sequence. In a few instances, PEST sequences have been implicated in proteol-
ysis of the target proteins by calpains, a subfamily of thiol proteases. Indeed, a specific calpain protease inhibitor, calpeptin, substantially increased total and cell surface wild-type ABCA1 levels but had no effect on the protein level of the PEST deletion mutant, suggesting that ABCA1 is a target of calpain proteolysis and that the PEST sequence helps to target calpain to cell-surface ABCA1. Further, purified μ-calpain protease added to permeabilized cells efficiently degraded wild-type ABCA1 but not the PEST deletion mutant, providing direct evidence that PEST-dependent degradation of ABCA1 is mediated by calpain protease. Calpeptin treatment also increased ABCA1 protein levels in primary mouse hepatocytes and mouse peritoneal macrophages, suggesting a physiological role of calpain protease in regulation of ABCA1 turnover.

Because apoA-I binding increased ABCA1 protein levels, we then tested whether apoA-I-mediated ABCA1 stabilization was mediated by inhibiting calpain proteolysis in a PEST sequence-dependent fashion. ApoA-I increased levels of wild-type ABCA1 but not of the PEST deletion mutant, and apoA-I pretreatment blocked the degradation of the wild-type ABCA1 by purified calpain protease added to the permeabilized cells. Together, these results have provided convincing evidence for a novel mode of regulation of ABCA1: apoA-I stabilizes ABCA1 by inhibiting PEST sequence-mediated calpain proteolysis. To evaluate the effect of apoA-I on ABCA1 as a potential mechanism for its anti-atherogenic role in vivo, we injected a bolus of apoA-I into mice and determined ABCA1 protein levels. Intravenous apoA-I injection resulted in an induction of ABCA1 protein in liver and peritoneal macrophages. However, hepatic and macrophage ABCA1 protein levels showed no change in apoA-I knockout or transgenic mice, suggesting that some form of chronic adaptation may occur as a result of sustained alterations in apoA-I levels. Therefore, these studies may provide a potential explanation for previously observed antiatherogenic effects of apoA-I infusion, that occurred even without sustained phosphorylation of the target protein often increases its degradation, which is reversed by inhibition of proteasome pathway, likely reflects an independent mechanism in the regulation of ABCA1 turnover. The cellular compartment in which ABCA1 ubiquitination occurs is unknown and endoplasmic reticulum or plasma membrane could both be potential sites. Although marked free cholesterol accumulation in macrophages leads to ABCA1 degradation, which is reversed by inhibition of proteasome pathway, the physiological significance of this pathway in regulation of ABCA1 turnover in vivo is still not clear. Interestingly, the cystic fibrosis transporter regulator (CFTR), another ABC transporter, has also been shown to be ubiquitinated and degraded by the proteasome pathway. Conformational maturation of wild-type CFTR in the endoplasmic reticulum is an inefficient process, where approximately 75% of newly synthesized CFTR molecules are degraded by cytoplasmic proteasomes shortly after synthesis. Defective protein folding of some CFTR mutants has been proposed as the cause for cystic fibrosis, and great efforts have been made to explore different mechanisms to help the folding of the mutant CFTR. Similarly, a recent study suggests that some ABCA1 missense mutations leading to Tangier disease also show defective ABCA1 presentation at the cell surface, perhaps reflecting defective protein folding.

**Regulation of ABCA1 Protein Turnover as a Result of Altered Lipid Metabolism**

In addition to apolipoproteins, ABCA1 turnover is also modulated by changes of cellular lipid metabolism. Wang and Oram reported that unsaturated fatty acid accelerate the degradation of ABCA1 without altering ABCA1 mRNA levels and thus decreases apoA-I-mediated lipid efflux, although the specific proteolytic pathway is not known. Because type 2 diabetes and insulin resistance are characterized by elevated fatty acids, low plasma HDL levels, and
increased risk of cardiovascular diseases, impaired ABCA1-mediated cholesterol efflux from macrophages may contribute to the enhanced atherosclerosis associated with these metabolic disorders.\textsuperscript{53} Indeed, Uehara et al\textsuperscript{54} reported that hepatic and macrophage ABCA1 expression were markedly decreased in diabetic mice and this was reversed by insulin treatment. Furthermore, they also showed that ABCA1 mRNA and protein levels were reduced by unsaturated, but not saturated, fatty acids in hepatoma and macrophage-like cell lines.\textsuperscript{54}

**Mechanisms of ABCA1-Mediated Lipid Efflux**

The molecular mechanism for ABCA1-mediated lipid efflux is still poorly understood, and several models have been proposed. Some studies have suggested that ABCA1 is localized in and promotes lipid efflux from a membrane lipid domain distinct from cholesterol- and sphingomyelin-rich rafts.\textsuperscript{55} Several other studies have suggested the opposite interpretation\textsuperscript{56} or suggested that both raft and nonraft domains contribute to ABCA1-facilitated lipid efflux to apoA-I depending on the cell types.\textsuperscript{57} Phospholipid and cholesterol efflux promoted by ABCA1 can be dissociated,\textsuperscript{25,58} and different cell lines have shown nonparallel apoA-I-mediated cholesterol and phospholipid efflux,\textsuperscript{59} suggesting distinctly regulated ABCA1-dependent cholesterol and phospholipid efflux pathways. Studies from our group suggest that this lipid efflux pathway is in part regulated by stearoyl CoA desaturase (SCD), a rate-limiting enzyme in the cellular lipid efflux pathway is in part regulated by stearyol CoA desaturase (SCD), a rate-limiting enzyme in the cellular lipid efflux pathway is in part regulated by stearyol CoA desaturase (SCD), a rate-limiting enzyme in the cellular lipid efflux pathway is in part regulated by stearyol CoA desaturase (SCD), a rate-limiting enzyme in the cellular lipid efflux pathway.

**Conclusion**

ApoA-I binds specifically to ABCA1, likely involving a direct molecular interaction. Binding of apoA-I to ABCA1 promotes cellular cholesterol and phospholipid efflux, probably as a result of a coordinated specific transmembrane transport of both lipids by ABCA1. ABCA1 expression is highly regulated, both on transcriptional and post-transcriptional levels. The interaction of apoA-I with ABCA1 modulates both forms of regulation. Thus, cholesterol efflux...
promoted by ABCA1 leads to decreased activation of LXR/RRX by oxysterols and ultimately decreases ABCA1 transcription and protein levels. In contrast, apoA-I and apoE have a positive effect on ABCA1 protein expression, and this is likely to be important in hepatocytes and macrophage foam cells. Intense interest has recently centered on the possibility that increasing macrophage cholesterol efflux could represent a novel approach to treatment of atherosclerosis. LXR/RRX target a battery of genes mediating cholesterol efflux, transport, and excretion, and LXR activators are antiatherogenic. However, LXR/RRX also increases transcription of SREBP1c and its target genes, causing fatty liver and hypertriglyceridemia. Therefore, calpain protease inhibitors, or small molecules that modulate the local interaction of ABCA1 with calpain protease at the plasma membrane, might be an alternative way to upregulate ABCA1 protein and function. Low-affinity small peptides that mimic the binding of lipid-free apolipoproteins to ABCA1 could stabilize the transporter while allowing lipid efflux to apoA-I or apoE.

References


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