Supporting Text

Effect of *Ce-imp-2* RNAi on brood size. Because the reduced number of eggs in a common population may merely reflect increased death of worms during the molt stages, we examined the brood size of individual ST2 worms on *Ce-imp-2* RNAi food. The ST2 strain expressing GFP in the CNS was used for easier detection of weak and dead worms at the late embryonic and early L_1 stages. Synchronized L_1 larvae (n = 10) were placed in separate *Ce-imp-2* dsRNA plates. The hatched larvae and eggs were inspected daily during 5 days; each day, the parent worms were transferred to fresh plates. Worms that developed incomplete shedding of cuticle (ISC) at the L_4-adult stage died, some of them having a low number of progeny hatched inside dead mothers. The surviving worms were able to lay eggs over the next 2 or 3 days, but with a mean brood size at least 3-fold less than control RNAi worms. About 80% of these progeny died at the late embryonic-early larvae stages.

Supporting Materials and Methods

RNA Interference (RNAi) by Double-Stranded RNA (dsRNA) Feeding. To conduct RNAi by feeding, we have subcloned the full-length 1.54-kb cDNA *Ce-imp-2* (yk671a5 clone) into the L4440 vector. We also subcloned *Ce-imp-2* cDNA fragments that include the 1329-bp middle region, and nonoverlapping fragments [5'- *Ce-imp-2* sequence (485 bp) and 3'-*Ce-imp-2* sequences (823 bp)] into L4440 (Fig. 5*B*). We believed that application of the different dsRNAs would exclude theoretically possible nonspecific RNAi effects and might provide a more detailed characterization of *Ce-imp-2*-regulated phenotypes on different stages of worm development. Identical phenotype defects were induced by expression of all these RNAi constructs; these results were reproduced in multiple experiments in three laboratories (Brudnick Neuropsychiatric Research Institute, Howard Hughes Medical Institute, and Laboratory of Molecular Brain Genetics). Sub-fragments of *Ce-imp-2* cDNA for RNAi by feeding were cloned into L4440 vector using primers 5'- TTTTGAATTCGACGGCTAGCAATGTCACAG-3', 5'-TTTTCTCGAGTCCACATGTCT TGATTCATC-3' (1329 bp) ; 5'-TTTTGAATTC

CTTTCGCGACATGGCTGA-3', 5'-TTTTCTCGAGTATAAGCGTATGTAG CCGCATTT -3' (485 bp); and 5'-TTTTGAATTCCCATTTG TTACCGCGTTTCT-3', 5'-TTTTCTCGAG GCCGGAGTCTACTTTCTTTCG-3' (823 bp) (Fig. 5*B*). cDNA for *Ce-lrp-1* gene corresponding to the extracellular part of the protein was subcloned into *Bam*HI-*Xho*I L4440 using primers 5'-TTTTGGATCCGCCGTACTTGCTCTCCATTC-3' and 5'- TTTTCTCGAGCCATCCAATCGACATTTTCC-3' (Fig. 5*B*). These constructs were transferred to the *Escherichia coli* strain HT115. The bacterial cultures were grown in 1 liter of LB-Amp to $OD_{600} \approx 1$, spun down, and resuspended in 50 ml of LB , containing Amp 60 μg/ml and 7% DMSO, and were frozen and stored in 5-ml aliquots at -70°C. For each experiment, a fresh aliquot was used. For dsRNA induction, 100 μl of isopropyl β-D-thiogalactoside (IPTG) (200 mg/ml) was added to each *E.coli* stock. Synchronized population of worms was obtained by using the standard hypochlorite method. L_1 stage larvae, hatched in M9 buffer, were placed on dsRNA agarose plates, and the phenotype of worms (P_0) was analyzed on the second and third days at room temperature. On the third day, eggs were collected using the hypochlorite treatment. Eggs were placed on dsRNA plates, and the phenotype of F_1 progeny was analyzed for the next two days.

