Supporting Information

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SI Experimental Procedures

Thin-layer Chromatography (TLC). For TLC assay of the deacylation of [^{3}H]octanoyl CoA, each 50-μl reaction contained 50 mM Hepes-NaOH (pH 7.0), 50 μg of membrane protein, 5 μg of recombinant proghrelin-His_8, and 1 μM [^{3}H]octanoyl CoA (11 dpm/fmol) in the absence or presence of 50 μM palmitoyl CoA. After incubation at 37°C for 5 min, each sample was chilled on ice, and 200 μM palmitoyl CoA was added to stop further deacylation of [^{3}H]octanoyl CoA. An aliquot of each sample (5 μl) was loaded onto a Polygram SIL G plate (Macherey-Nagel), and the plate was developed in a solvent system of chloroform/methanol/water at a ratio of 10/10/3 (vol/vol/vol). The plate (20 × 20 cm) was cut into eight slices from origin to front, and the radioactivity in each slice was quantified by liquid scintillation counting as described (1). Standards of [^{3}H]octanoyl CoA and [^{3}H]octanoate were included in parallel lanes to indicate their positions of migration (R_f values of 0.0 and 0.9, respectively).

For TLC identification of the transfer of [^{3}H]octanoyl from [^{3}H]octanoyl CoA to GSSFL-NH_2 or GSAFL-NH_2 pentapeptides, each 50-μl reaction mixture contained 50 mM Hepes-NaOH (pH 7.0), 500 μM of the indicated pentapeptide, 50 μM palmitoyl CoA, and 1 μM [^{3}H]octanoyl CoA (11dpm/fmol) in the absence or presence of 50 μg of membrane protein. After incubation at 37°C for 10 min, the reaction was stopped by addition of 1 ml of 2% acetonitrile/0.1% trifluroacetic acid and 2.5 μg of unlabeled octanoyl-ghrelin (1–5)-NH_2. Four assay tubes for each condition were pooled, loaded onto a 360-mg Sep-Pak C18-cartridge (Waters), and subjected to a step gradient of 20% (6 ml), 30% (12 ml), and 40% (3 ml) acetonitrile in 0.1% trifluoroacetic acid. The 20% and 30% fractions contained >90% of the [^{3}H]octanoyl CoA and [^{3}H]octanoate but none of the octanoyl-ghrelin (1–5)-NH_2, which was eluted in the 40% fraction. After evaporation of each 40% acetonitrile fraction under vacuum, the residual pellet was dissolved in 20 μl of 40% acetonitrile/0.1% trifluroacetic acid and spotted onto a Polygram SIL G plate, and the plate was developed in a solvent system of 1-butanol/acetic acid/water at a ratio of 4/1/1 (vol/vol/vol). After staining the TLC plate with 0.2% ninhydrin to denote the position of migration of octanoyl-ghrelin (1–5)-NH_2, each lane of the plate was cut into 12 or 14 sequential slices, and the radioactivity in each slice was quantified by liquid scintillation counting as described (1). The R_f value for octanoyl-ghrelin (1–5)-NH_2 was 0.8.

^{3}H-Autoradiography. The ^{3}H-labeled proghrelin generated in the *in vitro* octanoylation assay was precipitated with 200 μl of ice-cold acetone. After incubation at −20°C for 1 h, the mixture was centrifuged at 20,000 × *g* for 15 min. The resulting pellet was dissolved in 25 μl of SDS/PAGE loading buffer [0.1 M Tris-Cl at pH 6.8, 5% (wt/vol) SDS, 0.1 M DTT, and 5% (vol/vol) glycerol] and then subjected to 16% Tricine SDS/PAGE, followed by transfer to an Immobilon-P^{SQ} membrane and autoradiography for 5–7 days at −80°C as described (1).

