Subcellular localization of the mosquito sterol carrier protein-2 and sterol carrier protein-x

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Abstract Subcellular distribution of Aedes aegypti sterol carrier protein-2 (AeSCP-2) and AeSCP-x was studied using electron microscopy. In both cultured A. aegypti cells and in the larval midgut, AeSCP-2 was detected mostly in the cytosol, with some labeling mitochondria and nucleus, but not in membranous vesicles. The widespread distribution of AeSCP-2 in the midgut epithelium is consistent with its potential lipid transfer function in all phases of cholesterol absorption. In contrast, AeSCP-x was found mostly in the peroxisome. Differences in the subcellular distribution of AeSCP-2 and AeSCP-x suggest that these two members of the SCP-2 gene family are functionally distinct. Overexpression of AeSCP-2 in A. aegypti cells showed increased localization of AeSCP-2 to cytosol, mitochondria, and nucleus. This is the first report on the nuclear distribution of an SCP. Overexpression of AeSCP-2 resulted in increased cholesterol incorporation in cells, suggesting that AeSCP-2 enhances cholesterol uptake.—Lan, Q., and R. J. Massey. Subcellular localization of the mosquito sterol carrier protein-2 and sterol carrier protein-x. J. Lipid Res. 2004. 45: 1468–1474.

Supplementary key words Aedes aegypti cell line-2 • cholesterol • immunoelectron microscopy • overexpression • ribonucleic acid interference

The sterol carrier protein-2 (SCP-2) gene family includes SCP-2, SCP-x, 17β-hydroxysteroid dehydrogenase Type IV, SCP-2-like, metallo-β-lactamase, and stomatin (1). In vertebrates, SCP-2 and SCP-x are two proteins encoded by a single gene, the SCP-x/SCP-2 gene (2–4); therefore, vertebrate SCP-x and SCP-2 share exactly the same sequences of SCP-2 domains. Moreover, the vertebrate SCP-x is proteolytically cleaved in cytoplasm and peroxisomes to produce a 45 kDa thiolase and a 14 kDa SCP-2 (5, 6). Vertebrate SCP-x and SCP-2 have been shown to be functionally different in fatty acid metabolism, although amino acid sequences of the C-terminal portion of SCP-x and SCP-2 are exactly same. SCP-2 enhances palmitic acid esterification, but SCP-x does not (7). Vertebrate SCP-x locates exclusively in peroxisomes; SCP-2 is found in both peroxisomes and cytosol (1).

Mosquitoes appear to have two distinct genes encoding SCP-x and SCP-2, and express two small SCP-2 domain-containing proteins via posttranslational modification of the Aedes aegypti SCP-x (AeSCP-x) gene product and transcription of the AeSCP-2 gene (Q. Lan and V. Wessely, unpublished observations). The identity between AeSCP-2 and AeSCP-x C-terminus (AeSCP-xC) (the cleaved SCP-2 domain of AeSCP-xC) is only 24%. There is no precedent reported in vertebrates of two distinct genes for the production of low molecular weight SCP-2 domain-containing proteins. AeSCP-2 and AeSCP-x belong to the SCP-2 gene family, and both may be involved in cholesterol metabolism, though the function of each protein is not completely understood.

Insect SCP-x genes have the same domain architecture as that of vertebrate SCP-x, containing both a thiolase domain and an SCP-2 domain [see Ref. (8); Lan and Wessely, unpublished observations]. However, transcription of insect SCP-x genes is very different from the vertebrate SCP-x gene in that the SCP-2 domain of the insect SCP-x genes was not transcribed independently from that of SCP-x. Interestingly, the posttranslational modification of the full length AeSCP-x is conserved between invertebrates and vertebrates. The SCP-2 domain of AeSCP-x is proteolytically cleaved off to produce a 13 kDa protein containing only the SCP-2 domain (Lan and Wessely, unpublished observations).

