Apoptosis-induced release of mature sterol regulatory element-binding proteins activates sterol-responsive genes

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Abstract **It is well established that during the execution of the apoptotic cascade, activated caspase 3 releases sterol regulatory element-binding proteins (SREBP) from the membrane of the endoplasmic reticulum in a proteolytic reaction that is distinct from their normal sterol-dependent activation. However, it is not known whether these transcription factors are capable of activating sterol-responsive genes under such conditions. The construction of SRE expression vectors has permitted characterization of the apoptotic activation of SREBP. Cell lines stably expressing the plasma membrane marker CD32, or GFP, under the control of the SRE promoter were shown to modulate SRE gene expression on the basis of the levels of available sterols. However, during the induction of apoptosis, expression of CD32 and GFP was highly induced, even in the presence of ample sterols. Apoptotic induction of sterol-regulated genes was due to activation of caspase 3 and was impervious to treatment with sphingomyelinase, indicating that activation of SRE genes during apoptosis is sterol independent. Further characterization of this apoptotic response indicated that sterol-regulated genes are activated at an early stage in the apoptotic cascade, preceding the externalization of phosphatidylserine on the plasma membrane of apoptotic cells. These results suggest that activation of sterol-responsive genes early during apoptosis may play a role in the proper execution of this program.**—Higgins, M. E., and Y. A. Ioannou. **Apoptosis-induced release of mature sterol regulatory element-binding proteins activates sterol-responsive genes.** ***J. Lipid Res.*** **2001.** 42: **1939–1946.**

Supplementary key words annexin V • caspase 3 • CD32 • cholesterol • SREBP

Cholesterol is the predominant sterol of the plasma membrane in animal cells and is critical for cell growth and proliferation. Sphingomyelin is also an important component of plasma membranes, and in fact membrane cholesterol solubilization depends largely on its sphingomyelin content. The interactions between cholesterol and sphingomyelin appear to be critical for the regulation of cellular cholesterol levels (1, 2), and it has been suggested that sphingomyelin modulates the amount of cholesterol found in the various cellular pools (3, 4). In addition, these two molecules have been implicated in the formation of microdomains or rafts within membrane regions and are believed to function in signal transduction and membrane trafficking (5).

Cholesterol is obtained from plasma lipoproteins via endocytosis of the LDL receptor or synthesized de novo from acetyl-coenzyme A. The rate-limiting steps in the cholesterol biosynthetic and salvage pathways, HMG-CoA reductase (6) and the LDL receptor (7), respectively, are both transcriptionally regulated by sterol regulatory element binding proteins (SREBP) (8). These proteins belong to the c-Myc family of transcription factors and have been shown to play an important role in the regulation of genes involved in intracellular cholesterol homeostasis in mammalian cells. They are synthesized as 125-kDa precursor proteins that are inserted into the membrane of the endoplasmic reticulum (ER) envelope (9–11). In sterol-deficient cells, proteases cleave the precursor proteins, thus releasing the mature transcription factors (12), which can enter the nucleus and activate target genes by binding to the sterol regulatory element (SRE) sequences in their promoters.

It was demonstrated that apopain (caspase 3), a cysteine protease that plays a key role in the induction of apoptosis, cleaves the SREBP when activated during apoptosis (13). These observations have led to the hypothesis that cholesterol may be required in the early stages of apoptosis to maintain plasma membrane integrity (14). However, this cleavage of SREBP is distinct from the sterol-regulated cleavage, and it is not clear yet whether caspase 3-cleaved SREBP are biologically relevant and capable of activating sterol-regulated gene expression (13).

Abbreviations: ALLN, *N*-acetyl-leucinal-leucinal-norleucinal; ER, endoplasmic reticulum; GFP, green fluorescent protein; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding proteins.

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Studies described in this report demonstrate that SREBP released by apoptosis-induced proteolysis activate sterol-responsive genes during the early stages of apoptosis. Furthermore, activation of these genes by the apoptotic-cleaved forms of SREBP precedes one of the earliest phenotypes of apoptotic cell death, the externalization of phosphatidylserine onto the outer layer of the plasma membrane. These results suggest that activation of cholesterol-regulated genes upon induction of the apoptotic cascade may be an important step in the execution of the death program.

