Membrane Fluidity Is Regulated Cell Nonautonomously by Caenorhabditis elegans PAQR-2 and Its Mammalian Homolog AdipoR2

Rakesh Bodhicharla,1 Ranjan Devkota,1 Mario Ruiz, and Marc Pilon2
Department of Chemistry and Molecular Biology, University of Gothenburg, S-405 30, Sweden
ORCID ID: 0000-0003-3919-2882 (M.P.)

ABSTRACT

Maintenance of membrane properties is an essential aspect of cellular homeostasis of which the regulatory mechanisms remain mostly uncharacterized. In Caenorhabditis elegans, the PAQR-2 and IGLR-2 proteins act together as a plasma membrane sensor that responds to decreased fluidity by promoting fatty acid desaturation, hence restoring membrane fluidity. Here, we used mosaic analysis for paqr-2 and iglr-2, and tissue-specific paqr-2 expression, to show that membrane homeostasis is achieved cell nonautonomously. Specifically, we found that expression of paqr-2 in the hypodermis, gonad sheath cells, or intestine is sufficient to suppress systemic paqr-2 mutant phenotypes, including tail tip morphology, membrane fluidity in intestinal cells, cold and glucose intolerance, vitellogenin transport to the germline, germ cell development, and brood size. Finally, we show that the cell nonautonomous regulation of membrane homeostasis is conserved in human cells: HEK293 cells that express AdipoR2, a homolog of paqr-2, are able to normalize membrane fluidity in distant cells where AdipoR2 has been silenced. Additionally, suppression of the paqr-2 mutant has also shown to be able to share membrane components even when they are not in direct contact with each other, and that this contributes to the maintenance of membrane homeostasis in C. elegans and human cells.

KEYWORDS fatty acid; HEK293; lipoprotein; albumin; vitellogenin; mosaic analysis; fat-6; fat-7; paqr-2; iglr-2; adiponectin receptor

The Caenorhabditis elegans protein PAQR-2 is a member of the PAQR protein family and is homologous to the antidiabetic mammalian proteins AdipoR1 and AdipoR2 (Svensson et al. 2011; Devkota et al. 2017). Various lines of evidence, ranging from crystal structure determination to C. elegans genetics, suggest that PAQR-2 and its AdipoR homologs have seven transmembrane domains with their N terminus in the cytosol (Svensson et al. 2011; Tanabe et al. 2015; Vasiliauskaité-Brooks et al. 2017), have a hydrolases activity capable of using ceramides as substrates (Holland et al. 2011; Pei et al. 2011; Vasiliauskaité-Brooks et al. 2017) and are required for membrane homeostasis during cold adaptation (Svensson et al. 2011; Svensk et al. 2013) or upon a rigidifying challenge by exogenous saturated fatty acids (SFAs) (Svensk et al. 2016; Devkota et al. 2017). The paqr-2 mutant is also intolerant of glucose because it is readily converted to membrane-rigidifying SFAs by the dietary Escherichia coli (Devkota et al. 2017). Additionally, the paqr-2 mutant has reduced brood size, length, locomotion rate and life span, and a withered tail tip (Svensson et al. 2011). All of these phenotypes seem secondary to a primary membrane homeostasis defect since they are abrogated by mutations that result in increased unsaturated fatty acids (UFAs) production, by the inclusion of UFAs in the diet or by supplementation of the culture plate with membrane-fluidizing concentrations of nonionic detergents (NP-40 or Triton X-100) (Svensk et al. 2013, 2016; Devkota et al. 2017). Also, suppression of the paqr-2 mutant phenotypes is invariably associated with
improved membrane fluidity, which we have measured in vivo using fluorescence recovery after photobleaching (FRAP) (Svensk et al. 2016; Devkota et al. 2017).

Our previous work also identified IGLR-2 as a protein essential for PAQR-2 function: the two proteins are coexpressed strongly in the plasma membrane of the gonad sheath cells, physically interact with each other, and give identical phenotypes when mutated (except for the observation that IGLR-2 is required for high PAQR-2 expression on the gonad sheath) (Svensk et al. 2016). Based on a small-scale mosaic analysis, we previously showed that IGLR-2 expression in the hypodermis is sufficient to restore glucose tolerance, which suggests that IGLR-2 can act cell nonautonomously to maintain membrane fluidity systemically (Svensk et al. 2016). In this study, we used a more extensive mosaic analysis and tissue-specific expression studies to show that both PAQR-2 and IGLR-2 can act cell nonautonomously from several different tissues to maintain membrane fluidity throughout the worm. Additionally, we show that human cells that express AdipoR2 can remotely rescue membrane homeostasis in AdipoR2-deficient cells, suggesting that cell nonautonomous membrane homeostasis is evolutionarily conserved.