Mammalian Cell Lines and Western Blot Analysis. Cell lines with stable or transiently transfected constructs were maintained in appropriate media supplemented with 10% FBS (GIBCO/BRL); 1% penicillin/streptomycin, 2 mM L-glutamine at 37°C; and 5% CO_2. Human embryonic kidney (HEK) 293 cells and mouse fibroblasts were cultured in DMEM; PC12 rat pheochromocytoma cells in RPMI medium 1640; Chinese hamster ovary (CHO) cells in F12; and H4 (human neuroglioma) cells in Opti-MEM (GIBCO/BRL). Transfection was performed using LipofectAMINE PLUS Reagent (GIBCO/BRL). Twenty-four to 48 h after transfection, cells were briefly washed twice in cold PBS, lysed in modified RIPA buffer (50 mM Tris•HCl, pH 7.4/1% Nonidet P-40/0.25% sodium deoxycholate Na/150 mM NaCl/1 mM EDTA) supplemented with protease inhibitors (Roche Molecular Biochemicals) for 15 min at 4°C, and centrifuged at 20,800 × *g* for 10 min at 4°C. For coimmunoprecipitation (co-IP) experiments of hIMP1, HEK293 and H4 cells were cotransfected with hIMP1-c-myc and hIMP1-V5 plasmids.

Ten to 20 μg of protein extracts were mixed with SDS sample buffer (twice) (Invitrogen) containing reducing agent, centrifuged at 12,000 rpm for 5 min with or without prior boiling for 5 min, and loaded onto SDS polyacrylamide gel (PAAG) minigels. Prestained molecular weight marker was loaded into a separate well. Electrophoresis was run in 10–20% Tricine PAAG or 10% SDS PAAG for hIMP1 products and 8% Tris-Glycine SDS PAAG for NotchΔE and NOTCH1 intracellular domain (NICD) fragments in corresponding 1× SDS running buffer at 125 V. Electro-transfer onto poly(vinylidene difluoride) (PVDF) membranes was performed in a Tris-Glycine transfer buffer 12 mM Tris base, 96 mM glycine, 20% methanol. After transfer, membranes were washed in TBS-T buffer (50 mM Tris•HCl, pH 7.4/150 mM NaCl/0.05% Tween 20) three times for 5 min, incubated in blocking buffer (5% milk in TBS-T) at room temperature for 1 h, and hybridized with primary antibodies, 1:1,000–1:5,000 dilution in 10 ml of hybridization buffer (1% milk in TBS-T) at room temperature for 1 h, or 4°C overnight. The primary polyclonal and monoclonal antibodies against N-terminal-PS1 fragments and N-terminal hIMP1 or V5 and c-myc C-terminal targets of hIMP1 or Notch epitopes have been used to control efficiency of expression in each transfection experiment. After incubation with appropriate secondary antibodies, signal visualization was performed using ECL Western blotting detection reagent kit (Amersham Pharmacia) by exposure to an x-ray film.

Pulse-chase experiments. HEK293 cells were transfected with Notch, hIMP1, and PS1 constructs using LipofectAMINE PLUS Reagent (GIBCO/BRL). NotchΔE and NICD (obtained from R. Kopan, Washington University School of Medicine, St. Louis) were fused to c-myc epitopes. hIMP1 wild-type or mutant isoforms were cloned into cDNA3 or pcDNA4/c-myc-HisB. Twenty-four hours after transfection, cells were starved for 2 h in methionine and cysteine-free medium without serum, labeled with 0.1 mCi of [^{35}S]methionine and [^{35}S]cysteine for 30 min, chased for 1 h in DMEM medium supplemented with 10% FBS, and lysed in RIPA buffer with 1% Nonidet P-40 and protease inhibitor mixture. Protein extracts were immunoprecipitated with anti-c-myc-antibodies to Notch-C-terminal tag-epitopes and subjected to electrophoresis and autoradiography.