# Structure of Transcription Factor SREBP-2 and Importin-β Complex

Many proteins including histones, gene regulatory proteins, and RNA-processing proteins are imported into the nucleus from the cytosol. Nuclear import receptors bind to these proteins to be transported and enter the nucleus from the cytosol through the nuclear pore. Each protein family is transferred by a receptor protein that is specialized for the transport of a group of proteins, which have a similar fold.

A sterol regulatory element-binding protein (SREBP), a nuclear transcription factor, is a gene regulatory protein. SREBPs synthesized on ribosome particles are located on the endoplasmic reticulum (ER) membrane (Fig. 1). When cells are deprived of cholesterol, a membrane protein, designated the SREBP cleavage-activating protein (SCAP), is accumulated in the ER membrane to interact with SREBPs. SCAP escorts SREBPs to a post-ER component to reach the Golgi apparatus, which contains various proteases. Site-1 protease (S1P) catalyzes the first cut in the luminal loop, followed by a second cleavage at site 2 within the first membrane-spanning segment. This proteolytic process liberates a transcriptionally active N-terminal fragment of 480 residues from the membrane, designated the active form of a SREBP. The active form of a SREBP enters the nucleus and activates the transcription of a number of genes that control the synthesis and uptake of cholesterol (2). Thus, the transcriptional regulation of cholesterol metabolism is largely dependent on the nuclear transport of the active form of a SREBP. It was recently reported that SREBP-2 enters the nucleus through the direct interaction with importin-β via the helix-loop-helix-leucine-zipper (HLHZ) domain of SREBP [1,2] (Fig. 2). We have determined the structure of importin-β complexed with the active form of SREBP-2 [3].

The crystal belongs to the orthorhombic space group of $P2_12_12_1$ with unit cell dimensions of a = 101.0 (9), b =113.2 (8), c = 240.0 (4) Å. Diffraction experiments were carried out at beamline **BL44XU**. Then the SAD phasing method utilizing the anomalous scattering signal of Se atoms from the SeMet-substituted SREBP-2 crystal was applied. Phases were improved by solvent flattening and histogram matching using the DM program. However, the electron density map did not have sufficient quality for model building, particularly around the 100 residues of the N-terminus. Successive Fourier refinements showed a clear electron density that corresponds to the SREBP-2 dimer and the SREBP-2. Bulk solvent correction and anisotropic B-factor correction were applied during the course of the refinements. After each refinement cycle, the structural model was manually revised using the simulated annealed omit map. The final R- and free R-factors were 23.7% and 29.6%, respectively, in the resolution range of 20 ~ 3.0 Å (F > 0). The r.m.s. deviations for bond length and bond angle from the ideal values are 0.008 Å and 1.427 degrees, respectively. No residues are in the disallowed region in the Ramachandran plot.

This is the first structure in which the HLHZ domain in complex with a protein, but not DNA, is visualized and in which

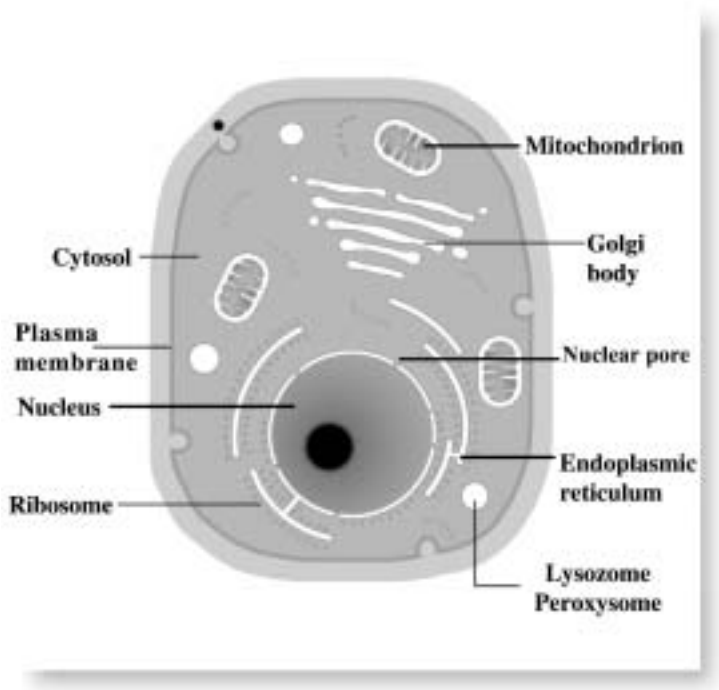


Fig. 1. Schematic drawing of an animal cell.

