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LAAT-1 is the Lysosomal Lysine/Arginine Transporter that Maintains Amino Acid Homeostasis

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Abstract

Defective catabolite export from lysosomes results in lysosomal storage diseases in humans. Mutations in the cystine transporter gene *CTNS* cause cystinosis, but other lysosomal amino acid transporters are poorly characterized at the molecular level. Here we identified the *C. elegans* lysosomal lysine/arginine transporter, LAAT-1. Loss of *laat-1* caused accumulation of lysine and arginine in enlarged, degradation-defective lysosomes. In mutants of *ctns-1* (*C. elegans* homolog of *CTNS*), LAAT-1 was required to reduce lysosomal cystine levels and suppress lysosome enlargement by cysteamine, a drug that alleviates cystinosis by converting cystine to a lysine analog. LAAT-1 also maintained availability of cytosolic lysine/arginine during embryogenesis. We showed that LAAT-1 is the lysosomal lysine/arginine transporter and suggested a molecular explanation for how cysteamine alleviates a lysosomal storage disease.

Defects in exporting hydrolytic degradation products from lysosomes cause lysosomal storage diseases such as cystinosis, which is characterized by intralysosomal accumulation of free cystine because of mutations in the lysosomal cystine transporter gene *CTNS* (cystinosin) (1-4). The most effective therapeutic agent for cystinosis, cysteamine (an aminothiol), converts lysosomal free cystine to cysteine and the mixed disulfide of cysteine-cysteamine, which is thought to be exported from lysosomes as a lysine analog through a lysine/cationic amino acid transporter (5-7). The molecular identity of the transporter remains unknown. Although biochemically detected, most mammalian lysosomal amino acid transporters have not been molecularly characterized (1).

From a forward genetic screen for *C. elegans* mutants with increased embryonic cell corpses, we isolated a recessive mutant *qx42* that accumulated many refractile corpse-like objects and lysotracker-positive puncta, suggestive of abnormal lysosomes (fig. S1, A to G). Using NUC-1::mCHERRY, which labels lysosomes (8, 9), or lysotracker staining, we found

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that *qx42* lysosomes were on average twice the volume of wild type (1.3 versus 0.5 μm^3; Fig. 1, A to F"' and fig. S1, H to K).

We next examined whether *qx42* affected lysosomal cargo degradation. Apoptotic cells are phagocytosed then degraded in lysosomes. Cell death and cell corpse engulfment were normal in *qx42* mutants (fig. S2). However, degradation of apoptotic cells in phagolysosomes (indicated by GFP::RAB-7 or NUC-1::mCHERRY) as measured by loss of HIS-24::GFP or H2B::GFP (which label chromatin in all somatic and germ nuclei, including cell corpses, respectively) was severely affected in *qx42* mutants, with HIS-24::GFP persisting >4 times as long as in wild type (Fig. 2A and fig. S2, L to O). Yolk lipoprotein is degraded throughout embryogenesis to nourish developing cells (10, 11). In *qx42* mutants, intestinal secretion of yolk reporter VIT-2::GFP and uptake by oocytes were normal (fig. S3, A to B'). However, *qx42* embryos accumulated significantly more VIT-2::GFP in enlarged puncta, which overlapped with NUC-1::mCHERRY, suggesting defective lysosomal yolk degradation (Fig. 2, B to D and fig. S3, C to H'). Cell surface proteins CAV-1 and RME-2, which are internalized and degraded in wild-type embryos, accumulated in enlarged lysosomes in *qx42* embryos (fig. S4) (12). Damaged organelles and protein aggregates are delivered via the autophagy pathway to lysosomes for degradation (13). Autophagy substrates SEPA-1 and T12G3.1 (the *C. elegans* homolog of mammalian p62) were cleared during embryogenesis in wild type, but persisted in late-stage *qx42* mutant embryos and overlapped with NUC-1::mCHERRY, indicating defective autolysosomal degradation (Fig. 2, E to G and fig. S5) (14, 15). Thus, *qx42* impairs lysosomal degradation of phagocytic, endocytic and autophagic cargoes.

