Title: Asymmetric Wnt pathway signaling facilitates stem cell-like divisions via the non-receptor Tyrosine Kinase FRK-1 in Caenorhabditis elegans.

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Abstract

Asymmetric cell division is critical during development, as it influences processes such as cell fate specification and cell migration. We have characterized FRK-1, a homologue of the mammalian Fer non-receptor tyrosine kinase, and found it to be required for differentiation and maintenance of epithelial cell types, including the stem cell-like seam cells of the hypodermis. A genomic knockout of frk-1, allele ok760, results in severely uncoordinated larvae that arrest at the L1 stage and have an excess number of lateral hypodermal cells which appear to have lost asymmetry in the stem cell-like divisions of the seam cell lineage. frk-1(ok760) mutants show that there are excess lateral hypodermal cells which are abnormally shaped and smaller in size compared to wildtype; a defect which could only be rescued in a manner dependent on the kinase activity of FRK-1. Additionally, we observed a significant change in the expression of heterochronic regulators in frk-1(ok760) mutants. However, frk-1(ok760) mutants do not express late, non-seam hypodermal GFP markers suggesting the seam cells do not precociously differentiate as adult-hyp7 cells. Finally, our data also demonstrate a clear role for FRK-1 in seam cell proliferation, as eliminating FRK-1 during the L3-L4 transition results in supernumerary seam cell nuclei that is dependent on asymmetric Wnt signaling. Specifically, we observe aberrant POP-1 and WRM-1 localization that is dependent on the presence of FRK-1 and APR-1. Overall, our data suggest a requirement for FRK-1 in maintaining the identity and proliferation of seam cells primarily through an interaction with the asymmetric Wnt pathway.
Introduction

Asymmetric division is critical component of stem cell populations ranging from organismal development to tissue maintenance during adulthood. Without asymmetric divisions early embryos would not progress toward cellular specification and adult cell types, such as blood, would lose the ability to maintain numbers through self-renewal. Particularly important to stem cell division is the capability to maintain the stem cell population via asymmetric division where one daughter cell is specified to become a certain cell type, while the other daughter cell becomes another stem cell.

In the nematode *Caenorhabditis elegans* a stem-cell like population, called seam cells, exists in the hypodermis and undergoes a series of asymmetric division after each larval molt, thus facilitating post-embryonic development (SULSTON AND HORVITZ 1977). Seam cells are critical for proper formation of the hypodermis, the secreted cuticle, as well as other cell types derived from seam cells such as neuroblasts and glial cells. The seam cells consist of three anterior sets, H0, H1 and H2, followed by six V cells and one T cell in the posterior (Figure 1). The V-cells undergo unique stem cell-like divisions during post-embryonic development that leads to one anterior daughter that fuses with the hypodermal syncytial cell, hyp7, and one posterior daughter that goes on to divide asymmetrically again at the next larval molt. Interestingly, within these V-cells, there is one symmetric division during the early L2 larval stage which precedes the asymmetric division later in L2. After the L4 molt, progressing to adulthood, the remaining seam cells then differentiate and exit the cell cycle (JOSHI et al. 2010).
Seam cell asymmetry has been well studied and shown to be regulated primarily by two interacting pathways: 1) heterochronic genes, which regulate the timing of seam cell division (ROUGVIE AND MOSS 2013), and 2) asymmetric Wnt pathway components, which function to specify the anterior and posterior daughter during each round of division (PHILLIPS AND KIMBLE 2009; SAWA 2012). The Wnt pathway has been closely associated with asymmetric cell division and cell polarity in cellular contexts ranging from embryonic development (MADURO 2009; SAWA 2012) to maintenance of the stem cell niche (CLEVERS et al. 2014). The *C. elegans* asymmetric Wnt pathway, termed the Wnt/β-catenin asymmetry (WβA) pathway, shares some attributes with the “canonical” Wnt/β-catenin pathway involved in cellular proliferation (among other functions). In the canonical pathway the presence of a Wnt signal binding the Frizzled receptor stabilizes cytoplasmic β–catenin, which then localizes to the nucleus to interact with TCF/LEF factors to activate target gene expression (CLEVERS 2006). Similarly, the *C. elegans* WβA pathway increases the levels of a β–catenin transcriptional activator, called SYS-1, which then binds the TCF homolog POP-1 and activates expression of Wnt target genes. To negatively regulate β-catenin in the absence of Wnt, both systems require a kinase, casein kinase 1α (CK1α, KIN-19 in *C. elegans*) and the protein scaffold, adenomatous polyposis coli (APC, APR-1 in *C. elegans*). However, in WβA the transcriptional activity of SYS-1 and POP-1 is asymmetric and coupled to cell division, such that only one of two daughter cells (the “signaled” daughter) displays elevated SYS-1 levels and target gene expression, compared to the “unsignedaled” daughter thereby asymmetrically changing cellular identity (HERMAN 2001; KIDD et al. 2005; HUANG et al. 2007; PHILLIPS et al. 2007).
The major difference between the WβA and the canonical pathways is that, in *C. elegans*, the asymmetric Wnt pathway also regulates POP-1/TCF nuclear localization, which is normally asymmetric between the two nuclei of recently divided cells affected by the presence of a Wnt signal (Lin *et al.* 1995; Rocheleau *et al.* 1997; Wildwater *et al.* 2011). Somewhat counter-intuitively, Wnt signaling lowers nuclear POP-1 levels in the same cell that has elevated SYS-1/β-catenin resulting in reciprocal β-catenin and TCF asymmetry (Thorpe *et al.* 1997; Lin *et al.* 1998; Huang *et al.* 2007; Phillips *et al.* 2007; Phillips and Kimble 2009; Sawa 2012). Current models suggest this lowering results in an optimal β-catenin:TCF ratio where, in the signalled daughter, most of the POP-1 is complexed with its SYS-1 coactivator, while in the unsignalled daughter, most of the POP-1 is unbound by β-catenin and represses target gene expression. Like SYS-1 asymmetry, POP-1 nuclear asymmetry has been shown to be required in myriad cell types including the intestinal and gonadal precursors as well as the hypodermal seam. The mechanism of POP-1 lowering is dependent on a distinct branch of WβA pathway that utilizes a second β-catenin, WRM-1. WRM-1 is elevated in the signalled nucleus where it binds and phosphorylates POP-1 with the Nemo-like kinase, LIT-1, to induce POP-1 nuclear export rather than transcriptional activation (Rocheleau *et al.* 1999; Shin *et al.* 1999; Lo *et al.* 2004; Yang *et al.* 2011). Thus the asymmetric regulation of the two β-catenins is critical to the asymmetric cell division and the subsequent cell fate decision. Interestingly, both WRM-1 and SYS-1 are kept low in the nucleus of the unsignalled daughter by APR-1/APC. APR-1 negatively regulates total cellular levels of SYS-1 in a manner consistent with its degradation (Baldwin and Phillips 2014), while also lowering nuclear WRM-1 by stabilizing its cortical localization (Mizumoto and Sawa 2007a; Sugioka *et al.* 2011). Although much has been reported on the importance of the Wnt
signaling influence on seam cell asymmetric division, certain aspects remain unclear, such as
the mechanism by which POP-1 asymmetry is established in the two daughter cells.