MATERIALS AND METHODS

Materials

All reagents were purchased from Sigma (St. Louis, MO) unless otherwise stated. DMEM and FBS were purchased from Mediatech (Herndon, VA) and HyClone (Logan, UT), respectively. L-Glutamine, gentamicin, G418, and the pGreen Lantern-1 plasmid were from GIBCO-BRL (Grand Island, NY). VectaShield was purchased from Vector Laboratories (Burlingame, CA). U18666A was from BioMol (Plymouth Meeting, PA). Restriction endonucleases, polymerases, and ligases were from New England BioLabs (Beverly, MA). The ApoAlert annexin V apoptosis kit was purchased from Clontech Laboratories (Palo Alto, CA). The fluorescein isothiocyanate (FITC)-conjugated fluorescent antibodies were from Roche Molecular Biochemicals (Indianapolis, IN). The mouse monoclonal antibody to SREBP-2 was obtained from Transduction Laboratories (Lexington, KY). The anti-human CD32 monoclonal antibody was produced by the hybridoma cell line IV.3 (generous gift of J. Unkeless, Mount Sinai School of Medicine, New York, NY). Oligonucleotides were synthesized with phosphoramidite chemistry on a 380B DNA synthesizer (Applied Biosystems, Foster City, CA).

Cell culture

HeLa cells (CRL-1651) were purchased from the American Type Culture Collection (Manassas, VA). Cell lines were maintained in complete DMEM (DMEM supplemented with 10% FBS, 2 mM L-glutamine, and gentamicin at 50 μg/ml) in a humidified incubator at 37°C and 5% CO_2.

Construction of the SRE expression plasmids and derivatives

All molecular biology manipulations were performed according to standard procedures (15). All vector constructs were confirmed by restriction digestion and dideoxynucleotide sequencing. For the construction of the SRE-containing plasmids, equimolar amounts of SRE primers, 5′-TCGAGGACATTTGAAA ATCACCCCACTGCAAACTCCTCCCCC TGCTAG GCGTGTAC GGTGGGAGGCCTG-3′ and 5′-TCGACAGGCCTCCCACCGTA CACGCCTAGCAGGGGGAGGAGTTTGCAGTGGGGTGATT T TCAAATGTCC-3′, were mixed together, boiled for 5 min, and allowed to cool to room temperature for 20 min. The double-stranded product was phosphorylated with T4 polynucleotide kinase and cloned in the sense orientation into the *Xho*I site of plasmid pUHD10-3 (generous gift of Dr. H. Bujard, University of Heidelberg, Heidelberg, Germany). The resulting product was digested with *Stu*I to remove the tet operator, and the larger fragment was gel purified. The eluted DNA was self-ligated, redigested with *Xho*I, and another phosphorylated double-stranded oligonucleotide was cloned into the *Xho*I site, resulting in plasmid pSRE++. Sequence analysis confirmed that both SRE elements were inserted in the plus (+) orientation. The pSRE++ plasmid was digested with *Bam*HI and a cassette containing the neomycin resistance gene driven by the simian virus 40 (SV40) promoter-enhancer was inserted to generate pSRE++.neo. Subsequently, a human CD32 cDNA fragment (generous gift of J. Unkeless, Mount Sinai School of Medicine) was inserted into the *Eco*RI site of SRE++.neo to generate pSRE++.CD32.neo.

Similarly, to generate the pSRE++.GFP plasmid, a green fluorescent protein (GFP)-encoding cDNA was excised from the pGreen Lantern-1 plasmid and was inserted into the pSRE++.neo plasmid. The integrity of the resulting vector, pSRE++.GFP.neo, was confirmed by restriction digestion.

Generation of HeLa-SRE-CD32 and HeLa-SRE-GFP cell lines

HeLa cells at 80% confluence were harvested by trypsinization and electroporated with 10 μg of either pSRE.CD32.neo or pSRE.GFP.neo plasmid DNA, using a GenePulser apparatus (Bio-Rad, Hercules, CA) at 250 V and 500 μF in complete DMEM. Stable clones were isolated in G418 antibiotic medium (500 μg/ml), expanded, and analyzed for sterol-inducible expression of CD32 or GFP after growth in high or low cholesterol-containing medium.

Western blot analysis

On day 0, cells were seeded in 60-mm dishes in complete medium. On day 1, the medium was refreshed with the following additions: *1*) none (control), *2*) 25 mM mevastatin, *3*) U18666A (2 μg/ml), or *4*) 25 mM mevastatin and U18666A (2 μg/ml). Three hours before harvesting on day 2, *N*-acetyl-leucinal-leucinal-norleucinal (ALLN, 25 μg/ml) was added to each dish. Cells were harvested with PBS-2 mM EDTA and centrifuged at 800 *g* for 10 min. The cell pellet was resuspended in buffer [150 mM NaCl, 50 mM Tris (pH 8.0), 1% Triton X-100, leupeptin (10 μg/ml), pepstatin A (5 μg/ml), ALLN (25 μg/ml), 1 μM phenylmethylsulfonyl fluoride, and 0.1 mM Pefabloc], incubated on ice for 10 min, and centrifuged at 800 *g* for 10 min. The supernatant was boiled for 5 min in SDS loading buffer, and proteins were separated on an 8% polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes with a Novex (San Diego, CA) Xcell II Blot module for 2 h at 20 V. Proteins were visualized by chemiluminescence according to the manufacturer recommendations (Roche Diagnostics, Indianapolis, IN).