The gene affected in *qx42, Y43H11AL.2*, encodes a conserved protein containing seven predicted transmembrane domains and two internal PQ (Proline-Glutamine) loop repeats, characteristic of lysosomal cystine transporters (LCTs) (16) (fig. S6F). Cystinosin, the archetypal LCT family member, is a lysosomal cystine transporter, abnormal function of which causes cystinosis (4). We named the *Y43H11AL.2* gene *laat-1* (*l*ysosomal *a*mino *a*cid *t*ransporter 1) based on similarity with LCT family proteins and cellular functions (see below). *qx42* has an A>T mutation in *laat-1* that creates a premature stop codon after Asn^{127}. Other independently isolated *laat-1* mutant alleles also caused enlarged lysosome and persistent cell corpse phenotypes (fig. S1, L to R and S2K). *laat-1* was expressed in various cell types in embryos, larvae and adults (fig. S7). GFP or mCHERRY fusion of LAAT-1, which fully rescued *qx42* defects (fig. S6, A to E), labeled membranes of NUC-1- or lysotracker-positive structures and overlapped with lysosomal membrane protein CTNS-1, the *C. elegans* homolog of human cystinosin (17), indicating that LAAT-1 localizes to lysosomal membranes (Fig. 1, G to H" and fig. S7, A to C"). LAAT-1(Δ299-304)::GFP, which lacks the C-terminal dileucine-based lysosomal sorting motif (18), stained plasma membranes instead of lysosomes and failed to rescue *laat-1(qx42)* mutant phenotypes, indicating that LAAT-1 function depends on its lysosomal localization (fig. S6, A to F and S7, D to E").

We examined lysosomes purified from *C. elegans* embryos (fig. S8) and found that loss of CTNS-1 caused cystine accumulation, suggesting that CTNS-1 mediates cystine efflux from lysosomes like human cystinosin (Fig. 3A). In *laat-1* mutant lysosomes, cystine levels were normal but lysine and arginine levels were 16 and 8 times as high as wild type, respectively, suggesting that LAAT-1 exports lysine and arginine from lysosomes (Fig. 3A and fig. S9A). Macrophage-like coelomocytes from *ctns-1* mutants contained huge granules (>6.5 μm in diameter), which accumulated endocytosed cargo CHERRY and were labeled by lysosomal membrane protein CUP-5 but not endosomal protein RME-8, indicating that they are enlarged lysosomes (19, 20) (Fig. 3, B and C and fig. S9B). Most wild-type and *laat-1(qx42)* coelomocytes contained small lysosomes (<4.5 μm) or 2-3 bigger ones (4.5 to 6.5 μm) (Fig.

3C). Cysteamine treatment of *ctns-1* mutants greatly reduced lysosomal cystine accumulation and almost completely suppressed the enlarged lysosome phenotype (Fig. 3, C and D). In *laat-1(qx42)ctns-1(ok813)* double mutants, however, cysteamine failed to deplete lysosomal cystine and suppress enlarged lysosomes, which accumulated high levels of cystine and the lysine analog mixed disulfide of cysteine-cysteamine (Fig. 3, C to E). These data strongly suggest that LAAT-1 transports lysine out of lysosomes.

We tested whether LAAT-1 or its human counterpart PQLC2 transported lysine and arginine using a whole cell-based transporter assay (4). Wild-type PQLC2::GFP localized to lysosomes in COS-7 cells, while PQLC2 (ΔLL)::GFP, which lacks the lysosomal sorting motif, associated with plasma membranes, indicating that PQLC2 is a lysosomal membrane protein like LAAT-1 (fig. S6F and fig. S9, C to H"). Expression of plasma membrane-targeted LAAT-1 [LAAT-1(Δ299-304)::GFP] or PQLC2 [PQLC2(ΔLL)::GFP] caused increased uptake of lysine and arginine, which was almost completely abolished when the invariant Pro in the first PQ loop was mutated to Leu (Fig. 3, F and G and fig. S6F). Uptake of histidine but not alanine, glutamic acid, cystine or cysteine was increased in LAAT-1- or PQLC2-expressing cells, suggesting specific transport of cationic amino acids (fig. S10). *laat-1* lysosomes did not significantly accumulate histidine, indicating that LAAT-1 is probably not the major histidine transporter in vivo (fig. S9A).

