MINIREVIEW

Mutant Mammalian Cells as Tools to Delineate the Sterol Regulatory Element-Binding Protein Pathway for Feedback Regulation of Lipid Synthesis

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The tools of somatic cell genetics have been instrumental in unraveling the pathway by which sterol regulatory element-binding proteins (SREBPs) control lipid metabolism in animal cells. SREBPs are membrane-bound transcription factors that enhance the synthesis and uptake of cholesterol and fatty acids. The activities of the SREBPs are controlled by the cholesterol content of cells through feedback inhibition of proteolytic processing. When cells are replete with sterols, SREBPs remain bound to membranes of the endoplasmic reticulum (ER) and are therefore inactive. When cells are depleted of sterols, the SREBPs move to the Golgi complex where two proteases release the active portions of the SREBPs, which then enter the nucleus and activate transcription of target genes. This processing requires three membrane proteins—a sterol-sensing escort protein (SCAP) that transports SREBPs from the ER to the Golgi and two Golgi-located proteases (S1P and S2P) that release SREBPs from membranes. The existence of all three proteins was revealed through analysis of mutant mammalian cells in tissue culture. Their cDNAs and genes were isolated by genetic complementation or by expression cloning. The somatic cell genetic approach described in this article should prove useful for unraveling other complex biochemical pathways in animal cells.

Key Words: cholesterol; fatty acids; feedback regulation; gene transcription; mammalian mutants; membranes; proteolytic processing; SCAP; somatic cell genetics; SREBP.

Recent years have witnessed important advances in the understanding of the mechanism by which mammalian cells regulate the synthesis of lipids and their uptake from plasma lipoproteins. Many of these insights have come from the isolation and study of mutant cell lines with defects in the regulation of lipid metabolism. These studies revealed a family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs) that control more than 20 genes whose products mediate the uptake and synthesis of cholesterol and unsaturated fatty acids (1–4). The activity of SREBPs is subject to feedback regulation by the end-products of these pathways, a mechanism that ensures an optimal concentration of lipids in cell membranes. SREBP action requires three proteins—a sterol-sensing escort protein and two membrane-bound proteases—whose existence and identities were revealed through studies of mutant Chinese hamster ovary (CHO) cell lines in tissue culture (5). In this article, we review the use of these mutant cell lines to unravel the feedback regulation of lipid metabolism in animal cells.

THE SREBP PATHWAY: MULTIPLE MEMBRANE PROTEINS AND MULTIPLE ORGANELLES

Figure 1 shows a model of the SREBP pathway and illustrates how this pathway is controlled by one of its...
end-products, cholesterol. Mammalian organisms produce three different SREBP proteins. SREBP-1a and SREBP-1c, which are produced from a single gene on human chromosome 17p11.2, differ only in their first exon. SREBP-2 is produced from a separate gene on human chromosome 22q13 (1). Newly synthesized SREBPs are embedded in membranes of the ER and nuclear envelope in a hairpin orientation. The SREBPs are composed of three domains: (1) an NH2-terminal domain of ~480 amino acids that faces the cytosol and functions as a transcription factor of the basic helix-loop-helix leucine zipper (bHLH-Zip) family, (2) a membrane-anchoring domain of 90 amino acids consisting of two membrane-spanning sequences that are separated by a hydrophilic loop of ~30 amino acids that projects into the ER lumen, and (3) a regulatory domain of ~590 amino acids that extends into the cytosol (1).

The activities of all three SREBP isoforms (SREBP-1a, -1c, and -2) are regulated by the cell’s sterol content. In sterol-depleted cells, two sequential cleavages release the NH2-terminal transcription factor domain of SREBPs from membranes (5) (Fig. 1). The first cleavage is catalyzed by a membrane-bound serine protease that cleaves SREBP at a leucine–serine bond in the sequence RXXLS (where X is any amino acid) within the luminal loop that links the two transmembrane domains (6). This cleavage generates the substrate for a second enzyme, a membrane-bound zinc metalloprotease, designated Site-2 protease (S2P), that cleaves the NH2-terminal bHLH-Zip domain at a site located within the membrane-spanning region. After the second cleavage, the NH2-terminal bHLH-Zip domain leaves the membrane, carrying three hydrophobic residues at its COOH-terminus. The protein enters the nucleus, where it activates target genes controlling lipid synthesis and uptake. When the cholesterol content of cells rises, the SCAP/SREBP complex is no longer incorporated into ER transport vesicles, SREBPs no longer have access to the S1P and S2P in the Golgi, and the bHLH-Zip domain cannot be released from the ER membrane.

