mTORC1 activates SREBP-2 by suppressing cholesterol trafficking to lysosomes in mammalian cells

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mTORC1 is known to activate sterol regulatory element-binding proteins (SREBPs) including SREBP-2, a master regulator of cholesterol synthesis. Through incompletely understood mechanisms, activated mTORC1 triggers translocation of SREBP-2, an endoplasmic reticulum (ER) resident protein, to the Golgi where SREBP-2 is cleaved to translocate to the nucleus and activate gene expression for cholesterol synthesis. Low ER cholesterol is a well-established trigger for SREBP-2 activation. We thus investigated whether mTORC1 activates SREBP-2 by reducing cholesterol delivery to the ER. We report here that mTORC1 activation is accompanied by low ER cholesterol and an increase of SREBP-2 activation. Conversely, a decrease in mTORC1 activity coincides with a rise in ER cholesterol and a decrease in SERBP-2 activity. This rise in ER cholesterol is of lysosomal origin: blocking the exit of cholesterol from lysosomes by U18666A or NPC1 siRNA prevents ER cholesterol from increasing and, consequently, SREBP-2 is activated without mTORC1 activation. Furthermore, when mTORC1 activity is low, cholesterol is delivered to lysosomes through two membrane trafficking pathways: autophagy and rerouting of endosomes to lysosomes. Indeed, with dual blockade of both pathways by Atg5$^{-/-}$ and dominant-negative rab5, ER cholesterol fails to increase when mTORC1 activity is low, and SREBP-2 is activated. Conversely, overexpressing constitutively active Atg7, which forces autophagy and raises ER cholesterol even when mTORC1 activity is high, suppresses SREBP-2 activation. We conclude that mTORC1 actively suppresses autophagy and maintains endosomal recycling, thereby preventing endosomes and autophagosomes from reaching lysosomes. This results in a reduction of cholesterol in the ER and activation of SREBP-2.

mTORC1 | SREBP-2 | cholesterol | autophagy | endosomal recycling

It is well-established that mTORC1 functions as a nutrient/energy/stress sensor (1). Activated mTORC1 promotes cell growth by inducing anabolic processes while inhibiting catabolic events, such as autophagy (1). For instance, nutrient-rich conditions activate mTORC1 to increase adipogenesis (2), to up-regulate mitochondrial biogenesis in muscle (1), and to promote hepatic lipogenesis by activating sterol regulatory element-binding proteins (SREBPs) (3), the master transcriptional regulators of lipid and sterol biosynthesis. SREBPs consist of three isoforms: 1a, 1c, and 2. SREBP-1a and -2 are the predominant forms in cultured cells. They activate fatty acid and cholesterol synthesis, respectively. SREBP-1c is expressed primarily in the liver, where it is regulated by multiple signals related to nutrient and energy status. With obesity and overnutrition mTORC1 is hyperactivated, resulting in persistent activation of SREBP 1c in the liver (4). This leads to overproduction of lipids and hence hepatic steatosis and hypertriglyceridemia. Furthermore, constitutively activated mTORC1 greatly elevates de novo lipid synthesis (5). mTORC1 also activates SREBPs including SREBP-2 in cultured fibroblasts (6). In these cells, mTORC1 promotes SREBPs translocation from the endoplasmic reticulum (ER) to the Golgi, where SREBP is proteolytically cleaved to translocate to the nucleus and acts as a transcription factor to activate target gene expression (6). SREBP translocation, proteolytic processing, and nuclear entry is also under the control of ER cholesterol levels (7). It is unclear whether and how mTORC1 influences ER cholesterol to regulate SREBPs.

In addition to activating SREBPs, mTORC1 is known to play a key regulatory role in two membrane trafficking events. Low mTORC1 activity triggers autophagy (8) and also reroutes cholesterol-rich endosomes, which are normally recycled (9), to lysosomes (10, 11). From there, amino acids and membrane components, including cholesterol, can be released for reuse. Thus, mTORC1 could influence ER cholesterol through these membrane trafficking events to modulate SREBPs.

Here we present evidence that, when mTORC1 is inactive, autophagosomes and rerouted endosomes are delivered to lysosomes. This results in cholesterol-rich lysosomes and hence an increase in ER cholesterol, which suppresses SREBP-2. Conversely, high mTORC1 activity suppresses autophagy and promotes endosome recycling, so that less membrane cholesterol reaches lysosomes and ER cholesterol is lower. This activates SREBP-2.