Materials and Methods

C. elegans strains and cultivation

The wild-type C. elegans reference strain N2, the transgene-carrying strains MD702 (bcls39 [lim-7p::ced-1::GFP + lin-15(+)]), OD95 (ltls37 [pAA64 pie-1p::mCherry::his-58 + unc-119(+)] IV; ltls38 [pie-1p::GFP::PH(PLCdelta1) + unc-119(+)]), and RT130 [pols23 (vit-2::GFP)], and the mutant alleles studied are available from the C. elegans Genetics Center (Minneapolis, MN). Unless otherwise stated, experiments were performed at 20°C, using the E. coli strain OP50 as food source, which was maintained on LB plates kept at 4°C, cooled NGM after autoclaving. The stocks kept frozen at −80°C. For glucose plates, stock solutions of 1 M glucose, were filter-sterilized, and then added to cooled NGM before being used to seed NGM plates (Sulston and Hodgkin 1988); stock solutions were filter-sterilized, and then added to cooled NGM after autoclaving. The assembled plasmid was injected into N2 worms at 5 ng/μl together with 3 ng/μl Pmyo-2::gfp marker, and PAQR-2 mutants. The low-pH-resistant strains were propagated using primers 5'-tgtccagcactcagatggagagtagatgggatgg-3' and 5'-ctcggtctattcttttgatttataaatgtgagagaacaaactagccgcag-3', and the paqr-2 coding sequence with GFP was amplified from the plasmid Pqaqr-2::pqaqr-2::GFP, using primers 5'-tgctcgcagatggagagtagatgggatgg-3' and 5'-ctcggtctattcttttgatttataaatgtgagagaacaaactagccgcag-3'. The assembled plasmid was injected into N2 worms at 5 ng/μl together with 3 ng/μl Pmyo-2::gfp marker.

For Punc-54:pqaqr-2::GFP, a muscle-specific transgene was constructed using a Gibson assembly cloning kit (NEB, Beverly, MA) by assembly of the following two DNA fragments: 2 kb upstream regulatory sequences from elt-3 was amplified from N2 genomic DNA using the primers 5’-ctcggtctattcttttgatttataaatgtgagagaacaaactagccgcag-3’ and 5’-ccgattcctcagatggagagtagatgggatgg-3’. The pqaqr-2 coding sequence with GFP was amplified from the plasmid Pqaqr-2::pqaqr-2::GFP, using primers 5’-tgctcgcagatggagagtagatgggatgg-3’ and 5’-ctcggtctattcttttgatttataaatgtgagagaacaaactagccgcag-3’. The assembled plasmid was injected into N2 worms at 5 ng/μl together with 3 ng/μl Pmyo-2::gfp marker.

For Pges-1:pqaqr-2::GFP, an intestine-specific transgene was constructed using a Gibson assembly cloning kit (NEB)
by assembly of the following two DNA fragments: 2 kb upstream regulatory sequences from \textit{ges-1} was amplified from \textit{N2} genomic DNA using the primers 5’-cgaatctcgtgacccccggg gatcaaatctatgtaagaggta-3’ and 5’-cgaatctcgtgacccccggg gatcaaatctatgtaagaggta-3’; and the \textit{paqr-2} coding sequence with GFP was amplified from the plasmid \textit{Ppaqr-2::paqr-2::GFP} using primers 5’-atggaggaagagtcgcttg aatcgccagaaccgcggaatcgcaaaaat-3’ and 5’-gaatctccgggg gctgagagatctgtaagaggta-3’. The assembled plasmid was injected into \textit{N2} worms at 5 ng/\mu l together with 40 ng/\mu l \textit{pRF4}.

For \textit{Plim-7::paqr-2::GFP}, a gonad sheath-specific transgene was constructed using a Gibson assembly cloning kit (NEB) by assembly of the following two DNA fragments: 2 kb upstream regulatory sequences from \textit{lim-7} was amplified from \textit{N2} genomic DNA using the primers 5’-tggatccgccctgtcta gattgaactctgtatggaatg-3’ and 5’-attcagctcatctctcctca taatcgccatcgggtg-3’; and the \textit{paqr-2} coding sequence with GFP was amplified from the plasmid \textit{Ppaqr-2::paqr-2::GFP} using primers 5’-atggaggaagagtcgcttg aatcgccagaaccgcggaatcgcaaaaat-3’ and 5’-gaatctccgggg gctgagagatctgtaagaggta-3’. The assembled plasmid was injected into \textit{N2} worms at 5 ng/\mu l together with 40 ng/\mu l \textit{pRF4}.