![FIG. 1. Model for the sterol-mediated proteolytic release of SREBPs from membranes. SCAP is a sensor of sterols and an escorter of SREBPs. When cells are depleted of sterols, SCAP transports SREBPs from the ER to the Golgi complex. Release of SREBPs from the membrane is initiated by Site-1 protease (S1P), a Golgi-located protease that cleaves SREBP in the luminal loop between its two membrane-spanning sequences. Once the two halves of SREBP are separated, a second Golgi protease, Site-2 protease (S2P), cleaves the NH2-terminal bHLH-Zip domain of SREBP at a site located within the membrane-spanning region. After the second cleavage, the NH2-terminal bHLH-Zip domain leaves the membrane, carrying three hydrophobic residues at its COOH-terminus. The protein enters the nucleus, where it activates target genes controlling lipid synthesis and uptake. When the cholesterol content of cells rises, the SCAP/SREBP complex is no longer incorporated into ER transport vesicles, SREBPs no longer have access to the S1P and S2P in the Golgi, and the bHLH-Zip domain cannot be released from the ER membrane.](image-url)
point that is three amino acids into the first transmembrane domain (7, 8). The released NH₂-terminal fragment migrates to the nucleus with three hydrophobic amino acids at its COOH-terminus. There, it activates transcription of the low-density lipoprotein (LDL) receptor gene and multiple other genes involved in the biosynthesis of cholesterol as well as fatty acids (1–4). When cholesterol levels rise in cells, SREBPs are no longer cleaved by S1P. As a result, cholesterol synthesis and uptake of LDL decrease, thereby maintaining cholesterol homeostasis in a classic feedback fashion (1).

Cleavage of SREBPs by S1P requires the action of SREBP cleavage-activating protein (SCAP), a polytopic membrane protein of 1276 amino acids (9) (Fig. 1). SCAP is divided broadly into two domains (10). The NH₂-terminal 730 amino acids consist of eight membrane-spanning segments with three N-linked oligosaccharide chains attached to the luminal loops. The COOH-terminal 546 amino acids project into the cytosol and contain five copies of a protein/protein interaction motif called the WD repeat. The WD repeat domain of SCAP binds to the COOH-terminal regulatory domain of SREBP, forming a complex that is required for SREBP cleavage by S1P (11).

A clue to the role of SCAP in the SREBP pathway emerged from the observation that the uncleaved precursor forms of SREBPs are found in the ER (6, 12), whereas the active form of S1P is located in a post-ER compartment (13, 14). Cleavage of SREBPs thus requires that the SREBPs be transported from the ER to a post-ER compartment, most likely the Golgi. This conclusion is supported by experiments in which S1P was artificially induced to relocate from the Golgi to the ER. Under these conditions, the SREBPs were cleaved by S1P in a reaction that no longer required SCAP and was no longer inhibited by sterols (13).

Recent biochemical and genetic experiments revealed that the role of SCAP in the SREBP pathway is to escort SREBPs from the ER to the Golgi and to render this transport sensitive to sterols (Fig. 2). This conclusion is supported by three lines of evidence. The first stems from an analysis of the N-linked oligosaccharides of SCAP. In cells replete with cholesterol, the N-linked oligosaccharide chains on SCAP are sensitive to hydrolysis by endoglycosidase H (Endo H), indicating that SCAP is located in the ER. Upon sterol depletion, the oligosaccharide chains on SCAP become resistant to Endo H as a consequence of modification by Golgi-specific enzymes (15, 16). Sterols block this ER-to-Golgi transport by inter-
acting, directly or indirectly, with an ~170-amino-acid segment within the polytopic membrane attachment domain of SCAP that has been termed the sterol-sensing domain. Domains with similar sequences have been identified in several other membrane proteins (3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase), NPC1, and Patched), all of whose actions are influenced by cholesterol (5).