Another mosquito gene, the AeSCP-2 gene, has been identified as the gene encoding a low molecular weight protein containing only the SCP-2 domain (9). Because AeSCP-2 represents the gene encoding the low molecular weight protein of SCP-2 domain, similar to the vertebrate SCP-2 gene, AeSCP-2 is identified to be the mosquito counterpart of the vertebrate SCP-2 (9). The major differences between mosquito AeSCP-2 and the vertebrate SCP-2 are that there is no peroxisome targeting sequence in the C-terminus of AeSCP-2 (9), and the coordinating

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Abbreviations: Aag-2, Aedes aegypti cell line-2; AeSCP-2, Aedes aegypti sterol carrier protein-2; AeSCP-xC, AeSCP-x C-terminal; dsRNA, double-stranded RNA; RNAi, RNA interference.

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ligand binding site is in the opposite orientation compared with the vertebrate SCP-2 (10).

To facilitate the functional study of AeSCP-2 and AeSCP-xC, we produced specific polyclonal antibodies of AeSCP-2 (9) and AeSCP-xC (Lan and Wessely, unpublished observations). Using those specific antibodies, we performed immunoelectron microscopic labeling with antibody in conjunction with colloidal gold. In the fourth instar midgut, AeSCP-2 was found in the cytosol within the gut epithelium, and AeSCP-x was heavily associated with peroxisomes. The subcellular distribution of AeSCP-2 and AeSCP-x suggests that AeSCP-2 and AeSCP-x are functionally different.

EXPERIMENTAL PROCEDURES

Chemicals and reagents

Chemicals and reagents were purchased from Sigma (Sigma, St. Louis, MO), Fisher Scientific (Pittsburgh, PA), and ICN Biomedicals, Inc. (Costa Mesa, CA), if their origins are not mentioned in the text.

Mosquitoes

The mosquitoes used in these experiments, A. aegypti, are taken from an inbred laboratory strain (Rockefeller). Larvae were reared at 26°C in 70–80% humidity in a light-dark cycle (14:10) with pellets of rabbit food. Larvae hatching during a 15-min period were collected and used in experiments. Under these conditions, development from first to fourth stages takes 3.5 days and another 64 h (mostly males) to 72 h (mostly females) to complete the fourth stadium. Adults were maintained at 24°C.

Mosquito cell line

A. aegypti cell line-2 (Aag-2) is maintained in Eagles’ medium supplemented with 5% fetal bovine serum (11) at 28°C under a 5% CO₂ atmosphere. Passages of cells were conducted every 7 days with a 1:5 dilution of cells.

Electron microscopy

The midgut from 24 h-old fourth instars was dissected out in cold insect saline solution (12). To maintain the morphological integrity of the midgut, food was not removed from the gut. The midgut and the cells were fixed in 4% formaldehyde, 0.1% glutaraldehyde in a 0.1 M NaPO₄ buffer (pH 7.4) for 4 h at 4°C. The fixed cells and midgut were rinsed in 0.1 M NaPO₄ buffer 4× (5 min). To further maintain integrity, the midgut tissues were embedded in 2% (w/v) molten agarose at 44°C, solidified on ice, and trimmed to 2 mm cubed. After trimming, the samples were dehydrated in a graded series of ethanol at room temperature (50–100%; v/v). Dehydrated samples were infiltrated and embedded using an LR White resin (Polysciences, Warrington, PA) at 48°C for 72 h.

Aag-2 cells were seeded onto a poly-L-lysine-coated coverslip in 35 × 10 mm culture dishes. After the culture reached confluence, the cells were washed twice with 2 ml cold phosphate-buffered saline (PBS). Cells on the coverslip were fixed, washed, and infiltrated as described above while on the coverslip. Half gelatin capsules were filled with resin, inverted on top of the infiltrated cells, and polymerized for 3 days at 48°C. The coverslip was “popped off” the hardened capsules by temporarily placing them on an aluminum block cooled to liquid nitrogen 2 temperature. The cells were sectioned on the face of the block through the monolayer (parallel to the former glass surface).

