Expanded View Figures

Figure EV1. Sequence alignments of yeast Lac1 and Lag1, and human CerS family members.

Secondary structural elements of yeast Lac1 are labeled above the sequence alignment. CH: cytosolic helix; TM: transmembrane helix; LH: luminal helix. The NTD is colored wheat. The TLC domain is colored marine with the Lag1p motif shown in yellow. The four red triangles indicate the predicted catalytic histidine and aspartate residues in the Lag1p motif. The Hox domains and acyl-CoA selective regions in human CerS are highlighted with wheat and dark pink boxes, respectively. The DxRSDxE dimerization motif is highlighted with a green box. The UniProt IDs for the aligned sequences are as follows: yLac1: P28496; yLag1: P38703; hCerS1: P27544; hCerS2: Q96G23; hCerS3: Q8IU89; hCerS4: Q9HA82; hCerS5: Q8N5B7; and hCerS6: Q6ZMG9. "y" for Saccharomyces cerevisiae (yeast) and "h" for Homo sapiens (human).

yLac1 MSTIKPSPSNNNLKVRSRPRRKSSIGKIDLGDTVPSLGTMFETKESKTAAKRRMQRLSEATKNDSDLVKKIWFSFREISY 80 yLag1 MTSATDKSIDRLVVNAKTRRNSSVGKIDLGDTVPGFAAMPESAASKNEAKKRMKALTGDSKKDSDLLWKVWFSYREMNY 80 hCerS1
hCerS2
hCerS5
yLac1 RHAWIAPLMILIAVYSAYFTSGNTTKTNVLHRFVAVSYQIGDTNAYGKGINDLCFVFYYMIFFTFLREFLMDVVIRPFAI 160 yLag1 RHSWLTPFFILVCVYSAYFLSGNTTKTNVLHRFVAISYQVDGTDSYAKGIKDLSFVFFYMIFFTFLREFLMDVVIRPFTV 160 hCerS1 AGPTGPEPMPSYAQLVQRGWGSALAAARGCTDCGWGLARRGLAEHAHLAPPELLLLALGALGWTALRSAATARLFRPLAK 87 hCerS2MLQTLYDYFWWERLWLPVNLTWADLED.RDGRVYAK.ASDLYITLPLALLFLIVRYFFLYVATPLAA 66 hCerS3MFWTFKEWFWLERFWLPPTIKWSDLED.HDGLVFVK.PSHLYVTIPYAFLLLIIRRVFEKFVASPLAK 66 hCerS4MFWTFKEWFWLERFWLPPTIKWSDLED.RDGRVYPH.PQDLLAALPLALVLLAMRLAFERFIGLPLSR 66 hCerS5MATAAQGPLSLLWGWLWSERFWLPPNYTWTELED.RDGRVYPH.PQDLLAALPLALVLLAMRLAFERFIGLPLSR 66 hCerS6MATAAQGPLSLLWGWLWSERFWLPENVSWADLEGPADGYGYPR.GRHILSVFPLAAGIFFVRLLFERFIAKPCAL 74 hCerS6MAGILAWFWNERFWLPHNYTWADLKN.TEATFPQ.AEDLYLAFFLAGIFFVRLFFFVRKPCAI 65
