INTERACTION OF CENTROSOMAL COMPONENT SPD-5 WITH WNT SIGNALS IN THE CONTROL OF CELL POLARITY IN *CAENORHABDITIS ELEGANS*

by

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Abstract

All multicellular organisms consist of a variety of cell types. One of the mechanisms to generate this cellular diversity is the asymmetric cell division, which requires the establishment of cell polarity. In *Caenorhabditis elegans* hermaphrodites, 807 of 949 somatic cell divisions are asymmetric. The centrosome and the Wnt signaling pathway both have been shown to regulate cell polarity and subsequently asymmetric divisions in many model organisms. However, it is not clear whether the Wnt signaling pathway manipulates the cell polarity through specific cellular organelles, such as the centrosome. To address this question, we examined a centrosomal component, SPD-5, to see whether it cooperates with the Wnt signaling pathway to regulate certain asymmetric cell divisions. We showed that SPD-5, which was originally found to be critical for the embryonic development, also played a role during certain post-embryonic cell divisions in *C. elegans*. Specifically the asymmetric divisions of seam cells that required SPD-5 function were also known to be regulated by the Wnt signaling pathway. Thus the stem-cell like seam cell divisions could be an intriguing system to study the interaction of centrosomes and the Wnt pathway. We found that SPD-5 was required for a successful cell division, similar to other centrosomal components. This suggests that SPD-5 still functions as a centrosomal component during *C. elegans* post-embryonic development. It has been shown that establishment of seam cell polarity relies on the asymmetric localization of certain Wnt pathway components. Interestingly, we found that SPD-5 was required for the proper localization of several Wnt components in a way that was independent of a key MTOC (microtubule-organizing center) member γ-tubulin. In addition, SPD-5 genetically interacted with the Wnt pathway components APR-1/APC and POP-1/Tcf to regulate asymmetric divisions of seam cells. These data suggest that SPD-5 interacts with the Wnt signaling pathway in controlling the polarity of seam cells. Overall, our results suggest a novel role of SPD-5 in cooperating with the Wnt signaling pathway to regulate cell polarity and asymmetric cell division, in addition to its function as a centrosomal component.

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Introduction

Asymmetric cell division that generates daughter cells with different fates is one of the key mechanisms to produce cellular diversity in multicellular organisms. In most of the cases, cells that undergo asymmetric divisions are polarized before the division. Two cues have been shown to trigger the polarization, one is a non-membrane bound organelle, the centrosome (Gönczy, 2005); the other is extracellular Wnt signaling (Eisenmann, 2005). In *Caenorhabditis elegans* as many as 807 of 949 somatic cell divisions are asymmetric, hence the short-living and transparent nematodes are an ideal model to study the cell polarity and the subsequent asymmetric divisions. The mechanisms behind the establishment of cell polarity by the centrosome and the Wnt signaling pathway are different among species. Here we review what is known about how the centrosome and Wnt signaling regulate cell polarity in different organisms.

Centrosome

More than 130 years have passed since the discovery of the centrosome (Flemming, 1875; Van Beneden, 1876) and we are still trying to understand the numerous functions of this cellular organelle. In animal cells, centrosomes function as microtubule-organizing center (MTOC), and were named according to their close location to the cell center (Boveri, 1888). However in certain eukaryotic cells that lack centrosomes, other structures function as the MTOC. For example, spindle pole bodies in

yeast (Knop, 1999) and nucleating sites in plants (Chan, 2003) perform the MTOC function. The centrosomes have been recognized as core structures of spindle poles and thus essential for the success of mitosis since its first description in sea urchin eggs (Boveri, 1914). Although centrosomes appear to have a central function during cell division, it has been shown that *Drosophila melanogaster* containing a null mutant of a centrosomal protein, Centrosomin, which caused the loss of functional centrosomes, could still develop into adults (Megraw, 2001). This suggests that centrosomes might perform diverse functions in different systems. In addition to functioning as the MTOC, the centrosome has been recently recognized as a docking site where multiple cell activities take place, such as cell cycle regulatory events (Kramer, 2004). Here we will focus on functions of centrosomes in *C. elegans* and make comparisons with other model organisms.

Major structure and key functions

The centrosome is a subcellular non-membrane bound organelle, which consists of a pair of perpendicularly oriented cylindrical centrioles and a mass of centrosome proteins, referred to as pericentriolar material (PCM) (Figure 1.1). The centrosome duplicates only once in one cell cycle (Kellogg, 1989). Thus normal cells contain either one or two centrosomes depending on their position in the cell cycle (Bettencourt-Dias, 2007). Because the centrosome serves as a MTOC, it performs microtubule-related functions, such as segregating chromosomes during cell division, maintaining cell shape, regulating cell polarity and motility, and localizing organelles and cargos (Schatten, 2008). In addition, centrosomes have been recently shown to control several cell cycle

regulatory events, such as entry into mitosis, cytokinesis, G1/S transition and monitoring DNA damage (Kramer, 2004).