Fluorescence microscopy

On day 0, cells were seeded on coverslips in complete medium. On day 1, the medium was changed to complete medium containing *1*) no addition (control), *2*) 10 μM camptothecin, or *3*) 10 μM camptothecin and neutral sphingomyelinase (100 μU/ml). After 4 h, cells were washed with chilled PBS and fixed in 3.7% paraformaldehyde for 20 min at room temperature. Immunofluorescence analysis was carried out on fixed cells as previously described (16). Hoechst 33258 (10 μg/ml) or propidium iodide (1.5 μg/ml) was added to cells as indicated. For annexin V detection of phosphatidylserine, cells were washed with chilled PBS and incubated with FITC (2 μg/ml)-conjugated annexin in 1× binding buffer for 20 min at room temperature. After a wash with chilled PBS, cells were fixed in 3.7% paraformaldehyde for 20 min at room temperature. CD32 expression was visualized with an anti-mouse rhodamine-conjugated antibody as described above.

DNA fragmentation assay

On day 0, cells were placed in complete medium. On day 1, the medium was changed to complete medium containing *1*) no addition (control), *2*) 1 μM camptothecin, or *3*) 1 μM camptothecin and neutral sphingomyelinase at 100 μU/ml. On day 2, the cells were harvested with PBS-2 mM EDTA and collected by

centrifugation. Cells were lysed in DNA fragmentation lysis buffer, and DNA fragmentation was quantitated as previously described (17).

RESULTS

Construction of sterol-inducible expression vectors

Vectors were engineered in which a cDNA encoding either the plasma membrane marker CD32 or GFP was placed under the control of a synthetic sterol-regulated promoter. To generate the pSRE plasmids, two copies of the SRE-42 element found in the LDL receptor gene promoter were inserted 22 bp upstream from the TATA box of a minimal human cytomegalovirus (CMV) promoter (**Fig. 1A**). A modified human IgG Fc γ receptor II cDNA (hFcRII, CD32) or a GFP cDNA was inserted in the polylinker under the control of the sterol-responsive promoter (Fig. 1B) to generate pSRE-CD32 and pSRE-GFP, respectively. Because CD32 is expressed only on B lymphocytes, granulocytes, and platelets (18), it is an ideal plasma membrane marker for expression and biopanning in all other cell types, whereas GFP is useful for cell sorting and qualitative analysis of positive cells. Finally, a cassette containing the neomycin resistance gene driven by the SV40 promoter-enhancer was inserted into these vectors for positive selection.

Expression of sterol-inducible vectors in HeLa cells

HeLa cells were transfected with either the pSRE-CD32 or the pSRE-GFP vector to generate stable cell lines that could respond to changes in cellular cholesterol levels. A number of clones were identified that expressed high levels of CD32 or GFP after growth in reduced sterol medium but completely suppressed the expression of these marker proteins after growth in sterol-rich medium. A CD32-expressing clone (HeLa-CD32) and a GFP-expressing clone (HeLa-GFP) that exhibited optimal sterol-inducible expression were chosen for all subsequent work.

Both cell lines expressed the markers in the sterol-dependent manner previously described for the LDL receptor, indicating that the cell lines could be used to monitor SRE-induced cellular responses. HeLa-CD32 or HeLa-GFP cells grown in high sterol medium effectively downregulated the expression of CD32/GFP (**Fig. 2**, FCS). In fact, continuous growth of these cells in medium containing soluble cholesterol effectively maintained the expression vector in the "off" state (data not shown). The expression level of CD32/GFP was only slightly upregulated when the de

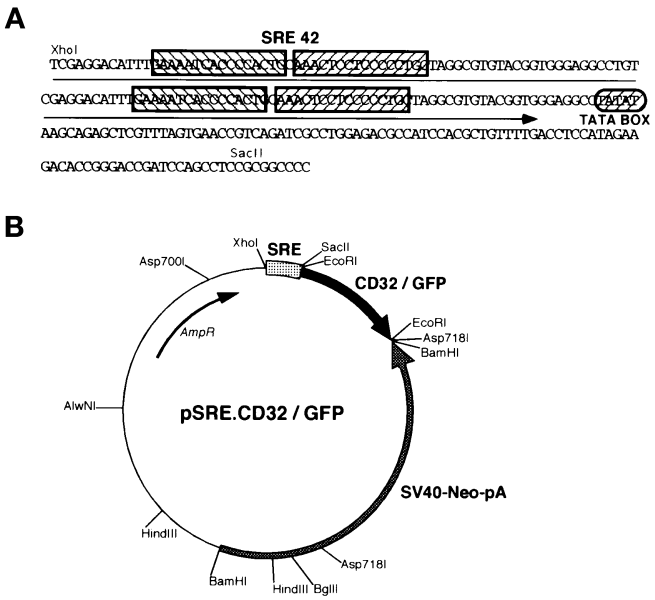

Fig. 1. Sterol-regulated mammalian expression vectors. A: Two copies of the SRE 42 element found in the LDL receptor gene were placed in front of a minimal CMV promoter (TATA box) in the (+) orientation (see Materials and Methods). B: The SRE-regulated promoter drives the expression of a cDNA encoding either a plasma membrane marker (CD32) or GFP. Stable expression is obtained by selection for the SV40-neo cassette.