An important aspect of asymmetric signaling is the presence of kinases that facilitate the
progression of the signal from the plasma membrane to the nucleus. Kinases such as LIT-1 and
KIN-19, have both been shown to be critical for proper asymmetric division within the seam cell
lineage. However, these two kinases have been reported to function in separates manners,
with the heterochronic genes regulating the LIT-1 influence on seam cell division in addition to
regulating WRM-1 and POP-1 (Takeshita and Sawa 2005; Mizumoto and Sawa 2007a; Harandi and
Ambros 2015). KIN-19 negatively regulates SYS-1 in the unsignaled daughter along with APR-1,
which promotes the hypodermal fate (Banerjee et al. 2010; Gleason and Eisenmann 2010;
Baldwin and Phillips 2014). We report here the requirement for a non-receptor tyrosine kinase,
FRK-1, that facilitates the proper asymmetric localization of POP-1, and thus identity, of each
seam cell daughter. FRK-1 is a homologue of the vertebrate Fer kinase, which is activated
downstream of pathways such as PDGF and EGFR, and has been shown to be involved in
adhesion, proliferation and activation of NFκB (Guo and Stark 2011; Lennartsson et al. 2013).
We have previously shown that the absence of FRK-1 during embryogenesis prevents proper
enclosure in a kinase independent manner (Putzke et al. 2005). Furthermore, we reported a role
for FRK-1 in regulating proliferation of C. elegans intestine, during embryogenesis, by
preventing hyperactivation of a Wnt response in a kinase-dependent manner (Putzke and
Rothman 2010). Importantly, in each case we showed that FRK-1 interacts with a third C.
elegans β-catenin, HMP-2, involved in cell adhesion but not WβA signaling, as well as APR-
1/APC.
Related to asymmetric cell division is the heterochronic gene regulation pathway which controls the timing of progression during post-embryonic development in *C. elegans*. More specifically, expression of microRNAs is activated and suppressed in a synchronized manner to regulate translation of mRNA, and thus modulate protein activity, based on the stage of development (reviewed in (Resnick et al. 2010)). Importantly, heterochronic regulation has been linked to asymmetric cell division within the seam cell lineage of *C. elegans* in a manner that general causes arrested or precocious seam cell division in the absence of specific miRNAs such as occurs with the function of *let-7* and *lin-4* (Feinbaum and Ambros 1999; Reinhardt et al. 2000). More recently, Harandi and Ambros demonstrated that there is a distinct interconnection between heterochronic genes and the WβA pathway, in that heterochronic genes regulate the ability of the WβA pathway to achieve asymmetric seam cell division (Harandi and Ambros 2015).

Here we characterize that loss of zygotic FRK-1 (via genomic knockout) results in loss of seam cell regulation, such that, although the animal arrests at the L1 stage of post-embryonic development, the seam cells within the V-cell lineage divide symmetrically, similar to the first division within the L2 stage of development. Mutant larvae show a marked decrease in heterochronic gene targets, due to aberrant expression of the heterochronic regulator miRNA, *lin-4*. In later larvae (L3-L4) we show that the absence of FRK-1 or APR-1 results in excess seam cell division that is sufficient to compensate for the loss of WRM-1 in a manner that is independent of SYS-1. Finally, we show that FRK-1 is asymmetrically localized in seam cell daughter nuclei parallel to that of POP-1. Loss of FRK-1 results in loss of POP-1 asymmetry, due to the loss of WRM-1 asymmetry and a shift in APR-1 nuclear/cytoplasmic ratio. Finally, we
present a novel mechanism by which FRK-1 functions to regulate POP-1 nuclear accumulation in dividing seam cells through APR-1 and WRM-1, in a SYS-1 independent fashion, the loss of which results in compromised seam cell identity and premature arrest.

Methods

Strains and alleles

The wild-type strain used was the Bristol N2 strain, as described by Brenner (BRENNER 1974). To examine the loss of frk-1 phenotype we used line VC558 containing allele (ok760). To examine the tissues in which the frk-1 gene region was active an extrachromosomal array of P_{frk-1::GFP}, consisting of 1.8kb of the frk-1 promoter region fused to a nuclear localized transcriptional GFP using pPD96.62 (gift from A. Fire) plus the rol-6(su1006) marker, on plasmid pRF4, to create JR2355. JR667 (P_{scm::GFP}), HS1417 (WRM-1::YFP), BTP118 [unc-76 V; osls13 (P_{apr-1::APR-1::VNS; unc-76 (+))], uiwls4 (p_{sys-1::mCherry::SYS-1}), Strains AP110 and AP112 contain extrachromosomal arrays consisting the frk-1 coding region (or (P_{scm::FRK-1(D308R)}) respectively ) fused to the scm promoter region contained in plasmid pMF1 (a generous gift from J. Rothman).

RNA-mediated interference (RNAi)

Primer pairs were made to the coding regions of frk-1, wrm-1, apr-1, hmp-2, sys-1, pop-1 and unc-22, and tagged with a T7 RNA polymerase promoter sequence. Each fragment was PCR-amplified from genomic DNA or cDNA. dsRNA was generated using a T7 reverse transcriptase
kit (Ambion). Newly transitioned L3 larvae were soaked with dsRNA or fed in IPTG-containing plates with OP50 expressing *frk-1* dsRNA (HT115; pLT61: T04B2.2) for 10 hours to ensure the L3-L4 seam cell divisions were captured. Larvae were analyzed using by fluorescence microscopy for nuclear GFP detection in seam cells. RNAi knockdown efficiency was determined via quantitative PCR with knockdown percentages ranging from 57-75% (i.e. gene expression ranged from 25-43% after knockdown depending on the target) when performed from the L3 to L4 transition (see Supplemental Figure 3).