Immunocytochemistry

Ultrathin sections (70 nm) were collected on a Reichert-Jung Ultracat E ultramicrotome and placed on Polysform (Ted Pella, Redding, CA)-coated 300 mesh nickel grids. PBS 10 mM sodium phosphate, 150 mM NaCl, pH 7.4 with 0.1% BSA, 0.1% cold water fish skin gelatin was used as an antibody dilution and washing buffer. No etching or pretreatment steps were taken. All steps, including washes, were done on 35 μl drops placed on parafilm. The grids were incubated on drops of buffer containing 4% BSA content for 30 min (as a blocking step), and washed twice (5 min each) in PBS buffer. Then, the grids were incubated overnight with drops on purified primary antibodies at 1:500 dilutions in PBS buffer at 4°C. Grids were washed 4× (5 min each) in PBS buffer. The washed grids were incubated for 4 h on drops of 15 nm gold-conjugated labeled goat anti-rabbit IgG diluted with buffer at 1:100. Grids were washed in buffer 4× (5 min each). Immunolabeled samples were fixed for 5 min in 4% glutaraldehyde in 0.1 M NaPO₄ buffer, pH 7.4. The grids were rinsed in distilled water twice (5 min each) and poststained in uranyl acetate and Reynolds lead citrate. The samples were viewed on a Philips CM120 transmission electron microscope and documented with a SIS MegaView III digital camera.

Affinity-purified chicken anti-sheep IgG antibody (Immunology Consultants Laboratory, Newberg, OR) was conjugated to 9 nm colloidal gold and used as a secondary immunocytochemical marker against sheep anti-bovine catalase (BIODESIGN Int., Saco, ME). Conjugation was performed according to British Biocell International technical booklet “Gold Colloids” (13).

The two primaries were mixed at the 1:500 dilution and incubated overnight. Individual gold labeling steps were performed afterward (i.e., 15 nm anti-rabbit, 4 h, rinsed; anti-sheep, 4 h, rinsed). Grids were washed in buffer 4× (5 min each). Immunolabeled samples were fixed for 5 min in 4% glutaraldehyde in 0.1 M NaPO₄ buffer, pH 7.4. The grids were rinsed in distilled water twice (5 min each) and poststained in uranyl acetate and Reynolds lead citrate. The samples were viewed on a Philips CM120 transmission electron microscope and documented with a SIS MegaView III digital camera.

A set of sections was incubated with preimmune serum from the same rabbit that primary antibodies were obtained as negative controls for immunoelectron microscopy, and no labeling was observed with preimmune serum (data not shown).

The quantification of labeling density included 3 to 4 independent sections (8-12 μm²) randomly chosen for quantitative analysis of the labeling. All of the gold particles were counted in the selected section and derived the data for labeling/μm². Data for labeling in cytoplasm were counts excluding only the nuclear; data for cytosol were counts excluding all organelles.

Cholesterol incorporation in Aag-2 cells

AeSCP-2 overexpression vector was constructed by inserting the entire coding sequence of AeSCP-2 into the pBluescript vector (14). High quality plasmid DNA was purified using Maxprep column (QIAGEN, Valencia, CA). The quantity of the DNA was determined by UV spectrum measurement at 260 nm.

To confirm that any effects of AeSCP-2 on cholesterol uptake are mediated by AeSCP-2, we also repressed AeSCP-2 overexpression in Aag-2 cells by RNA interference (RNAi). We designed two 21 bp primers for AeSCP-2 gene silencing. The primers matched to the N-terminal portion of the AeSCP-2 sequence were targeted. An 8-bases T7 primer sequence was added to the sense- and the antisense-targeted sequences (5’-aatggcgatgtggggaaacccgctgcc-3’ and 5’-aagtttccgaagctgtactgtc-3’) for in vitro transcription. High quality double-stranded RNA (dsRNA) was produced using an RNAi construction kit (Silencer™, Ambion,
Austin, TX). The quantity of AeSCP-2 dsRNA was determined by UV spectrum measurement at 260 nm.