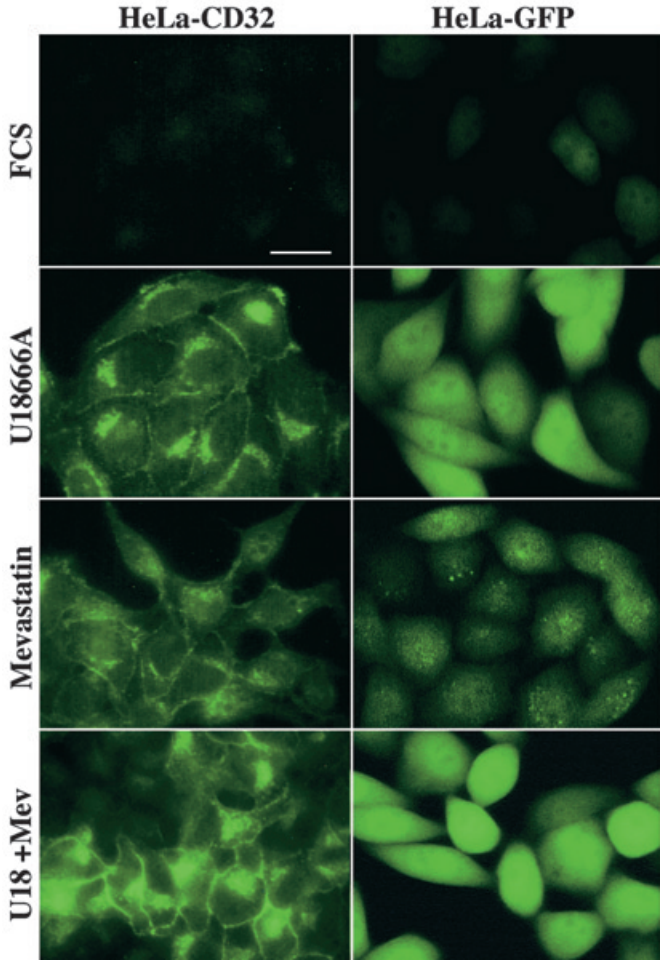

Fig. 2. Sterol-regulated expression of CD32 or GFP in stable cell lines. Both CD32-expressing (HeLa-CD32) and GFP-expressing (HeLa-GFP) cell lines exhibited sterol-regulated expression of the marker genes. In sterol-rich medium, expression of the genes is not induced (FCS), but in the presence of U18666A, an inhibitor of lysosomal cholesterol efflux, both cell lines turn on the expression of the marker gene. Mevastatin, an inhibitor of de novo synthesis of cholesterol, also induces expression, albeit at a lower level. The addition of both inhibitors, however, causes a dramatic increase in the expression of both markers (U18+Mev). Magnification bar: 10 μm.

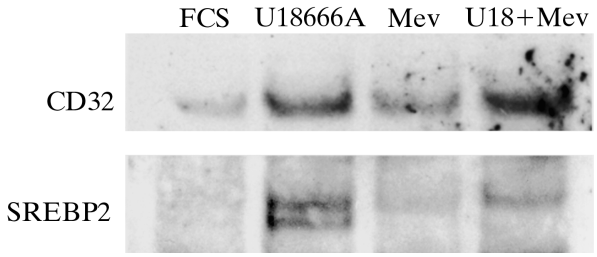

Fig. 3. Immunoblot of CD32 and SREBP-2 expression in HeLa-CD32 cells. HeLa-CD32 cells were grown in the presence of 10% FCS and either of the inhibitors U18666A and mevastatin (see legend of Fig. 2). In sterol-rich medium (FCS), the mature form of SREBP-2 is absent, along with little, if any, expression of CD32. U18666A and mevastatin, however, cause an increase in the levels of mature SREBP-2, with a concomitant increase in CD32 expression.

novo biosynthetic pathway was blocked by inhibiting HMG-CoA reductase with mevastatin (Fig. 2, mevastatin). Treatment of cells with U18666A, a potent inhibitor of lysosomally derived cholesterol (19), caused a significant increase in CD32/GFP expression (Fig. 2, U18666A), indicating that U18666A is an effective inducer of these sterol-regulated markers. Simultaneous treatment of cells with both inhibitors caused a dramatic increase in CD32/GFP expression even though the cells were bathed in a high cholesterol medium (Fig. 2; U18+Mev).

