How sterols regulate protein sorting and traffic

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Students of cell biology and physiology are in for a treat with the discoveries, reported in this issue of PNAS by Radhakrishnan *et al.* (1) and Sun *et al.* (2), that cholesterol and an enigmatic but potent derivative, 25-OH cholesterol, exert their effect on gene expression by changing the conformation of two membrane protein sensors that guide a transcription factor precursor into the machinery responsible for vesicular traffic from the endoplasmic reticulum (ER). Over the past dozen years or so, the prolific team of Michael Brown and Joseph Goldstein has elucidated a control pathway in which the production of a transcription factor responsible for the expression of HMG-CoA reductase, a key branch point enzyme in the biosynthesis of cholesterol, among other targets, is controlled by the cholesterol-regulated proteolytic maturation of a transcription factor precursor, sterol regulatory element-binding protein (SREBP) (3). In cells grown with excess exogenous cholesterol, SREBP is synthesized as a membrane protein precursor housed in the ER. On cholesterol starvation, SREBP precursor is mobilized and transported via a vesicle carrier to the Golgi apparatus, where two site-specific proteases act to liberate the N-terminal cytosolic domain of SREBP. This domain constitutes a transcription factor that is directed to the nucleus, where it engages genes controlled by the sterol regulatory element (SRE) promoter sequence.

Sterol Sensors

The key questions have been: how do cholesterol and 25-OH cholesterol cause SREBP precursor to be retained in the ER, and what happens to liberate SREBP when sterol levels decline? Control molecules that govern this sorting decision have been teased apart by an elegant combination of genetics and biochemistry. Two ER membrane proteins, Scap and Insig, appear to be the key regulators. Scap is a multispanning protein, of which a portion comprising transmembrane (TM) helices 2–6 was shown previously to bind rather specifically to cholesterol, but not, for example, to 25-OH cholesterol (4). Although the sterol sensing domain of Scap does not bind 25-OH cholesterol, the two sterols work independently to tether Scap to Insig in a manner that is reversed by sterol deprivation. Contact with SREBP

is mediated by a C-terminal cytosolic domain of Scap, but this interaction appears to be independent of sterol. Thus, the crucial regulatory step is control of lateral contact between Insig and the complex of Scap-SREBP.

The mystery of 25-OH cholesterol is resolved by Radhakrishnan *et al.* (1). Using a special detergent, Fos-choline 13, which they also used to purify a functional form of the sterol-binding domain of Scap, the group purified a recombinant form of one of two mammalian Insigs and showed that it binds 25-OH cholesterol but not cholesterol. Thus, Scap and Insig together cover the range of sterol molecules known to control HMG-CoA gene expression in mammalian cells. Extensive mutagenesis of the predicted TM helices of Insig identified two helices containing residues that influence 25-OH cholesterol binding and/or contact with Scap bound to cholesterol. Unfortunately, stoichiometry measurements suggest that only a small fraction of the purified Insig is active in sterol binding, in which case the defective mutant proteins may be substantially altered.

Sterols and Protein Sorting in the Endoplasmic Reticulum

How does sterol influence the access of Scap-SREBP to the vesicular traffic machinery? Cargo capture and protein sorting are intimately coupled to the polymerization of the COPII coat, which is responsible for all anterograde traffic of membrane and secretory proteins from the ER (5). This process begins when Sar-1, a small GTP-binding protein, is recruited to the ER membrane through a transient interaction with Sec12, an ER resident guanine nucleotide exchange factor. Activated Sar-1-GTP extends an amphipathic N terminus to anchor in the ER membrane and then recruits Sec23/24, a heterodimer that contains a built-in GTPase activating protein as part of the Sec23 subunit. The activated complex samples ER membrane proteins by collision to discriminate cargo from ER resident proteins. Cargo bound to the activated complex is then clustered by the assembly of a scaffold complex comprising a heterotetramer of Sec13/31. The key sorting event in transport of membrane proteins from the ER involves the recognition of a sorting signal on the cargo molecule by the Sec24 subunit. Most cargo proteins that display

such a sorting signal are recognized by the Sec24 subunit during an early stage in the polymerization of COPII on the ER surface. However, in the case of SREBP and a few other proteins whose ER exit is regulated, this contact with Scap-SREBP must somehow be spatially controlled by the ligand-bound form of the cargo protein.

