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Leveraging the withered tail tip phenotype in *C. elegans* to identify proteins that influence membrane properties

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ABSTRACT

The properties of cellular membranes are critical for most cellular functions and are influenced by several parameters including phospholipid composition, integral and peripheral membrane proteins, and environmental conditions such as temperature. We previously showed that the *C. elegans paqr-2* and *iglr-2* mutants have a defect in membrane homeostasis and exhibit several distinct phenotypes, including a characteristic tail tip defect and cold intolerance. In the present study we report that screening for novel mutants with these 2 defects can lead to the identification of genes that are important contributors to membrane properties. In particular we isolated 3 novel alleles of *sma-1*, the *C. elegans* homolog of βH spectrin, and 2 novel alleles of *dpy-23*, which encodes the *C. elegans* homolog of the AP2 μ subunit. We also show that *sma-1* and *dpy-23* act on membrane properties in pathways distinct from that of *paqr-2* and *iglr-2*.

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Introduction

Membranes are critical for most cellular processes, and their properties, such as composition and fluidity, must be tightly regulated so as to achieve what may be called "membrane homeostasis."[1-4] We have previously shown that *C. elegans* mutants lacking *paqr-2* or *iglr-2* have defects in membrane fluidity accompanied by 3 obvious phenotypes: a characteristic tail tip morphology defect, inability to grow at low temperatures, and inability to grow in the presence of glucose.[5-7]

The tail tip defect is particularly interesting for several reasons: 1) it is the only *paqr-2* and *iglr-2* mutant phenotype that is readily visible in worms cultivated under standard conditions at 20°C; 2) This phenotype is suppressed by the inclusion of small amounts of nonionic detergent (e.g. 0.05% NP40) in the culture plates; and 3) it affects a distinct and limited number of cells, namely the 4 hypodermal cells of the tail tip, and is very distinct from the tail tip phenotype of the *cdh-3* mutant in which the terminal hypodermal cell exhibits a range of defects including kinked and forked appearances.[8] Here, we characterize the *paqr-2* tail tip phenotype in some more detail and, to identify other genes that influence membrane properties, we report the results of a screen for *paqr-2* genocopiers, i.e. novel mutants that exhibit the same phenotypes as the *paqr-2* mutant.

Results

The tail tip phenotype in hermaphrodites and males

The hermaphrodite tail tip of *paqr-2* mutants is short and withered (Fig. 1A–B). The tail tip in the hermaphrodite is composed of 4 hypodermal cells: the binucleate hyp10 cell makes up the extreme tip of the tail, which is in contact with hyp11 on the dorsal side and hyp9 and hyp8 on the ventral side and anteriorly to hyp10. These cells are born during early embryogenesis and acquire their tapered shapes during the elongation phase of development, which is then maintained throughout the life of the healthy hermaphrodite.[9,10] The hermaphrodite tail tip defect in *paqr-2* mutants is post-developmental and manifests itself as the tail tip grows in size during the larval stages; this was confirmed using the adherence junction marker *ajm-1::GFP* to monitor the shape of the tail hypodermal cells during development. Specifically, the hyp8-10 cells are present and properly shaped in early *paqr-2* larvae, and begin to show signs of deformation at the L3

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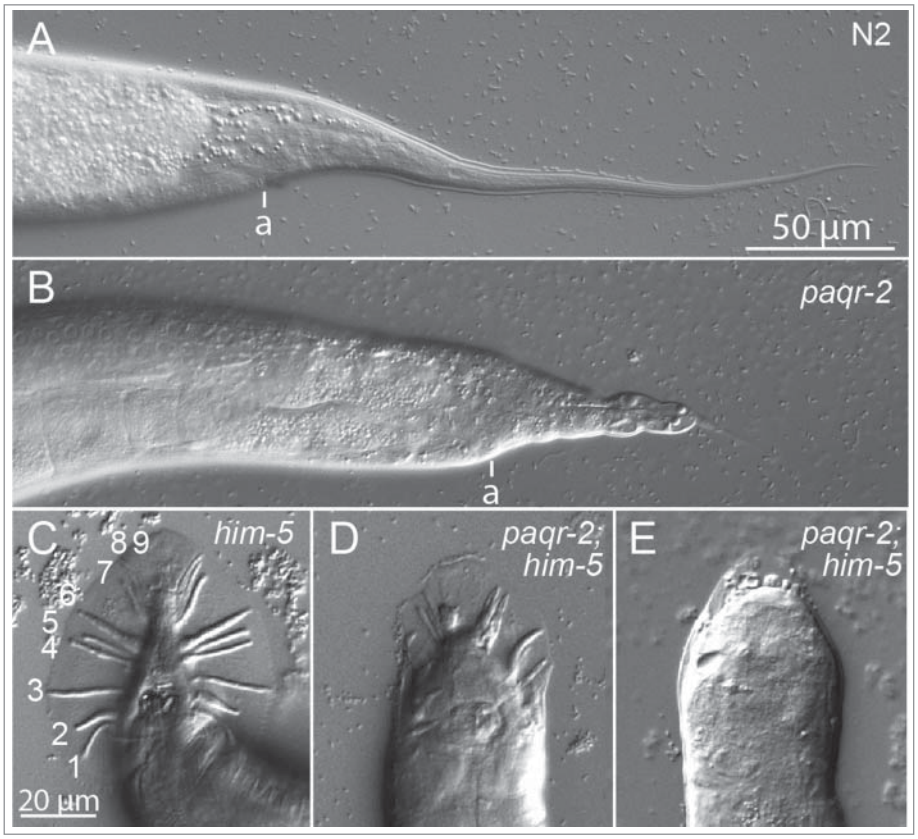

Figure 1. *paqr-2* mutant defects in the hermaphrodite tail tip and male tail. (A) Hermaphrodite tail tip in an adult wild-type worm. Note the long tapered shape of the tail tip posterior to the anus (a). (B) Tail tip of an adult hermaphrodite *paqr-2* mutant. Note the deformed and truncated shape. (C) Normal male tail in an adult *him-5* worm, with the 9 rays indicated on the left side of the tail. Note also the smooth fan held uniformly spread by the rays. (D-E) Deformed male tail in 2 adult *paqr-2; him-5* mutant worms. The worm in (D) has grossly deformed rays and fan, while the worm in (E) has no visible rays and nearly no fan at all.

stage, which becomes increasingly more severe in L4 larvae and adult worms (Fig. 2).