Centrioles are hollow cylinders that consist of nine microtubule triplets, doublets or singlets, depending on the cell type. *C. elegans* centrioles are made of nine singlet microtubules (Albertson, 1984; Callaini, 1990), while vertebrate centrioles are composed of nine triplets (Marshall, 2001; Preble et al., 2000). Microtubules universally consist of polymerized α- and β-tubulin dimers. However, different numbers of α- and β-tubulin homologs are present in different eukaryotic organisms. For instance, the human genome contains at least 15 α-tubulin and 21 β-tubulin genes; while the *C. elegans* genome has 9 α-tubulin (*tba-1~9*) and 4 β-tubulin (*tbb-1,2,4,6)* genes (Dutcher, 2003). The function of centrioles is to partially aid in the recruitment of the PCM, and maintain the structural integrity of centrosomes. However, the existence of the centrosomal material without centrioles in animal oocytes implies that centrioles are not as essential as the PCM for MTOC function.

Centriole proteins directly associate with centrioles. In *C. elegans,* all known centriole proteins are shown to regulate the duplication of centrioles. In each cell cycle, the centriole duplicates once and only once. The duplication requires a protein kinase ZYG-1 and four coiled-coil proteins SPD-2, SYS-4, SYS-5 and SYS-6 in *C. elegans* embryo (O'Connell, 2000 and 2002; Kirkham, 2003; Leidel, 2005; Delattre, 2004). ZYG-1 triggers the initiation of the centrosome duplication at the G1 to S transition in *C. elegans.* A Polo-Like Kinase, PLK4 is the functional ortholog of ZYG-1 in other organisms (also known as SAK in *Drosophila melanogaster*), although their structures differ (Azimzadeh, 2010). The recruitment of ZYG-1 depends on SPD-2, which localizes

both to the centrioles and the PCM. Physical interaction of SYS-4, SYS-5 and SYS-6 are required for the centriole assembly (Azimzadeh, 2010).

Pericentriolar material (PCM) is an amorphous scaffolding lattice that contains many coiled-coil proteins. These proteins are not like centriole proteins that directly associate with centrioles, instead they surround the centrioles. In human over 500 proteins were identified by mass spectroscopy to be associated with centrosomes (Andersen, 2003). However, only a small number of these proteins are related to centrosome function, thus can be termed as PCM, such as components of the γ-tubulin ring complexes that anchor microtubules. Most of these proteins might be anchored by the PCM, and thus only use the centrosome as a docking station to perform biological activities, such as mitogen activated protein kinase (MAPK) (Sun, 2002).

The core component of PCM is the γ-tubulin ring complex, which is responsible for the centrosome function as a MTOC to nucleate microtubules in eukaryotes. In *C. elegans*, γ-tubulin ring complexes are composed of only three key proteins, γ-tubulin, a homologue of α- and β-tubulin, and two γ-tubulin complex proteins, CeGrip-2 (*gip-2)* and CeGrip-1 (*gip-1)*. While in *Drosophila melanogaster* embryos and *Xenopus lsaevis* eggs, γ-tubulin complex contains at least three other proteins: GCP4, GCP5, GCP6 and the human γ-tubulin ring complex contains even more components than that, such as MOZART1 and 2 (mitotic-spindle organizing proteins associated with a ring of γ-tubulin 1) (Kollman, 2011).

Essential PCM proteins generally function to recruit γ-tubulin and other PCM components into centrosomes. These include the coiled-coil proteins: pericentrin (Delaval, 2010) in mammals, SPD-2 and SPD-5 in *C. elegans* (Oegema, 2006); enzymes: protein

phosphatase 4 (PP4) in both *Drosophila* and *C. elegans*, and Aurora A kinase in a variety of organisms (Meraldi, 2002).

SPD-5 is an essential component of the centrosome

SPD-5 (SPindle Defective) is predicted to contain 1198 amino acids and consists of 11 coiled-coil domains (Hamill, 2002). SPD-5 is an essential PCM component and regulates both duplication and maturation of the centrosome during embryonic development (Dammermann, 2004). Particularly in controlling centrosome maturation, SPD-5 genetically interacts with SPD-2 and DHC-1, and is required for the centrosomal localization of γ-tubulin, AIR-1 (Aurora A kinase) and ZYG-9, a centrosomal MT-stabilizing protein related to XMAP-215 in *Xenopus* (Matthews, 1998). In *spd-5* mutant embryos, the PCM fails to form, and centrosomal asters are not observed (Hamill, 2002). The requirement of SPD-5 in centriole duplication is likely due to its function to concentrate γ-tubulin around the parent centriole (Dammermann, 2004). It was shown recently that SPD-5 is essential for the proper placement of microtubules in the centrosome (O'Toole, 2012). However, since the current studies of SPD-5 only focused on the embryo, whether SPD-5 functions consistently during the post-embryonic development is unknown.

Other centrosome functions

Other functions of centrosomes, in addition to the MTOC, include cell cycle regulatory events, cytokinesis and DNA damage regulation; which are largely studied in vertebrates (Kramer, 2004). For these activities, centrosomes generally function as docking stations where regulatory molecules are modified. By anchoring components of

cellular pathways, centrosomes help to physically link these molecules, and provide spatial control of their interactions.