laat-1 mutants were viable but developed slowly (Fig. 4A). External supplements of both lysine and arginine completely rescued retarded embryonic development (Fig. 4B and fig. S11, A and B) but did not reverse the enlarged lysosome or defective yolk degradation phenotypes in *laat-1* mutants (fig. S11C). Thus, loss of *laat-1* affects lysosomal export of lysine/arginine, which limits their cytoplasmic availability and thereby retards embryonic development. When deprived of amino acids, eukaryotic cells trigger the amino acid response (AAR) pathway through activation of GCN2 protein kinase, leading to repression of global protein synthesis (21). Consistent with this, *laat-1* embryos showed reduced protein synthesis, which was efficiently rescued by supplementing lysine and arginine (Fig. 4C and fig. S11D) (22). The AAR pathway is essential for survival during amino acid deprivation (23, 24). *gcn-2(ok871)* embryos developed normally but died when *laat-1* was defective. The synthetic lethality was completely rescued by supplying both lysine and arginine but not glycine (Fig. 4D). Thus, loss of *laat-1* limits cytosolic lysine and arginine, causing embryonic lethality when the GCN-2-mediated AAR pathway is impaired (fig. S11E).

We have identified LAAT-1 and its human homolog PQLC2 as the lysosomal lysine/arginine transporter. Cysteamine treatment significantly reduced lysosomal free cystine and efficiently suppressed the enlarged lysosome phenotype in *ctns-1(lf)* single mutants but not *laat-1(lf) ctns-1(lf)* double mutants, which accumulated the lysine analog mixed disulfide of cysteine-cysteamine in lysosomes, suggesting that LAAT-1 (and probably PQLC2) may mediate cystine depletion by cysteamine. It is thus important to examine whether loss of *PQLC2* affects mammalian lysosome function and causes lysosome-related diseases. Our finding that defective lysosomal export of lysine/arginine leads to retarded embryonic development reveals the role of lysosomal amino acid transporters in maintaining cytosolic amino acid availability during embryonic development, providing insights into the pathogenesis of lysosomal transport disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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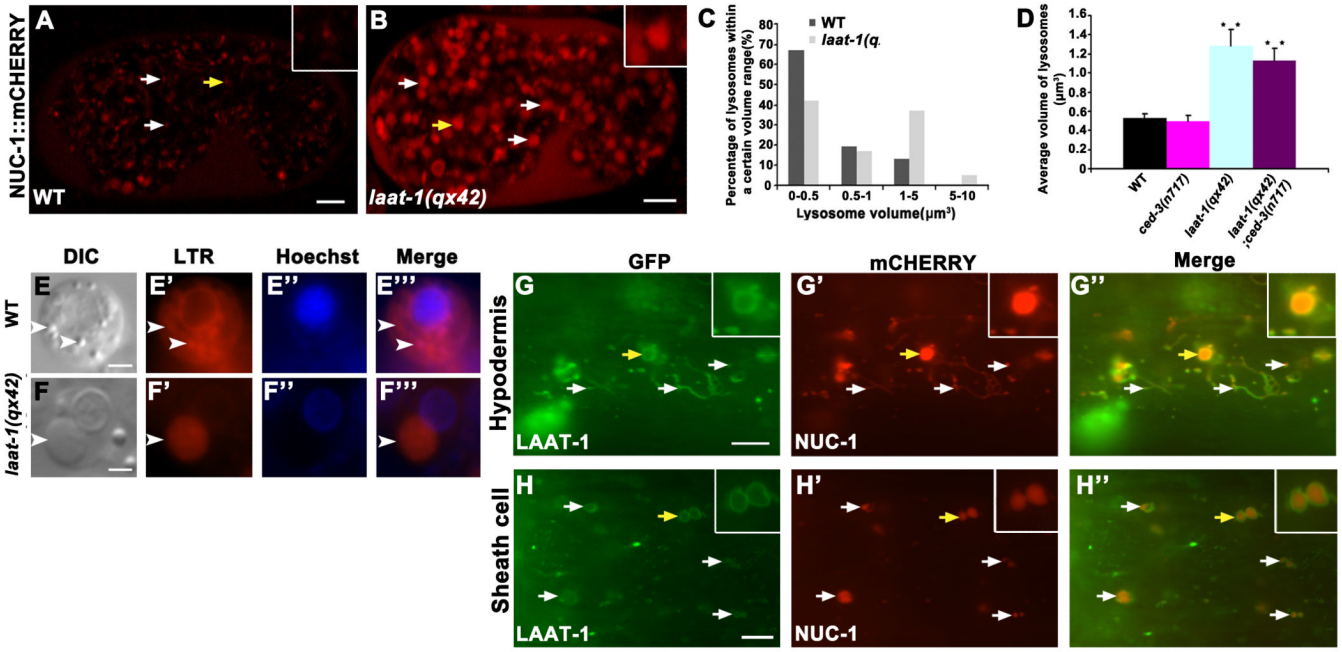