In previous work, the Brown and Goldstein laboratory established a role for COPII proteins in the sterol-deprivation-dependent packaging of Scap-SREBP into transport vesicles by using a cell-free vesicle budding reaction that recapitulates the authentic sorting reaction (6, 7). Further work identified the sorting signal, MELADL, located near TM domain 6 of the largest cytosolically exposed loop of Scap (8). Mutation of amino acid residues within this signal retards the traffic of SREBP independent of sterol levels in the cells. Sun *et al.* (2) probe the contact between loop 6 of Scap and a complex of COPII proteins, Sec23/24, in cells and membranes harboring wild-type or mutant sensor proteins (2). Epitope-tagged Sec23 binds to Scap in membranes from sterol-deprived cells in a reaction that is blocked by an antibody directed to a peptide containing the MELADL signal. This same antibody blocks the sterol-deprivation-dependent traffic of GFP-tagged Scap from the ER to the Golgi complex in living cells.

It makes perfect sense that the sorting machinery would interact with the sorting signal under conditions where Scap and SREBP must be transported from the ER, but how exposed is the MELADL sequence under conditions where Scap-SREBP is retained in the ER, i.e., in the presence of sterol? Several additional experiments show that the signal is not sequestered by Insig. Anti-MELADL antibody binds to and coprecipitates Scap and Insig from detergent-solubilized membrane isolated from cells loaded with 25-OH cholesterol. Thus, at least the epitope associated with the sorting signal is accessible even when Scap is unavailable for sorting. However, a more subtle conforma-

Author contributions: R.S. wrote the paper.

The author declares no conflict of interest.

See companion articles on pages 6511 and 6519.

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tional change in Scap is suggested by the effect of sterol on the covalent labeling of select cysteine residues in the exposed loop 6 domain. Cholesterol or 25-OH cholesterol treatment causes a change in the exposure of a cysteine residue inserted between the MELADL sorting signal and the preceding TM domain 6 of Scap. This change depends on the presence of Insig, although the magnitude of Insig-dependence is most pronounced in samples taken from cells exposed to 25-OH cholesterol. This subtle conformational change is confirmed by an Insig- and sterol-dependent alteration in the protease accessibility of an arginine residue located on the other side of loop 6, closer to TM domain 7.

The alteration produced by sterol and the exact positioning of the MELADL signal in relation to TM domain 6 of Scap has a profound effect on the interaction of Scap with the Sec23/24 complex and traffic of SREBP to the Golgi complex. Even small insertions or deletions positioned between MELADL and TM6 abolish binding to COPII and transport. Thus, we now have the closest look yet at exactly how sterol binding or withdrawal from the sensor proteins influences the placement of a traffic sorting signal in contact with a COPII subunit responsible for cargo protein packaging into a transport vesicle.

Applications and Other Regulated Sorting Events

The subtle conformation change in loop 6 of Scap caused by cholesterol in the binding pocket of this sensor, or 25-OH cholesterol in the binding pocket of Insig, must somehow be registered on COPII, most likely on a surface of Sec24 in close apposition to the ER membrane. Genetic and structural experiments with yeast COPII proteins have shown that Sec24 contains at least three distinct binding sites for separate cohorts of cargo molecules (9, 10). How-

ever, a formal possibility remains that the Scap sorting signal is decoded by Sar-1 or Sec23. COPII polymerizes on the surface of synthetic lipid vesicles under conditions where the basic concave face of the basic Sec23/24 heterodimer makes intimate contact with acidic phospholipids (11–13). The known cargo binding pockets are displayed along the lateral surface of the Sec24 subunit and not on the concave face that is in direct contact with the

Small insertions or deletions positioned between MELADL and transmembrane 6 abolish binding to COPII and transport.

ER surface. The MELADL signal probably makes contact at one of these or possibly a new site along this lateral surface.

In mammals, four paralogs of Sec24, two paralogs of Sec23, and two paralogs of Sar-1 may expand the repertoire of cargo selection at the ER by creating many different combinations of these core COPII subunits. Such cargo discrimination has been documented for two of the three Sec24 proteins in yeast (14). Two human diseases, Anderson's disease, which blocks chylomicron secretion in enterocytes of the absorptive epithelium (15), and craniofacial dysplasia, which affects craniofacial bone morphogenesis (16), display defects in one paralog of Sar-1 and one paralog of Sec23, respectively.