**Antibodies and immunofluorescence**

MH27 is a mouse monoclonal antibody that recognizes epithelial apical junctions (PRIESS AND HIRSH 1986). NE2-1B4 is a mouse monoclonal antibody that recognizes an antigen expressed only in seam cells (SCHNABEL 1991). P4G4 is a monoclonal mouse antibody recognizing POP-1 (LIN et al. 1998). Immunofluorescence was imaged via Nikon A1 confocal microscope and a Zeiss Axio Imager D2 compound fluorescent microscope equipped with Zeiss Zen software. Raw image data was exported into TIFF format and analyzed using ImageJ software. Nuclei of seam cells were identified via DIC images and then mean protein fluorescence in fluorescent images was quantified using ImageJ. Background fluorescence was normalized for each experiment by using the same channels and exposures to image seam cells in N2 wild-type worms (N = 24 for each exposure setting). All statistical comparisons were performed with either Student’s T-test in Microsoft Excel or the Mann-Whitney Test and VassarStats software (LOWRY 2010). Bar graphs show error bars that are standard error of the mean.
Results

A fusion between the \textit{frk-1} promoter region and nGFP shows the \textit{frk-1} promoter to be active primarily in epithelial cell types, including the hypodermis, intestine and germline uterine and spermathecal lining. Specifically, \textit{frk-1} expression is observed in the developing seam cells during embryogenesis and larval development (Figure 2 A, B). We previously reported that FRK-1 protein localizes primarily to the plasma membrane in these cells types, with the exception of mitotically active cells, in which case FRK-1 is also observed localized to the nucleus (\textit{Putzke et al.} 2005).

To address the mechanism by which FRK-1 functions during development in \textit{C. elegans}, we obtained a genomic knockout of \textit{frk-1}, allele \textit{ok760}. The \textit{frk-1(ok760)} allele disrupts \textit{frk-1} expression by deleting 1500 basepairs of genomic region that correspond to 910 basepairs of promoter region as well as the first two exons and the second intron of the \textit{frk-1} gene (Figure 2C). The \textit{ok760} deletion results in no detectable transcription of \textit{frk-1}, when compared to wildtype larvae (not shown), thus eliminating the possibility of expression from \textit{frk-1} due to transposition to another location in the genome as a result of the mutation process.

Homozygous \textit{frk-1(ok760)} mutants exhibit lethal larval arrest at the L1 stage with no progression to L2. The mutants remain alive for three to four days prior to death. In addition to L1 arrest, homozygous mutants are severely uncoordinated, being able to move their head region with very little body movement posterior to the pharynx. Death is likely not due to an inability to feed after hatching as we have observed bacteria in the intestine of \textit{frk-1(ok760)} mutant larvae after being exposed to plates growing GFP expressing OP50 (not shown).
Although the nature of the phenotype in these mutants is complex, there are likely multiple tissues affected by the mutation, thus resulting in the uncoordinated nature and early larval arrest, in addition to the seam cell division defect upon which this study focused.

Based on our previous studies showing that FRK-1 is required for proper patterning of the hypodermal seam cells (Putzke et al. 2005), we investigated whether the seam cells in frk-1(ok760) mutants were morphologically normal. In the absence of frk-1, seam cells appear to have lost their asymmetry, as both daughter cells are small, similar in size to the anterior seam cell in a wildtype larva, suggesting that these cells may have both taken on an anterior identity (Figures 2D – small box and 3C).

With the observed phenotype of the frk-1(ok760) homozygous mutants, we then tested the hypothesis that larval progression was halted in the mutants primarily due to improperly functioning seam cells. We first observed seam cell function by looking at seam cell numbers and expression of seam cells markers, normally observed in wildtype embryos. When newly hatched frk-1(ok760) mutant larvae are stained with MH27, which marks plasma membrane in epithelial cell types, the seam cells, especially the V seam cells, appear misshapen (Figure 3C, A – wildtype). When the frk-1(ok760) allele is crossed with a transgenic line expressing GFP exclusively in seam cells, homozygous mutants show an increase in seam cell proliferation that occurs primarily within the V1-V4 seam cells (Figure 3F, E – wildtype). In these cases, the V1-V4 cells divide without division of the other seam cells and remain as a pair without the anterior cell fusing with the hypodermal syncytium), as is normally observed in wildtype larvae (average in WT=4.3, average in ok760 mutants=7.7, p=2.6x10^{-8}, n=25 animals per condition). The seam cell GFP expression in the anterior daughter persists until fusion with the hypodermis in
wildtype (Figure 3E – white arrowheads) while it persists indefinitely in the ok760 mutant
(Figure 3F – white arrowheads).

In addition, NE2-1B4, a marker of functional seam cells is primarily absent in frk-1(ok760) homozygotes, suggesting that the seam cells are not functioning properly even when present (Figure 3D, B – wildtype). We then examined a series of genes that are normally expressed in functioning seam cells and the surrounding hypodermis, and have been shown to facilitate proper asymmetry and division during embryonic and larval progression: lin-14, lin-28, lin-41, elt-3, egl-18, nhr-72 and nhr-81 (AMBROS AND HORVITZ 1987; MOSS et al. 1997; GILLEARD et al. 1999; KOH AND ROTHMAN 2001; YAMAMOTO et al. 2011). In the case of markers such as lin-14 and lin-28, we observed a near complete loss of expression in the frk-1 mutants, with other seam determining genes, such as lin-41, egl-18, nhr-72 and nhr-81 being significantly reduced (or nearly absent) when compared to wildtype (Figure 3G). The marker, elt-3, is expressed in non-seam hypodermal cells, suggesting that regulation of hypodermal development in mutant larvae is disrupted beyond the seam cells. Interestingly, lin-14 and lin-28 are heterochronic genes, suggesting that timing of division may be inappropriately altered in frk-1 mutants, however it is more likely that the aberrant expression of heterochronic genes is due to upstream gene expression changes and not genuine defects in the heterochronic pathway.

We then tested the hypothesis that the excess number of seam cells, combined with the apparent loss of asymmetry with the seam cells, was due to precocious differentiation of the seam cells, which would be supported by the premature loss of heterochronic gene expression (lin-14 and lin-28). When we performed quantitative RT-PCR with lin-4 and let-7, two miRNAs known to regulate developmental progression (Moss 2007), on newly hatched L1s, we observed
an increase of lin-4 expression in excess of sixteen-fold compared to wildtype (Supplemental Figure 2), further suggesting that the heterochronic gene regulation was initiated prematurely in the absence of FRK-1. We did not, however, observe let-7 expression in newly hatched frk-1(ok760) mutants (Supplemental Figure 2), implying that although early heterochronic regulation may be precocious, later regulation, directed by let-7 expression beginning in L4, is not contributing to the misregulation of seam cells. Indeed, when we crossed the frk-1(ok760) mutation into a variety of transgenic lines expressing GFP under the regulation of genes that are active in late larval to adult seam cells (col-19, col-49, col-38, bli-1)(Jackson et al. 2014), we found that there was no GFP expression in the frk-1 mutants (n=50 per transgenic line)(Supplemental Table 1). The only instances in which we observed expression was in two constructs that include expression in seam cells ranging from embryonic to L4 or adulthood (grd-10 and egl-18). Together these data suggest that the seam cells in frk-1 mutants may be misregulated, but are not precociously differentiating. With these observations we pursued the idea that seam cells were not functional due to lack of asymmetry and thus, loss of specific identity, in the absence of FRK-1.