Results

Inactivation of mTORC1 Promotes Cholesterol Trafficking to the ER Through Lysosomes. We first established that, in mouse embryonic fibroblasts (MEFs), mTORC1 activity, assessed by P-S6K1 and P-S6, is high in full growth medium with serum (Fig. 1*A*, lane 1) or in the presence of amino acids (control) (Fig. 1*A*, lane 3) but diminished in the presence of mTORC1 inhibitor Torin 1 (Fig. 1*A*,

Significance

Through unknown mechanisms mTORC1 triggers translocation of SREBP-2, an endoplasmic reticulum (ER) resident protein, to the Golgi to produce mature SREBP-2, which translocates to the nucleus to act as a transcription factor. Low ER cholesterol is a well-known inducer of SREBP-2 activation. We thus investigated whether mTORC1 activates SREBP-2 by reducing ER cholesterol levels. We report that, in cultured mammalian cells, an increase in mTORC1 activity is accompanied by a decrease in ER cholesterol and by SREBP-2 activation. Conversely, a decrease in mTORC1 activity coincides with higher ER cholesterol and lower SERBP-2 activity. We demonstrate that, by suppressing autophagy and by maintaining endosomal recycling, mTORC1 actively prevents membrane-derived cholesterol from reaching lysosomes, thereby reducing cholesterol ER and activating SREBP-2.

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The authors declare no conflict of interest.

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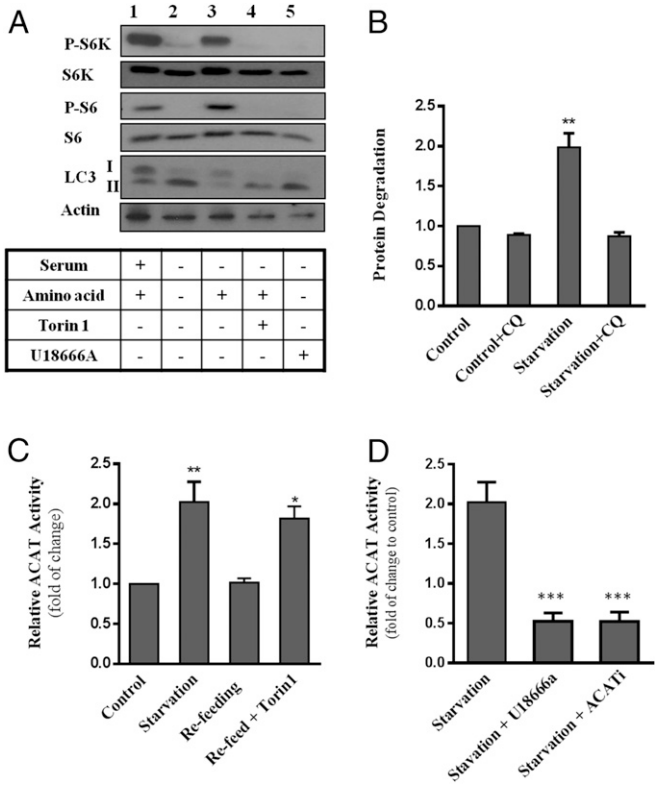

Fig. 1. mTORC1 regulates cholesterol trafficking from the lysosomes. (*A*) MEFs were incubated in serum-containing medium (control) for 4 h (lane 1) or medium without serum and amino acids (starvation) for 4 h (lane 2) or starvation medium plus 1 μM U18666A (lane 5). Some of cells were in starvation medium for 4 h and then switched to medium containing amino acids (refeeding) for 4 h, with or without Torin 1 (250 nM) (lanes 3 and 4). Cells were then lysed, subjected to SDS/PAGE and immunoblotted with indicated antibodies. Data are representative of at least three experiments. (*B*) MEFs were grown in normal serum medium containing [^{14}C]L-valine for 3 d and then shifted to fresh medium containing 0.1% BSA for 1 h. Cells were then subjected to 4 h incubation with control or starvation medium for 4 h, with or without chloroquine (30 μM). Medium was then collected and analyzed for TCA-soluble [^{14}C]L-valine as described in *Materials and Methods*. Results are expressed as fold increase of cellular protein degraded in 4 h in starvation medium, relative to that in control medium. Data are the average ± SEM of three independent experiments. (*C* and *D*) MEFs were subjected to control or starvation medium for 4 h or starvation 4 h followed by 4 h in refeed medium; [^{3}H]oleate was added to cells during the last 30 min to measure ACAT activity. Results are represented as fold increase of CE formation, relative to cells in control medium. Data are the average ± SEM of three independent experiments. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0001$.