Aag-2 cells were seeded at 2 ml of 5 × 10⁶ cells/ml in 35 × 10 mm culture dishes. After overnight incubation, culture medium was removed, and cells were washed once with 2 ml of transfection medium (without serum and antibiotics). One milliliter of transfection medium containing 3 μg/ml AeSCP-2 overexpression vector and 20 μg/ml lipofectin (Invitrogen) was added into the cell cultures. Mock-transfected cells were treated with a transfection medium containing only 20 μg/ml lipofectin. Cells were incubated in the transfection medium for 8 h, and the transfection medium was replaced with 2 ml of fresh medium and incubated at 28°C.

For RNAi experiments, AeSCP-2 dsRNA was cotransfected at 40 nM concentration with the AeSCP-2 overexpression vector as described above. After 8 h, 2 ml of fresh medium were added into the transfection medium, and cells were incubated at 28°C. Culture medium was removed at 36 h after transfection, and 2 ml of fresh steroid-free medium were added (15). After overnight culture in a steroid-free medium, the medium was replaced with 1 ml of steroid-free medium containing 0.33 μCi [3H]cholesterol/ml (40 Ci/mM, ICN) and incubated for 12 h. Cells were washed twice with 2 ml cold PBS, and the total cellular lipids were extracted as described (16). The quantity of cellular [3H]cholesterol was measured in a liquid scintillation counter (Packard, Billerica, MA). The content of cellular [3H]cholesterol is described as disintegrations/min/mg total protein.

Western blotting analysis

Selected animals were cleaned in sterile water, and excess water was blotted off using clean Kim wipes. Five to six whole animals were homogenized in 150 μl lysis buffer [0.25M Tris-HCl, pH 8.0/0.2% Triton X-100/1 mM dithioerythritol/5 mM EDTA/10 mM β-mercaptoethanol/1 mM phenylmethylsulfonyl fluoride/protease inhibitor cocktail (Sigma)], and centrifuged at 12,000 g at 4°C for 15 min. Supernatants containing soluble proteins were stored at −80°C. Protein concentrations were determined using a bicinchoninic acid (BCA) kit (Pierce, Rockford, IL).

Cultured cells in 35 × 10 mm dishes were washed twice with 2 ml of cold PBS (pH 7.4). Cells were scraped off with a rubber scraper in 1 ml of cold PBS, transferred to a clean 1.5 ml Eppendorf tube, and collected by centrifuging at 5,000 g for 5 min at 4°C. The cell pellets were homogenized in 150 μl lysis buffer, and supernatants containing soluble proteins were stored at −80°C. Protein concentrations were determined using a BCA kit.

Western blotting analysis was performed as described (14), using either SDS 15% PAGE or SDS 4–20% gradient PAGE (ISC BioExpress, Kaysville, UT). The protein blots were incubated with 1:1,000 dilution of rabbit polyclonal anti-AeSCP-2 or anti-AeSCP-xC antibodies. The goat anti-rabbit horseradish peroxidase conjugated secondary antibody (Jackson ImmunoResearch Laboratory, West Grove, PA) was used at 1:2,000 dilution. A 3,3’-diaminobenzidine solution (0.3 mg/ml and 0.03% hydrogen peroxide in PBS) was used to visualize the bound antibodies, which were developed within 5 min at room temperature.

RESULTS

Specific antibodies against recombinant AeSCP-2 and AeSCP-xC (the cleaved SCP-2 domain of AeSCP-x) were

![Fig. 1.](image1) Antibody specificity of anti-Aedes aegypti sterol carrier protein-2 (AeSCP-2) (A) and AeSCP-x (B) sera to the recombinant proteins. Lane 1: Protein molecular weight markers. Lane 2: Recombinant AeSCP-2. Lane 3: Recombinant AeSCP-x C-terminal (AeSCP-xC). Lane 4: Bacterial GST. The proteins were analyzed on 4–20% SDS-PAGE. In each lane, 100 ng protein was loaded. Primary antibodies were diluted at 1:3,000. Secondary antibody was diluted at 1:5,000. A: Western blotting using anti-AeSCP-2 serum as the primary antibodies. B: Western blotting using anti-AeSCP-xC serum as the primary antibodies.