Since it has been previously reported that there is kinase-independent activity of the Fer-related kinases, and we were able to demonstrate this specifically in C. elegans intestinal proliferation (Putzke and Rothman 2010) we investigated whether the larval arrest and seam cell proliferation defects might be rescued in the presence of a kinase inactive FRK-1. In the presence of wildtype FRK-1, we observed a significant rescue of the larval lethality, with approximately 90.1% of larvae progressing beyond the L3 stage (n=237/263), compared to frk-1(ok760) homozygous larvae containing the empty vector, in which all individuals arrested at
the L1 stage (n=126/126)(Figure 4A). The majority of \textit{frk-1(ok760); FRK-1 (WT)} rescued animals
died as L4 or adults, with none viable enough to produce progeny, likely due to mosaicism of
the rescuing construct. Larvae expressing the kinase inactive (KD –kinase dead) version of FRK-1
were able to progress beyond the L1 stage 34.9% of the time but none progressed beyond L2,
strongly suggesting that the kinase activity of FRK-1 is required during post-embryonic
development (n=65/186)(Figure 4A). We then examined whether seam cell proliferation was
dependent on FRK-1 kinase activity by performing the same rescue experiment as mentioned
above, but in the presence of the seam cell marker GFP (used in Figure 3). Similar to viability
rescue, in the presence of wildtype FRK-1, 88.1% of larvae exhibited normal seam cell numbers,
when compared to the \textit{ok760} allele alone (Figure 4B). In the presence of the kinase inactive
FRK-1 (KD), only 16.4% of embryos showed normal seam cell proliferation, compared to 94.4%
in wildtype embryos. These data demonstrate that the kinase activity is required not only for
viability, but for proper proliferation within the seam cell lineage, during post-embryonic
development.

Additionally, to determine whether the seam cell defect could be rescued by expressing
\textit{frk-1} exclusively in the seam cells, we fused the \textit{frk-1} coding region to the seam cell promoter
used in the P\textsubscript{scm::}GFP construct (see methods). Indeed, when wildtype \textit{frk-1} was expressed in
the seam cells of the \textit{frk-1(ok760)} homozygous mutant, the larval lethality was abrogated such
that most larvae progressed beyond the L1 stage (61.2%, n=134), with over half of the larvae
progressing to L3 or beyond when compared to either the \textit{frk-1(ok760)} alone or \textit{frk-1(ok760); P}\textsubscript{scm::}GFP (negative control), in which 97% or more of the larvae arrested at the L1 stage (n= 147 and 163 respectively)(Figure 4C). This occurred in a kinase-dependent manner such that
expressing the kinase inactive (KD) version of FRK-1 was unable to compensate for the loss of endogenous *frk-1*, with 90.9% of *ok760* larvae arresting at the L1 stage (n=177). Similar to the rescue from the *frk-1* promoter the construct was not able to stably rescue any *frk-1(ok760)* homozygous individuals to viable, reproducing adults (again likely due to the mosaic nature of the extrachromosomal array). Importantly, we were able to observe rescue of the precocious proliferation in the seam cell lineage, observed in *frk-1* mutants, with the seam cell-exclusive expression of *frk-1* (Figure 4D). As with viability, the wildtype *frk-1* expressed solely in seam cells was able to return the number of V1-4 seam cells to normal levels (avg = 4.35), while the kinase inactive version was unable to do so (avg = 7.70), with seam cell numbers being similar to *frk-1(ok760)* alone or with the P_{scm}::GFP control (avg = 8.15)(n = 50 for each rescue condition).

With an established connection between FRK-1 and seam cell proliferation, we examined whether FRK-1 might be participating in the asymmetric seam cell division regulatory pathway. Using the P_{scm}::GFP transgenic line we performed RNA interference (RNAi) on L3 larvae and observed seam cell proliferation during progression to the L4 stage. We chose this stage as it is a stage in which to consistently monitor seam cell divisions with ease of manipulation compared to earlier stages. Additionally, we suspect FRK-1 functions in the developmental decisions of many tissues, so waiting until these non-seam lineages have terminally differentiated bypasses this role and instead allows us to focus on the tissue under study. However, because of this we needed to use RNAi instead of the *frk-1(ok760)* allele as the homozygous mutants do not progress beyond the L1 stage. In *frk-1(RNAi)* individuals there are excess number of seam cells, with the average being 29.2 (n = 84)(Figure 5A and B), when
compared to animals exposed to \textit{unc-22}(RNAi) which does not affect seam cell numbers (14.5, 
n = 72). The Wnt asymmetry pathways components affect seam cell proliferation such that loss
of POP-1, via RNAi, results in excess numbers averaging 45.3 cells per larva (n = 73)(Figure 5A
and B), whereas loss of WRM-1 causes loss of seam cells, with less than 10 per larva (average =
8.6 per larva, n = 62)(Figure 5B).

Our previous studies showed FRK-1 interacting with asymmetric Wnt pathway elements
by preventing excess $\beta$-catenin activity during intestine proliferation within the E-lineage
(\textsc{Putzke and Rothman} 2010). It has also been demonstrated that the asymmetric Wnt pathway,
functioning through factors such as WRM-1, APR-1, PRY-1 and SYS-1, affects asymmetry and
proliferation of seam cells (\textsc{Phillips et al.} 2007; \textsc{Gleason and Eisenmann} 2010; \textsc{Baldwin and Phillips}
2014). Thus, we tested the hypothesis that FRK-1 may be functioning in a similar manner in
lateral seam cells as it does in the intestine. Eliminating either APR-1, FRK-1 or POP-1 via RNAi
resulted in a significant increase in the number of seam cells, (22.2, 29.8, and 45.3
respectively)(n= 53, 58, 49)(Figure 5B). When eliminated in tandem, \textit{apr-1, pop-1 (double RNAi)}
yielded an average of 52.9, whereas \textit{frk-1, pop-1 (double RNAi)} resulted in 61.3 seam cells.
(n=45 for each case). Although the increase is significant (\textit{pop-1 RNAi} alone versus \textit{frk-1, pop-1}
or \textit{apr-1, pop-1 double RNAi}), it points to an additive rather than synergistic effect, suggesting
they are acting in the same pathway as opposed to separate signaling mechanisms.