lane 4). Furthermore, 4-h amino acid removal (starvation) lowered mTORC1 activity (Fig. 1*A*, lane 2), and triggered lysosomal protein degradation (Fig. 1*B*), a hallmark of autophagy (12, 13). Autophagy is known to deliver membrane-rich organelles and cell debris to lysosomes, which release amino acids and, inevitably, membrane lipids including cholesterol. Because lysosome-derived cholesterol is intimately linked to ER cholesterol (14), we next assessed ER cholesterol by whole-cell ACAT (acyl-CoA cholesterol acyltransferase) activity assay. ACAT is an ER resident enzyme that converts free cholesterol (hereafter referred to as cholesterol) to cholesteryl ester (CE). Its activity (i.e., the amount of CE formed) is generally governed by cholesterol availability in the ER membrane (15). We observed that ACAT activity was elevated by amino acid starvation (Fig. 1*C*), suggesting high levels of cholesterol in the ER. However, cells with activated mTORC1 [in amino acid-containing medium (control) or in medium resupplied with amino acids for another 4 h after starvation (refeed)] exhibit lower ACAT activity, indicative of low ER cholesterol. However, if mTORC1 activity is blocked by Torin1, ACAT activity is high even with refeeding (Fig. 1*C*). This suggests mTORC1 plays a direct role to lower ER cholesterol in refeeding.

The source of the higher ER cholesterol during starvation is likely the lysosomes: U18666A, which specifically inhibits the exit of cholesterol from lysosomes (16), blocked the increase of ACAT activity during starvation (Fig. 1*D*). U18666A at the dose used here (1 μM) had no effect on mTORC1 activity (Fig. 1*A*, lane 5). Indeed, we detected cholesterol accumulation in the lysosomes of cells in starvation medium and in the presence of U18666A (Fig. S1). As expected, specific inhibitor ACATi verified that ACAT is solely responsible for the CE formed during starvation (Fig. 1*D*). Additionally, the levels of cellular [^{3}H]oleate, which forms [^{3}H]CE with ER cholesterol in the ACAT assay, and ACAT activities in isolated ER membrane (microsome fraction) were identical among cells treated with different nutrient conditions or inhibitors (Fig. S2 *A* and *B*). Thus, the changes in whole-cell ACAT activity most likely reflect changes in cholesterol content in the ER membrane and this change in ER cholesterol is related to lysosomal cholesterol release.

Similar correlations between mTORC1 activity, protein degradation, and ACAT activity can be seen in HEK cells (Fig. S3 *A*–*C*), another mammalian cultured cell line responsive to nutrient conditions (6). Again, U18666A lowered ACAT activity during starvation, Torin 1 maintained high ACAT activity during refeeding, and $ACAT_i$ blocked CE formation during starvation (Fig. S3*C*).

We conclude that starvation not only increases lysosomal protein degradation but also triggers cholesterol release from lysosomes, leading to higher ER cholesterol levels.

mTORC1 Activates SREBP-2 by Inhibiting Cholesterol Trafficking from Lysosome to the ER. We next established that, in MEFs, this increase in ER cholesterol, during starvation or with mTORC1 inhibition, is of sufficient magnitude to affect SREBP-2. First, as seen by immunofluorescence staining, SREBP-2 is predominantly in the nucleus in normal growth medium or after amino acid refeeding (Fig. S4*A*, first and third columns), indicating activation. During starvation, however, SREBP-2 is excluded from the nucleus, consistent with its inactivated state (Fig. S4*A*, middle column). Second, the magnitude of the increase in ACAT activity during starvation is similar to that observed in cells treated with 10 μg/mL 25-hydroxy-cholesterol (25HC) (Fig. S4*B*); 25HC at this concentration indeed suppressed SREBP-2 activity (Fig. S4*C*).

Mature SREBP-2, produced by low ER cholesterol, is expected to enter the nucleus and bind sterol-responsive elements (SRE) in target promoters to initiate transcription of genes involved in cholesterol synthesis (7). We observed that transcriptional expression of HMG-CoA synthetase was low during starvation (Fig. 2 *A*, *i*, first bar), when mTORC1 activity is low (Fig. 1*A*, second lane) and ACAT activity (thus ER cholesterol) is high (Fig. 1*C*, second bar). Also, as shown in Fig. 1, Torin 1 and U18666A changed ER cholesterol. We reasoned that SREBP-2 transcriptional activity could change accordingly. Consistent with its inhibition of the rise in ER cholesterol, U18666A abrogated the suppression of HMG-CoA synthetase expression by starvation (Fig. 2 *A*, *i*, fourth bar). Adding back amino acids (refeeding) increased HMG-CoA synthetase expression (Fig. 2 *A*, *i*, second bar) in a manner dependent on mTORC1 activity, as Torin 1 blocked this effect (Fig. 2 *A*, *i*, third bar). The regulation of other SBEBP-2–dependent genes followed a similar pattern, including SREBP-2 itself (17) (Fig. 2 *A*, *ii*–*iv*) (primers are listed in Table S1).

Significantly, the expression of SREBP-2-targeted genes correlated positively with mTORC1 activity and negatively with ACAT activity (Fig. 2*B*) in HEK cells, in agreement with observations made in the MEFs.