The male tail is a specialized structure composed of many cells born post-embryonically, and its development involves cell migrations, fusions, differentiation into neurons and 9 pairs of bilateral rays embedded in a cuticular fan, as well as spicules, hooks and other structures used in mating.[9,11] Development of the male tail is relatively fragile and male tail defects are found in mutants affecting the TGF-β pathway,[12] the Wnt pathway,[13] cuticular glycoproteins[14] and several other pathways. The tail fan and rays are often missing or deformed also in *paqr-2* mutant males (Fig. 1C–E).

Screen for paqr-2 *genocopiers*

We performed a forward genetic screen of 80 000 mutagenized haploid genomes for *paqr*-2 genocopiers, i.e., mutants that exhibit a tail tip defect and cold sensitivity similar to that of *paqr-2* mutants. The term "genocopier" is derived from "genocopy" which is defined as a "genetic mutation that results in a phenotype known to be caused by another mutation or process."[15] Ten candidate *paqr-2 g*enocopier mutants were selected for further studies because they most resembled *paqr-2* for these 2 phenotypes. These were outcrossed 6 times and their genomes sequenced, and outcrossed at least 8 times prior to detailed phenotypic characterization. We previously reported that 5 of the picked mutants were 2 alleles of *paqr-2* itself (*et35* and *et36*) and 3 alleles of *iglr-2* (*et34, et37* and *et38*), and that these mutants had identical phenotypes in all aspects, including tail tip defect, cold sensitivity, excess saturated fatty acids, glucose sensitivity, impaired regulation of membrane fluidity and suppression by detergents or mutations that promote fatty acid desaturation.[7] The five other mutants picked in the screen correspond to 3 alleles of *sma-1* (*et39, et40* and *et44*) and 2 alleles of *dpy-23* (*et42* and *et43*) (Fig. 3A–B). These novel *sma-1* and *dpy*-23 alleles are here described in details, with the null *paqr-2(tm3410)* and *iglr-2(et34)* alleles used for comparison purposes.

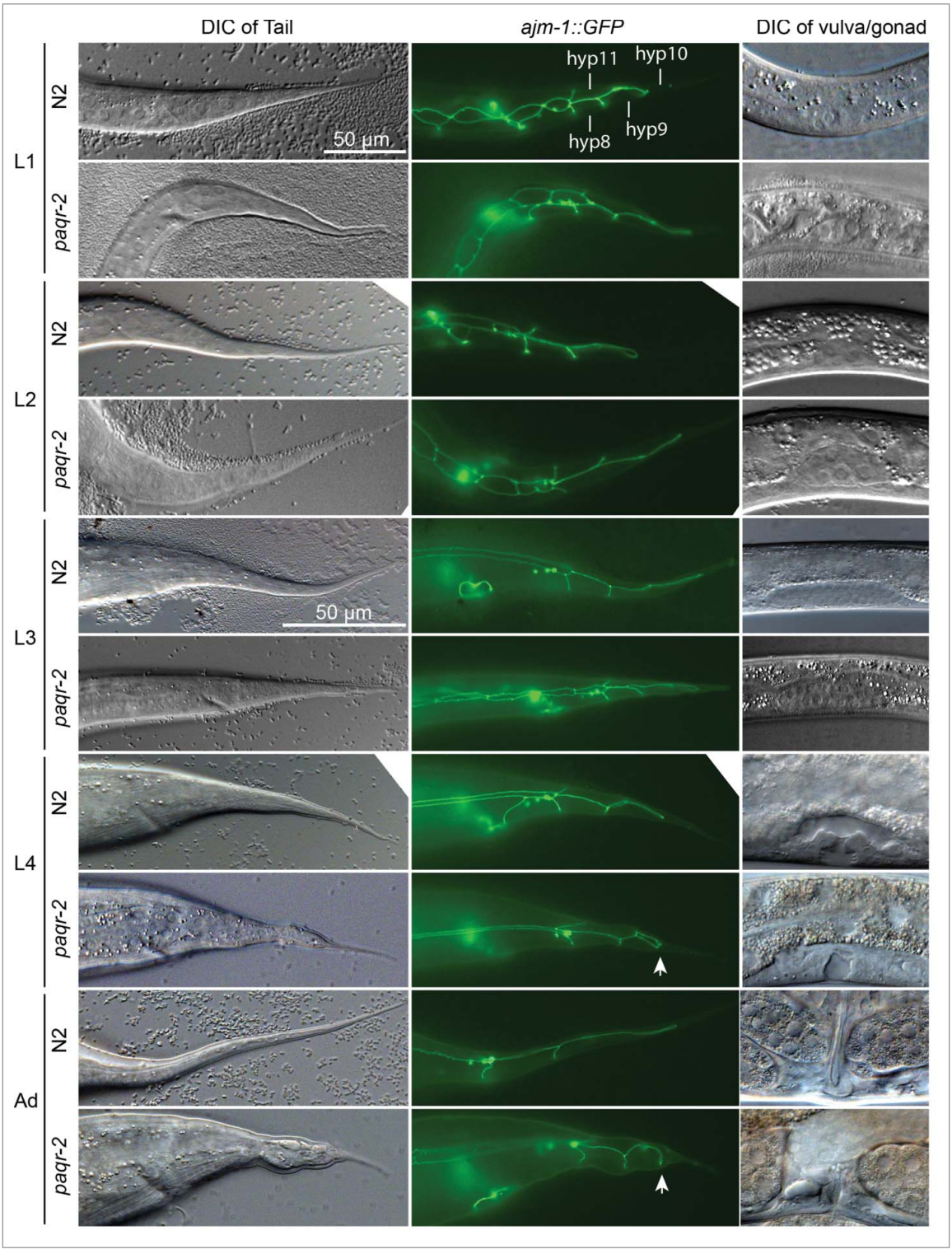

Figure 2. Hermaphrodite tail tip in wild type and *paqr-2* mutants during post-embryonic development. The tail hypodermal cells were monitored in wild-type (N2) or *paqr-2* mutants carrying the *ajm-1::GFP* transgene during larval development, and the stage of the worms were verified by scoring the gonad/vulva, as shown in the right-most column. The hyp8-11 cells are indicated in the wild-type L1 larva. Note that the *paqr-2* mutant shows no tail tip defect in the L1 and L2 stages, but begins to exhibit deformations in hyp10 at the L3 stage, which worsens and affects also hyp8-11 in later stages (arrows).