The second line of evidence for SCAP's role as an escort protein is morphologic, involving the use of a green fluorescent protein (GFP)–SCAP fusion protein to visualize the transport of SCAP in living cells (17). When CHO cells, permanently transfected with GFP–SCAP, were incubated in the presence of sterols, the GFP–SCAP fluorescence was confined to the ER. Within 30 min of sterol deprivation, GFP–SCAP was detectable in structures corresponding to the Golgi complex, as determined by colocalization with Golgi mannosidase II. Time-lapse photography showed that GFP–SCAP moved to the Golgi in vesicles that behaved similar to those that transport other membrane proteins from ER to Golgi. A video showing this sterol-dependent transport of SCAP between ER and Golgi can be viewed at http://www.cell.com/cgi/content/full/102/3/315/DC1.

The third line of evidence is based on the results of an in vitro system designed to measure the incorporation of SCAP into vesicles that bud from ER membranes (17). When ER membranes were isolated from sterol-depleted cells, SCAP entered budding vesicles in a reaction that required nucleoside triphosphates and cytosol. When membranes were isolated from sterol-treated cells, the vesicles also formed, and they continued to carry cargo such as the G protein of vesicular stomatitis virus and p58, but SCAP was no longer incorporated. When cells were treated acutely to deplete sterols, the kinetics of induction of SCAP budding (measured in vitro) matched the kinetics of SCAP exit from the ER in living cells expressing GFP–SCAP (17). These findings provide strong support for the hypothesis that the movement of SREBP from ER to Golgi depends on SCAP and that sterols suppress cleavage by blocking the incorporation of the SCAP/SREBP complex into ER-derived vesicles. The in vitro budding system provides an opportunity to determine the biochemical basis for the regulated incorporation of the SCAP/SREBP complex into ER-derived vesicles.

The sterol-regulated exit of SCAP from the ER appears to represent the first example to date of an ER exit process that is under metabolic control. The importance of this process is underscored by the fact that sterol-regulated transport of SCAP is the central event mediating the cholesterol feedback process in mammalian cells.

**Identification of the key molecules in the SREBP pathway** that mediate cholesterol feedback depended on the use of mutant cell lines with alterations in cholesterol homeostasis. These cell lines permitted the verification of the central role of proteolysis in SREBP action and the identification and cloning of the cDNAs for S1P, S2P, and SCAP. Before discussing the somatic cell genetics of the SREBP pathway, we first present a historical perspective on the use of mutant mammalian cells as tools to expose molecules that maintain cholesterol homeostasis.

In 1973–1974, studies of the altered regulation of HMG CoA reductase in cultured fibroblasts from patients with homozygous familial hypercholesterolemia led to the discovery of the LDL receptor and its role in controlling cholesterol homeostasis (18–20). Two years later, the essential role of lysosomes in the LDL receptor pathway was revealed through the study of fibroblasts from patients with cholesterol ester storage disease and Wdlman's syndrome, both of which have defects in lysosomal acid lipase (21, 22).

These early studies relied on the use of naturally occurring human mutants. To expand the use of genetics, several laboratories devised schemes to induce mutations in Chinese hamster fibroblasts or ovary cells and to isolate cell lines with defects in lipid metabolism or in its regulation. Chang *et al.* (23) reported the first such somatic cell mutant in 1977. They isolated a line of CHO cells that required cholesterol for growth. Biochemical characterization of this auxotrophic mutant showed a functional defect in the 4α-methylsterol oxidase enzyme that normally demethylates lanosterol (24). Between 1975 and 1984, our group isolated three mutant strains of Chinese hamster V79 and CHO cells that failed to suppress cholesterol biosynthesis and LDL receptor activity in the presence of 25-hydroxycholesterol. We called these cells sterol regulatory-defective (SRD-1, -2, and -3) cells (25, 26). Similar sterol-resistant cell lines were reported from the laboratories of Kandutsch (27), Sinensky (28, 29), and Chang (30, 31).