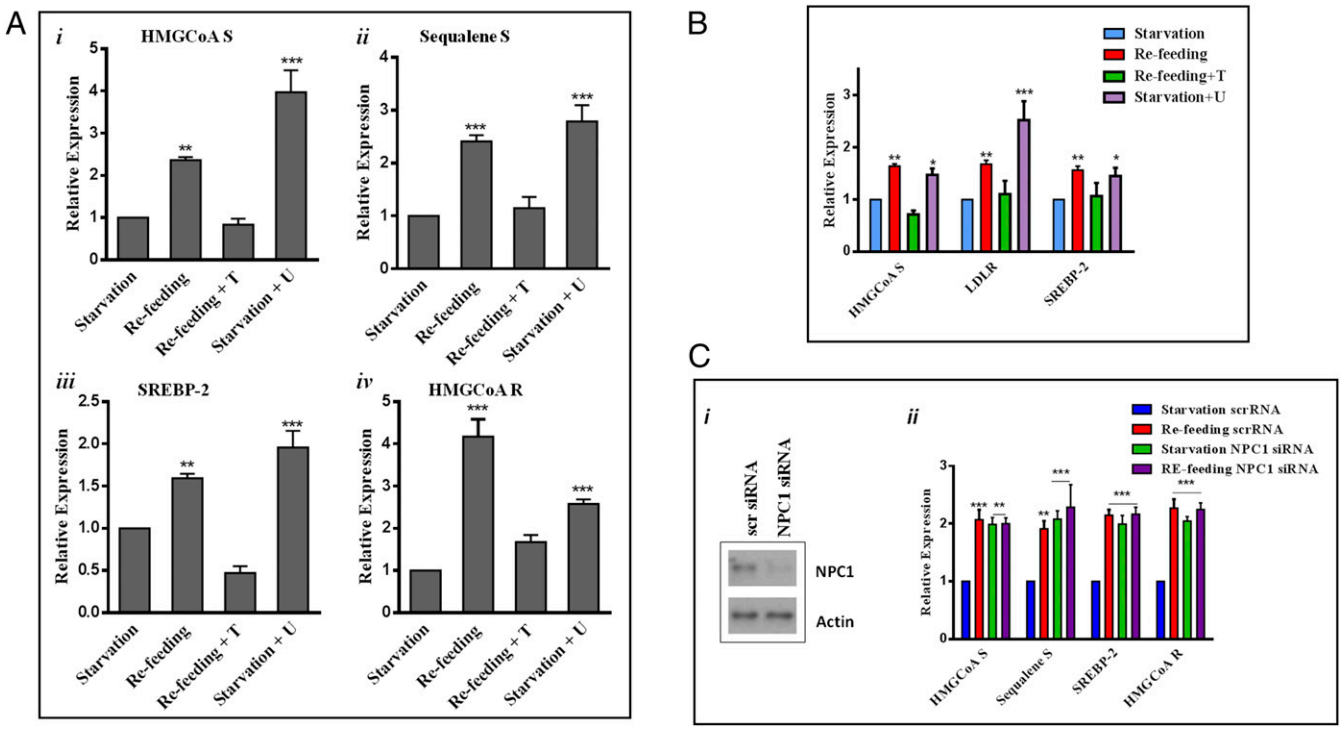

Fig. 2. mTORC1 regulates SREPB-2 transcriptional activity through cholesterol trafficking via lysosomes. (*A*) Relative expression of SREBP-2 target genes in MEF cells subjected to the conditions indicated. mRNA levels were determined by real-time qPCR and normalized to 18S mRNA. Data are presented as the fold increase in gene expression relative to cells in starvation medium and represent the average ± SEM of four independent experiments. (*B*) Relative expression of SREBP-2 genes in HEK293T cells (*i–iv*). Data are presented as the fold increases in transcription activity relative to cells in starvation medium and represent averages of four independent experiments with SEM. (*C*, *i*) Western blotting showing MEFs transfected with either scrambled or NPC1 siRNA. (*C*, *ii*) Gene expression, assessed by RT-PCR, in cells transfected with scrambled or NPC1 siRNA. Results show fold increases of transcriptional activity relative to starvation. Data are the average ± SEM from four independent experiments.*$P < 0.05$, **$P < 0.005$, ***$P < 0.0001$.

Also, we observed effects similar to those of U18666A on SREBP-2 target gene expression by silencing NPC1 (Fig. 2 *C*, *i*), a protein necessary for cholesterol exit from lysosomes and the pharmacological target of U18666A (18, 19). Because lysosomes cannot release cholesterol without NPC1, starvation no longer suppressed SREBP-2 targeted genes (Fig. 2 *C*, *ii*, green bars): Their expression levels were comparable to those of refed cells (Fig. 2 *C*, *ii*, purple bars). This again supports the notion that lysosomal-derived cholesterol is crucial for the suppression of SREBP-2 activity to lower the expression of its target genes, when mTORC1 activity is low.