Separation of Desacyl-Ghrelin from Octanoyl-Ghrelin by Reverse-Phase Chromatography. Peptides were extracted from INS-1 cells with acetic acid and HCl. After centrifugation and evaporation, the residue was dissolved in 2% acetonitrile/0.1% trifluroacetic acid, loaded onto a 360-mg Sep-Pak C18-cartridge (Waters), and eluted with a step gradient of 20%, 40%, and 80% acetonitrile as previously described (1).

Immunoblot Analysis. Immunoblot analysis was performed as previously described (1). Bound primary antibodies (see figure legends for concentrations) were visualized by chemiluminescence (SuperSignal West Pico Developing Kit; Pierce) using a 1:10,000 dilution of either donkey anti-rabbit IgG or donkey anti-mouse IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch). Membranes were exposed to Phoenix Blue x-ray film for 5 s to 1 min at room temperature.

1. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL (2008) Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132:387–396.

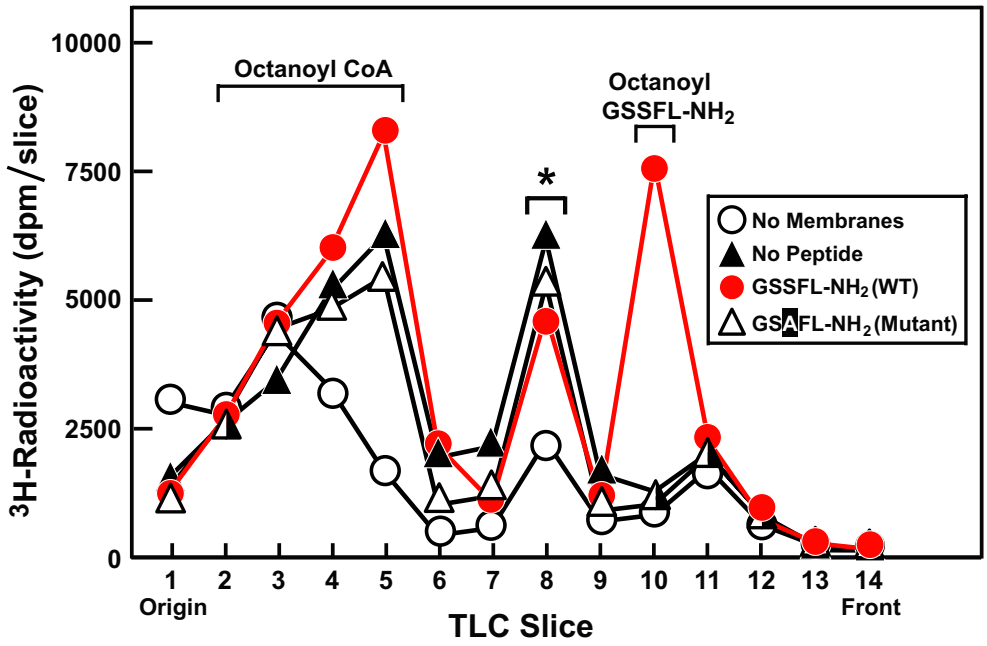

Fig. S1. Octanoylation of ghrelin pentapeptide and quantification by TLC. Each 50-μl reaction mixture contained 50 μg of membrane protein, 50 μM palmitoyl CoA, 1 μM [^{3}H]octanoyl CoA (11 dpm/fmol; 5.5×10^5 dpm per tube), and the indicated ghrelin pentapeptide (500 μM). After incubation at 37°C for 10 min, the reaction mixtures from four tubes were pooled and subjected to TLC analysis, after which each lane was cut into 14 sequential slices and counted by liquid scintillation as described in *SI Experimental Procedures*. The data in the figure correspond to Experiment A in Table 1. The positions of migration of residual [^{3}H]octanoyl CoA (not completely removed in the 20% and 30% acetonitrile fractions) and authentic octanoyl-GSSFL-NH_2 (Peptide International) are indicated. The identity of the middle peak of radioactivity, denoted by an asterisk, has not been characterized but is likely to represent the membrane-dependent incorporation of the [^{3}H]octanoyl group into an endogenous substrate.