For \textit{Plim-7::fat-6::GFP}, a gonad sheath-specific \textit{fat-6} transgene was constructed using a Gibson assembly cloning kit (NEB) by assembly of the following two DNA fragments: the \textit{fat-6} coding sequence was amplified from \textit{N2} genomic DNA using the primers 5’-ATGACGTTAAAATCTGTC-3’ and 5’-ATCCAGTCTTGAAGACCTAT-3’; and the \textit{lim-7} promoter with vector sequence was amplified from \textit{Plim-7::paqr-2::GFP} plasmid using primers 5’-ATCCAGTCTTGAAGACCTAT-3’ and 5’-TGAAACGAGTTT TTACGCTCATAAACTGCCATCGGTGTA-3’. The assembled plasmid was verified by sequencing. The plasmid was injected into \textit{fat-6} mutant worms at 5 ng/\mu l together with 40 ng/\mu l \textit{pTG96}.

For \textit{Plelt-3::fat-6::GFP}, a hypodermis-specific \textit{fat-6} transgene was constructed using a Gibson assembly cloning kit (NEB) by assembly of the following two DNA fragments: the \textit{fat-6} coding sequence was amplified from \textit{N2} genomic DNA using the primers 5’-ATGACGTTAAAATCTGTC-3’ and 5’-CCCGGGGTTCGGTTTGC-3’; and the \textit{elt-3} promoter with vector sequence was amplified from \textit{Plelt-3::paqr-2::GFP} plasmid using primers 5’-GCAAGCCGAAACGCCCGGTTTA TAAATCAAAGAATAGA-3’ and 5’-GAACGAAGTTTTACCTGTA CATCCAGCTTGCATGACAGGCT-3’. The assembled plasmid was verified by sequencing. The plasmid was injected into \textit{fat-6} mutant worms at 5 ng/\mu l together with 40 ng/\mu l \textit{pTG96}.

**Mosaic analysis**

The plasmid \textit{pTG96} carrying the \textit{sur-5::gfp(NLS)} was co-injected into the gonad syncytium of wild-type worms at a concentration of 50 ng/\mu l together with 5 ng/\mu l of \textit{pGLR-2} or \textit{pPAQR-2} to establish a transgenic line. The extrachromosomal array was then crossed into the \textit{iglr-2} or \textit{paqr-2} mutant background, and these transgenic worms were bleached and their eggs allowed to hatch overnight in M9 to produce synchronized L1s that were transferred to NGM plates containing 20 mM glucose. Worms that grew into adults were scored 72 hr later.

**Growth and tail tip scoring assays**

For length measurement studies, synchronized L1s were plated onto test plates seeded with \textit{E. coli}, and worms were mounted then photographed 72 hr later. The length of \textit{>20} worms was measured using ImageJ (Schneider et al. 2012). Quantification of the withered tail tip phenotype was done on synchronous 1-day-old adult populations, \textit{i.e.}, 72 hr post L1 (\textit{n} \geq 100) (Svensk et al. 2013).

**FRAP in \textit{C. elegans} and HEK293 cells**

FRAP experiments in \textit{C. elegans} were carried out using a membrane-associated prenylated GFP reporter (pGL-1P::GFP-CAAX) expressed in intestinal cells, as previously described, using a Zeiss LSM700inw laser scanning confocal microscope with a 40x water immersion objective (Mörck et al. 2009; Svensk et al. 2016; Devkota et al. 2017). For FRAP in mammalian cells, HEK293 cells were stained with BODIPY 500/510 C1, C12 (4,4-difluoro-5-methyl-4-bora-3a,4a diaza-s-indacene-3-dodecanonic acid; BODIPY-C12) (Invitrogen, Carlsbad, CA). FRAP images were acquired with an LSM880 confocal microscope equipped with a live cell chamber (set at 37° and 5% CO2) and ZEN software (Zeiss, Thornwood, NY) with a 40x water immersion objective as previously described (Devkota et al. 2017).

**Vitellogenin assay**

Vitellogenin was analyzed in \textit{N2}, \textit{paqr-2}, and \textit{iglr-2} worms using the \textit{pwl23} (vit-2::GFP) transgene. Synchronized \textit{vit-2::GFP}, \textit{paqr-2::GFP} and \textit{iglr-2::GFP} worms were spotted on control plates and incubated at 20°. After 72 hr of incubation at 20°, they were washed off the plate and mounted on agarose pads, and then observed with a Zeiss Axiophot microscope at 400x magnification. Worms were scored based on wild type and defective vitellogenin accumulation (\textit{n} \geq 100).

**Germline morphology**

Germline morphology was analyzed in \textit{N2}, \textit{paqr-2}, and \textit{iglr-2} worms using \textit{4’,6’-diamidino-2-phenylindole hydrochloride} (DAPI) staining. Synchronized worms were spotted on control plates and after 96 hr of incubation at 20°, they were washed and fixed with (\textit{-20°}) methanol for 5 min. The supernatant was then removed and the worms washed twice with PBST. The fixed samples were then stained in 100 ng/ml DAPI in PBST for 30 min, washed twice with PBST and mounted on agarose pads, and then observed and photographed using a Zeiss Axiophot microscope.