In another application of somatic cell genetics, in 1982 our laboratory took advantage of Endo's discovery of the reversible HMG CoA reductase inhibitor, compactin (32), to isolate a mutant line of CHO cells (designated UT-1) that grow in the presence of compactin, owing to a more than 100-fold overproduction of HMG CoA reductase, as a result of amplification and enhanced transcription of the gene encoding the enzyme (33, 34). The availability of these cells allowed us to clone the first two cDNAs for enzymes in the cholesterol synthetic pathway, namely, HMG CoA reductase
and HMG CoA synthase (37, 38). These cDNAs were among the earliest isolates of transcripts derived from “housekeeping” genes (39). All subsequent cDNAs for mammalian and yeast HMG CoA reductases were isolated through the use of this original cDNA, or its descendants, as probes.

At the other extreme, in 1983 our laboratory isolated a line of mutant CHO cells (designated UT-2) that are auxotrophic for mevalonate because they fail to produce any detectable HMG CoA reductase protein or mRNA (40). Another mevalonate auxotroph, Mev-1, which lacks HMG CoA synthase, was isolated by Sinensky and co-workers (41). Another useful cell line is the AC29 line isolated in 1988 by Chang and co-workers (42). These mutant CHO cells have two defects: (1) they are resistant to suppression of LDL receptor and HMG CoA reductase activity by sterols and (2) they fail to activate the acyl-CoA:cholesterol acyltransferase (ACAT) enzyme when sterols are added. ACAT is the enzyme responsible for the synthesis of cholesteryl oleate and other cholesteryl esters when cells accumulate excessive cholesterol. Using a clever selection protocol, the Chang laboratory subsequently used the mutant AC29 cells as recipients for expression cloning of a cDNA encoding ACAT-1 (43).

In 1987, Faust and Krieger (44) isolated a mutant CHO cell line, designated Met-18-b2 cells, which manifest a markedly enhanced rate of mevalonate uptake. In 1992, our laboratory constructed an expression cDNA library from these mutant cells, divided it into pools, and transfected it into cultured HEK-293 cells, which were subsequently assayed for mevalonate uptake (45). The techniques of sib selection and expression screening permitted the isolation of a novel cDNA encoding a membrane transporter that was responsible for the high rate of mevalonate uptake in the Met-18-b2 cells (45). This protein was then shown to be a mutated version of a transporter for lactate, pyruvate, and other monocarboxylates (46). The transporter gene in Met-18-b2 cells had undergone a point mutation, resulting in the substitution of cysteine for phenylalanine in the 10th membrane-spanning region. This single mutation had the remarkable effect of changing the specificity of the protein from 3-carbon monocarboxylates to 5-carbon mevalonate. These studies provided a powerful new tool: a transfectable cDNA encoding a mevalonate transporter that endowed cells with the ability to take up [3H]mevalonate with high efficiency. The cDNA subsequently proved useful in identifying new prenylated proteins (47). This work also provided the first molecular description of a family of monocarboxylate transporters that plays a crucial role in the Cori cycle of fuel homeostasis by mediating the uptake and efflux of lactate and pyruvate in red blood cells, liver, muscle, kidney, and other organs (46, 48, 49).

Table I lists the somatic cell mutants that proved essential in the initial cloning of key molecules in cholesterol homeostasis and in the subsequent analysis of their functional roles during the interval from 1982 to 1999. In addition to the mutants listed in Table I, Chang's laboratory identified two mutant lines of CHO cells, called CT-60 and CT-43 cells, that showed abnormalities in the trafficking of cholesterol within cells (50). These mutant cells, now known to harbor mutations in the NPC1 gene (51), should prove invaluable in dissecting the mechanism by which the NPC1 protein mediates the transport of cholesterol from endosomes and lysosomes to the plasma membrane.

### USE OF MUTANT CELLS TO UNRAVEL THE SREBP PATHWAY

Two distinct mutant phenotypes in CHO cells have proved useful in the identification of molecules in the SREBP pathway: (1) sterol-resistant cells, which fail to suppress LDL receptors and enzymes of cholesterol synthesis (35, 36) and HMG CoA synthase (37, 38). These cDNAs were among the earliest isolates of transcripts derived from “housekeeping” genes (39). All subsequent cDNAs for mammalian and yeast HMG CoA reductases were isolated through the use of this original cDNA, or its descendants, as probes.