![Fig. 2.](image2) Antibody specificity to AeSCP-2 (A) and AeSCP-x (B) in mosquito cell extracts. Lane 1: Protein extract from Aedes aegypti cell line-2 (Aag-2) cells. Lane 2: Protein extract from AeSCP-2 overexpressing Aag-2 cells via transfection of AeSCP-2 expression vectors. Twenty micrograms of total protein were loaded in each lane. Primary antibodies were diluted at 1:1,000. Secondary antibody was diluted at 1:3,000. A: Western blotting using anti-AeSCP-2 serum as the primary antibodies. Proteins were analyzed on a 4–20% SDS-PAGE gel. B: Western blotting using anti-AeSCP-xC serum as the primary antibodies. Proteins are analyzed on a 15% SDS-PAGE gel.
produced in rabbits using recombinant proteins [see Ref. (9); Lan and Wessely, unpublished observations]. Anti-AeSCP-2 antibodies only recognized AeSCP-2 recombinant proteins (Fig. 1A, lane 2), and anti-AeSCP-xC antibody reacted only to recombinant AeSCP-xC (Fig. 1B, lane 3). Purified anti-AeSCP-2 and anti-AeSCP-xC antibodies do not have cross-reactivity to each other’s prospective proteins. Anti-AeSCP-2 antibodies detected a 13 kDa protein band in proteins extracted from Aag-2 cells (Fig. 2A, lane 1) and Aag-2 cells overexpressing AeSCP-2 (Fig. 2A, lane 2). Anti-AeSCP-xC antibodies recognized a 62 kDa protein band, the full length AeSCP-x (Fig. 2B, lanes 1 and 2) and did not show immunoreactivity to the overexpressed AeSCP-2 (Fig. 2A, lane 2; Fig. 2B, lane 2).

SCP-2 in Aag-2 cells

In Aag-2 cells, subcellular distribution of AeSCP-2 and AeSCP-x was examined using an immunoelectron microscopic technique. Secondary antibody, in conjunction with colloidal gold, was used to visualize the cellular localization of AeSCP-2 and AeSCP-x. AeSCP-2 had broad distribution patterns, mostly in the cytosol, with some stains in the mitochondria and the nucleus (Fig. 3A). In AeSCP-2 overexpressing Aag-2 cells, AeSCP-2 distribution was clearly increased in cytosol, mitochondria, and nucleus (Fig. 3B). AeSCP-2 overexpression in Aag-2 cells led to an overall more than 2.2-fold increase of labeling in the cells ($P = 0.013$) and the same proportion increase of nuclear labeling ($P = 0.075$; Fig. 3C). However, AeSCP-2 was not associated with peroxisomes, because the labeling did not appear in membranous vesicles of moderate electron density (Fig. 3A, B). The result is consistent with the fact that AeSCP-2 does not have a peroxisome targeting sequence (9). This result is different from findings in vertebrate SCP-2 studies, showing that the vertebrate SCP-2 associates heavily with peroxisomes (17, 18).

SCPs in the larval midgut

Subcellular distribution of AeSCP-2 in the larval midgut epithelium had a very similar pattern as that of Aag-2 cells, with notably no apparent association with peroxisomes. AeSCP-2 was detected throughout the cytosol (Fig. 4A, arrows), even within the microvilli (Fig. 4A). There was limited labeling in mitochondria (Fig. 4A). More than 98% of the AeSCP-2 labeling in the cytoplasm of midgut epithelium was in the cytosol (Fig. 5), which was not associated with membranous structures. It was noticed that the visceral muscle cell had only a few AeSCP-2 labels (Fig. 4C), which was in sharp contrast to the midgut epithelium (Fig. 4A–C). Interestingly, there were noticeable stains in the nucleus (Fig. 4B), which is consistent with the observation of the Aag-2 cell (Fig. 3). The apparent nuclear localization of AeSCP-2 is in contrast to the vertebrate SCP-2 that does not show nuclear localization (17, 19).