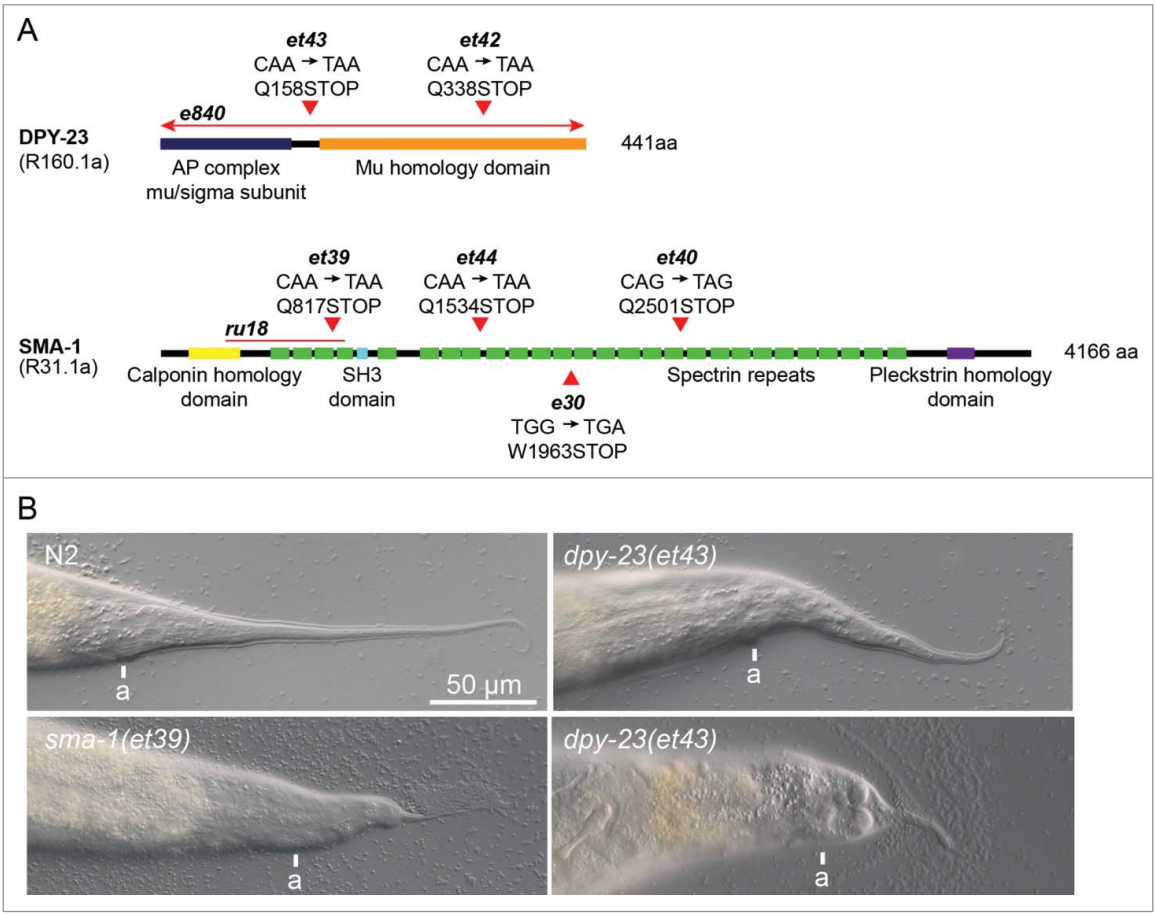

Figure 3. Illustration of the *dpy-23* and *sma-1* mutations. (A) Structural overview of the DPY-23 and SMA-1 proteins with the location of the novel alleles described in the present study (*et* alleles). *dpy-23(e840), sma-1(ru18)* and *sma-1(e30)* were used as reference alleles. Note that *dpy-23(e840)* and *sma-1(ru18)* are deletion alleles; the red bars indicate the extent of the deletions. (B) The wild-type (N2) tail tip and 3 examples of mutant tail tips are shown. Note that the tail tip phenotype is variable, especially in the *dpy-23* mutants where the defect ranges from subtle to severe. The approximate position of the anus is indicated (a).

Experimental confirmation of the paqr-2 *genocopiers*

The fact that we isolated multiple independent alleles of each gene is in itself evidence that the correct molecular mutations were identified. Several evidences support the identification of the novel *sma-1* alleles: 1) they caused a Sma phenotype; 2) the novel *et39, et40* and *et44* mutants display a tail tip phenotype similar to that of the reference *sma-1(ru18)* and *sma-1(e30)* alleles (Fig. 4A–B); and 3) the new alleles fail to complement *sma-1(ru18)* and *sma-1(e30)* for both the size and tail tip defects. Similarly, the novel *dpy-23* mutant alleles showed growth defects indistinguishable from the reference *dpy-23(e840)* mutant, and were rescued by a wild-type *dpy-23* transgene (Fig. 4C–D). We conclude that the *et39, et40* and *et44* are undoubtedly novel alleles of *sma-1*, while *et42* and *et43* are undoubtedly novel alleles of *dpy-23*.

The sma-1 *and* dpy-23 *mutants are not complete* paqr-2 *genocopiers*

The newly isolated mutants were evaluated using several assays in order to determine the degree to which they may be true *paqr-2* genocopiers. When considering the tail tip defect, it is clear that the *paqr-2* and *iglr-2* mutants are very similar both in terms of the nature of the defect and its penetrance.[7] The *sma-1* alleles also have a tail tip defect but differ from the *paqr-2* and *iglr-2* mutants in that the penetrance is lower (Fig. 5A), and the shortened tail is smoother (see Fig. 3B). The *dpy-23* mutants have a low penetrance of tail tip defect (Fig. 5A), and the defect is more subtle than in *paqr-2, iglr-2* or *sma-1* mutants. In conclusion, the *paqr-2* and *iglr-2* mutants have an identical tail tip phenotype that differs from that of the *sma-1* and *dpy-23* alleles.