**Germline apoptosis**

Apoptotic cells was analyzed in \textit{N2}, \textit{paqr-2}, and \textit{iglr-2} worms using the \textit{bcl39} [\textit{Plim-7::ced-1::gfp; lin-15(+)}] transgene (Zhou et al. 2001). Synchronized \textit{bcl39}, \textit{paqr-2};\textit{bcl39} and
Igdr-2; bcls39 worms were spotted on control plates and incubated at 20°C. After 72 hr of incubation at 20°C, they were washed off the plate and mounted on agarose pads, and then observed and photographed using a Zeiss Axioskop microscope. Worms were scored by counting the number of apoptotic cells in the germline (n ≥ 20).

Germline membrane morphology

Germline membrane morphology was analyzed in N2, паqr-2, and igdr-2 worms using the. lts38 (pie-1p::GFP::PH (PLCdelta1) + unc-119(+)) transgene (McNally et al. 2006). Synchronized worms were spotted on control plates and incubated at 20°C. After 72 hr of incubation at 20°C, they were washed off the plate and mounted on agarose pads, and then observed and photographed using a Zeiss Axioskop microscope.

Cultivation of HEK293

HEK293 were grown in DMEM containing glucose 1 g/liter, pyruvate, and Glutamax and supplemented with 10% fetal bovine serum, 1% nonessential amino acids, HEPES 10 mM, and 1% penicillin and streptomycin (all from Life Technologies) at 37°C in a water-humidified 5% CO2 incubator. Cells were subcultured twice a week at 90% confluence. Cells were cultivated on treated plastic flask and multidish plates (Nunc). For FRAP experiments, HEK293 were seeded in μ-dishes (35 mm, high) containing culture-insert four wells (Ibidi) and precoated with 0.1% porcine gelatin (Sigma, St. Louis, MO).

Small interfering RNA in HEK293 cells

The following predesigned small interfering RNAs (siRNAs) were purchased from Dharmacon: Adipor2 J-007801-10, nontarget D-001810-10 and Δ9 stearoyl-CoA desaturase (SCD) J-005061-07. Transfection of 25 nM siRNA was performed in complete media using Viromer Blue according to the manufacturer’s instructions “IIX” (Lipocalyx). Knockdown gene expression was verified 48 hr after transfection.

Quantitative PCR in HEK293 cells

Total cellular RNA was isolated using an RNaseasy Kit according to the manufacturer’s instructions (Qiagen, Valencia, CA) and quantified using a NanoDrop spectrophotometer (ND-1000; Thermo Scientific). Complementary DNA was obtained using a RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo) with random hexamers. Quantitative PCR was performed with a CFX Connect thermal cycler (Bio-Rad, Hercules, CA) using Hot FIREpol EvaGreen qPCR SuperMix (Solis Biodyne) and standard primers. Samples were measured as triplicates. The relative expression of each gene was calculated according to the ΔΔCT method (Livak et al. 2001). Expression of the housekeeping gene PPIA was used to normalize for variations in RNA input. Primers used were as follows: Adipor2, forward (TCATCTGTGCTGGCCGATT) and reverse (CTATCTGCTATGGTGCGG); PPIA, forward (GTCTCCTTTGAGCCTGTTGCAAG) and reverse (GGAGAACATGCCAGGGACCC); and SCD, forward (TTCGTTGCACCTTTCTCTTGC) and reverse (TGGTGGTAGTTGTGGAAGCC).

HEK293 fatty acid treatment

Palmitic acid (PA; Sigma) was dissolved in sterile DMSO (Sigma) then mixed with fatty acid-free BSA (Sigma) in serum-free medium for 20 min at room temperature. The molecular ratio of BSA to fatty acid was 1–2.65 (when using 200 μM PA) or 1–5.3 (when using 400 μM PA). Cells were then cultivated in this serum-free media containing the fatty acids for 24 hr prior to FRAP analysis.

Laurdan dye measurement of membrane fluidity in HEK293 cells

Live HEK293 cells were stained with Laurdan dye (6-dodecanoyl-2-dimethylaminophosphalene) (Thermo Fisher) at 10 μM for 45 min. Images were acquired with an LSM880 confocal microscope equipped with a live cell chamber (set at 37°C and 5% CO2) and ZEN software (Zeiss) with a 40× water immersion objective. Cells were excited with a 405 nm laser and the emission recorded between 410 and 461 nm (order phase) and between 470 and 530 nm (disordered phase). Pictures were acquired with 16 bits image depth and 1024 × 1024 resolution, using a pixel dwell of ~1.02 μsec. Images were analyzed using ImageJ software (Schneider et al. 2012), following published guidelines (Owen et al. 2011).

