Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	020
Title	Activity and Topology Determination of *In vitro* Expressed Membrane Proteins
Research Unit	Cell-Free Protein Product
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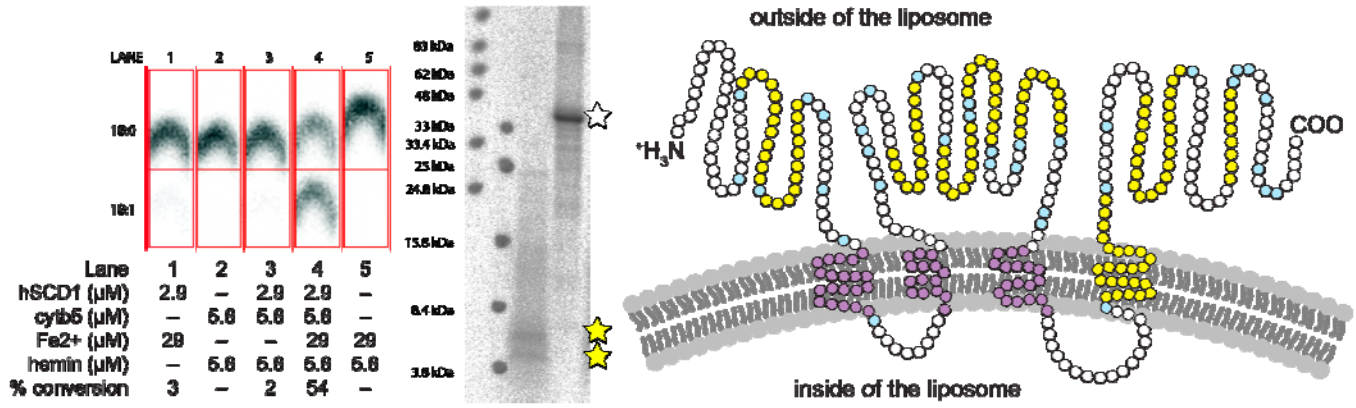

Summary

We report reconstitution of a three-component membrane protein complex using wheat germ cell-free technology [1]. Proteoliposomes containing the peripheral membrane protein cytochrome b_5 (CytB5) and the integral membrane protein human stearoyl-CoA desaturase isoform 1 (hSCD1) were produced in a cell-free expression reaction supplemented with 100 nm unilamelar liposomes derived from soy bean total lipid extracts. Purification of hSCD1/CytB5 proteoliposomes was accomplished by single step gradient floatation. Prior to initiation of stearoyl-CoA desaturation by addition of CytB5 reductase, NADH and stearoyl-CoA, the hSCD1 diiron and CytB5 heme cofactors were reconstituted by incubation with Fe^{2+} and hemin chloride. Conversion of stearoyl-CoA (18:0) to oleoyl-CoA (18:1) was monitored by the addition of a [U-^{14}C]-stearoyl-CoA tracer, resolved by thin-layer chromatography, and visualized on a phospho-imager (Figure A).

Investigation of the topology of hSCD1 proteoliposomes produced *in vitro* is done in collaboration with Professor Yaeta Endo (Ehime University, Matsuyama, Japan) and Professor Lloyd Smith (University of Wisconsin-Madison). Initial identification of hSCD1 topology was performed by protease protection of [^{14}C]-leucine labeled protein. Floated proteoliposomes with (Fig. B, lane 3, yellow stars) and without (lane 4, white star) proteinase K pretreatment were visualized by autoradiography. Two fragments were protected from proteolysis, in agreement with the predicted topology containing ~5.5 and 6.5 kDa internal fragments. A more detailed analysis of topology was accomplished using an HPLC-ESI-MS/MS. Purified hSCD1 proteoliposomes were digested by trypsin during an 18-h time-course and loaded directly for analysis. As visualized in Figure C, 8 diagnostic fragments (yellow spheres) were observed, bracketed by trypsin cut sites (cyan spheres). Seven of these fragments were cytoplasmic peptides, while one, which was detected only at the final time point, was a predicted transmembrane domain. These data strongly support the utility of wheat germ cell-free technology to produce active and correctly folded integral membrane proteins.

Publication:

[1] Goren M.A. and Fox B.G. Wheat germ cell-free translation, purification, and assembly of a functional human stearoyl-CoA desaturase complex. *Prot Exp Purif* 62(2):171-8.

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