yLac1 RIHVTSKHRIKRIMEQMYAIFYTGVS 186 yLag1 yLNVTSKHRIKRIMEQMYAIFYTGVS 186 hCerS1 RCcLQPRDAAKMPESAWKFLFYLGS 112 hCerS2 LINIKEKTRLRAPPNATLEHFYLTSGKQPKQVEVELLSRQSGLSGRQVERWFRRRNQDRPSLIKKFREASWRFTFYLIA 146 hCerS3 SFGIKETVR.KVTPNTVLENFFKHSTRQPLQTDIYGLAKKCNLTERQVERWFRSRRNQERPSRLKKFQEACWRFAFYLMI 145 hCerS4 WIGVRQQTRRQVKPNATLEKHFLTEGHRPKEPQLSLLAAQCGLTLQQTQRWFRRRNQDRPQLTKKFCEASWRFIFYL 3 146 hCerS5 CIGIEDSGPYQAQPNATLEKVFISITKYPDKKRLEGLSKQLDWNVKKIQCWFRHRRNQDKPPTLTKFCESMWRFIFYL 3 146 hCerS6 ALNIQANGPQIAPPNAILEKVFTAITKHPDEKRLEGLSKQLDWDVRSIQRWFRQRRNQEKPSTITRFCESMWRFSFYL 70 154
Hox domain Hox domain TM4 TM5
yLac1 GPFGIYCMYHSD.LWFFNTKAMYRTY.PDFTNPFLFKVFYLGQAAFWAQQACILVLQLEKPRKDHNELTFHHIVTLLLIW 264 yLag1 GPFGLYIMYHSD.LWLFKTKPMYRTY.PVITNPFLFKIFYLGQAAFWAQQACVLVLQLEKPRKDYKEUVFHHIVTLLLIW 264 hCerS1 WSYSAYLLFGTDYPFFHDPPSVFYDWTPGMAVPRDIAAAYLLQGSFYGH.SIYATLYMDTWRKDSVVMLLHHVVTLILIV 191 hCerS2 FIAGMAVIVD.KPWFYDMKKVWEGY.PIQSTIPSQYWYYMIELSFYW.SLLFSIASDVKRKDFKEQIIHHVATIILIS 221 hCerS3 TVAGIAFLYD.KPWLYDLWEVWNGY.PKQPLLPSQYWYYILEMSFYW.SLLFRLGFDVKRKDFKEQVIHHVATIILIS 221 hCerS4 FVGGLSVLYH.SSNLWAPVMCWDRY.PNQTLKPSLYWWYLLELGFYL.SLLFRLGFDVKRKDFKEQVIHHFVAVILMT 221 hCerS6 FTYGVRFLKK.TPWLWNTRHCWYNY.PYQPLTTDLHYYYILELSFYW.SLMFSQFTDIKRKDFLMFVHLVTIGLIS 229
(TM6)TM7
yLac1 SYVFHFTKMGLPIYITMDVSDFLLSFSKTLNYLDSGLAFFSFAIFVVAWIYLRHYT.NLKILWSVLTQF 333 yLag1 SSYVFHFTKMGLAIYITMDVSDFFLSLSKTLNYLNSVFTPFVFGLSVFWIYLRHVV.NIRILWSVLTQF 333 hCerS1 SSYAFRYHNVGILVIFLHDISDVQLEFTKLNIYFKSRGGSYHRLHALAADLGCLSFGFSWFWFRLYWFPLKVLYATSHCS 271 hCerS2 FSWFANYIRAGTLIMALHDSSDYLLESAKMFNYAGWKNTCNNIFIVFAIVFIITRLVILPFWILHCTLVYP 292 hCerS3 FSWCANYIRSGTLVMIVHDVADIWLESAKMFSYAGWTQTCNTLFFIFSTIFFISRLTVFPFWILYCTLILP 291 hCerS4 FSYSANLLRIGSLVLLLHDSDYLLEACKMVNYMQYQQVCDALFLIFSFVFFYTRLVLFPTQILYTTYYES 292 hCerS5 FSYINNMVRVGTLIMCLHDVSDFLLEACKMVNYMKYQRLCDTLFVIFSAVFMVTRLGIYPFWILNTTLFES 300 hCerS6 FSYVNNMARVGTLIMCLHDVSDFLLEAAKLANYAKYQRLCDTLFVIFSAVFMVTRLGIYPFWILNTTLFES 291
[]
yLac1 RTEGNYVLNFATQQYKCWISLPIVFVLIGALQLVNLYWLFLIFRVLYRILW.RGILKD.DRSDSESDEESDESSTTPTDS 411 yLag1 RHEGNYVLNFATQQYKCWISLPIVFVLIAALQLVNLYWLFLILRILYRLIW.QGIQKD.ERSDSDSDSSESDEESDESSTTPTDS 411 hCerS1 LRTVPD
yLac1TPTKKDI418yLag1350hCerS1LRNGLVKDKRF350hCerS2AKSRPLANGHPILNNHRKND380hCerS3GKEMDCLKNGLRAERHLIPNGQHGH383hCerS4QLKNGAAGGPRPAPTDGPRSRVAGRLTNRHTTAT394hCerS5CDSSSSNGANRVNGHMGGSYWAEE392hCerS6PHTATTTNGTSGTNGYLLTGSCSMDD384

Figure EV1.