Taking these results together, we conclude that activation of mTORC1 coincides with low ER cholesterol, indicated by ACAT activity, and with activation of SREBP-2. Conversely, suppression of mTORC1, by starvation or pharmacological inhibition, is accompanied by high ER cholesterol, due to increased cholesterol release from lysosomes. This suppresses the transcription of SREBP-2 target genes.

mTORC1 Regulates Autophagy and Endosomal Recycling to Govern Cholesterol Supply to the Lysosomes, Thereby Regulating SREBP-2. Lysosomes are primarily digestive organelles that degrade macromolecules and cell debris delivered to them via various mechanisms. The products, such as amino acid and cholesterol, are then released for reuse. Given that mTORC1 controls cholesterol availability to ER through lysosomes, mTORC1 may regulate the delivery of cholesterol-containing material to lysosomes. We thus searched for potential sources of cholesterol that can reach lysosomes when mTORC1 activity is low.

We first considered lipid droplets, a rich source of CE. CE would have to be hydrolyzed by lipases in the lysosomes to produce cholesterol. However, we found that lalistat 1, a lysosomal lipase inhibitor (20), had a limited impact on nutrient-dependent changes in ER cholesterol (Fig. S5*A*). This suggested that cholesterol-containing membranes were more likely the source of cholesterol. mTORC1 is known to suppress autophagy (8). In the absence of mTORC1 activity, autophagy sends membrane-rich autophagosomes, a potential source of cholesterol, to lysosomes. Hence, we investigated MEFs lacking Atg5 ($Atg5^{-/-}$). Although defective in LC3-defined autophagy (Fig. 3*A*), $Atg5^{-/-}$ MEFs responded to starvation normally with an increase in ER cholesterol (Fig. 3*B*) and in protein degradation (Fig. 3*C*), agreeing with a previous report (21). However, there was no alteration of SREBP-2 target gene expression patterns in $Atg5^{-/-}$ MEFs, relative to that in WT MEFs (discussed below). We then considered that other entities besides autophagosomes might also deliver cholesterol-rich membranes to lysosomes during starvation when mTORC1 activity is low.

Endosomes are derived from cholesterol-rich plasma membrane. In normal proliferating cultured cells, such as fibroblasts, endosomes deliver nutrients (LDL, iron, etc.) to lysosomes while efficiently recycling to the plasma membrane (22). However, we recently discovered a significant rerouting of endosomes to lysosomes when mTORC1 activity is low (10) (also see Fig. S5*B*, WT MEF). This suggests that mTORC1 may actively maintain endosomal recycling and thus divert endosomes away from lysosomes, in addition to suppressing autophagy. When mTORC1 is low, endosomes are delivered to lysosomes, even when autophagosomes were blocked by Atg5 knockout (Fig. S5*B*, $Atg5^{-/-}$). This could lead to high ER cholesterol and suppression of SREBP-2.

To test this possibility, we expressed dominant-negative (DN) rab5 in $Atg5^{-/-}$ MEFs. DN rab5 is known to block endocytosis