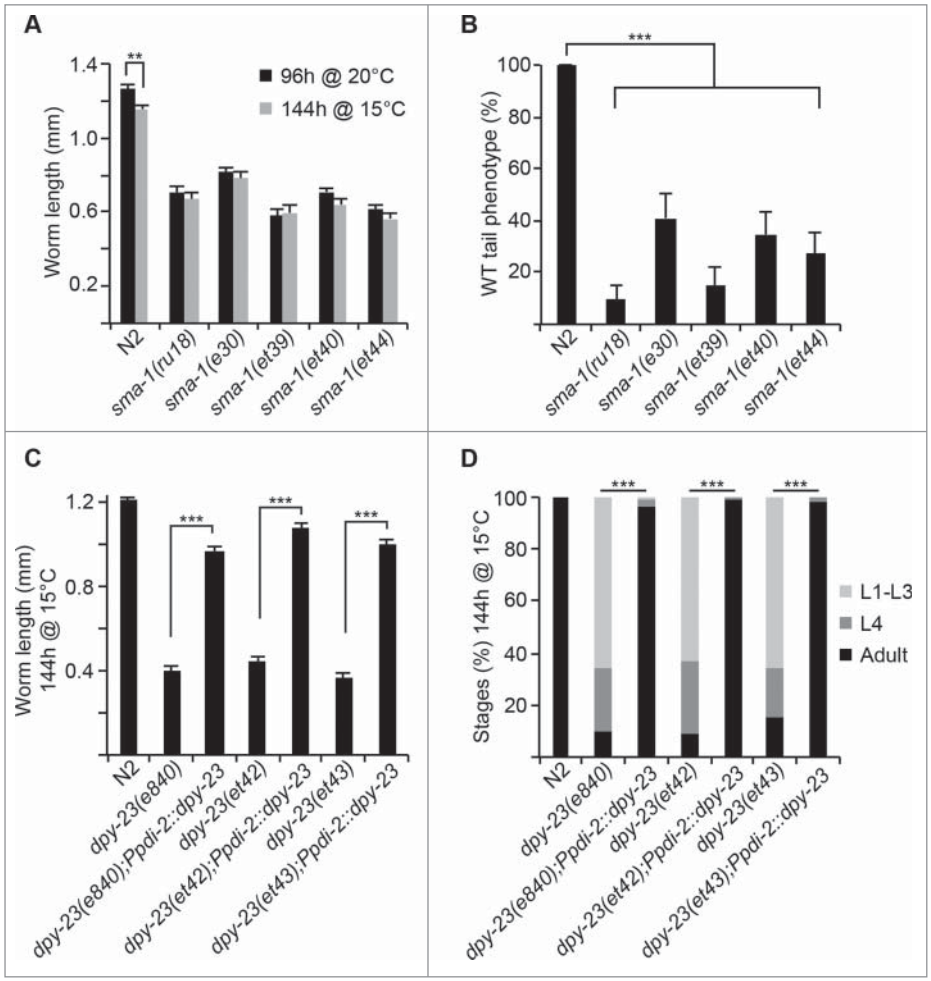

Figure 4. Experimental confirmation of the *sma-1* and *dpy-23* alleles. (A-B) Shows that the novel *sma-1* alleles, *et39, et40* and *et44* have growth and tail tip defects similar to those of the reference alleles *ru18* and *e30*. (C-D) Shows that expression of a wild-type *dpy-23* transgene in hypodermal cells can significantly rescue the growth and development defects at 15°C in the *dpy-23(e840)* reference alleles and in the novel *et42* and *et43* alleles. ** $p < 0.01$; *** $p < 0.001$.

Three assays were used to evaluate the temperature sensitivity of the mutants: measuring their length, scoring their developmental stage after cultivating synchronized L1s for 96 hrs at 20°C or 144 hrs at 15°C, and scoring their ability to produce eggs at 15°C, 20°C and 25°C. The results for the growth and developmental staging assays (Fig. 5B–D) show that the *paqr-2* and *iglr-2* mutants have the clearest cold sensitivity phenotype, while the *sma-1* and *dpy-23* are less cold sensitive. In terms of fertility (Fig. 5E), the *paqr-2* and *iglr-2* mutants are all infertile at 15°C and most fertile at 25°C, while the *sma-1* and *dpy-23* alleles are most fertile at 20°C (with the exception of the *sma-1(et44)* allele which is slightly more fertile at 25°C than 20°C). In conclusion, the *paqr-2* and *iglr-2* mutants have identical temperature sensitivity profiles, clearly benefiting from a warmer cultivation temperature (25°C), and differ from the *sma-1* and *dpy-23* alleles which thrive best at 20°C. All the mutant strains have an abnormally small brood size (Fig. 5F).

Small amounts of nonionic detergent can suppress the tail tip phenotype of the *paqr-2* and *iglr-2* mutants,[6,7] but had no beneficial effect on the *sma-1* or *dpy-23* mutants (Fig. 6A). The *paqr-2* and *iglr-2* mutants are glucose intolerant, being completely unable to grow in the presence of 20 mM glucose[7]; this was not the case for the *sma-1(et39)* and the *dpy-23(et43)* mutants which showed no special sensitivity to glucose (Fig. 6B).

In summary, based on the tail tip phenotype, temperature sensitivity, brood size and suppression by nonionic detergents and glucose sensitivity, we conclude that only *iglr-2* is a true *paqr-2* genocopier. The *sma-1* and *dpy-23* alleles are clearly different from each other phenotypically and differ from *paqr-2* in most or all of these traits. This is especially true for the 2 *dpy-23* alleles where the