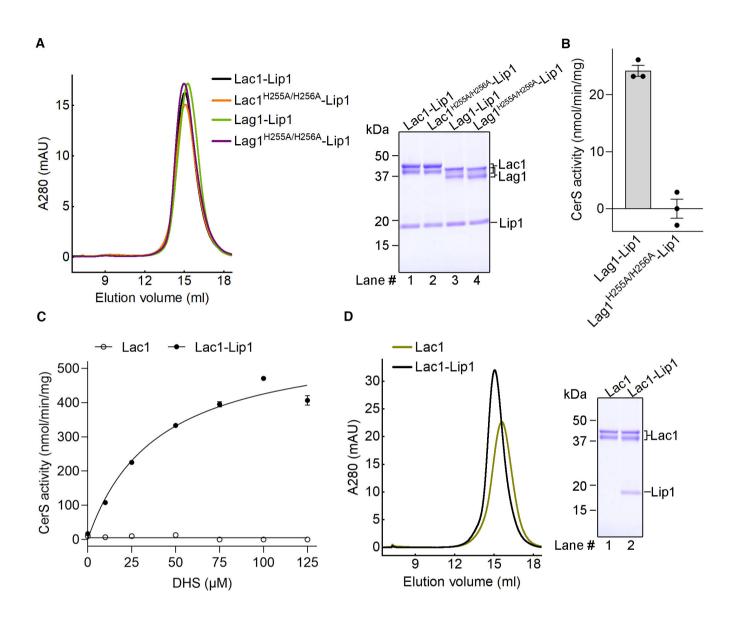


Figure EV2. Biochemical characterization of the Lac1-Lip1 and Lag1-Lip1 complexes.

- A Size exclusion chromatography (SEC) profiles and Coomassie blue-stained SDS-PAGE gel of the purified Lac1-Lip1 and Lag1-Lip1 complexes.
 B Enzymatic activity of the WT Lag1-Lip1 complex and the catalytic mutant Lag1^{H255A/H256A}-Lip1. Each data point is the average ± SEM of three independent experiments.
- C CerS activity versus DHS concentration for the Lac1 alone protein and the Lac1-Lip1 complex. The activity curve of the Lac1-Lip1 complex follows a Michaelis-Menten equation with a K_m of 39.44 \pm 6.29 μ M for DHS and a V_{max} of 593.3 \pm 34.5 nmol/min/mg. Each data point is the average \pm SEM of three independent experiments.
- D SEC profile and Coomassie blue-stained SDS-PAGE gel of the Lac1 alone protein and the Lac1-Lip1 complex.

Figure EV3. Features of Lip1 dimer.

- A Overall structure of Lip1 dimer.
- В Lip1 dimer interface.
- SEC profiles and Coomassie blue-stained SDS-PAGE gel of the Lip1 cysteine mutants. C
- D Normalized CerS activity of the Lip1 cysteine mutants. Each data point is the average ± SEM of three independent experiments.
- E SEC profile and Coomassie blue-stained SDS-PAGE gel of the Lip1 dimer interface variant.
- Normalized CerS activity of the Lip1 dimer interface variant. Each data point is the average \pm SEM of three independent experiments. F

