Squalene monoxygenase (SM), which synthesizes a cholesterol precursor, is degraded when cholesterol levels in the endoplasmic reticulum (ER) membrane are high, but the signal for degradation was not known. In this issue of JBC, Brown and co-workers identify an N-terminal domain in SM that interconverts in a cholesterol-sensitive manner between a membrane-binding amphipathic helix and a soluble degradation-prone segment, providing the first example of a cholesterol-degron collaboration.

Sterols such as cholesterol play critical roles in maintaining membrane fluidity and organization in eukaryotic cells and are precursors of steroid hormones, vitamin D, and bile acids. Cholesterol is essential for cell viability, but concentrations must be maintained within an appropriate range to avoid toxicity and disease. Cells have a number of ways to maintain the cholesterol balance (1): When the level of cholesterol is too high, it can be pumped out of cells or transported to intracellular storage depots called lipid droplets. The enzymes required for cholesterol production and uptake can also be regulated at the transcriptional or protein level. Central to all these responses, however, is the ability to sense intracellular cholesterol levels. A number of cholesterol-sensing mechanisms have been identified, including the ability of a 100-amino acid sequence in the enzyme squalene monoxygenase to regulate protein levels in a cholesterol-sensitive manner (2). However, the molecular details of this process were not clear. Brown and co-workers (3) have now discovered that a 12-amino acid sequence mediates this mechanism, defining a new way by which cells sense cellular cholesterol levels.

Proteins that control cellular cholesterol metabolism sense cholesterol levels in the endoplasmic reticulum (ER) membrane, which is also the site of cholesterol synthesis. This may seem surprising because the ER membrane is one of the most cholesterol-poor membranes in cells; most free cholesterol is in the plasma membrane and late secretory compartments. However, cholesterol continuously cycles between these cholesterol-rich membranes and the ER (4) and small changes in plasma membrane cholesterol levels can significantly alter ER membrane cholesterol levels (5).

Mechanisms of sensing cholesterol can be divided into two broad categories. Some proteins have motifs that directly bind cholesterol or oxidized cholesterol derivatives called oxysterols. For example, a number of integral membrane proteins have a conserved sterol-sensing domain (SSD) located in the membrane that probably directly binds cholesterol and other sterols. One of the best-known SSD-containing proteins, SCAP, plays a central role in sensing cholesterol levels in the ER and regulating cholesterol synthesis and uptake (6).

Some cholesterol-sensing proteins like squalene monoxygenase (SM) do not seem to directly bind cholesterol in membranes but instead respond to changes in the physical properties of membranes brought about by altered cholesterol levels. SM converts the alkene squalene into squalene epoxide as one of the rate-limiting steps in cholesterol synthesis, making it an ideal point in the pathway for feedback control. Brown and co-workers previously found that when cellular cholesterol levels are high, SM is degraded by the ubiquitin-proteasome system, decreasing the rate of cholesterol production (2). The N-terminal 100 amino acids of SM mediate this cholesterol-dependent degradation, undergoing a conformational change when cholesterol levels are high (7). However, the molecular details of this conformational change and the mechanism by which it promotes degradation were not clear.

To explore these questions, Brown and co-workers (7) now use alanine-scanning mutagenesis to investigate the N-terminal domain further. It was known that the domain does not contain a transmembrane region but does have a portion that is buried in the membrane, termed a re-entrant loop. The authors now show that, in addition to the re-entrant loop, a 12-residue sequence from Gln62-Leu73 is required for cholesterol-dependent degradation of SM (3). A variety of biophysical techniques were used to demonstrate that this region forms an amphipathic helix when it binds membranes. This is a common feature of many amphipathic helices, which are generally unfolded when not membrane-associated and only form a stable helix when they bind a bilayer. Importantly, the portion of SM that forms an amphipathic helix only binds membranes with low cholesterol; addition of cholesterol reduces the affinity of the helix for the membrane, with release leading to unraveling of the helix to reveal a disordered sequence that targets SM for degradation (Fig. 1).

How does the amphipathic helical region of SM sense ER membrane cholesterol? It seems unlikely that it specifically binds cholesterol because it has only a few hydrophobic residues and, like most amphipathic helices, probably does not penetrate deeply into the membrane. Indeed, the authors...
showed that hydrophobic regions from other proteins with no homology to SM were able to replace the amphipathic helix domain in SM, suggesting that membrane binding is sufficient to stabilize SM (2). The hydrophobic surface of amphipathic helices can interact with the interior of the bilayer (8), suggesting that changes to the structural properties of the membrane due to increasing cholesterol levels could disfavor the binding of amphipathic helices. Specifically, it has been suggested that amphipathic helices with affinity for the ER membrane tend to bind bilayers with lipid packing defects (9), which increase the exposure of the hydrophobic core in the bilayer to the aqueous phase. Determining how changes in cholesterol levels in the ER membrane alter its physical properties is therefore an important task for understanding this new mechanism of cholesterol sensing. It will also be interesting to determine whether proteins in addition to SM use similar mechanisms of sensing changes in membrane cholesterol levels.

The N-terminal 100 residues of SM, which contain the cholesterol-sensitive amphipathic helix and the re-entrant loop, form a degron, a domain that regulates protein degradation. Another new finding of the work from Brown and co-workers (10) is that this region is similar to the well-characterized degron in Deg1, part of the yeast transcription factor MATα2. As a result, the findings regarding SM may have wider implications for proteins unconnected to cholesterol synthesis. Indeed, the amphipathic helix-forming portion of the Deg1 degron is not known to respond to cholesterol levels in membranes but may unfold in response to changes in membrane packing, suggesting that similar degrons could regulate protein stability in response to changes in membrane properties and lipid composition. It will be interesting to see how membrane regulation of degrons unfolds in future research.

Figure 1. Model of cholesterol-dependent membrane binding by the amphipathic helix of SM. A 12-residue sequence near the N terminus of SM forms an amphipathic helix that binds the ER membrane when cholesterol levels are low, possibly mediated by lipid-packing defects in the membrane. Increasing cholesterol may restore lipid packing, disrupting the helix-membrane interaction; once away from the structure-inducing environment, the protein sequence becomes unstructured, causing SM to be degraded by the ubiquitin-proteasome system.

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