HEK293 transwell plate experiments

HEK293 cells were grown in transwell permeable supports (polyester membrane, 0.4 μm pore size) (Costar, Cambridge, MA) and are referred to as donor cells in the text. Then, medium in the upper chamber was replaced by serum-free medium supplemented with 0.5% BSA and [9,10-3H(N)]-palmitic acid (Perkin Elmer, Norwalk, CT) at a specific activity of 2 μCi for 4 hr. The medium in the lower was serum-free medium. Media were collected (loading medium), the donor cells were washed with serum-free medium and then moved to new wells preseeded with HEK293 cells (acceptor cells) and cultured with serum-free medium with ±0.5% BSA or 10% fetal bovine serum. Finally, after 24 hr, the transferring media was collected and cells were recovered with TrypLE. Sample activity was measured with a TRI-CARB 4810TR 110 V Liquid Scintillation Counter (Perkin Elmer).

Statistics

Error bars for worm length measurements show the SEM, ANOVA and Dunnett’s multiple comparisons test were used to identify significant differences between worm lengths. Error bars for the frequency of the tail tip defect show the 95% confidence interval and significant differences determined using Z-tests. t-tests were used to determine significance in FRAP experiments. All experiments were repeated at least twice with similar results. Asterisks are used in the figures to indicate various degrees of significance, as follows: * P < 0.05, ** P < 0.01, *** P < 0.001.
Data availability

The complete mosaic analysis data are presented in the Supplemental Material, Table S1, and three figures are provided as supplemental materials (Figures S1–S3). Supplemental material available at Figshare: https://doi.org/10.25386/genetics.6608843.

Results

Mosaic analysis identifies sufficient sites of action for paqr-2 and iglr-2

We performed an extensive mosaic analysis to identify tissues where expression of paqr-2 and iglr-2 are sufficient to rescue the mutant phenotypes. In C. elegans, transgenes are typically retained as extrachromosomal arrays that are not always segregated to both daughter cells during cell division, resulting in genetic mosaics. If the array carries a GFP marker, one can easily determine which cells are transgenic in a mosaic individual and later correlate sites of expression with phenotypes (Yochem et al. 1998). The results of our mosaic analyses show that expression of paqr-2 and iglr-2 in either hypodermis or gonad sheath cells is sufficient to suppress the glucose sensitivity of the mutants (Figure 1, Figure S1, and Table S1). In particular, five worms that had paqr-2 expression in gonad sheath cells had no expression in the hypodermis, lacking the extrachromosomal transgene from the ABp lineage and carrying it in just a few nonhypodermal cells of the ABA lineage (Figure 1A). These worms were most likely rescued because of the expression of paqr-2 in gonad sheath cells, where both PAQR-2 and IGLR-2 are predominantly expressed (Svensk et al. 2016). In many other mosaic paqr-2 and iglr-2 mutant worms, expression of the corresponding wild-type transgene was entirely lost from the P1 lineage but retained in hypodermal cells of the AB lineage, suggesting that expression in the hypodermis is also sufficient to rescue the mutant.

Tissue-specific expression confirms the cell nonautonomous activity of paqr-2

We were intrigued by the possibility that localized expression of paqr-2 could rescue systemic phenotypes cell nonautonomously. To explore this further, we generated several tissue-specific paqr-2 expression transgenes (Figure 2A) and scored their ability to suppress paqr-2 mutant phenotypes. We found that paqr-2 expression in either hypodermis or gonad sheath cells is sufficient to suppress glucose toxicity (Figure 2, B and C), cold intolerance (Figure 2B), tail morphology (Figure 2, D and E), and brood size (Figure 2F). We also found that expression of paqr-2 specifically in the intestine itself (which could not be scored in our mosaic analysis because it obscures expression in other tissues; Yochem et al. 1998) could also rescue these phenotypes (Figure 2, A–E). Importantly, membrane fluidity in the intestine was normalized by hypodermis-specific paqr-2 expression (Figure 2, G and H). Altogether these results provide very strong evidence that paqr-2 can act cell nonautonomously to support systemic membrane homeostasis in C. elegans.