Identification of the COPII subunit that makes contact with the MELADL

signal and where exactly this binding pocket is located remains for future work. The definition of this binding site, through genetic or structural analysis, could provide an opportunity for drug design. A small molecule inhibitor of this interaction may be useful as a drug that reduces the expression of the set of genes controlled by cholesterol. Drug design may be challenging if this binding pocket is shared with other cargo proteins.

Regulated traffic from the ER has been reported in a few other instances, but among these, the best understood is the transport of another transcription factor precursor, ATF6 (17). ATF6 is made as a membrane-bound precursor in the ER that is matured in the Golgi complex by the same proteases responsible for processing of SREBP. Unlike SREBP, ATF6 senses a stress signal that triggers the unfolded protein response (UPR). Indeed, the active transcription factor produced by maturation of the ATF6 precursor is responsible for the expression of genes that reinforce the UPR in mammalian cells. Recombinant mapping experiments have identified two signals on the C-terminal luminal domain of ATF6 precursor (18). One signal is required for traffic of the precursor from the ER in the presence of an inducing agent (e.g., a reducing agent, DTT, or a reagent that discharges Ca^{2+} from the ER, thapsigargin), and another signal required for ER retention recruits BiP, an ER luminal chaperone that facilitates protein folding, to the C-terminal domain. It appears that the displacement of BiP initiates the regulated transport of ATF6. Presumably, some other protein in contact with the luminal sorting signal is activated to mobilize ATF6 exposing a cytoplasmic signal, possibly on ATF6, to the COPII transport machinery. ATF6 thus represents an interesting alternative example with unique features that may not be shared with the sterol-regulated process.

1. Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL (2007) *Proc Natl Acad Sci USA* 104:6511–6518.
2. Sun L-P, Seemann J, Goldstein JL, Brown MS (2007) *Proc Natl Acad Sci USA* 104:6519–6526.
3. Goldstein JL, DeBose-Boyd RA, Brown MS (2006) *Cell* 124:35–46.
4. Radhakrishnan A, Sun L-P, Kwon HJ, Brown MS, Goldstein JL (2004) *Mol Cell* 15:259–268.
5. Lee MCS, Miller EA, Goldberg J, Orci L, Schekman R (2004) *Annu Rev Cell Dev Biol* 20:87–123.
6. Nohturfft A, Yabe D, Goldstein JL, Brown MS, Espenshade PJ (2000) *Cell* 102:315–323.
7. Espenshade PJ, Li W-P, Yabe D (2002) *Proc Natl Acad Sci USA* 99:11694–11699.
8. Sun L-P, Li L, Goldstein JL, Brown MS (2005) *J Biol Chem* 280:26483–26490.
9. Mossessova E, Bickford LC, Goldberg J (2003) *Cell* 114:483–495.
10. Miller E, Beilharz TH, Malkus PN, Lee MCS, Hamamoto S, Orci L, Schekman R (2003) *Cell* 114:497–509.
11. Bi X, Coprina RA, Goldberg J (2002) *Nature* 419:217–277.
12. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T (1998) *Cell* 93:263–275.
13. Matsuoka K, Schekman R, Orci L, Heuser JE (2001) *Proc Natl Acad Sci USA* 98:13705–13709.
14. Shimoni Y, Kurihara T, Ravazzola M, Amherdt M, Orci L, Schekman R (2000) *J Cell Biol* 151:973–984.
15. Jones B, Jones EL, Bonney SA, Patel HN, Mensenkamp AR, Eichenbaum-Voline S, Rudling M, Myrdal U, Annesi G, Naik S, et al. (2003) *Nat Genet* 34:29–31.
16. Boyadjiev SA, Fromme JC, Ben J, Chong SS, Nauta C, Hur DJ, Zhang G, Hamamoto S, Schekman R, Ravazzola M, et al. (2006) *Nat Genet* 38:1192–1197.
17. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL (2002) *Mol Cell* 6:1355–1364.
18. Shen J, Chen X, Hendershot L, Prywes R (2002) *Dev Cell* 3:99–111.