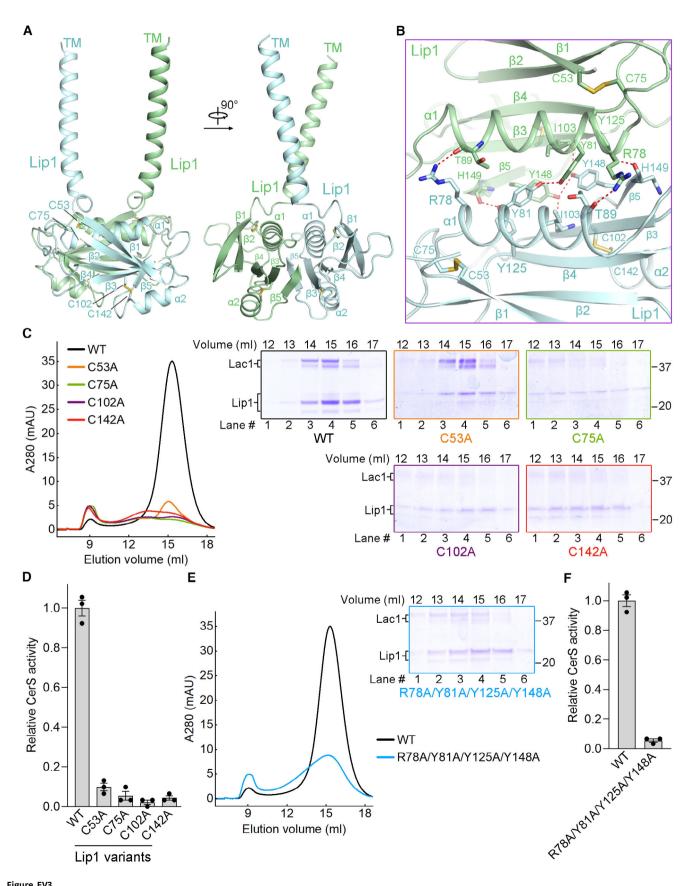


Figure EV3.

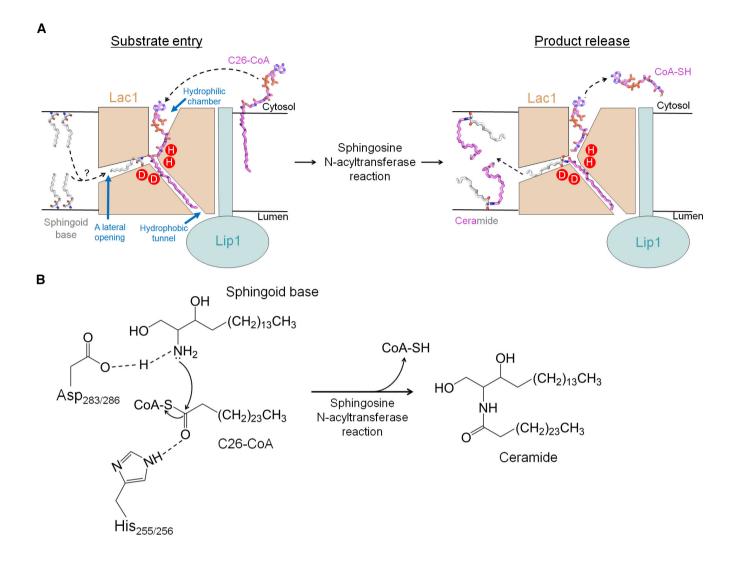


Figure EV4. Proposed working mechanism of yeast CerS.

- A A hypothetic model for yeast CerS-catalyzed ceramide formation. The C26-CoA substrate enters the reaction chamber within Lac1 probably through the cytosolic side of the membrane. The CoA moiety of C26-CoA is located in the hydrophilic reaction chamber, while the acyl-chain moiety is positioned in the hydrophobic tunnel. Once the sphingoid base substrate from either leaflet of the membrane enters a lateral opening on Lac1, it approaches the catalytic histidine and aspartate residues near the thioester bond of C26-CoA, preparing for the sphingosine N-acyltransferase reaction. After the reaction, the product of free CoA-SH is released to the cytosol, whereas the hydrophobic product ceramide is released to either leaflet of the membrane. For simplicity, only one Lac1-Lip1 heterodimer is illustrated.
- B Proposed catalytic mechanism. Asp283 or Asp286 of Lac1 functions as a general base to activate the amino group of the sphingoid base for nucleophilic attack on the carbonyl carbon of C26-CoA, which is coordinated by His255 or His256. The CerS-catalyzed acyl transfer reaction promotes the N-acylation of sphingoid base for ceramide production and releases free CoA-SH.

Figure EV5. Human CerS structural models predicted by AlphaFold.

- A The AlphaFold-predicted human CerS structures (hCerS1AF-hCerS6AF). The N-terminal regions and Hox domains of hCerSs are colored light gray. The TLC domains are colored green with the acyl-CoA selective regions highlighted in dark pink. The predicted catalytic histidine and aspartate residues in the Lag1p motifs are shown in sticks.
- B Structural superposition of the hCerS5AF (colored green) and the Lac1-Lip1 heterodimer (colored wheat and light cyan). The N-terminal region of hCerS5AF severely clashes with the TM of Lip1.