Having observed a genetic interaction with POP-1, we then investigated whether FRK-1
and WRM-1 might be functioning together to regulate seam cell proliferation. When FRK-1 and
WRM-1 are knocked down simultaneously, a modest, but significant, change was observed with
seam cell numbers increasing to 18.7 (n = 41), which is consistent with a rescue of \textit{wrm-1}(RNAi)
to wildtype seam cell numbers, compared to loss of WRM-1 alone (8.6, n= 55)(Figure 5B). Interestingly, when the expression of FRK-1 and APR-1 are knocked down the number of seam cells increases to 34.3 (n= 47). However, the increase due to the loss of either (or both) FRK-1 and APR-1 was abrogated by loss of WRM-1, strongly suggesting that regulation of POP-1-mediated seam cell asymmetry and proliferation functions through FRK-1, APR-1 and WRM-1.

To determine whether the supernumerary seam cells in the absence of FRK-1 was due to participation of other β-catenin homologues, we tested the loss of both SYS-1, a major contributor to seam cell asymmetry, and HMP-2, a β-catenin homologue not previously associated with seam cell identity or proliferation. Loss of neither hmp-2 nor sys-1 (both via RNAi) resulted in a significant change in seam cell number, with their averages being 16.3 and 15.1, respectively (n= 42, 38)(Figure 5B). When either β-catenin homologue was eliminated along with frk-1, the subsequent increase in seam cell number was not significantly different than elimination for frk-1 alone (frk-1, sys-1(double RNAi) avg = 25.4, n=45, frk-1, hmp-2(double RNAi) avg =28.2, n=52). These results suggest that the increase we observed were specific for an interaction between frk-1, apr-1 and the β-catenin homologue, wrm-1.

With an established connection between seam cell proliferation and the asymmetric Wnt pathway, we next tested the hypothesis that POP-1 asymmetry in the anterior and posterior nucleus of the newly dividing seam cells was perturbed in the absence of FRK-1. It has been long established that POP-1 is localized asymmetrically in seam cell nuclei, such that the anterior nucleus has a higher level of POP-1 than the posterior nucleus (LIN et al. 1998). Although we previously reported that FRK-1 is nuclear in mitotically active cells in embryos (PUTZKE et al. 2005), the subcellular localization of FRK-1 in mitotically active seam cells is not
known. Interestingly, we found that, under wildtype conditions, in actively dividing seam cells (L1 to L2), that FRK-1 mimics POP-1 asymmetric localization such that both proteins exhibit higher levels in the anterior nucleus and low levels in the corresponding posterior (Figure 6A).

In the absence of FRK-1 (frk-1(ok760) mutants), POP-1 asymmetry is lost, with both seam cell nuclei exhibiting low levels of POP-1 (n=20). Quantitation of the signal intensity for each protein shows the asymmetric localization for POP-1 and FRK-1 is significant between the two nuclei (Figure 6B). In stark contrast, the loss of FRK-1 results in no quantitative difference between levels of POP-1 between the anterior and posterior nucleus in all twenty cell divisions that were analyzed.

After establishing the FRK-1 is involved in regulation seam cell division through the asymmetric Wnt pathway we then tested the hypothesis that subcellular localization of pathway components was perturbed, resulting in the loss of POP-1 asymmetry in seam cell nuclei. We first examined localization of APR-1 (APR-1::YFP) and SYS-1 (mCherry::SYS-1) in frk-1(ok760), observing the initial seam cell division at the L1 stage. In wildtype larvae APR-1 localizes to the cytoplasm with punctae on the plasma membrane, as well as some nuclear localization, with no quantitative difference between the anterior and posterior nuclei (Supplemental Figure 1A, B, E, F). This APR-1 asymmetry can be seen as an anterior crescent in sections that bisect the cell (Supplemental Figure 1A, left column), or as asymmetric puncta that localize to the basal surface of cortical sections (Supplemental Figure 1B, left column). However, SYS-1 is normally nuclear localized with an asymmetry that favors stronger localization in the posterior nucleus (Supplemental Figure 1A, B, middle column). In the absence of FRK-1, there is no change in the nuclear localization, nor the anterior-posterior asymmetry,
of SYS-1 in dividing seam cells (Supplemental Figure 1C, D, middle column). Additionally, we did not observe a significant shift in APR-1 localization occurs in the absence of FRK-1, when compared to wildtype larvae (Supplemental Figure 1E, F).

The lack of any shift in localization of APR-1 from the anterior nucleus led us to speculate that WRM-1 localization might be perturbed when FRK-1 is not present. We then performed *frk-1(RNAi)* on early larvae, examining WRM-1::YFP localization during the L1-L2 seam cell divisions. Normally, although distributed in both the cytoplasm and the nucleus, WRM-1 is asymmetrically localized between the seam cell nuclei, with highest levels being in the posterior nucleus of dividing seam cells (n=24)(Figure 6C). However, loss of normal FRK-1 activity causes a loss of WRM-1 asymmetry such that the anterior and posterior nuclei are no longer significantly different with respect to WRM-1 levels (n=22)(Figure 6C, D). These data suggests that FRK-1 is working in conjunction with WRM-1 (and likely APR-1), but independently of SYS-1, to alter POP-1 levels, and thus, perturb seam cell divisions during post embryonic development.

**Discussion**

Asymmetric cell divisions, and the timing thereof, in the seam cells of the hypodermal lineage is critical during post-embryonic development. There are two major pathways in the seam cells that drive division: 1) the non-canonical Wnt pathway that initiates asymmetric division within the seam cells, and 2) heterochronic gene expression that regulate the timing of the divisions during larval progression. Within the asymmetric Wnt pathway, it has been well
established that the regulation of POP-1 localization between the two daughter nuclei of a dividing seam cell, in an unequal manner, determines the identity of those daughters (Lin et al. 1998; Herman 2001; Kidd et al. 2005; Phillips et al. 2007). More recently, the interplay between the two pathways has been shown to be important in controlling the switch between symmetric and asymmetric divisions (Harandi and Ambros 2015), thus allowing for the proper number of hypodermal cells to form and organize during development.

In this study we have characterized the role of a non-receptor tyrosine kinase, FRK-1, that is crucial for proper seam cell division during post-embryonic development in C. elegans. We report that in the absence of FRK-1, beyond the general arrest at the L1 larval stage, the seam cell division that occurs during L1 has been inappropriately switched from asymmetric to symmetric. We further show that identities of the resulting daughter cells appear to be compromised, as genes regulating seam cell development are significantly altered.