Figure 1. *laat-1* mutants accumulate enlarged lysosomes

(**A** to **F”’**) Enlarged lysosomes indicated by NUC-1::mCHERRY [(A) and (B), arrows] or lysotracker red (LTR) [(E) to (F”’), arrowheads] were observed in a *laat-1(qx42)* embryo (B) or cell [(F) to (F”’)] but not wild type [(A), (E)-(E”’)]. Lysosome volumes are quantified in (C) and (D). Average lysosomal volumes (±SEM, n=100) in different strains are shown in (D). ** $P<0.0001$. (**G** and **H**) Fluorescent images of hypodermal (G) or sheath cells (H) in wild-type animals expressing LAAT-1::GFP and NUC-1::mCHERRY. In (A), (B), and (G) to (H”), insets show *x4* magnification of lysosomes indicated by yellow arrows. Scale bars: 2 μm in (E), (F); 5 μm in other panels.

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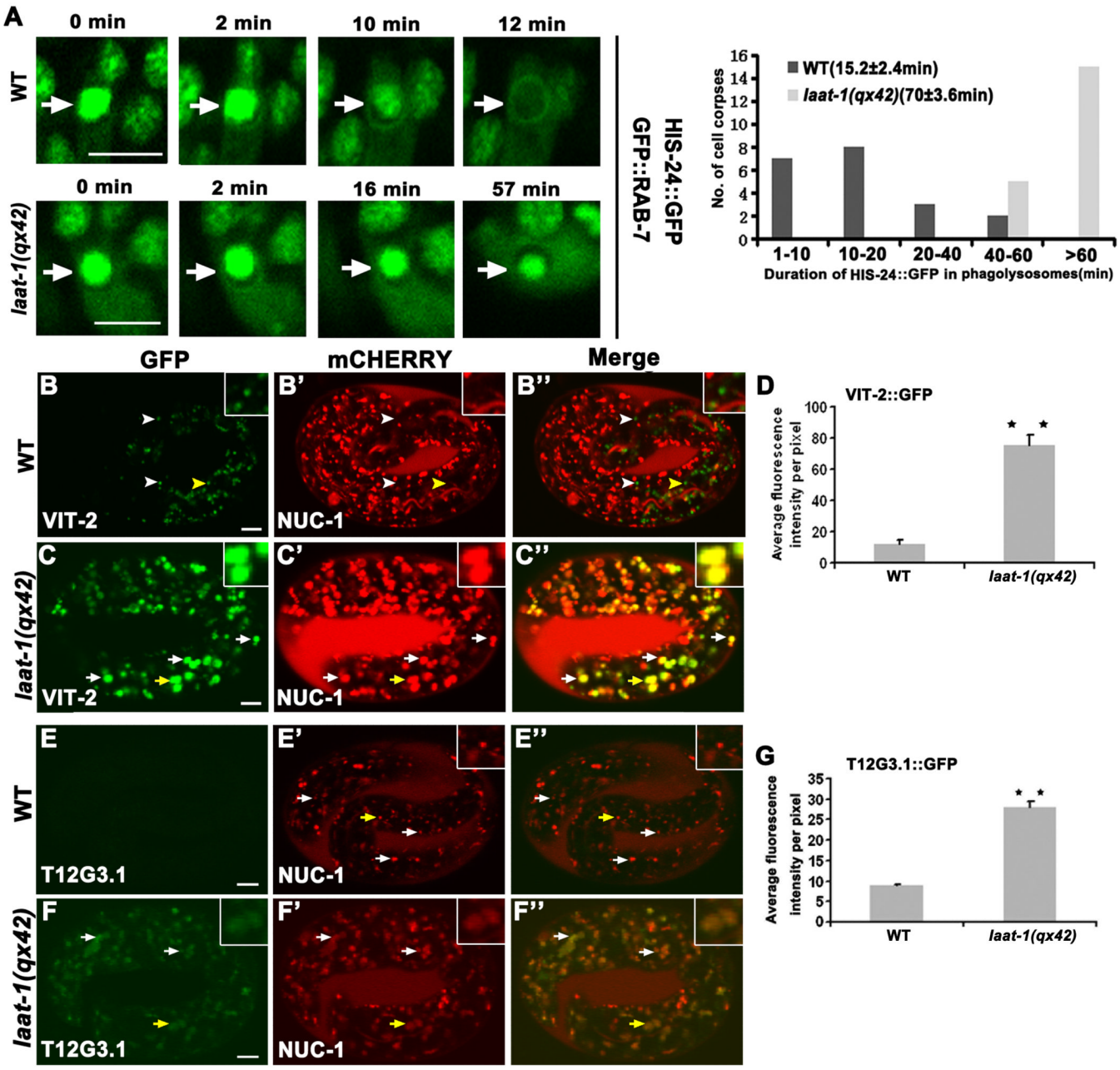

Figure 2. *laat-1* mutants are defective in lysosomal degradation of various cargoes

(A) Fluorescent images of wild-type and *laat-1(qx42)* embryos expressing HIS-24::GFP and GFP::RAB-7 at different time points. Arrows indicate phagolysosomes. Quantification is shown in the right panel with average duration (±SEM) shown in parentheses. (**B** to **G**) Confocal fluorescent images of wild-type [(B) and (E)] or *laat-1(qx42)* [(C) and (F)] embryos expressing NUC-1::mCHERRY and VIT-2::GFP [(B) and (C)] or T12G3.1::GFP [(E) and (F)]. Arrows indicate overlapping GFP and mCHERRY; arrowheads indicate non-overlapping GFP. Structures indicated by yellow arrows or arrowheads are magnified *x4* in the insets. Quantifications are shown in (D) and (G). At least 10 embryos were scored in each strain. Data are shown as mean±SEM. ***P*<0.0001. Scale bars: 5 μm.

