Supplementary material

Plasmid constructions

LAC1, *LAG1*, and *LIP1* were cloned into pESC-URA vector (Stratagen, 2μ, *URA3*), which allows simultaneous expression of two-N-terminal-tagged proteins *(*c-*myc* and Flag epitopes). To construct pSTS30b (#1121), a 1.2 kbp fragment of *LAC1* without the start codon was amplified by PCR using the primers 5'-AGATCTCGACAATAAAGCCAAGCCCTTC- and 5'-TTAATTAATCAAATATCCTTTTTCGTTGGAGTAC. The fragment was cloned into pESC in frame with the Flag tag using BglII and PacI as restriction sites. *LAG1* was amplified with the primers 5'-CTCGAGATGACATCAGCTACGGACAAATC and 5'-GCTAGCTTATTCACACTTTTCCTTAGATTCTTC providing XhoI and NheI as restriction sites and the 1.2 kbp fragment was cloned in frame with the c-*myc* tag into pESC yielding pSTS30a (#1119) or into pSTS30b yielding pSTS30 (#1120). A Flag tagged version of Lag1p was created by amplifying its gene by the primers 5'-CGGCCGAGATCTACATGACATCAGCTACGGACAAATCTATCGAT and 5'-CGCGCGTTAATTAATTATTCACACTTTTCCTTAGATTCTTCATT and the fragment was cloned into pESC using BglII and PacI as restriction sites, yielding pSTS30c (#1548). *LIP1* was amplified from genomic DNA by the primers 5'-CGGAGATCTCATCTCAACCCACTCCCATCATAAC and 5'-CGGTTAATTAATCACATGTGATAAATTGTGG (restriction sites BglII and PacI) or 5'-CGGCTCGAGTCTCAACCCACTCCCATCATAAC and 5'-CGGGCTAGCTCACATGTGATAAATTGTGC (restriction sites XhoI and NheI) and the fragments were cloned into pSTS30a or pSTS30b, respectively, yielding plasmids # 1559 and #1560, respectively. A truncated version of *LIP1* was created by amplifying this gene by the primers 5'-CGCGCGAGATCTCAAAGCCAAAACCAAAAATT and 5'-CGGTTAATTAATCACATGTGATAAATTGTGG and the fragment was cloned into pESC using BglII and PacI as restriction sites, yielding pESC(Flag-ΔNT-LIP1), #1552. The corresponding protein misses the 14 first amino-acids. A tandem tag version (GST-Flag) of Lac1p was created by amplifying LAC1 with the primers 5'-GCGCTCTAGAAGATTACAAGGATGACGACGATAAG and 5'-CGCGCCCAAGCTTTCAAATATCCTTTTTCGTTGGAGTACT. The fragment was inserted into the vector pEG(KT) (2μ, *URA3*) using XbaI and HindIII as restriction sites.

(C26) Acyl-CoA synthesis

The protocol was adapted from Guillas *et al*. (Guillas et al., 2001). Briefly, 43.6 mg of C26 fatty acid resuspended in 4 ml of benzene were dried three times under a stream of nitrogen. 6 ml of dry benzene and 3 ml of oxalyl chloride were then added to the fatty acid residue and incubated for 90 minutes at room temperature with gentle agitation and under a flux of nitrogen. Liquid was evaporated by a stream of nitrogen and the residue was washed three times with 4 ml of dry benzene and dried with nitrogen. 100 mg of CoA lithium salt dissolved in 10 ml of tetrahydrofuran(THF)-aqueous 150 mM $NaHCO_3$ (2.2:1.0, adjusted to pH 8.8 with NaOH) were added to the acyl chloride and incubated for 2 hours at 37°C with vigorous agitation and under nitrogen. The reaction was stopped by adding 150 µl of 10% $HClO_4$. Most of the THF was evaporated with nitrogen. The residue was then washed by repeated resuspension and centrifugation (18,000 × g for 15 minutes at 4°C): twice with 18 ml of 1.3% $HClO_4$, once with 30 ml of acetone, twice with 25 ml of anhydrous ether and finally once with 25 ml of THF. The residue was dried under dry nitrogen, resuspended in 0.5 mM Zwittergent 3-16, and stored under N_2 at -20°C. The concentration of C26 fatty acyl- CoA was evaluated by absorbance at 260 nm (ε=16,800).