Through investigating the aberrant divisions within the *frk-1* V1-4 seam cells we found that POP-1 asymmetry is lost, such that each daughter nucleus shows low levels of POP-1. Since FRK-1 nuclear localization parallels POP-1 in the seam cells, we investigated localization of WRM-1 and found it too lost the asymmetric nuclear localization immediately following division, with an increase of WRM-1 in the anterior nucleus when FRK-1 is lost. Yang et al. demonstrated that POP-1 is phosphorylated through LIT-1 and bound by WRM-1 for export from the nucleus (Yang et al. 2011). The same study found that SYS-1-bound POP-1 inhibits WRM-1 binding, thereby preventing nuclear export. Additionally, it has also been reported that APR-1 is likely involved in exporting WRM-1 from the seam cell nuclei (Takeshita and Sawa 2005; Mizumoto and Sawa 2007a; Gleason and Eisenmann 2010). FRK-1 has been shown to interact with
both APR-1 and WRM-1 previously in regulating cellular identity and proliferation (Putzke et al. 2005; Putzke and Rothman 2010). Our data contribute significantly to these previous studies by supporting a model whereby FRK-1 facilitates POP-1 export via WRM-1.

Interestingly, in the absence of FRK-1 we observed no significant change in SYS-1 or APR-1 levels in seam cell nuclei, which is required for proper POP-1 activity in asymmetric cell divisions (Kidd et al. 2005; Banerjee et al. 2010). It has been reported that another kinase, KIN-19, interacts with SYS-1 in preventing seam cell hyperplasia (Banerjee et al. 2010), and that KIN-19 also controls the asymmetric localization of an APR-1 population that is capable of regulating WRM-1/POP-1 branch of the pathway (Baldwin and Phillips 2014). We also observe no combined effect on the number of seam cell divisions when eliminating sys-1 and frk-1 simultaneously via RNAi, further supporting a model in which the export of POP-1 is uncoupled from POP-1 nuclear transcriptional activation of target genes by SYS-1. It is clear that the increased seam cell proliferation we observed in the absence of FRK-1 is due to aberrant signaling through the asymmetric Wnt pathway primarily involving WRM-1 and POP-1, but not SYS-1, which regulates POP-1 activity but not localization.

Our study shows that FRK-1 acts in conjunction with components of the asymmetric pathway, and may have an overall effect that confounds heterochronic gene expression. Heterochronic genes are also affected in the absence of KIN-19, suggesting a complex interplay between the uncoupled pathways leading to POP-1 export and activity. Although our data show that there is altered expression of some heterochronic genes in the absence of FRK-1, we were not able to determine whether this was directly due to loss of FRK-1 interacting with the transcription factors responsible for activating those genes, or if it was an indirect effect due to
a compromised state of the seam cells (and possibly other hypodermal cells). Alternatively, it could be due to loss of POP-1 asymmetry but maintenance of SYS-1 asymmetry that leads to misregulation of heterochronic gene expression.

Harandi and Ambros recently hypothesized that weak POP-1/APR-1 activity leads to the symmetric division in the early L2 stage (Harandi and Ambros 2015). The fact that we observe that the L1 seam cell division appears symmetric, similar to early L2, led us to hypothesize that there is an interruption in the binary fate decision of seam cells in the absence of FRK-1. Although our data imply a possible precocious L2 seam cell division in L1 due to loss of asymmetry, our gene expression data show that could be due to inappropriate expression of heterochronic regulators, such as lin-4, which Harandi and Ambros hypothesize is likely contributing to lowering POP-1 activity. However, this is confounded by the fact that we also observe a loss of lin-28 expression, which could release inhibition of lin-46 and thus counteract the effects of lin-4 activity on POP-1. Therefore, we submit that the conflicting gene expression patterns observed in frk-1(ok760) mutants is not due to a heterochronic phenotype but is an indicator of disrupted upstream Wnt signaling, which results in compromised seam cell identity.

A significant contributing factor to normal seam cell asymmetry and the reaction to the Wnt signal, may be the level of EGL-18. Gorrepati, et al., reported that EGL-18 overexpression in a Wnt-sensitized background is capable of activating seam cell marker expression in head and ventral hypodermis, and that EGL-18 is required for seam cell fate specification (Gorrepati et al. 2013). It has also been previously reported that frk-1 acts upstream of elt-18 and elt-3 in regulating hypodermal cell specification during embryonic development (Putzke et al. 2005). Here, we demonstrate that, in addition to a decrease in expression of certain seam cell
regulating genes, there was significantly less expression of both *egl-18* and *elt-3* in *frk-1(ok760)* mutants. Being that it is a non-seam, hypodermis marker, lower levels of *elt-3* suggest a broader hypodermal specification role for FRK-1. However, the lower levels of *elt-18* expression again points to a compromised identity of both seam cell daughters, likely prompting an arrest in subsequent divisions. Thus, consistent with both studies, our data shows that FRK-1 expression is required for proper seam cell maintenance and subsequent division during larval development.

Our data further support previous studies suggesting separate pools of active APR-1 that are involved in exporting WRM-1, and thus regulating POP-1 activity via localization in seam cell nuclei, than those regulating SYS-1 levels through KIN-19 (Baldwin and Phillips 2014). We present here a model in which nuclear FRK-1, present in dividing seam cells of the V-lineage during post-embryonic development, interacts with the WRM-1 complex to regulate POP-1 accumulation in the nucleus. Normally, when seam cells divide, there is a higher level of FRK-1 in the nucleus of the anterior daughter compared to the posterior daughter nucleus shortly after division (Figure 7 top). It is reasonable to hypothesize that, based on our data, FRK-1 interacts with the nuclear pool of WRM-1 and/or POP-1 to facilitate POP-1 accumulation in the nucleus of the anterior daughter, while the low levels of FRK-1 in the posterior daughter allow for POP-1 export, via APR-1/WRM-1. This appears to be directed toward WRM-1, and independent of SYS-1, such that in the absence of FRK-1 we observe altered subcellular localization of both WRM-1 and POP-1 but not SYS-1 or APR-1 (Figures 5 and 6). Importantly, our genetic data points to an interaction between FRK-1, APR-1 and WRM-1 that facilitates nuclear export of WRM-1 without significantly altering nucleo-cytoplasmic ratios of APR-1.
(Supplemental Figure 1). Thus, in the absence of FRK-1, there is a compromised seam cell fate, due to the accumulation of WRM-1 in both recently divided seam cell nuclei, without altering normal SYS-1 asymmetry, resulting in loss of asymmetric division (Figure 7 bottom).