In the larval midgut epithelium, AeSCP-x was localized primarily to membranous vesicles of moderate electron density (Fig. 6A, arrows). Catalase is a well-known peroxisomal enzyme and localizes to the peroxisomal matrix (20). Results from double-labeling experiments indicate that AeSCP-x was colocalized with catalase in the peroxisome using the anti-bovine catalase polyclonal antibody (Fig. 6B). Sixty five percent of AeSCP-x labeling was in the peroxisome (Fig. 5). There was some AeSCP-x labeling in the cytosol, mitochondria, and nucleus in the midgut epithelium (Figs. 5, 6). Because the anti-AeSCP-x antibodies were made against the SCP-2 domain of AeSCPx, the results indicate that AeSCPx and the proteolytic product, AeSCPxC (the cleaved SCP-domain of AeSCPx), localize mostly to peroxisomes (Fig. 6B). Therefore, AeSCPx showed a very similar cellular distribution pattern as that of the vertebrate SCP-2 (17, 18).

Both AeSCP-2 and AeSCP-x are expressed at high levels in fourth instar midgut during the feeding stage [see Ref. (9); Lan and Wessely, unpublished observations]. However, AeSCP-2 labeling has higher density ($158.07 \pm 38.87$ labeling/$\mu m^2$) than that of AeSCP-x ($19.70 \pm 12.16$ labeling/$\mu m^2$).
ing/H9262/h2 in the cytoplasm, which indicates that AeSCP-2 levels were higher than AeSCP-x in the larval midgut epithelium.

Potential biological function of AeSCP-2

There is ample data on the function of the vertebrate SCP-x and SCP-2, especially on the vertebrate SCP-2 (1, 21). However, AeSCP-2 represents a different low molecular weight, SCP-2 domain-containing protein from that of the cleaved SCP-2 domain product of AeSCP-x. Whether AeSCP-2 has a similar function as that of the vertebrate SCP-2 regarding cholesterol metabolism is unknown. The function of AeSCP-2 was investigated using Aag-2 cells. Aag-2 cells were transfected with an AeSCP-2 expression vector (Fig. 2A, lane 2). Overexpression of AeSCP-2 in Aag-2 cells enhanced cholesterol uptake by about 19% within 12 h compared with mock transfected cells (Fig. 7, control and AeSCP-2; P = 0.094). AeSCP-2-mediated cho-

Fig. 4. Immunoelectron microscopic labeling of AeSCP-2 in the midgut epithelium of a fourth instar. A: On the lumen side of the epithelium. Bar = 1 μm. B: In the nucleus of the epithelium. Bar = 0.5 μm. C: In the visceral muscle and on the basal side of the epithelium, 45,000× magnified. Arrows indicate some of the colloidal gold labeling. BM, basal membrane; Mv, microvillus; VM, visceral muscle.

Fig. 5. Distribution of AeSCP-x and AeSCP-2 in the cytoplasm of the fourth instar midgut epithelium. Labeling density was counted from a total of four independent sections (8–12 μm²/each), excluding nucleus. Error bars represent mean ± SD.
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Cellular distribution of sterol carrier proteins

We have discovered that there are two distinct low molecular weight, SCP-2 domain-containing proteins encoded by two genes in yellow fever mosquitoes: AeSCP-2 and AeSCP-xC (the cleaved SCP-2 domain product from AeSCP-xC). AeSCP-2 and AeSCP-xC have 31% and 44% identity to the vertebrate SCP-2 [see Ref. (9); Lan and Wessely, unpublished observations], respectively. Whether these two distinct low molecular weight, SCP-2 domain-containing proteins in mosquitoes are functionally redundant or distinct is unknown.