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### TABLE I

**Somatic Cell Mutants Used to Clone cDNAs That Encode Key Proteins in Cholesterol Homeostasis**

<table>
<thead>
<tr>
<th>Mutant cell line</th>
<th>cDNA cloned from mutant cells</th>
<th>Function of encoded protein</th>
<th>Year of cDNA cloning</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT-1 HMG CoA reductase</td>
<td>Cholesterol synthesis</td>
<td>1982 (33, 35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UT-1 HMG CoA synthase</td>
<td>Cholesterol synthesis</td>
<td>1986 (37, 38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC29</td>
<td>ACAT</td>
<td>Cholesterol esterification</td>
<td>1993 (43)</td>
<td></td>
</tr>
<tr>
<td>Met-18-b2 Mev</td>
<td>Transports mevalonate into cells</td>
<td>1994 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-RA SCAP (D443N)</td>
<td>Sterol sensor and SREBP escorter</td>
<td>1996 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M19 Site-2 protease</td>
<td>Cleaves SREBPs at Site-2</td>
<td>1997 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRD-12 Site-1 protease</td>
<td>Cleaves SREBPs at Site-1</td>
<td>1998 (63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRD-13A SCAP</td>
<td>Sterol sensor and SREBP escorter</td>
<td>1999 (56)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MEV is a mutant version of the wild-type monocarboxylate transporter in which a single amino acid substitution changes the transporter's specificity from lactate and pyruvate (wild-type) to mevalonate (mutant) (46, 48).

* SCAP (D443N) is a mutant version of the wild-type SCAP in which a single amino acid substitution confers a dominant phenotype of sterol resistance (9).
synthesis in response to incubation with LDL or oxygenated sterols, such as 25-hydroxycholesterol, and (2) sterol-requiring cells (cholesterol auxotrophs), which fail to induce LDL receptors and the enzymes of cholesterol synthesis in response to sterol deprivation. Both of these phenotypes result from defects in the regulated processing of SREBPs, and both permitted the identification of crucial mediators of this process.

The selection procedure used to isolate the sterol-resistant class of mutants is straightforward. Mutagenized CHO cells are cultured in the continual presence of 25-hydroxycholesterol, which is toxic to normal cells because it blocks SREBP processing, thereby stopping cholesterol synthesis, but it cannot replace cholesterol in cell membranes. Mutant cells grow in the presence of 25-hydroxycholesterol because they resist suppression of SREBP processing and therefore cholesterol synthesis.

The selection for the sterol-requiring class of mutants (cholesterol auxotrophs) is more involved. We employ an approach modified from that pioneered by Chang (23, 24). In this approach, mutagenized cells are grown in medium supplemented with free cholesterol and fatty acids so that cells auxotrophic for these lipids will survive. Periodically, the cells are starved for cholesterol and then fed LDL prior to a brief (3–5 h) exposure to amphotericin B. This polyene antibiotic binds to cholesterol present in the outer leaflet of the plasma membrane, forming pores that kill the cell (52). Cells with defects in SREBP processing can neither synthesize cholesterol nor utilize the LDL receptor to take up LDL. The plasma membrane of these cells is depleted of cholesterol during the brief incubation in medium with LDL as the sole source of exogenous cholesterol. Therefore, they survive selection with amphotericin. Cells that can synthesize cholesterol or that can utilize the LDL receptor to take up LDL are replete with cholesterol and succumb to amphotericin. Figure 3 shows the growth pattern of various sterol-resistant and sterol-sensitive CHO mutant cell lines.