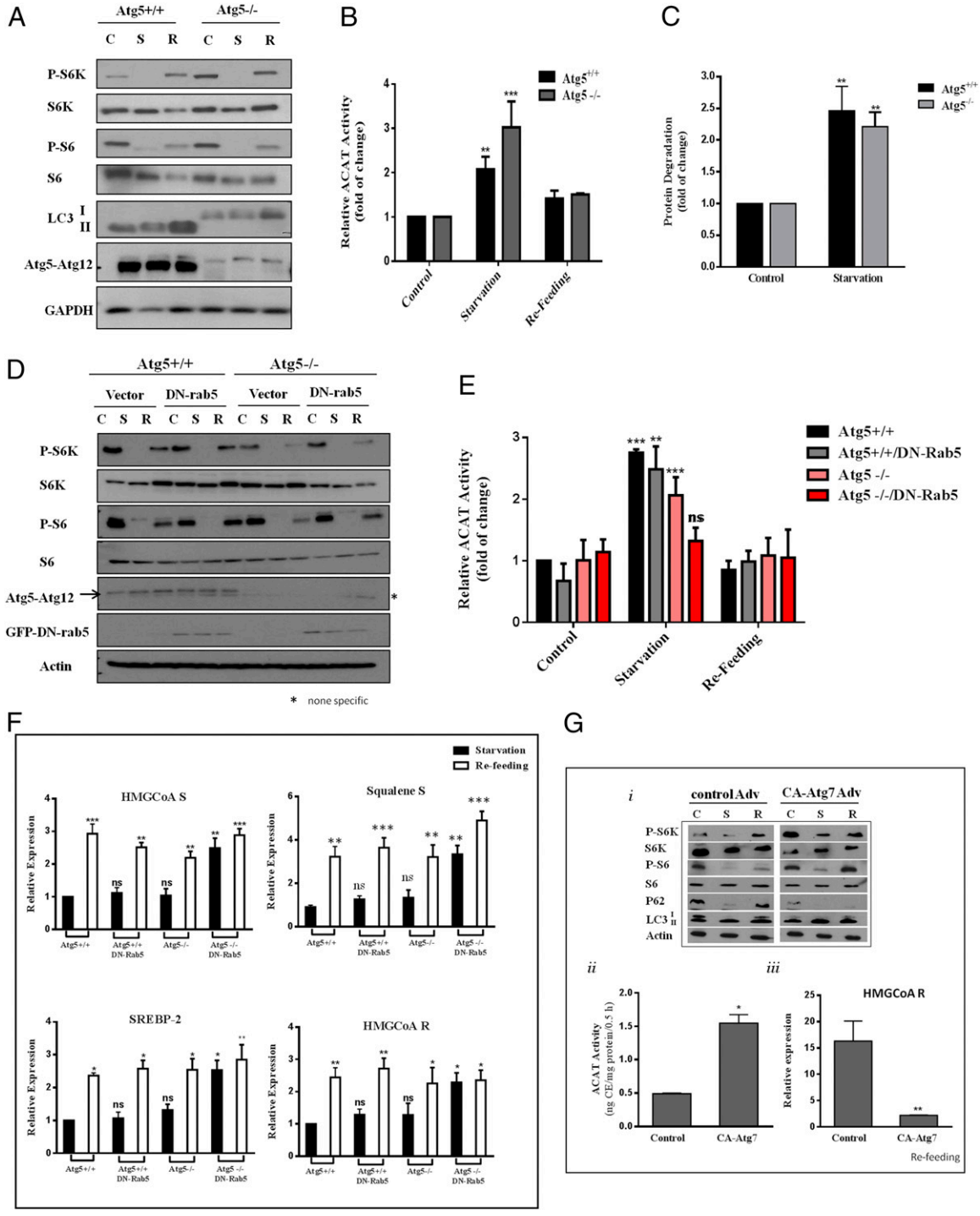

Fig. 3. Combination of autophagy and rerouted endosomal trafficking contributes to cholesterol trafficking from the lysosome to the ER to suppress SREBP-2. (*A*) Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs were incubated in control (C), starvation (S), and starvation/refeeding (R) medium as in Fig. 1*A*. Cells were then lysed, subjected to SDS/PAGE, and Western-blotted with indicated antibodies. (*B*) ACAT activity was measured in Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs. Results represent the fold increase of CE formation in relative to cells in control medium. Data are the average ± SEM of three independent experiments. (*C*) Protein degradation in Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs. Data represent the average ± SEM of three independent experiments. (*D*) Atg5 Tet-off–inducible MEFs were grown in growth medium for 4 d in the presence or absence of doxycycline (10 ng/mL). Some cells were transfected with either a control vector or DN rab5 DNA on day 2. Cells were then subjected to starvation or starvation/refeed as in Fig. 1*A*; cells were lysed and protein was analyzed by SDS/PAGE followed by Western blotting with indicated antibodies. (*E*) ACAT activity in Atg5 Tet-off–inducible MEFs with or without DN rab5 transfection. Data are the average ± SEM of three independent experiments. (*F*) Relative expression of SREBP-2 target genes in Atg5 Tet-off–inducible MEFs with or without DN rab5 transfection. Result shows fold increase of gene expression in cells after refeeding relative to cells in starvation. Results are the average ± SEM of four independent experiments. (*G*, *i*) Western analysis of WT MEFs transfected with CA-Atg7 or control adenovirus for 1 d then grown for 3 d. (*G, ii*) ACAT activity of cells transfected with CA-Atg7 or control adenovirus. Data represent the cells in the refeeding condition. (*G*, *iii*) HMGCoA R mRNA expression in MEFs transfected with CA-Atg7 or control adenovirus. Data are the average ± SEM of three independent experiments. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0001$. ns, not significant.

and as well as the fusion of early endosomes (23). DN rab5 or Atg5$^{-/-}$ or in combination had little effect on mTORC1 activity upon nutrient conditions (Fig. 3*D*). The rise of ER cholesterol during starvation is minimally affected by blocking either pathway alone (Atg5$^{-/-}$ or DN rab5, Fig. 3*E*, gray and pink bars). However, when both pathways are blocked simultaneously (DN rab5-expressing Atg5$^{-/-}$ MEFs), ER cholesterol failed to rise during starvation (Fig. 3*E*, red bars). This correlated with high SREBP-2 activity during starvation (Fig. 3*F*, Atg5$^{-/-}$ + DN rab5).