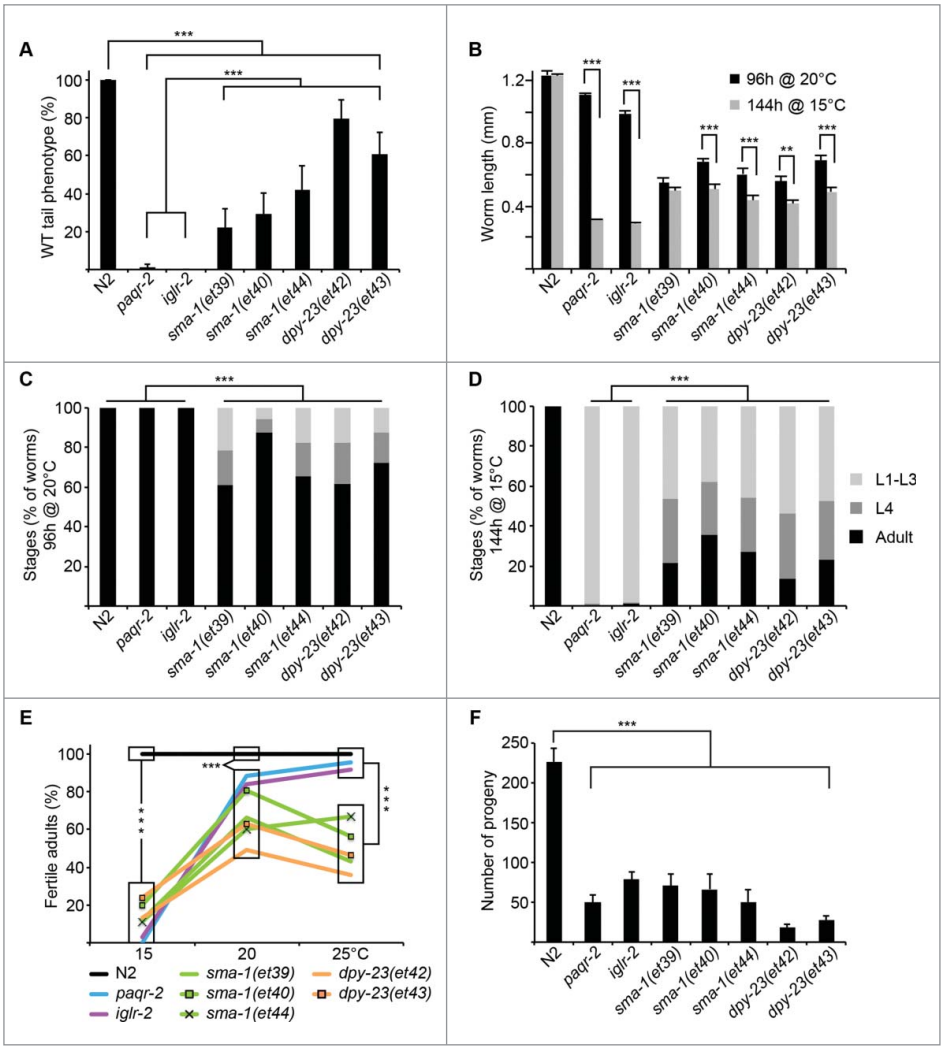

Figure 5. *sma-1* and *dpy-23* are not as good *paqr-2 g*enocopiers as *iglr-2*. The penetrance of the tail tip defect (A) and severity of the cold tolerance defect (B-D) is very similar for the *paqr-2* and *iglr-2* mutants, but less severe in the *sma-1* and *dpy-23* mutants. (E) All mutants studied are poorly fertile at 15°C, but differ in that the *paqr-2* and *iglr-2* mutants are most fertile at 25°C, while the *sma-1* and *dpy-23* mutants favor 20°C (except for *sma-1(et44)* which was slightly more fertile at 25°C). (F) The brood size at 20°C was low in all mutants, and especially low for the *dpy-23* mutant alleles. ** $p < 0.01$; *** $p < 0.001$.

tail tip phenotype is subtle and of low penetrance; the *dpy-23* mutants are also characterized by "jowls," a swelling of the head at the level of the nerve ring which has been described elsewhere.[16]

Genetic interaction studies show that sma-1 *acts in parallel to* paqr-2

In spite of several phenotypic differences, the *sma-1* and *dpy-23* mutants do have tail tip defects and significant cold sensitivity, i.e. the very reasons for which they were picked as candidate *paqr-2 g*enocopiers. To explore the possibility that *sma-1* and *dpy-23* may be in the *paqr-2* pathway we performed gene interaction experiments. A first experiment examined the double mutants. The *paqr-2(tm3410);dpy-23(et43)* double mutant is very sick and difficult to maintain (data not shown), suggesting that the 2 genes act in separate pathways. This double mutant was not further studied. The *paqr-2(tm3410);sma-1(et39)* double mutant is also much more severe than either single mutant, growing very poorly at 20°C (Fig. 7A–B). We also examined whether the potent *paqr-2* suppressor *nhr-49(et8)* could suppress the *sma-1* mutant phenotypes. This was not the case: the *sma-1(et39);nhr-49(et8)* double mutant actually had a worse cold adaptation defect than the *sma-1(et39)* single mutant (Fig. 7C), and also showed no amelioration of the tail tip defect (Fig. 7D–E). This suggests that *sma-1* and *paqr-2* cause somewhat similar phenotypes but likely act in separate pathways.

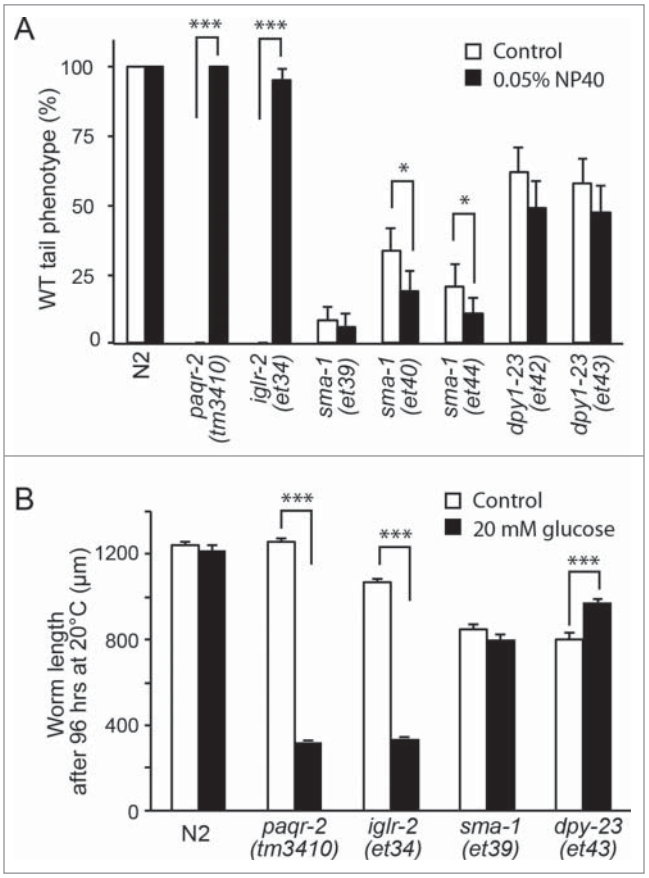

Figure 6. *sma-1* and *dpy-23* mutants are not rescued by detergents and are not glucose sensitive. (A) The inclusion of 0.05% NP40 completely rescues the tail tip defect in the *paqr-2* and *iglr-2* mutants but has no beneficial effect on the *sma-1* and *dpy-23* mutants. (B) The *paqr-2* and *iglr-2* mutants are unable to grow in the presence of glucose, which has no effect on the *sma-1* or *dpy-23* mutants. $^{*}p < 0.05$; $^{***}p < 0.001$.