Table II shows the members of the two classes of mutant cell lines that we have used in our analysis of the SREBP pathway. The sterol-resistant class of mutants is so far composed of mutations in either of two genes, both of which produce a dominant phenotype. Type 1 mutants are sterol-resistant because they pro-
duce a truncated form of SREBP-2 that is constitutively active as a transcription factor even when cells are incubated with sterols. Three mutant cell lines of this type, SRD-1, SRD-2, and SRD-3, have been isolated in our laboratory (Table II). Each of these cell lines has a similar but molecularly distinct recombination between the large intron following codon 460 of SREBP-2 and another gene (53, 54). The resulting mutant mRNAs encode truncated SREBP-2 proteins whose sequence terminates at codon 460, which lies between the bHLH-Zip domain and the first transmembrane domain. Since these truncated SREBP-2 proteins are never attached to membranes, they proceed to the nucleus directly without requiring proteolysis. Thus, they are immune to sterol-mediated downregulation and confer a 25-hydroxycholesterol-resistant phenotype. The importance of these Type 1 mutants is that they verified the central role of SREBP in the transcriptional control of cholesterol homeostasis and provided genetic evidence for the regulated proteolysis model of SREBP function.

Type 2 mutants of the sterol-resistant class (Table II) include the 25-RA cell line (isolated in T. Y. Chang’s laboratory) and three other CHO cell lines (SRD-4, SRD-8, and SRD-9; isolated in Dallas), all of which produce a dominantly acting mutant form of SCAP (9). Remarkably, although they were derived independently, three of the four cell lines of this class harbor the same mutation, namely, the substitution of an asparagine at position 443 for an aspartate (D443N) in SCAP (53, 54). The fourth Type 2 cell mutant contains a Y298C substitution in SCAP (15). As discussed above, SCAP plays a central role in the feedback regulation of cholesterol homeostasis via its sterol-regulated escort function in transporting SREBPs from the ER to the Golgi. Both the D443N and the Y298C mutations in SCAP lie within the sterol-sensing domain of the protein (5) and render SCAP/SREBP transport resistant to sterol-mediated inhibition.

The Type 2 mutations of the sterol-resistant class enabled the discovery of SCAP by virtue of the fact that the mutant SCAP has a dominant effect, i.e., it produces sterol resistance even in the presence of a wild-type SCAP allele. This sterol resistance could be transferred to wild-type cells using pooled cDNAs derived from a Type 2 mutant cell line. Once the mutant SCAP cDNA was identified, its wild-type version was cloned. Their cDNA sequences were identical except for a single basepair difference, which produced a single amino acid substitution, as mentioned above. When this mutation was produced artificially in a wild-type cDNA, the cDNA conferred sterol resistance when transfected into wild-type cells. These studies revealed the existence of SCAP for the first time, and they pointed to SCAP as the target for sterol-mediated regulation. The fact that SCAP is absolutely required in SREBP processing was established by the subsequent isolation of a mutant CHO cell line that was auxotrophic for cholesterol, owing to a deficiency of SCAP (56) (discussed below).

The other class of CHO cell mutants, the recessive cholesterol auxotrophs, has also taught us much about SREBP processing (Table II). Chang and colleagues (57, 58) originally isolated several cell lines with this phenotype, one of which is designated M19. These investigators showed that M19 cells manifest three phenotypic abnormalities: (1) failure to induce cholesterol biosynthetic enzymes (such as HMG CoA synthase and HMG CoA reductase) upon sterol deprivation, thus explaining their cholesterol auxotrophy; (2) failure to induce LDL receptors, indicating a global failure to induce sterol-repressed genes upon sterol deprivation;
and (3) requirement for unsaturated fatty acids, in addition to cholesterol, for growth. All three of these defects were subsequently traced to a single abnormality: failure of the S2P to release the NH2-terminal fragment of SREBPs from cell membranes (8). Through complementation of this mutation, we were able to isolate a cDNA encoding S2P. A detailed chronology of these studies is given below.

In 1996 our laboratory showed that two independently derived cholesterol auxotrophic CHO cell lines, the M19 cells of Hasan and Chang (58) and our own SRD-6 cells (59), exhibited normal cleavage of SREBPs at Site-1 following sterol deprivation. However, cleavage at Site-2 did not occur, and the NH2-terminal fragments of SREBPs accumulated in a membrane-bound intermediate form (60). Hasan and Chang (58) had shown earlier that the defect in M19 cells is recessive and that it could be corrected by transfecion of human genomic DNA. Through repeated transfection of genomic DNA from one corrected M19 cell done to another, we were eventually able to purify the human gene encoding S2P (8). The wild-type S2P gene was shown to encode a Zn2+ metallocproteinase that is embedded in the membrane. Remarkably, genes encoding similar proteins are found in species as evolutionarily divergent from mammals as Archaea (8, 61). The defect in Site-2 proteolysis and the consequent failure of SREBPs to enter the nucleus explain the failure of M19 cells to produce cholesterol and LDL receptors.