Western blot analysis of HeLa-CD32 cell lysates demonstrated that CD32 expression levels correlated with the levels of mature SREBP (**Fig. 3**). There was negligible expression of mature SREBP-2 and CD32 in HeLa-CD32 cells maintained in a high cholesterol medium (FCS), whereas inhibition of cholesterol egress from the endosomal/lysosomal system with U18666A resulted in the appearance of mature SREBP-2 and high levels of CD32 (Fig. 3). This high level of expression persisted when the cells were treated with both mevastatin and U18666A. Taken together, these results indicate that these vectors can be used as monitors or "sensors" of cellular cholesterol levels or, alternatively, as high level sterol-inducible expression vectors.

Cholesterol-independent activation of sterol-responsive genes during apoptosis

During the characterization of the above-described cell lines, it was observed that cells exhibiting signs of apoptosis also expressed the SRE-regulated markers in the absence of sterol induction. Previous reports demonstrated that during apoptosis, activated caspase 3 cleaves SREBP (13, 14). However, it was not determined whether caspase 3-cleaved SREBP were capable of activating SRE-responsive genes, because the caspase 3 cleavage site is different from that produced by sterol induction. To address this question, HeLa-GFP cells were treated with the topoisomerase inhibitor camptothecin to induce apoptosis. Expression of the sterol-responsive marker GFP could be clearly seen in apoptotic cells even though the presence of ample sterols in the growth medium should have suppressed the SRE response (**Fig. 4A**, arrows). Cells expressing GFP displayed the characteristic morphological features of apoptosis, including membrane blebbing and nuclear and cytoplasmic condensation. Staining these cells with propidium iodide revealed a condensed, bright-red nuclear morphology (Fig. 4B, arrows), whereas in the absence of any SRE-driven GFP expression in the nonapoptotic cells, the DNA appears as a diffuse red fluorescence (Fig. 4B). To ensure that the SREBP cleavage was not due to sterol regulation, neutral sphingomyelinase was added to the LDL-enriched culture medium. Treatment of cultured cells with neutral sphingomyelinase abolishes sterol-regulated maturation of SREBP

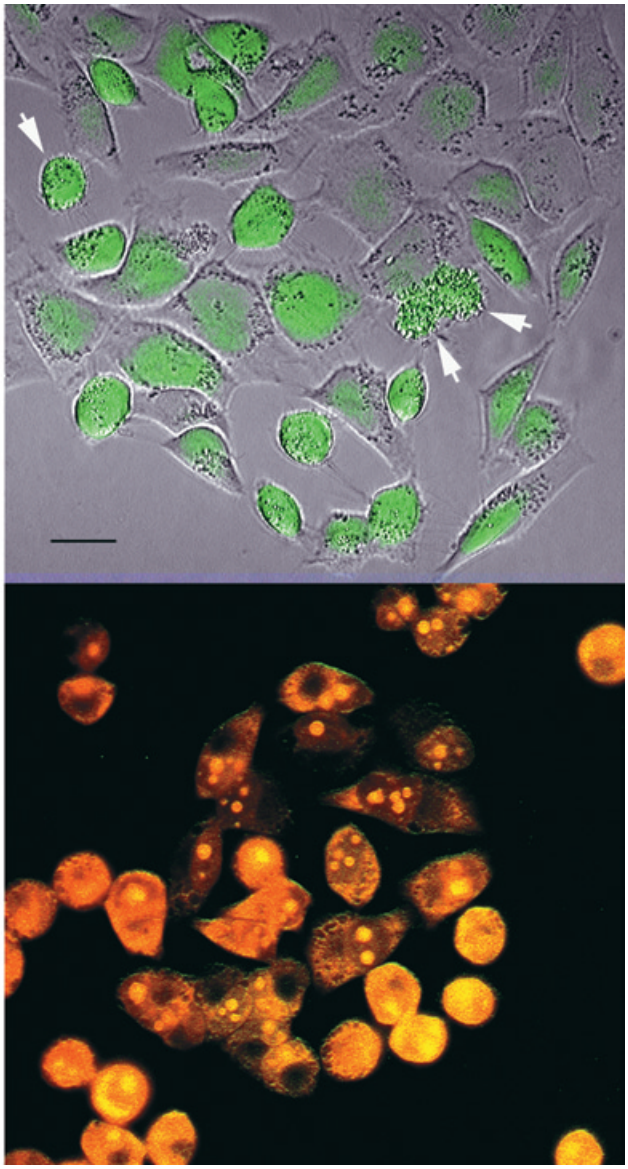

Fig. 4. HeLa-GFP cells treated with camptothecin to induce apoptosis. HeLa cells grown in sterol-rich medium to suppress sterol-regulated expression of GFP were treated with camptothecin to induce apoptosis. A: Sterol-independent induction of GFP expression is seen in many cells that also exhibit signs of apoptotic cell death, such as membrane blebbing (arrows). B: Cells were stained with propidium iodide to detect nuclei undergoing apoptosis. Arrows indicate cells exhibiting the characteristic condensed nuclear morphology of apoptotic cells. Magnification bar: 10 μm.