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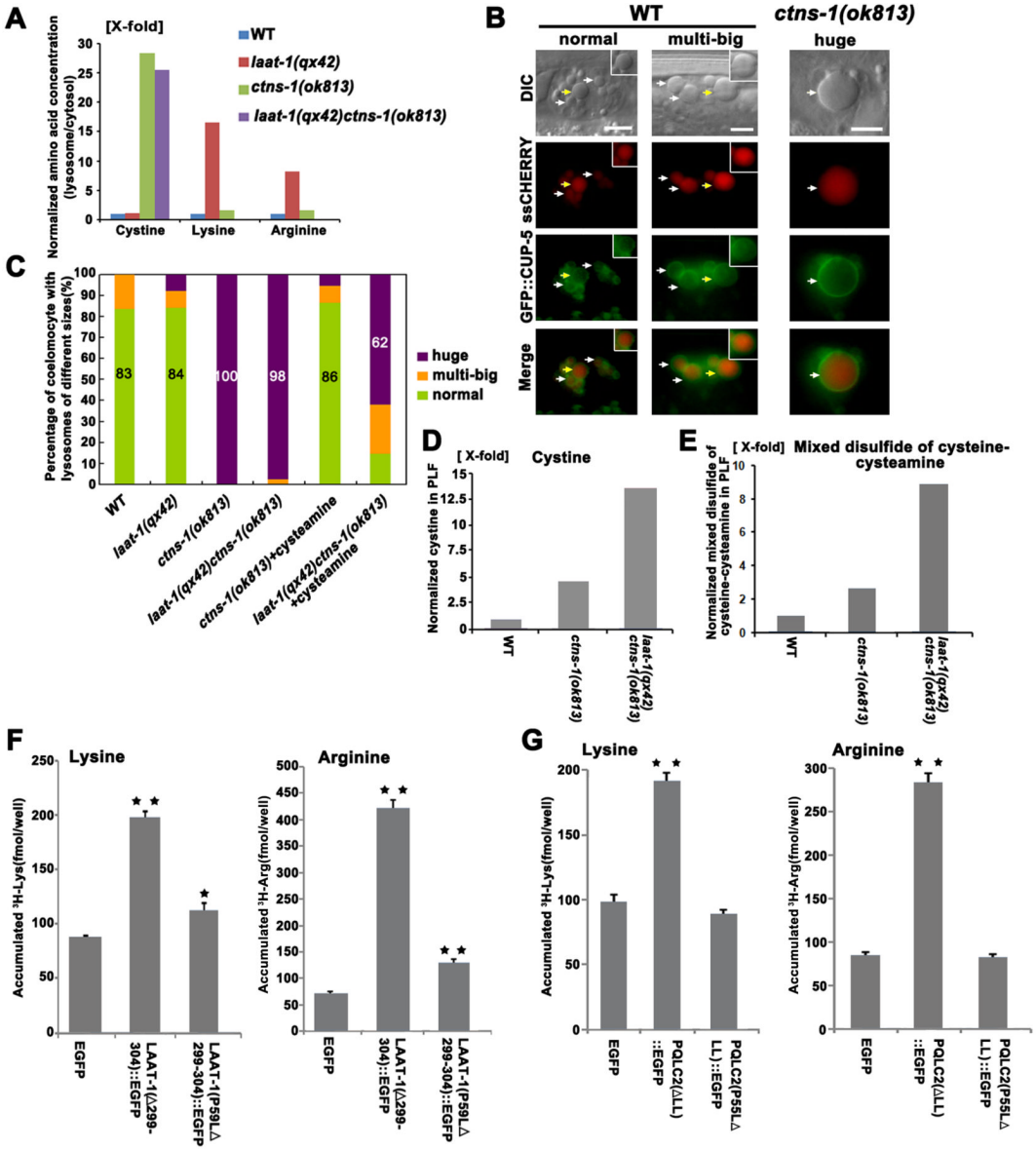

Figure 3. LAAT-1 is a lysosomal lysine and arginine transporter

(**A**) The ratio of amino acid concentration in lysosomal versus cytosolic fractions prepared from embryonic lysates was determined and normalized as 1 fold in wild type (*y*-axis). (**B**) DIC and fluorescent images of wild-type and *ctns-1(ok813)* coelomoctyes expressing secreted CHERRY (ssCHERRY) and the lysosomal marker GFP::CUP-5. Lysosomes are labeled by CHERRY and CUP-5 (arrows). Insets show lysosomes indicated by yellow arrows. Scale bars: 5 μm. Quantification is shown in (**C**). (**D** and **E**) Cystine (D) and mixed disulfide of cysteine-cysteamine (E) was determined in purified lysosomal fractions (PLF) after cysteamine treatment and normalized as 1 (fold) in wild type. (**F** and **G**) Lysine and arginine uptake was determined in (F) LAAT-1- or (G) PQLC-2-expressing COS-7 cells. Data are shown as mean±SEM. $^{**}P<0.0001$,$^{*}P<0.05$; all other points had $P>0.05$. Data in (A), (D), (E), (F), and (G) are representative of at least 3 independent experiments.