The function of a protein can be partly inferred by analyzing the localization of the protein. The results from immunoelectron microscopy confirm that AeSCP-2 and AeSCP-x are distinct proteins with different cellular distribution. AeSCP-x is restricted mostly within the peroxisomes (Figs. 5, 6). Because peroxisomes are heavily involved in lipid metabolism (22), it is hypothesized that AeSCP-x may aid in lipid metabolism in mosquitoes, which is similar to the function of the vertebrate SCP-x (23). It is unclear, however, where proteolysis of AeSCP-x occurs within the midgut epithelium. Based on the fact that over two-thirds of AeSCP-x are proteolytically cleaved (Lan and Wessely, unpublished observations), and most of AeSCP-xC (cleaved SCP-2 domain of AeSCP-x) are labeled in peroxisomes (Figs. 5, 6), it is suggested that the bulk of AeSCP-x is proteolytically cleaved in the peroxisome.

In vertebrates, the only reported low molecular weight SCP-2 domain-containing protein is the vertebrate SCP-2 transcribed from the SCP-x/SCP-2 gene (1). In mosquitoes, there is a second small SCP-2 domain-containing protein, AeSCP-2, which is not transcribed from AeSCP-x gene (Lan and Wessely, unpublished observations). It has been reported that AeSCP-2 binds to cholesterol (9) and C16 fatty acid (10). In this study, it has been shown that 98% of AeSCP-2 localizes to cytosol in the cytoplasm (Figs. 3, 4, 5), which is markedly different from the vertebrate SCP-2 (17, 19). Because AeSCP-2 is not associated with peroxisomes, AeSCP-2 is unlikely to be involved in peroxi-

Fig. 7. The effect AeSCP-2 has on cholesterol uptake in Aag-2 cells. Inset: Western blot analysis of AeSCP-2 in Aag-2 cells. Lane 1: Protein molecular weight marker. Protein was analyzed on 15% SDS-PAGE. Lane 2: Mock transfected cells. Lane 3: AeSCP-2 overexpressing cells. Lane 4: AeSCP-2 RNA interference (RNAi)-treated AeSCP-2 overexpressing cells. Rabbit anti-AeSCP-2 antibody was diluted 1:3,000. Ten micrograms of total protein were loaded per lane. Error bars represent mean ± SD (N = 3). AeSCP-2, AeSCP-2 overexpressing cells. AeSCP-2/RNAi, AeSCP-2 overexpressing/AeSCP-2 double-stranded RNA-treated cells; control, mock transfected cells.
somal lipid metabolisms. Surprisingly, AeSCP-2 also localizes to the nucleus (Figs. 3, 4B), and overexpression of AeSCP-2 increased the presence of AeSCP-2 in the nucleus (Fig. 3B, C). In contrast, the vertebrate SCP-2 or SCP-x is not found in the nucleus (17). It is unclear whether AeSCP-2 is targeting cholesterol or fatty acids into the nucleus. Nuclear distribution of a lipid carrier has been reported for the liver fatty acid-binding protein, which targets fatty acids to the nucleus (24).

To shed light on the possible function of AeSCP-2, overexpression and knockdown of AeSCP-2 in cultured cells were examined in terms of cholesterol incorporation. The results showed that AeSCP-2 is partially responsible for the accumulation of cholesterol in cells. AeSCP-2 expression is high in the midgut during feeding stages (9). Early studies have indicated that midgut and possibly foresert are the sites of cholesterol absorption in insects (25–27). Therefore, it is highly possible that AeSCP-2 in the larval midgut is involved in cholesterol uptake.

In summary, it is clear that AeSCP-2 and AeSCP-x, mosquito SCPs, have distinct cellular distribution patterns in cultured cells and in the larval midgut epithelium. Because the high similarities between AeSCP-x and vertebrate SCP-x in terms of posttranslational modification and cellular localization, AeSCP-x may have very similar functions as that of vertebrate SCP-x. On the other hand, AeSCP-2, a unique, low molecular weight, SCP-2 domain-containing protein, may have functions different from vertebrate SCP-2 due to its subcellular distribution

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