Discussion

By screening 80 000 mutagenized haploid genomes, we isolated 10 candidate *paqr-2* genocopiers that had a tail tip morphology defect as well as a defect in their ability to grow at 15°C. Among the mutants were 2 novel alleles of *paqr-2* and 3 alleles of the gene *iglr-2*; these mutants exhibited remarkable similarities in their phenotypes, which suggests that they act together in the same pathway.[7] The other alleles, described in the present study, affected either *sma-1* or *dpy-23* and showed several phenotypic differences compared to each other or to the *paqr-2* and *iglr-2* mutants; *sma-1* and *dpy-23* likely impact the tail tip and cold adaptation via separate pathways. *sma-1* encodes an βH-spectrin homolog important for epithelial cell sheet morphogenesis, a process during which the SMA-1 spectrin network is remodeled under the apical membrane surface and is essential to organize actin filaments.[17,18] Spectrins are essential components of the membrane cytoskeleton where they interact with phospholipids and provide a structural platform for the stabilization and activation of various types of membrane proteins.[19] *dpy-23* (also known as *apm-2*) encodes the *C. elegans* μ subunit of the clathrin adaptor AP2 that mediates the endocytosis of membrane proteins and functions at the plasma membrane as an interaction hub for transmembrane cargoes (e.g., Wnt), accessory proteins, and clathrin.[16,20] It is therefore not surprising that the *dpy-23* mutant exhibits several membrane-related phenotypes, including the tail tip defect, "jowls" and perhaps even the dumpy phenotype. Based on these observations and also on the known molecular functions of the implicated genes, we propose a simple model by which at least 3 separate pathways act on the membrane properties to account for the observed phenotypes (Fig. 8). It is interesting that several alleles of *paqr-2, iglr-2, dpy-23*, and *sma-1* were isolated in our screen: it suggests that short body length may be a common outcome in mutants with membrane property defects. Note however that while all these mutants are shorter than wild-type, this is clearly not sufficient to cause the observed tail tip defect because mutants in dozens of other genes known to cause Sma or Dpy phenotypes were not picked.

The hermaphrodite tail may be particularly sensitive to membrane defects for several reasons. It is a rather large structure with a very narrow and elongated shape, hence far from thermodynamic equilibrium, and composed of only 4 cells. The tail tip is extremely thin over a rather long stretch and any defect in the properties of the cell membranes or the associated membrane cytoskeleton is likely to result in readily visible deformations. An additional Achilles heel for the tail tip is its relative isolation from the rest of the worm: any metabolite or building block, for example fatty acids or even phospholipids, that are turned over and equilibrated throughout the worm are less likely to do so efficiently in the narrowest end of the worm, i.e., the tail tip. Finally, defects in this structure seem to be well tolerated by worms, and indeed is such a subtle defect that it is easily missed; indeed, previous articles on *sma-1* or *dpy-23* did not mention a tail tip defect.[16-18,20] We conclude that screening for mutants with tail tip defects may be a useful approach to identify genes important for the regulation of membrane properties, but that this approach is not stringent enough to selectively identify components of the *paqr-2* pathway.