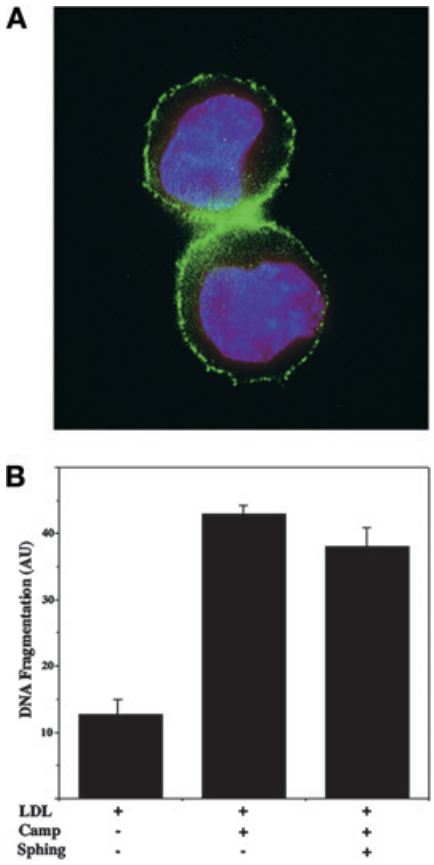

Fig. 5. Sphingomyelinase treatment does not prevent apoptosis or SRE-induced expression of CD32. A: HeLa-CD32 cells were treated with camptothecin in the presence of sphingomyelinase to induce apoptosis while at the same time inhibit sterol-dependent activation of SREBP-2. Under these conditions, cells still express the marker CD32, shown here decorating the plasma membranes of these cells. B: To demonstrate that sphingomyelinase does not affect the progression of camptothecin-induced apoptosis, a cell death assay was carried out. Apoptotic cell death is seen in cells treated with camptothecin irrespective of sphingomyelinase treatment.

(1) because cholesterol is translocated to the ER after depletion of plasma membrane sphingomyelin. Cells grown in sterol-rich medium and treated with both neutral sphingomyelinase and camptothecin still expressed the SRE marker CD32 (**Fig. 5A**), and to confirm that these cells were apoptotic, a DNA fragmentation assay was performed. As shown in Fig. 5B, cells treated with camptothecin showed a significant increase in DNA fragmentation, which was not affected by the addition of sphingomyelinase to the growth medium. These results indicate that the apoptosis-induced cleavage of SREBP can activate the expression of SRE-responsive genes in a sterol-independent manner.

SREBP activation by caspase 3 at an early point in the apoptotic cascade

Although these results demonstrated that SRE-responsive genes are activated during apoptosis, it is not clear at which point in the apoptotic cascade SREBP are activated. To determine when SREBP maturation occurs, cells were grown in sterol-rich medium with or without camptothecin to induce apoptosis. Cells were stained with the DNA-binding dye Hoechst 33258, and then stained for CD32 expression to assess SREBP activation. As shown in **Fig. 6A**, cells grown in sterol-rich medium had diffuse chromatin staining and negligible CD32 expression. After the addition of camptothecin, CD32 expression became apparent, although the nuclear envelope appeared intact with no signs of chromosomal breakdown (Fig. 6B). As before, cells grown in sterol-rich medium and treated with neutral sphingomyelinase failed to prevent SREBP maturation when camptothecin was added (Fig. 6C). Treatment of cells with camptothecin and the caspase 3 peptide aldehyde inhibitor Ac-DEVD-CHO prevented activation of the SREBP response (Fig. 6D), indicating that under these conditions, CD32 expression is caspase 3 dependent. These results suggest that the activation of SREBP by caspase 3 occurs at an early stage during the apoptotic cascade.