Current models of seam cell asymmetric division focus on the coordination of the asymmetric Wnt pathway for proper division and subsequent advancement to the next larval stage. Previous models consist of variations on cytoplasmic localization of the β-catenin homologues along with the APC homologue, APR-1 (MIZUMOTO AND SAWA 2007b; BALDWIN AND PHILLIPS 2014; GORREPATI et al. 2015); and that it is the shuttling of these proteins, in the present of a polarizing Wnt signal, that facilitates POP-1 (TCF) export from the nucleus in the posterior daughter cell (SUGIOKA et al. 2011). However, a continuing question in this area remained: is there a nuclear mechanism that sensitizes WRM-1 to export from the nucleus in the anterior daughter? Our data clarifies this question by supporting a role for FRK-1, due to the parallel localization between FRK-1 and POP-1 in seam cell daughter nuclei, in normally preventing WRM-1 from exporting POP-1 in the posterior daughter by functioning primarily in the nucleus and not in the cytoplasm of each seam cell daughter. However, whether FRK-1 modifies the WRM-1 complex or POP-1 to prevent the export of POP-1 in the anterior daughter remains to be determined. [Note: regarding the fate of each daughter cell in our model: we labeled each nucleus on the bottom of Figure 7 as “compromised” since we were not able to confirm that the posterior cell retains it posterior identity in the complete absence of FRK-1, as opposed to the low levels of FRK-1 it normally contains in wildtype.]

Importantly, unlike the kinase-independent roles of FRK-1 activity during embryonic development (PUTZKE et al. 2005), FRK-1 regulation of seam cell division in larvae appears to be
kinase-dependent. This suggests that either the Wnt pathway components, such as WRM-1, might be direct targets of FRK-1 phosphorylation in establishing export of the complex from the seam cell nuclei during POP-1 export in the posterior daughter, or perhaps, to prevent anterior accumulation of the WRM-1 in the anterior daughter. Our data are consistent with a prior study that demonstrated FRK-1 regulation of cell division within the endodermal lineage; however, that study did not test whether the endodermal proliferation influence of FRK-1 was kinase dependent (PUTZKE AND ROTHMAN 2010). In addition to FRK-1 in C. elegans, kinase independent activities of Fer kinase were shown by Craig et al., in which a kinase-inactivating knock-in resulted in viable mice (CRAIG et al. 2001). Furthermore, similar kinase inactive roles have been shown for the Fps/Fes kinase in mice (SENIS et al. 2003), providing additional evidence of the potential for kinases to acquire functions within the cell beyond their phosphorylation capabilities.

Finally, our observations in C. elegans, consistent with our prior studies, suggest that normally FRK-1 might phosphorylate a β-catenin homologue in C. elegans in order to stabilize it at the cortical membrane or adherens junctions (PUTZKE et al. 2005; PUTZKE AND ROTHMAN 2010). There have been conflicting reports with Fer kinase in mammalian systems which showing that Fer kinase activity leads to both stabilization and/or destabilization of cadherin complexes. This is in part due to the fact that β-catenin phosphorylation on Tyr654 regulates association with E-cadherin, while phosphorylation on Tyr142 facilitates interactions with α-catenin. For instance, Piedra et al., showed that although the cadherin complex was disrupted with increased Fer activity, β-catenin was still associated with E-cadherin, with the disruption of the complex being between β-catenin and α-catenin (PIEDRA et al. 2003). In contrast, Xu et al., demonstrated that
the presence of Fer phosphorylates PTP1B, which then dephosphorylates β-catenin, thus stabilizing the cadherin complex (Xu et al. 2004). The same study also showed that in the presence of a kinase inactive Fer the cadherin complex destabilizes, due to the subsequent phosphorylation of β-catenin. More recently, two studies have further supported the role of Fer directly phosphorylating β-catenin at Tyr142 (Chang et al. 2014) as well as causing indirect dephosphorylation of β-catenin at Tyr654 (Lee et al. 2008). Thus, the destabilization of cadherin complexes can occur through either dissociation of the β-catenin/α-catenin interaction (when Fer phosphorylates Tyr142 on β-catenin), or via the lack of Fer activity on PTP1B (or SHP2 as shown by Lee et al., 2008), resulting in disruption of the E-cadherin/β-catenin interaction (due to lack of dephosphorylation at Tyr654 on β-catenin).

Although we do not know whether FRK-1 directly phosphorylates any β-catenin homologues in C. elegans, such as HMP-2 and WRM-1, we previously reported that they interact with FRK-1(Putzke et al. 2005; Putzke and Rothman 2010). Furthermore, the possibility of a direct interaction is supported by a report from Sumiyoshi, et al., which showed that Src, another non-receptor tyrosine kinase, of which Fer is a sub-family member, is capable of phosphorylating HMP-2, thereby causing HMP-2 to dissociate from the cortical membrane (Sumiyoshi et al. 2011).

The issue of Fer/FRK-1 phosphorylation of cortically associated β-catenin is further complicated by two major issues: 1) it has been shown that Fer phosphorylation of plakoglobin (a β-catenin homologue in vertebrates) has the opposite effect of a direct Fer phosphorylation of β-catenin in mammalian cells (i.e. stabilizes cadherin junctions but also upregulates β-catenin/Tcf4 transcriptional activity)(Miravet et al. 2003) and 2) C. elegans has four β-catenin
homologues (with diverging functions) and no reported plakoglobin homologues. Thus, it is possible that the activity of FRK-1 in *C. elegans* modifies different β-catenin homologues depending on the interactions at the cortical membrane. However, our data suggests a novel role in which FRK-1 is interacting with β-catenin (in this case WRM-1), not at the cellular cortex but in the nucleus. In this context FRK-1 may be phosphorylating WRM-1 (or the WRM-1 complex) in a way that facilitates WRM-1 export from the nucleus (Figure 7). Alternatively, FRK-1 may function to destabilize any interaction between WRM-1 and POP-1, thereby preventing WRM-1 accumulation in the nucleus. Although this model describes a novel mechanism for FRK-1, it is not completely unexpected in that prior studies have shown Fer/FRK-1 to translocate to the nucleus of actively dividing cells (Ben-Dor et al. 1999; Putzke et al. 2005).

Proper cell division within differentiating stem cell lineages is crucial for morphogenetic movements during development. When cell divisions occur in an unregulated manner, consequences ranging from loss of cellular identity to improper tissue size and function arise as a detriment to the organism. The Fer kinase homologue in *C. elegans*, FRK-1, is a crucial component in regulating the asymmetric divisions within the stem cell-like divisions of the hypodermal seam cells during post-embryonic development through the asymmetric Wnt pathway. It will be of interest to future studies to determine whether FRK-1 asymmetric localization in seam cell nuclei occurs in a Wnt-dependent manner. Furthermore, whether FRK-1 is interacting directly with the β-catenin homologue, WRM-1, remains to be seen, but investigating this interaction may further elucidate whether targets are merely shuttled by FRK-1 or modified as bona fide phosphorylation targets of this non-receptor tyrosine kinase.
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Figure Legends

Figure 1. Seam cell asymmetric divisions during post-embryonic development in *C. elegans*.