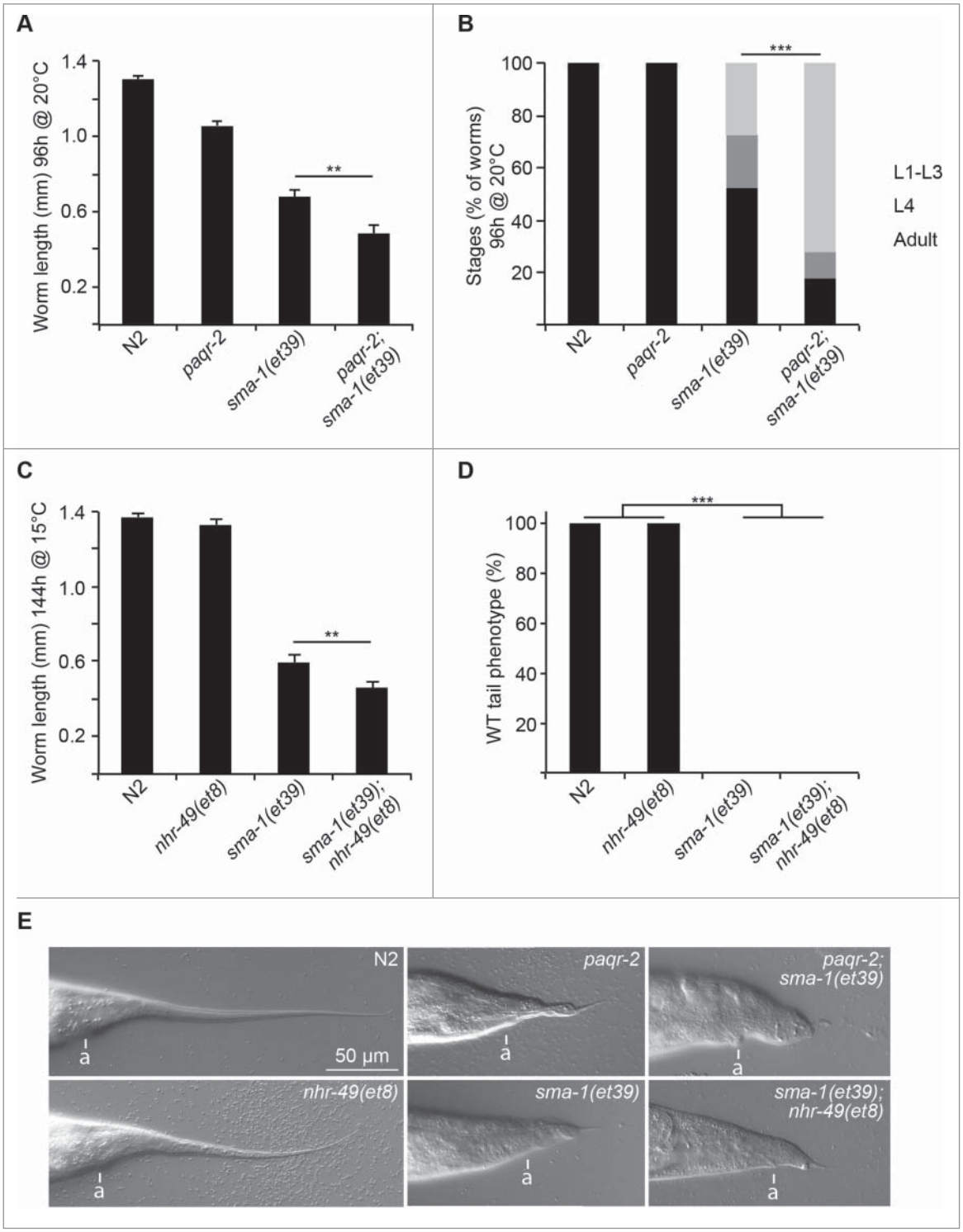

Figure 7. Genetic interactions between *paqr-2, sma-1(et39)* and the *paqr-2* suppressor *nhr-49(et8)*. (A-B) The double mutant *paqr-2;sma-1(et39)* is more severe than either single mutant in terms of growth and development at 20°C. (C-D) *nhr-49(et8)* did not suppress the growth or tail tip defects of the *sma-1(et39)* mutant, with representative tail tip images shown in (E). $^{**}p < 0.01$; $^{***}p < 0.001$.

Materials and methods

C. elegans *strains and transgenes*

The wild-type reference strain was the *C. elegans* Bristol variety strain, N2. Unless otherwise stated, strains were obtained from the *C. elegans* Genetics Center (CGC; MN, USA), and experiments were performed using the *E. coli* strain OP50 as food source, which was maintained by passaging either on NGM plates or liquid cultures in LB medium

using standard protocols.[21] *jcIs1*, which carries an *ajm-1::gfp* transgene that expresses a AJM-1::GFP fusion protein localized to adherens junctions[22] was obtained from the *C. elegans* Genetics Center. *Ppdi-2::dpy-23* expressed the wild-type *dpy*-23 gene under the hypodermal *pdi-2* promoter[20] and was a kind gift from Gian Garriga.

Mutagenesis and screen for paqr-2 *genocopiers*

As previously described,[7] 2 strategies were used to isolate novel mutants that are phenotypically similar to *paqr-2(tm3410)* among the F2 progeny of EMS-mutagenized worms. In the first strategy, we isolated mutants with a tail tip defect then searched among those for mutants that also had the cold sensitivity phenotype. Approximately 11 000 mutagenized haploid genomes were screened in this way, yielding 9 promising mutants (alleles *et34, et35, et37-et43)*. In the second strategy, we isolated mutants with the cold sensitivity phenotype then searched among those for mutants that also had a tail tip defect. Approximately 70 000 mutagenized haploid genomes were screened in this way, yielding 1 mutant (allele *et36)*. Each mutant was outcrossed 4-6 times prior to whole-genome sequencing, and a minimum of 8 times prior to careful phenotypic characterization.

Whole genome sequencing and mutant identification

The genomes of the novel mutants outcrossed 4 or 6 times were sequenced to a depth of 25-40x and differences between the reference N2 genome and that of the mutants were identified as previously described.[6] For each novel mutant, one or 2 mutation clusters, i.e. small genomic areas containing several mutations, were identified, which is in accordance to previous reports. These candidate mutations were tested experimentally as described in the text.

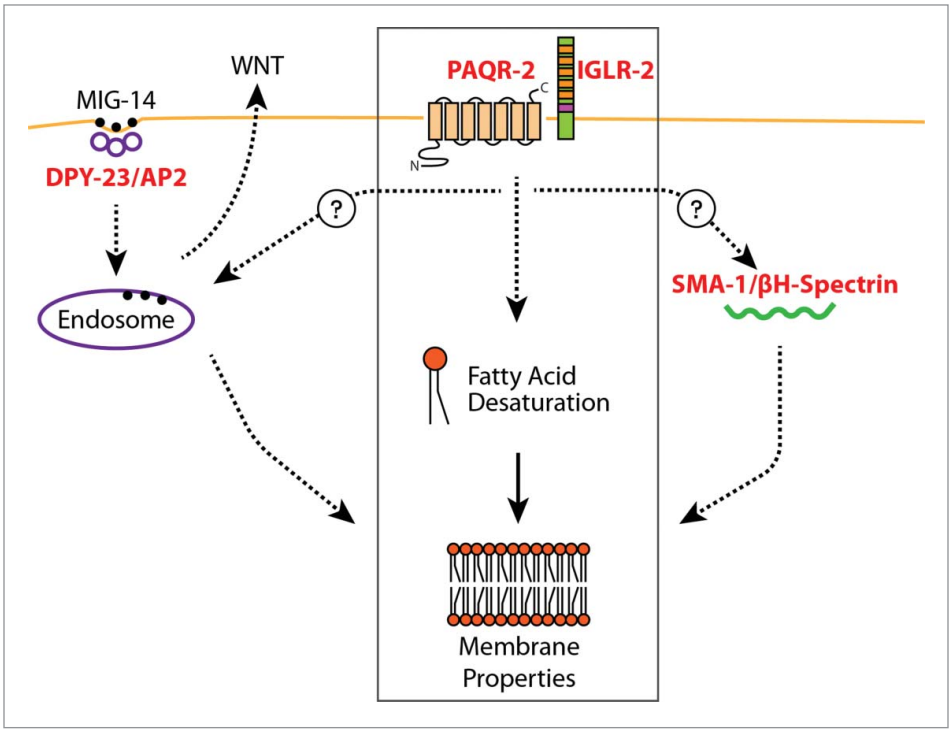

Figure 8. Model in which DPY-23 and SMA-1 act in pathways distinct from that of PAQR-2 and IGLR-2. The results presented here show that DPY-23 and SMA-1 are important for tail tip morphology and cold adaptation via pathways that are at least partly parallel to PAQR-2 and IGLR-2. Specifically, DPY-23 encodes an AP2 μ subunit important for clathrin-based endocytosis, hence recycling of membrane components or secretory cargo (e.g. WNT) via endosomes, while SMA-1 encodes a βH spectrin that is a component of the membrane cytoskeleton and hence directly influences the biophysical properties of membranes. PAQR-2 and IGLR-2 act together in a separate pathway that regulates membrane properties by influencing the relative abundance of saturated and unsaturated fatty acids in the phospholipids. Note that DPY-23 could play a role in recycling or membrane localization of PAQR-2, but that it must also have functions separate from PAQR-2 given the distinct phenotypes of the mutants.