Similar results were observed with HEK cells, expressing DN Atg5 or DN rab5 or both (Fig. S6*A*). Only dual blockade of both pathways prevented ER cholesterol) from increasing (Fig. S6*B*, red bars) and, as a result, SREBP-2-dependent gene expression was high regardless of nutrient conditions (Fig. S6*C*).

Furthermore, we overexpressed constitutive-active (CA) Atg7 in MEFs (Fig. S6*D*). This forces autophagy even when mTORC1 activity is high, as reported previously (24). Little p62 remained in CA Atg7-expressing cells, even in refeeding medium (Fig. 3 *G*, *i*). Indeed, CA Atg7 raised ER cholesterol in refed cells (Fig. 3 *G*, *ii*), compared with control virus-infected cells. This correlated with suppression of HMG-CoA reductase gene expression (Fig. 3 *G*, *iii*).

Overall, our data support the notion that, when mTORC1 is low, lysosomes obtain cholesterol from Atg5-related autophagy and from rerouted endosomes. Lysosomal exit of the cholesterol to the ER raises ER cholesterol and suppresses SREBP-2 activity. When both endocytosis and autophagy are blocked, as in DN rab5-expressing Atg5$^{-/-}$ MEFs, SREBP-2 activity is no longer suppressed, even though mTORC1 is inactivated by starvation. Conversely, with forced autophagy, ER cholesterol is high and SREBP-2 activity is suppressed, even when mTORC1 is high.

Discussion

The present study demonstrates that mTORC1 plays a significant role in regulating cholesterol trafficking to lysosomes. mTORC1 is usually activated in proliferating cells and also some cancer cells (25). High mTORC1 activity (Fig. 4*A*) has two effects on membrane trafficking: (*i*) suppressing autophagy and (*ii*) maintaining endosomal recycling to the plasma membrane. The net effect is that membrane organelles and cell debris do not reach lysosomes. Little cholesterol is then available to be released from lysosomes to the ER. This activates SREBP-2 translocation, processing, and eventual transcriptional regulation. However, low mTORC1 activity (Fig. 4*B*) (*i*) triggers autophagy that directs cholesterol, as part of cell membrane debris, to lysosomes and (*ii*) redirects cholesterol-rich endosomes to lysosomes. Lysosomes then become enriched in cholesterol, which leads to the rise of the ER cholesterol levels and suppression SREBP-2. In the cell types we used here, lipid droplets do not contribute to this process. However, these organelles could play a role in lipid-rich cells, such as hepatocytes or adipocytes (24). It remains to be seen whether the regulatory pathways we have observed in cultured cell models occurs in vivo.

SREBP-1a and -2 are the predominant isoforms in cultured cells and they activate fatty acid and cholesterol synthesis, respectively. SREBP-1c is expressed primarily in the liver, where it regulated by multiple signals related to nutrient and energy status. For example, insulin activates liver SREBP-1c through mTORC1 (26). Our objective here was to determine whether the regulation of cholesterol trafficking by mTORC1 would affect the functions of SREBP-2. We concentrated our studies on genes in the cholesterol biosynthesis pathway under the regulation of SREBP-2. We present a mechanism for the interaction between mTORC1 activity and cholesterol trafficking, which governs the expression of genes normally targeted by SREBP-2. Within this particular context, our data indicate mTORC1 modulates autophagy and endosomal recycling to regulate SREBP-2 activity. We note that FAS, a typical SREBP-1a–controlled gene, displayed responses similar to those of SREBP-2 targeted genes. More in-depth studies, however, will be necessary to determine whether SREBP-1a activity is similarly regulated.