importin-β is observed to bind to the dimeric form of proteins (Fig. 3). Importin-β utilizes characteristic long helices, the significance of which are not understood, in a manner analogous to a pair of chopsticks to pick up SREBP-2. Importin-β alters its conformation, showing a pseudo two-fold symmetry on its surface structure, to accommodate a symmetric dimer molecule. This structure provides a model for dimeric cargo recognition by importin-β, and suggests that transcriptional regulation by SREBPs including proteolysis, nuclear import and SRE recognition is efficiently and accurately performed without the dissociation/reassociation reactions of SREBP dimers.

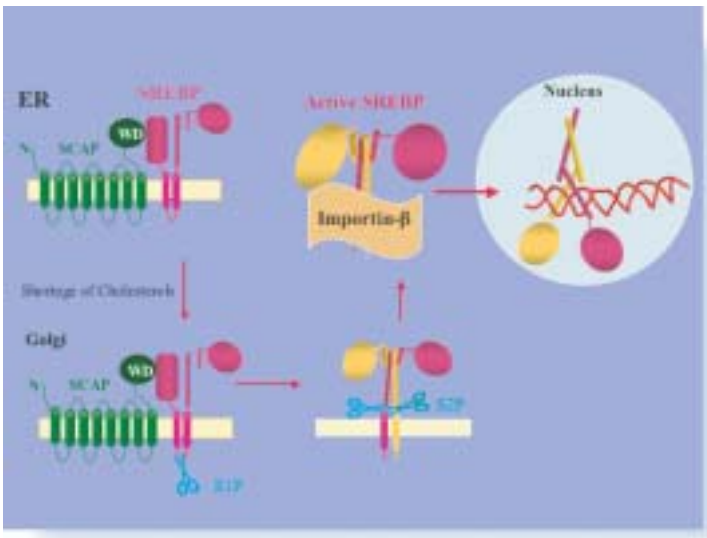


Fig. 2. Nuclear import of SREBP from ER.

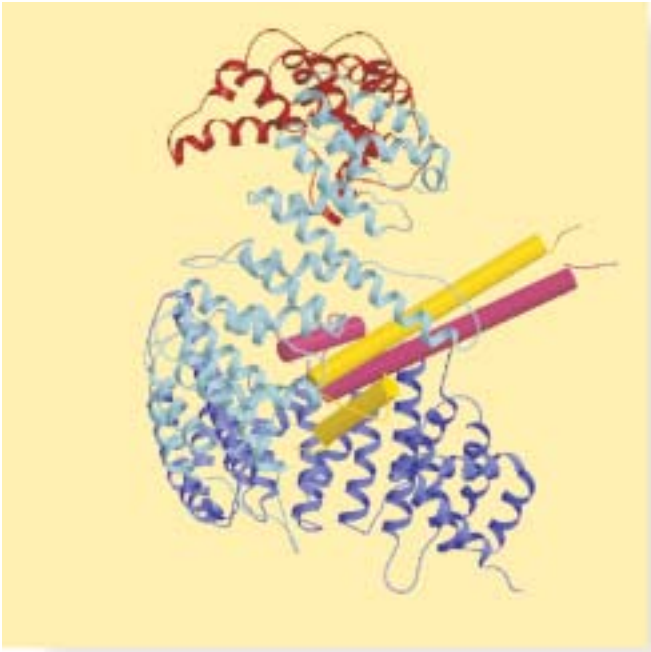

Fig. 3. Structure of importin-β complexed with the HLHZ domain of SREBP-2. Importin-β and SREBP-2 are depicted in ribbons and sticks, respectively.

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**References**

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