Fertility assay

To determine the percentage of fertile worms, the worms were synchronized by bleaching. The following day, 50 L1 worms were placed on a plate (3 plates for each genotype). The plates were cultivated at 15, 20 and 25°C. The worms were scored daily for pregnancy; pregnant adults and dead worms were removed until no worms were left.

Other methods

Generation of transgenic animals, self brood size assay, 15°C growth assay, scoring of tail tip phenotype and preparation of glucose plates were performed as previously described.[6]

Statistics

Error bars for worm length measurements show the standard error of the mean, and *t-tests* 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 the significanct differences in the tail tip, staging and fertility assays were determined using *Z-tests*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 2008; 9:112-24; PMID:18216768; http://dx.doi.org/10.1038/nrm2330

[2] McIntosh TJ, Simon SA. Roles of bilayer material properties in function and distribution of membrane proteins. Annu Rev Biophys Biomol Struct 2006; 35:177-98; PMID:16689633; http://dx.doi.org/10.1146/annurev.biophys.35.040405.102022

[3] Holthuis JCM, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. Nature 2014; 510:48-57; PMID:24899304; http://dx.doi.org/10.1038/nature13474

[4] Apaja PM, Lukacs GL. Protein homeostasis at the plasma membrane. Physiology (Bethesda) 2014; 29:265-77; PMID:24985330

[5] Svensson E, Olsen L, Mörck C, Brackmann C, Enejder A, Faergeman NJ, Pilon M. The adiponectin receptor homologs in *C. elegans* promote energy utilization and homeostasis. PLoS ONE 2011; 6:e21343; PMID:21712952; http://dx.doi.org/10.1371/journal.pone.0021343

[6] Svensk E, Ståhlman M, Andersson C-H, Johansson M, Borén JN, Pilon M. PAQR-2 Regulates Fatty Acid Desaturation during Cold Adaptation in *C. elegans*. PLoS Genet 2013; 9:e1003801; PMID:24068966; http://dx.doi.org/10.1371/journal.pgen.1003801

[7] Svensk E, Devkota R, Ståhlman M, Ranji P, Rauthan M, Magnusson F, Hammarsten S, Johansson M, Borén JN, Pilon M. *Caenorhabditis elegans* PAQR-2 and IGLR-2 Protect against Glucose Toxicity by Modulating Membrane Lipid Composition. PLoS Genet 2016; 12: e1005982; PMID:27082444; http://dx.doi.org/10.1371/journal.pgen.1005982

[8] Pettitt J, Wood WB, Plasterk RH. cdh-3, a gene encoding a member of the cadherin superfamily, functions in epithelial cell morphogenesis in *Caenorhabditis elegans*. Development 1996; 122:4149-57; PMID:9012534

[9] Nguyen CQ, Hall DH, Yang Y, Fitch DH. Morphogenesis of the *Caenorhabditis elegans* male tail tip. Dev Biol 1999; 207:86-106; PMID:10049567; http://dx.doi.org/10.1006/dbio.1998.9173

[10] Chisholm AD, Hsiao TI. The *Caenorhabditis elegans* epidermis as a model skin. I: development, patterning, and growth. Wiley Interdiscip Rev Dev Biol 2012; 1:861-78; PMID:23539299; http://dx.doi.org/10.1002/wdev.79

[11] Sulston JE, Albertson DG, Thomson JN. The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev Biol 1980; 78:542-76; PMID:7409314; http://dx.doi.org/10.1016/0012-1606(80)90352-8

[12] Morita K, Chow KL, Ueno N. Regulation of body length and male tail ray pattern formation of *Caenorhabditis elegans* by a member of TGF-β family. Development 1999; 126:1337-47; PMID:10021351

[13] Zhao X, Yang Y, Fitch DHA, Herman MA. TLP-1 is an asymmetric cell fate determinant that responds to Wnt signals and controls male tail tip morphogenesis in *C. elegans*. Development 2002; 129:1497-508; PMID:11880358

[14] Ko FC, Chow KL. Mutations with sensory ray defect unmask cuticular glycoprotein antigens in *Caenorhabditis elegans* male tail. Dev Growth Differ 2000; 42:69-77; PMID:10831045; http://dx.doi.org/10.1046/j.1440-169x.2000.00487.x

[15] Zori RT, Williams CA. Phenocopy versus genocopy. Am J Med Genet 1991; 40:248-8; PMID:1897581; http://dx.doi.org/10.1002/ajmg.1320400224

[16] Gu M, Liu Q, Watanabe S, Sun L, Hollopeter G, Grant BD, Jorgensen EM. AP2 hemicomplexes contribute independently to synaptic vesicle endocytosis. Elife 2013; 2:e00190; PMID:23482940; http://dx.doi.org/10.7554/eLife.00190

[17] Praitis V, Ciccone E, Austin J. SMA-1 spectrin has essential roles in epithelial cell sheet morphogenesis in *C. elegans*. Dev Biol 2005; 283:157-70; PMID:15890334; http://dx.doi.org/10.1016/j.ydbio.2005.04.002

[18] McKeown C, Praitis V, Austin J. *sma-1* encodes a betaH-spectrin homolog required for *Caenorhabditis elegans* morphogenesis. Development 1998; 125:2087-98; PMID:9570773

[19] Machnicka B, Czogalla A, Hryniewicz-Jankowska A, Bogusławska DM, Grochowalska R, Heger E, Sikorski AF. Spectrins: a structural platform for stabilization and activation of membrane channels, receptors and transporters. Biochim Biophys Acta 2014; 1838:620-34; PMID:23673272; http://dx.doi.org/10.1016/j.bbamem.2013.05.002

[20] Pan C-L, Baum PD, Gu M, Jorgensen EM, Clark SG, Garriga G. *C. elegans* AP-2 and retromer control Wnt signaling by regulating *mig-14*/Wntless. Dev Cell 2008; 14:132-9; PMID:18160346; http://dx.doi.org/10.1016/j.devcel.2007.12.001

[21] Sulston JE, Hodgkin JA. Methods. In: Wood WB, editor. The Nematode *Caernorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1988. pages 587-606.

[22] Mohler WA, Simske JS, Williams-Masson EM, Hardin JD, White JG. Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. Curr Biol 1998; 8:1087-90; PMID:9768364; http://dx.doi.org/10.1016/S0960-9822(98)70447-6