During post-embryonic development, *C. elegans* larvae undergo a series of molts, each of which is associated with carefully timed seam cell divisions. In the V1-4, 6 cells the divisions are nearly all symmetrical where the anterior daughter becomes part of the hyp7 syncytium. The posterior daughter then self-renews as another stem cell-like seam cell. The exception to the asymmetric divisions is in early L2 where the V1-4, 6 cells undergo one symmetrical division prior to dividing again in an asymmetric manner. The V5 cell divides asymmetrically as well, but gives rise to series of neuronal postdeirid cells in the L2 division, whereas the remaining cell divisions parallel V1-4, 6. Thus, excluding the V5 postdeirid cells, the remaining cells differentiate at adulthood into hypodermal cells (as hyp7 or seam).

Figure 2. The *frk-1* gene is active in seam cells throughout development and results in early larval arrest when deleted from the genome. *frk-1p::nGFP* fusion shows *frk-1* promoter activity in early seam cells at 1.5 fold (black arrows) and persists in seam cells through adulthood (seam cells -white arrows, other nuclei shown are non-seam hypodermal nuclei, as *frk-1* is expressed in multiple epithelial cell types)(A, B). PCR of the *ok760* allele in *frk-1* resulting in approximately 1500 basepairs of DNA deleted corresponding to 910 basepairs of promoter and the first two exons of the *frk-1* coding region (C). Presence of a truncated transcript expressed from the remaining *frk-1* sequence was not detected (data not shown). *frk-1(ok760)* mutants arrest at the L1 stage with an apparent loss of asymmetric division of the seam cell lineage (D). Wildtype seam cells are asymmetric post-division with a larger posterior daughter (inset – top), while *frk-
1(ok760) mutants contain two similar sized daughters (smaller like the anterior daughter in a wildtype individual)(inset – bottom).

**Figure 3.** The absence of FRK-1 results in aberrant seam cell function and inappropriate division. *frk-1(ok760)* mutants do not have regularly shaped seam cells (A,C -MH27) and lack alae (B,D - NE21B4) at the L1 stage, suggesting non-functional seam cells. Additionally, there are supernumery seam cells, especially in V1-V4, (E,F - *scm::GFP*). Wildtype larvae (E - lateral view) show single cells post-division (when anterior daughter GFP has mostly subsided – white arrowhead, posterior nucleus – orange arrowhead), while *frk-1(ok760)* mutants (F - lateral view) show arrested daughter pairs with GFP persistence in the anterior daughter. *frk-1* mutants exhibit significant loss of heterochronic gene larkers as well as seam specific markers (G).

**Figure 4.** FRK-1 activity in post-embryonic development is kinase dependent. *frk-1(ok760)* mutants are rescued by wildtype *frk-1* in a kinase-activity dependent manner (A,B). *frk-1(ok760)* mutants are rescued to adulthood with wildtype *frk-1* but not kinase inactive *frk-1* (A). Similarly, wildtype, but not kinase inactive, *frk-1* is sufficient to rescue proliferation defects within the seam cells of *frk-1(ok760)* mutants (B). Rescue to adulthood is sufficient with *frk-1* activity confined exclusively to seam cells, via expression of *frk-1* from the *scm* promoter (C). As with the expression from the *frk-1* promoter, expression from the *scm* promoter rescues seam cell proliferation in a kinase dependent manner (D – V1-4 cells are shown at 30 hours post fertilization. At this stage, in wildtype, the asymmetric L2 divisions are complete and the cells exhibit asymmetric GFP expression).
Figure 5. Elimination of \textit{frk-1} by RNAi at the L3-L4 transition results in excess seam cell divisions (A-top), approximately half as much as \textit{pop-1} elimination (A – middle), when compared to control RNAi (\textit{unc-22}, A – bottom). Removal of \textit{frk-1} or \textit{apr-1} results in supernumerary seam cell proliferation that is additive with \textit{pop-1} elimination. The defects in seam cell proliferation observed with loss of \textit{frk-1} and \textit{apr-1} are \textit{wrn-1} dependent but independent of \textit{sys-1} (B).

Figure 6. FRK-1 nuclear localization parallels that of POP-1 in seam cells nuclei post-division. FRK-1 is nuclear localized and asymmetric in an anterior-posterior manner in dividing seam cells within the V1-4 population (A - middle) similar to POP-1 (A – top), with higher levels in the anterior daughter. POP-1 asymmetry is lost in the absence of FRK-1, with the both daughters containing low levels of POP-1 (A – bottom). Quantification of FRK-1 and POP-1 asymmetry in seam cell daughters similar in wildtype compared to POP-1 in \textit{frk-1(ok760)} mutants (B - FITC signal in (A) is normalized to DAPI for quantitation). WRM-1 is normally asymmetric in dividing seam cell nuclei (top), but becomes more symmetric in the absence of \textit{frk-1} (bottom)(C). Quantitation of WRM-1::YFP localization in the anterior and posterior daughter nuclei shows a significant loss of asymmetry immediately post division (* indicates that it is $p<0.05$, with all other comparisons $p>0.05$).

Figure 7. Model for FRK-1 regulation of Wnt asymmetry in dividing seam cells. During normal seam cell divisions, high levels of FRK-1 in the anterior nucleus influence POP-1 accumulation, while low levels of FRK-1 facilitate export from the posterior seam cell (top). FRK-1 activity is likely via modification of the WRM-1 complex or perhaps through POP-1 to prevent a WRM-1/POP-1 interaction. Additionally, FRK-1 appears to act in parallel with SYS-1 in either case. In the absence of FRK-1, the WRM-1 complex is improperly localized, with no change in SYS-1,
compromising the fate of each daughter cell (bottom), resulting in a symmetric division with an anterior cell size but more posterior gene expression pattern. Thus, the cells in the bottom frame are labeled “compromised” because it cannot be assumed that the posterior fate is the default setting in the absence of FRK-1, since normally there are low levels of FRK-1 in the posterior nucleus, as opposed to none.
Figure 1. Seam cell asymmetric divisions during post-embryonic development in *C. elegans*.
Figure 2. The \textit{frk-1} gene is active in seam cells throughout development and results in early larval arrest when deleted from the genome.
Figure 3. The absence of FRK-1 results in aberrant seam cell function and inappropriate division.
Figure 4. FRK-1 activity in post-embryonic development is kinase dependent.
Figure 5. Elimination of *frk-1* by RNAi at the L3-L4 transition results in excess seam cell divisions.
Figure 6. POP-1 and WRM-1 lose anterior-posterior asymmetry in the absence of FRK-1.
Figure 7. Model for FRK-1 regulation of Wnt asymmetry in dividing seam cells.