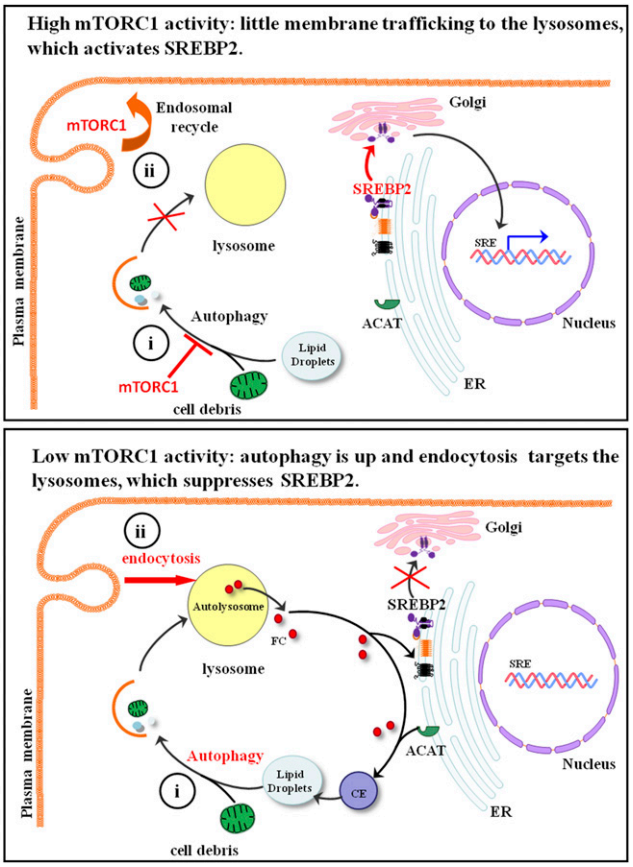

Fig. 4. Model of mTORC1/autophagy/endocytosis dependent regulation of SREBP-2 transcriptional activities during (*A*) high mTORC1 activity (growth condition) and (*B*) low mTORC1 activity (starvation condition).

It was reported that mTORC1 prevents the nuclear entry of lipin-1 (6). In the absence of mTORC1, nuclear translocated lipin-1 promotes degradation of mature SREBP proteins, potentially by an autophagy-related process (6). Less-mature SREBP then reduces target gene expression. Our study here emphasizes the role of mTORC1 in the regulation of SREBP-2 activity by assessing target gene expression, which is a consequence of the translocation and proteolytic cleavage of SREBP-2 and its entry to the nucleus. Lipin-1 may represent an additional layer of regulation over SREBP transcriptional activity. When both autophagy and endocytosis are blocked, SREBP-2 activity remains high even without active mTORC1 (implicating lipin-1 nuclear localization). This is consistent with the idea that lipin-1 is not capable of degrading mature SREBP-2 in the nucleus in the absence of autophagy, as suggested previously (6). Future studies will be required to understand the role of lipin-1 in these situations.

It should be noted that we do not directly measure ER cholesterol in live cells here. ER cholesterol concentration is inferred by ACAT activity instead. ER membranes only have 5% cholesterol. This makes them sensitive to cholesterol changes in the cells but also difficult to directly measure (27). Nevertheless, cholesterol accumulation in the lysosomes of starved cells (filipin staining) supports the subsequent rise of cholesterol in the ER, as indicated by whole-cell ACAT assay.

The current study does not define the exact route by which cholesterol moves from the lysosomes to the ER. However, it is accepted that cholesterol is mostly in equilibrium among different cellular pools in live cells. Any "newly" appeared cholesterol is

rapidly incorporated into many types of cellular membranes by both vesicular and nonvesicular means, including vesicular transport, carrier proteins, or membrane–membrane contact sites (28). Interestingly, an elegant study recently found that cholesterol released from the lysosomes appears exclusively in the plasma membrane, probed by a cholesterol-bind domain 4 of anthrolysin O (ALOD4) (29). This could imply the existence of a specific cholesterol-transport mechanism from the lysosomes to the plasma membrane. Alternatively, ALOD4 bound on the plasma membrane may have created a powerful sink for newly released cholesterol, thereby preventing it from reaching other cellular membranes. Regardless, this study refreshingly reminds us of the delicate balance of cholesterol levels among different membrane pools: As little as 1% trapping of cholesterol by ALOD4 on the plasma membrane can lower ER cholesterol and activate major cellular pathways such as SREBP. It also reflects cells' capacity to rapidly rebalance cholesterol among cellular pools after perturbation, which inevitably includes ER membrane and regulates related pathways. Our study is perhaps such an example: by altering the membrane trafficking through the lysosomes, cholesterol redistributes and regulates SREBP-2.

In summary, we present evidence that mTORC1, through its regulation of autophagy and endosomal membrane trafficking, directs the delivery of cholesterol to lysosomes and thereby modulates ER cholesterol. This process controls SREBP-2 activation and transcriptional activity.

Materials and Methods

WT, Atg5$^{-/-}$, and Atg5 (Tet-Off) MEFs were generously provided by N. Mizushima, University of Tokyo, Tokyo. TSC1/2$^{-/-}$ MEFs were provided by K. L. Guan, University of California, San Diego, La Jolla, CA and HEK cells (HEK293T) by J. Bell, University of Ottawa, Ottawa. All cell lines were grown and maintained in DMEM (Thermo Fisher Scientific) supplemented with 1% antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin; Life Technologies) and 10% FBS (Wisent) at 37 °C in a 5% CO_2 incubator. Amino acid starvation was performed using RPMI 1640 modified without L-glutamine, without amino acids, and without glucose (USBiological) supplemented with 25 mM glucose and 1% penicillin/streptomycin. Refeeding was performed using regular RPMI1640 medium (Life Technologies).

Additional information on materials and methods can be found in *SI Materials and Methods*.

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