When we and others used the amphotericin strategy to select for CHO cells auxotrophic for cholesterol, only mutations in the S2P gene were recovered (57, 59, 62). The explanation for this result became clear through genomic analysis of parental CHO cells. For unknown reasons, these cells harbor only a single functional copy of the gene encoding S2P (8). The preponderance of S2P mutations could be understood in light of the far greater likelihood of mutating a single copy compared to the chance of mutating two functional copies.

Encouraged by the success with the M19 cells, we next attempted to isolate mutants with defects in S1P in hope of using these cells to isolate an S1P cDNA. To avoid recovering an excess of S2P mutations, we prepared a line of wild-type CHO cells stably transfected with additional copies of the S2P gene (62). Assuming that these cells had two copies of the S1P gene and therefore required two independent mutations to inactivate both alleles, we employed a two-step selection process. The first step was designed to enrich for cells with defective activation of LDL receptor gene transcription. Using a fluorescence-activated cell sorter, we screened mutagenized cells that had been allowed to take up LDL containing a fluorescent dye. We reasoned that cells with a mutation in one allele of S1P might have a partial deficiency of nuclear SREBPs and therefore a partial deficiency of LDL receptors. The fluorescence-activated cell sorter permitted isolation of cells with a partial, but not necessarily complete, deficiency of LDL receptors. The population of cells that expressed low LDL receptors was then subjected to selection with amphotericin B, which selects for more complete loss of SREBP function. This approach yielded multiple mutants auxotrophic for cholesterol, and analysis of their SREBP processing indicated defects in Site-1 cleavage (56, 62).

With these mutant cells, we used a combined transient expression/complementation cloning strategy to isolate a cDNA encoding S1P. The cells were transfected with a plasmid construct encoding a fusion protein comprising placental alkaline phosphatase (PLAP) flanked by cleavage sites for signal peptidase and S1P, followed by the second membrane-spanning segment and the COOH-terminus of SREBP-2. In transfected wild-type cells deprived of sterols, cleavage by signal peptidase and S1P leads to secretion of PLAP into the culture medium. In the transfected mutant cells, no secretion of PLAP was observed, owing to a failure to cleave the S1P site. By cotransfecting this construct with pools of cDNA from wild-typehamster cells, we were able to identify a cDNA that restored the secretion of PLAP (63). This cDNA turned out to encode S1P.

In addition to multiple independent auxotrophic cell lines with defects in each copy of the gene encoding S1P, the experiment described above also yielded cells lacking functional SCAP. These SCAP-deficient cells showed the same profound defects in proteolytic processing of SREBPs as did the S1P-deficient cells. Figure 4 shows an immunoblot analysis of cell fractions from the S1P− and SCAP− mutants. Both mutants show a complete absence of cleavage of SREBP-1 and SREBP-2. As shown in the membrane fraction, the S1P− mutants lack S1P protein, and the SCAP− mutants lack SCAP protein (Fig. 4, left). As shown in the nuclear extract fraction, both mutants fail to cleave both SREBP-1 and SREBP-2 (Fig. 4, right).

CONCLUSION AND PERSPECTIVES

The experiments described in this review, from our laboratory and others, reveal the power of the somatic cell genetic approach for the study of regulated lipid metabolism in mammalian cells. These studies have disclosed a variety of genes and proteins that are crucial for ensuring the homeostasis of cell membranes (Table I). The studies in the lipid system appear to be the most successful use of mammalian somatic cell genetics with the possible exception of the studies revealing the pathway for the processing of carbohydrate chains of glycoproteins (64–66). In both systems the key has been the design of stringent selection protocols that permit the isolation of very rare mutants and
revertants (on the order of 1 in $10^8$ to $10^9$ cells for simultaneous hits in both alleles of the same locus).

With the recent advances in the complete sequencing of mammalian genomes, somatic cell genetic techniques should become even more useful in elucidating complex metabolic pathways.

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