Expression of paqr-2 in body wall muscles partially rescued some mutant phenotypes, including tail morphology and brood size (Figure 2, A–E), suggesting that even this tissue is able to provide some systemic benefits to membrane homeostasis. However, muscle-specific expression was not able to suppress glucose intolerance and cold sensitivity, which is consistent with the mosaic analysis results (Figure 1A).

fat-6 is required for paqr-2 function in the hypodermis

The primary defect in paqr-2 mutants appears to be an inability to enhance fatty acid desaturation when cellular membrane becomes too rigid, and Δ9 desaturases appear especially important for this process because many paqr-2 suppressor mutations act by promoting their activity (Svensk et al. 2013). In particular, we previously showed that the paqr-2 mutant has reduced expression of the intestinal Δ9 desaturase Pfat-7::GFP reporter (Svensk et al. 2013), which we again confirmed in this study (Figure 3A). Of all the transgenes tested, only paqr-2 driven from its own promoter was able to significantly raise the levels of Pfat-7::GFP expression (Figure 3A). This is somewhat surprising given that these same tissue-specific paqr-2 transgenes are able to at least partially suppress most paqr-2 phenotypes. There are three Δ9 desaturases in C. elegans, namely fat-5, fat-6, and fat-7, and these show functional redundancies: single mutants are phenotypically wild type and only the triple mutant is lethal (Brock et al. 2007). fat-6 and fat-7 share a very high degree of sequence identity, predominantly carry out the desaturation of stearate (18:0), and their inhibition using RNA interference causes a significant reduction in de novo lipogenesis accompanied by reduced membrane turnover (Dancy et al. 2015). Both fat-6 and fat-7 are required for the suppression of paqr-2 phenotypes by mutations that promote fatty acid desaturation (Svensk et al. 2013). However, at least two differences exist between fat-6 and fat-7: fat-6 is expressed in both the intestine and hypodermis (Brock et al. 2006) (Figure 3, B–E), whereas fat-7 expression seems restricted to the intestine (Brock et al. 2006); and the paqr-2;fat-6 double mutant is viable but sterile whereas the paqr-2;fat-7 double mutant is viable and fertile (Svensson et al. 2011). Since we did not observe upregulation of Pfat-7::GFP in the paqr-2 mutants rescued with various tissue-specific paqr-2 transgenes, we decided to explore the possibility that rescue of paqr-2 may be dependent on fat-6. We found that paqr-2 mutant worms carrying the wild-type Ppaqr-2 construct on an extrachromosomal array show a weaker suppression of the tail tip defect when either fat-6 or fat-7 is mutated, implicating both genes as paqr-2 effectors (Figure 3F). Next, we found that hypodermis-specific expression of paqr-2 is synthetic sterile with the fat-6 but not with the fat-7 mutation when the worms are challenged with 20 mM glucose (Figure 3G). Similarly, expression of paqr-2 specifically in gonad sheath cells rescues the growth of paqr-2 or paqr-2;fat-7 mutants on normal plate and plates containing glucose, but does not rescue fertility of the paqr-2;fat-6 double mutant (Figure S2, A and B). In contrast, intestine-specific expression of paqr-2 suppresses the...
glucose intolerance of the \textit{paqr-2} mutant even when either \textit{fat-6} or \textit{fat-7} is mutated (Figure 3G). \textit{fat-6} was also not required for the suppression of the brood size defect by intestine-specific expression of \textit{paqr-2} (Figure 3H). These results show that \textit{fat-6} is required for the ability of hypodermis-specific expression of \textit{paqr-2} to rescue the tail and glucose tolerance, but dispensable for the intestine-specific expression of \textit{paqr-2} to rescue these traits as well as brood size. The simplest interpretation is that hypodermis-specific or gonad sheath cell-specific expression of \textit{paqr-2} requires \textit{fat-6} to provide systemic phenotypic rescue.

Intestine-specific expression of \textit{paqr-2} is not critically dependent on either \textit{fat-6} or \textit{fat-7} because both are expressed and functionally redundant in that tissue (Brock et al. 2006).

We used tissue-specific expression of \textit{fat-6} to further investigate its site of action. In these experiments, we found that hypodermis-specific expression of both \textit{paqr-2} and \textit{fat-6}, driven by the \textit{elt-3} promoter is sufficient to suppress the infertility, glucose intolerance, and the tail tip defects in \textit{paqr-2};\textit{fat-6} double mutant (Figure S2, C–E). On the other hand, gonad sheath cell-specific expression of \textit{paqr-2} and \textit{fat-6} did not rescue the fertility
defect of the paqr-2;fat-6 double mutant (Figure S2, D and E).
Altogether, these results suggest that paqr-2;fat-6-dependent lipid metabolism in the hypodermis is sufficient to rescue the worms systemically but that the gonad sheath cells may have different requirements to fulfill this function.

**Vitellogenin transport defects in the paqr-2 and iglr-2 mutant**

The above results suggest that the hypodermis, and perhaps other tissues as well, can produce UFAs that are throughout the worm to achieve cell nonautonomous and systemic membrane homeostasis. One key lipid transport mechanism in *C. elegans* is that of the vitellogenins, which are lipoprotein-like proteins secreted into the pseudocoelom by the intestine, transported through the gonad sheath cells and ultimately endocytosed by maturing oocytes via receptors of the LDL receptor superfamily such as RME-2 (Kimble and Sharrock 1983; Grant and Hirsh 1999; Hall et al. 1999).