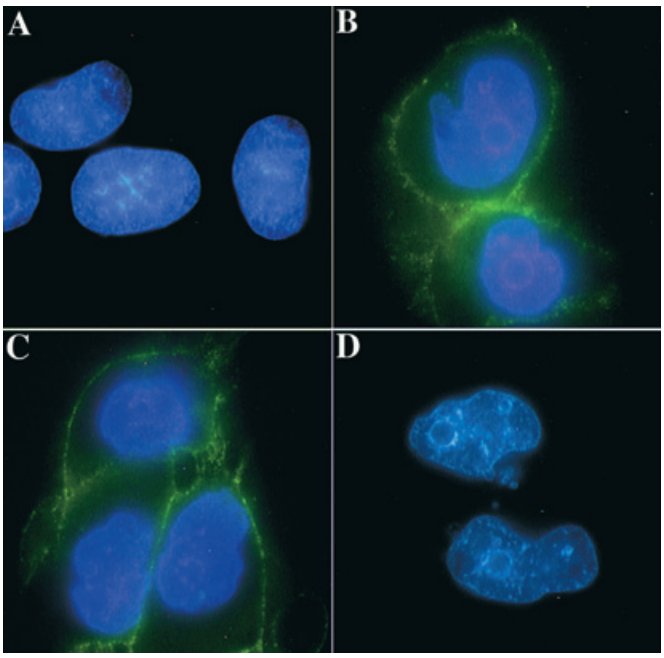

Fig. 6. Hoechst 33258 staining of HeLa-CD32 cells treated with camptothecin. A: Cells grown in sterol-rich medium show no signs of apoptosis or CD32 expression. B: Cells treated with camptothecin begin to express the marker CD32 before exhibiting any changes in nuclear morphology. C: Simultaneous treatment with both sphingomyelinase and camptothecin to prevent sterol-dependent activation of SREBP-2 has no effect on CD32 expression. D: Addition of the caspase 3 inhibitor Ac-DEVD-CHO prevents the apoptotic activation of SREBP-2 and consequently prevents the expression of CD32.

SREBP activation as a marker for apoptosis preceding the externalization of phosphatidylserine on the plasma membrane

One of the earliest characterized events in apoptosis involves cell surface changes, including the externalization of phosphatidylserine from the inner leaflet onto the outer layer of the cell membrane (20, 21). Annexin V is a calcium-dependent, phospholipid-binding protein that preferentially binds phosphatidylserine (22). Many studies have

used annexin V to detect the change in phosphatidylserine orientation as one of the earliest indicators of apoptosis. To further evaluate the timing of SREBP activation during the apoptotic program, HeLa-CD32 cells grown in sterol-rich medium were induced to undergo apoptosis by treatment with camptothecin. After induction, the cells were stained with FITC-conjugated annexin V and also analyzed for surface expression of CD32. As shown in **Fig. 7**, cells expressed CD32 before the exposure of phosphatidylserine could be detected by annexin V. At stages during which annexin V was barely visible on the cell surface (Fig. 7B, arrows), there was clear and strong SRE-induced CD32 expression on the cell surface (Fig. 7A, arrows). In fact, cells turn on the expression of CD32 before many of the visible morphological changes of apoptotic death become apparent. These results suggest that the expression of SRE-responsive genes may be used as an early marker of apoptosis, preceding even the exposure of phosphatidylserine on the outer leaflet of the plasma membrane.