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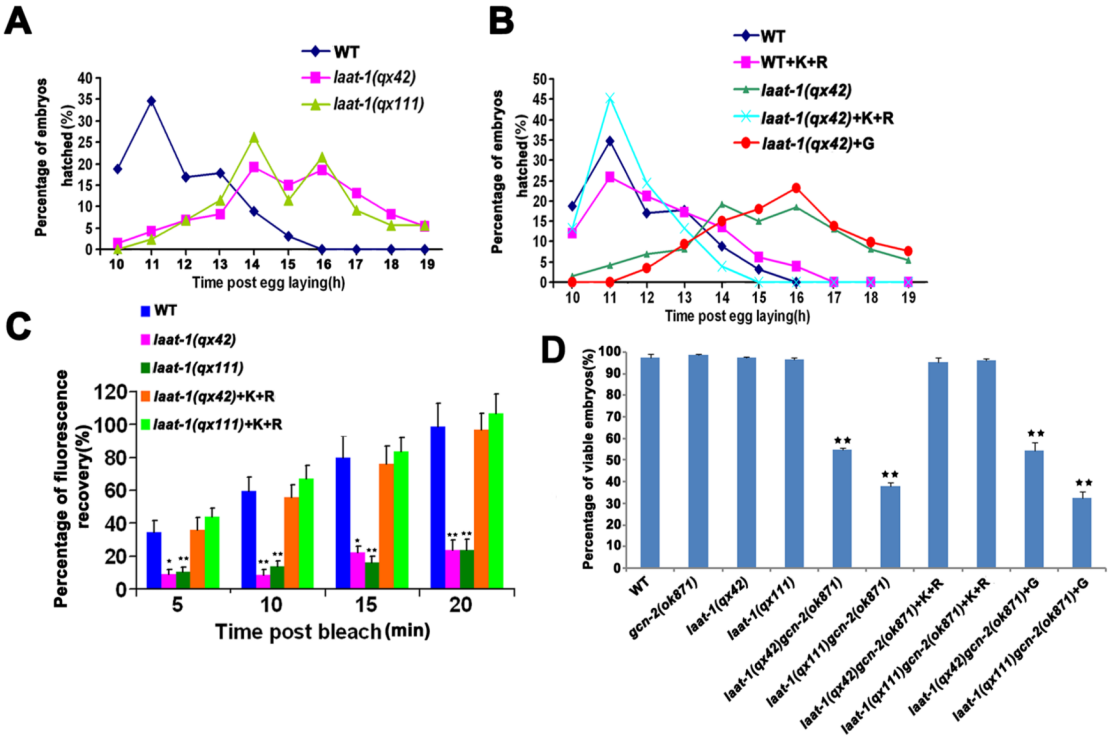

Figure 4. LAAT-1 maintains lysine and arginine availability for normal embryonic development

(**A** and **B**) Retarded embryonic development in *laat-1* mutants is rescued by external lysine and arginine supplements. At least 88 embryos were examined. (**C**) Protein synthesis rates determined by fluorescence recovery after photobleaching in wild-type, *laat-1(qx42)* and *laat-1(qx111)* embryos expressing $P_{laat\text{-}1}$mCHERRY with or without externally supplied lysine and arginine. At least 20 embryos were quantified in each strain/treatment. (**D**) Loss of *laat-1* and *gcn-2* causes synthetic embryonic lethality. The *y*-axis indicates the percentage of viable embryos in each strain/treatment. 3 independent experiments were performed with at least 95 embryos examined in each. In panels (C) and (D), data are shown as mean±SEM. $^{**}P<0.0001$. In panels (B) to (D), lysine (K) and arginine (R) were supplied at 100 mM each and glycine (G) was supplied at 200 mM.

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