We therefore explored a possible connection between the functions of *paqr-2* and *iglr-2* in membrane homeostasis and the lipid transport roles of vitellogenins. A careful examination of the gonads revealed that the *paqr-2* and *iglr-2* mutants
have an abundance of abnormal germ cell nuclei in the pachytene zone (Figure 4, A–N), and an excess of apoptotic bodies (Figure 4, O–U) in the germline (which could be due to excess apoptosis or defects in phagocytosis of apoptotic corpses). This is accompanied by a dramatic accumulation of vitellogenins in the pseudocoelom of the mutants (Figure 4, V–Y); this defect in lipid transport likely contributes to the low brood size and increased germline apoptosis in these mutants. Note that there are no visible germline defects in paqr-2 mutant worms at the L4 stage, i.e., before yolk production begins (Figure S2F). Importantly, and just as with the intestinal membrane fluidity defect, the vitellogenin uptake defect in the paqr-2 mutants can also be rescued cell nonautonomously by expression of paqr-2 specifically in intestine, hypodermis, or muscle, or cell-autonomously by expression of paqr-2 in the gonad sheath cells. We conclude that at least some forms of lipid transport, namely the transport of vitellogenins into the gonads, depends on the function of paqr-2 and iglr-2 and may contribute to the phenotypes of these mutants.

**Cell nonautonomous membrane homeostasis is conserved in human cells**

We previously showed that the human AdipoR1 and AdipoR2 proteins are functional orthologs of the *C. elegans* PAQR-2: they regulate membrane composition and fluidity in HEK293 cells (derived from human embryonic kidneys) and are necessary to prevent membrane rigidification by the SFA PA (Devkota et al. 2017). We now examined whether the cell nonautonomous regulation of membrane homeostasis may also be conserved between *C. elegans* and humans. For this purpose, we made use of four-quadrant partitioned culture dishes to cultivate HEK293 cells in serum-free conditions but containing albumin, and used siRNA to inhibit the human AdipoR2 in some quadrants, then removed the partition and later measured membrane fluidity of cells in all quadrants using FRAP. Note that the FRAP method is reliable in mammalian cells: it has been used in numerous studies (Reits and Neefjes 2001; Staras et al. 2013), it documents membrane rigidity that correlates with SFA content in HEK293 cells (Devkota et al. 2017), and it matches results obtained with a separate method, namely the Laurdan dye method (Owen et al. 2011) (Figure S3, A–F). The results show that AdipoR2-expressing cells can act at a distance to maintain membrane fluidity in remote PA-challenged cells where AdipoR2 is silenced (Figure 5, A–D). Inhibiting the SCD gene in HEK293 cells also impairs their ability to prevent rigidification by PA, which is consistent with SCD being a downstream target of AdipoR2 (Figure 5, E and F), just as Δ9 desaturases act downstream of PAQR-2 in *C. elegans*. Additionally, SCD-expressing cells are also able to cell nonautonomously prevent membrane rigidification by PA in distant cells where SCD has been silenced (Figure 5, E and F). It therefore seems likely that AdipoR2-positive cells respond to the rigidifying PA challenge in a SCD-dependent manner to produce fluidizing UFAs that they can share with distant cells with which they have no physical contact. Note that the serum-free culture conditions used are likely stressful to these cells adapted to cultivation in the presence of serum (which contains cholesterol), and that the use of PA likely causes other effects besides membrane rigidification, including ER stress and...
inflammation (Peter et al. 2009). We therefore sought to more directly test whether mammalian cells can exchange lipids over significant distances and without cell-cell contact. Using transwell plates, we found that donor cells pre-treated with ^3H-labeled PA are able to deliver it to naïve acceptor cells from which they are separated by a membrane barrier, and that this transport is dependent on the presence of albumin or whole serum in the culture media (Figure S3, G–L).

**Discussion**

Our extended mosaic analysis and tissue-specific expression experiments demonstrate convincingly that *paqr-2* and *iglr-2* can function cell nonautonomously and systemically when expressed in large tissues such as the hypodermis, gonad sheath, intestine, and body wall muscles. Our observations suggest a model where the PAQR-2/IGLR-2 complex can sense membrane rigidity in any large membranous tissue to induce local production of UFAs that are then shared with the
rest of the worm (Figure 6). This model presumes extensive flux of fatty acids among all tissues and thus that, for example, expression of the PAQR-2/IGLR-2 complex specifically in the hypodermis will cease promoting fatty acid desaturation only when fluidity is normalized throughout the entire worm.