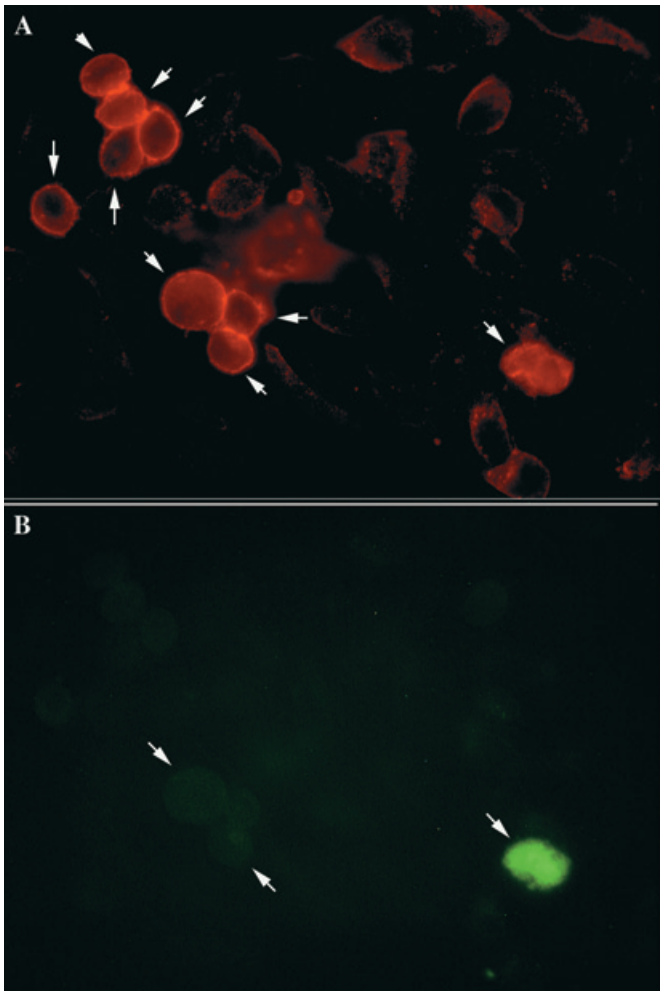

Fig. 7. SRE-CD32 expression precedes externalization of phosphatidylserine at the plasma membrane. HeLa-CD32 cells were treated with camptothecin in a sterol-rich medium. A: Expression of CD32 visualized with a rhodamine-labeled anti-CD32 monoclonal antibody reveals numerous cells expressing CD32 (arrows). B: The same field of cells was also stained with annexin V to visualize the externalization of phosphatidylserine. Only a few cells (arrows) show weak staining with annexin V.

DISCUSSION

In this study, vectors carrying sterol-responsive marker genes were used to generate stable cell lines in which the expression was dependent on cellular sterol levels. These vectors were used to demonstrate that the mature SREBP released during apoptosis can activate sterol-responsive genes in a sterol-independent manner and that this activation occurs early in the apoptotic program, before the earliest documented detectable changes.

The role of the cholesterol biosynthetic pathway and its derivatives in apoptosis is somewhat controversial. It has been suggested that cholesterol biosynthesis during the early stages of cell death may be necessary to maintain plasma membrane integrity in apoptotic cells (14), and thus the activation of sterol-responsive genes during apoptosis may not be an artifact of mass proteolysis. The data in this report also do not exclude the possibility of a cholesterol requirement for other cellular events during the apoptotic process. Alternatively, because the cholesterol biosynthetic pathway is critical for the generation of multiple products essential for normal cellular function, one of those products might be critical for the proper execution of the apoptotic program. An emerging theme in apoptosis is the propagation of the death signal to the appropriate cellular location (23), and disruption of the cholesterol biosynthetic pathway could result in inappropriate or aberrant signaling to various cellular compartments. Inhibitors of HMG-CoA reductase induce apoptosis in a variety of cell types (24) by the induction of cytochrome *c* release and caspase 3 activation (25). In fact, cholesterol starvation leads to cell arrest at G_2 and eventual apoptotic death (26). However, some evidence suggests that isoprenoids, not cholesterol, are necessary for proper cell function during programmed cell death (27), and still others have reported that oxysterols induce apoptosis in a variety of cell types (28–32). Apomine, a synthetic mimetic of farnesol, was shown to be a potent inhibitor of cell proliferation and an inducer of apoptosis via a caspase 3-dependent pathway (33). Clearly, the precise role of sterol responsive genes in apoptosis requires further clarification and appreciation of the interplay between phospholipids, sphingolipids, and sterols (34). A tempting hypothesis for the role of cholesterol in the proper execution of apoptosis can be derived from work demonstrating the scrambling of membrane lipids, including the externalization of phosphatidylserine at the plasma membrane (35), before the execution of apoptosis. This lipid scrambling requires the hydrolysis of sphingomyelin at the plasma membrane by an unidentified neutral sphingomyelinase. Scrambling-deficient cells exhibit aberrant apoptotic morphology, as they are unable to undergo the structural changes of the plasma membrane necessary for proper execution of apoptosis (35). Because cholesterol is a major component of the plasma membrane and, as men-

tioned above, associates with sphingomyelin, it may thus be important in facilitating the hydrolysis of sphingomyelin leading to the proper execution of membrane scrambling and cell death.

Programmed cell death is a fundamental biological process used by multicellular organisms to eliminate unwanted cells. Together with cell proliferation and differentiation, apoptosis is responsible for the arrangement and maintenance of almost all tissues. The connection between cholesterol metabolism and embryogenesis has proven to be critical in mammalian development (36, 37). Cholesterol activates the signaling of hedgehog proteins that are responsible for the patterning behavior of many multicellular organisms (38). Disruptions in cholesterol synthesis can lead to many developmental deformities. In addition, there is a growing awareness that inappropriate initiation or regulation of apoptosis may be associated with a variety of neurodegenerative diseases. Malfunctions in the apoptotic cascade have been proposed to contribute to the pathogenesis of such diseases as Alzheimer's disease and Parkinson's disease.

Although the significance of the activation of cholesterol-regulated genes during apoptosis is not yet established, it is becoming clear that cholesterol plays a far greater role in cell growth and differentiation than is currently known. Cholesterol influences signaling paths that guide development. Because apoptosis is a developmental tool, it is tempting to speculate that sterols serve as molecules that impact the functioning of critical signaling proteins during development. Thus, it is probable that such activation plays a role in the proper execution of programmed cell death. As these studies demonstrate, the role of sterol-regulated genes in the regulation of cell growth, differentiation, and death needs to be evaluated.

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