We have one strong experimental support for this model: hypodermis-specific paqr-2 expression, but not intestine-specific paqr-2 expression, requires fat-6 for systemic rescue of glucose tolerance (Figure 3, G and H). Because fat-6 is the only desaturase expressed in the hypodermis (Brock et al. 2006), this result suggests that UFA production in the hypodermis is responsible for the systemic rescue in worms expressing paqr-2 only in the hypodermis, a site of fat storage (Hellerer et al. 2007; Zhang et al. 2010). This conclusion is further strengthened by the fact that hypodermis-specific expression of both paqr-2 and fat-6 is sufficient to rescue all phenotypes of the paqr-2;fat-6 double mutant (Figure S2, C–E). The transport of lipids among C. elegans tissues is likely mediated via the pseudocoelomic fluid and may involve vitellogenins, other lipoprotein-like proteins such as DSC-4, or other pathways (Branicky et al. 2010). That lipids are in constant flux among C. elegans tissues should come as no surprise given that nearly 80% of phospholipids are replaced daily in an adult C. elegans, mostly using dietary fatty acids as building blocks, and thus implying extensive trafficking between the intestine and other tissues (Dancy et al. 2015).

Although the evidence is rather strong that phospholipids can be exchanged among C. elegans tissues, it is possible that, at least in some cases, signaling also contributes to systemic membrane homeostasis. For example, the PAQR-2/IGLR-2 complex could sense membrane rigidity in one tissue then signal to the intestine to promote fatty acid desaturation in that organ. The intestine would, in turn, provide more UFAs to all cells of the worm, presumably via the pseudocoelom.

Speculating further, it is possible that activation of the presumed PAQR-2 hydrolase activity (Holland et al. 2011; Pei et al. 2011; Vasiliauskaité-Brooks et al. 2017) in large membranes causes the production of a signaling molecule that can diffuse to the intestine where it promotes fatty acid desaturation. One merit of this hypothesis is that it relies on the intestine for the production and distribution of fatty acids, a well-documented role for this organ. Signaling between the intestine and other tissues has also been documented. For example, the gonad is able to communicate to the intestine to regulate vitellogenin production (Balklava et al. 2016) whereas the intestine can regulate germline sex determination via specific signaling fatty acids (Tang and Han 2017). Similarly, the hypodermis can modulate lipid accumulation in the intestine via a sma-3 dependent signal (Clark et al. 2018). This signaling hypothesis may explain why expression of paqr-2 and fat-6 did not rescue the fertility in the paqr-2; fat-6 double mutant: systemic paqr-2-dependent membrane homeostasis from the gonad sheath cells may depend on a signal reaching fat-6–expressing tissues such as the hypodermis or intestine.

Our results with human HEK293 cells show that the cell nonautonomous nature of membrane homeostasis regulation is evolutionarily conserved from nematodes to mammals.
esters and a surface monolayer of phospholipid, consisting of a droplet of triacylglycerols and/or cholesteryl esters, which make up 10% of blood fatty acids (van der Vusse 2009). Fatty acid esters, which make up 90% of blood fatty acids, are transported in blood by lipoprotein particles homologous to the C. elegans vitellogenins. These particles are made of a core consisting of a droplet of triacylglycerol and/or cholesteryl esters and a surface monolayer of phospholipid, cholesterol, and specific proteins (apolipoproteins), e.g., apolipoprotein B-100 in the case of LDL.

An additional interesting finding in this study was the detection of several germline defects (vitellogenin uptake defect, increased apoptosis, defects in membranes of maturing oocytes), which all likely contribute to the low brood size of the paqr-2 and iglr-2 mutants. The accumulation of VIT-2::GFP in the pseudocoelom of the paqr-2 and iglr-2 mutants likely reflects a defect of the gonad to take up vitellogenins; this defect leads to increased production of vitellogenins by the intestine because of feedback mechanism between the germline and intestine (Balklava et al. 2016). This does not necessarily imply a primary gonad sheath membrane defect: others have shown that omega-6 PUFAs are essential for germline development (Chen et al. 2016) and to regulate the balance of lipid stores between the soma and germ cells (Lynn et al. 2015), and their production, or that of other regulatory lipids, may be defective in the paqr-2 and iglr-2 mutants.

The most important finding of this study is that membrane homeostasis is achieved cell nonautonomously both in a whole organism (C. elegans) and among cultivated human cells. Physiologically, there are clear benefits to cell nonautonomous maintenance of membrane homeostasis, including achieving “ordered heterogeneity,” a fundamental property of biological systems whereby tissues achieve healthy homogeneity even though they are composed of heterogeneous cells experiencing various levels of stresses or mutations (Rubin 2006). It will be interesting to explore the relevance of these findings for human physiology. For example, tumor cells with abnormal lipid metabolism could affect the membrane composition of neighboring healthy cells. Conversely, the cell nonautonomous nature of membrane homeostasis may contribute to the robustness of whole organisms in response to dietary or disease-state challenges, with impaired tissues or cells continuing to have functional membranes because of healthy neighbors.

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