The first evidence that the centrosome plays a role in cell cycle regulation was the observation that microinjection of centrosomes into G2-arrested starfish oocytes induced a G2 to M transition (Picard, 1987). The kinase activity of cyclin B1-Cdk1 complex is required for entry into mitosis. Cdk1 activation relies on the upstream regulator phosphatase Cdc25B, which is phosphorylated and activated by Aurora-A in centrosomes (Kramer, 2004). Therefore, the initial activation of cyclin B1-Cdk1 takes place at centrosomes and spreads out to induce cytoplasmic and nuclear mitotic events, such as formation of mitotic spindles and chromosome condensation (Jackman, 2003). The ablation of centrioles does not prevent mitosis entry (Hinchcliffe, 2001; Khodjakov, 2001), but the disruption of proteins associating with centrosomes (such as Aurora-A) does (Kramer, 2004). These observations suggest that the PCM or proteins associate with centrosomes might perform as docking stations, instead of centrioles.

The direct evidence of centrosome function in cytokinesis was observed in 2001, when 30-50% of vertebrate somatic cells, in which centrosomes were ablated by a laser, exhibited cytokinesis failure and produced binucleated cells (Khodjakov, 2001). A high frequency of cytokinesis failure was also observed in an acentriolar *Drosophila* cell line (Piel, 2001). Although the molecular mechanism behind this phenomenon is not clear, there is evidence suggesting that centrosomes serve as docking stations for cytokinesis regulatory proteins (Schatten, 2008).

Supernumerary centrosomes and cancer

While removal of centrosomes causes defects in various cellular activities, such as formation of mitotic spindles, cell cycle progression, cytokinesis (detailed above); supernumerary centrosomes (more than two centrosomes) have also been shown to induce mitotic defects. Upon the discovery of the centrosome, supernumerary centrosomes have been linked to multipolar mitosis, further considered a potential cause of cancer (Boveri, 1914). Since then, a variety of cancer cell lines have been observed to contain different percentages of cells with supernumerary centrosomes (Kramer, 2005; Nigg, 2002; Lingle, 1998; Giehl, 2005; Pihan, 1998). Cancer cells with supernumerary centrosomes frequently form multipolar spindle poles, which are associated with either cytokinesis failure or severe aneuploidy. Furthermore, the stage of cancer, which is a description of the extent the cancer has spread, was shown to associate with frequency of centrosome amplification (Kawamura, 2004). Particularly, the higher the cancer stage, the more often the supernumerary centrosomes were observed. However, whether supernumerary centrosomes are real causes of cancer or just byproducts is still controversial.

Cell polarity and Asymmetric cell division

All multicellular organisms consist of a variety of cell types, which are ultimately derived from the single-cell zygote. To generate this diversity, cells can either divide into identical cells, which later become different through exposure to diverse environmental cues; or through asymmetric cell divisions that directly generate different cells. The significance of asymmetric cell division during development has been studied in many organisms, including *C. elegans, Drosophila,* and humans. In most cases, asymmetric cell

division partitions cell fates by the segregation of protein or RNA-based cell fate determinants unequally into two daughter cells. This process requires establishment of cell polarity, which directs asymmetric segregation of cell fate determinants along the body axis. In some cases, asymmetric cell divisions also generate cells of different sizes, which involve the regulation of the cytoskeleton network to polarize the cell (Gönczy, 2005). Key players of asymmetric cell division in diverse organisms are conserved, such as the *par* genes (Partitioning-defective). Here, we will compare the mechanisms and molecules involved in the control of asymmetric divisions in *C. elegans*, *Drosophila*, and vertebrates.

C. elegans

C. elegans is an ideal organism for studying cell polarity and asymmetric cell division for three reasons: first, 807 of 949 somatic cell divisions are asymmetric, which provide various cells for study; second, it is transparent through its life cycle, which makes it possible to observe development at the cellular level in living animals; third, it has a small number of cells and the complete cell lineage is known. The cues that function to induce the polarity include the sperm centrosome in the zygote, and extrinsic signals, in particular WNT signaling, as described below.

The centrosome triggers cell polarity in the zygote

The mechanism governing cell polarity and thus asymmetric division in the *C. elegans* zygote (called P_0) has been intensively studied (Figure 1.2). P_0 divides to a large anterior daughter cell called AB that produces hypodermis, neurons, anterior pharynx and some other cell types, and a small posterior daughter cell called P1 that generally produces muscle, hypodermis, neurons, the majority of the pharynx and the germ line

cells (Gönczy, 2005). The anterior-posterior (AP) axis and associated cell polarity is established around 25 minutes after fertilization (Goldstein, 1996). The initiation of the process is triggered by the sperm centrosome. The *C. elegans* oocyte lacks the centrosome and is generally symmetric. The sperm donates not only the genetic material but also a pair of centrioles, which gather the PCM to form the centrosome in the P_0 zygote (Gönczy, 2005). Right after sperm entry, the entire zygote cortex undergoes a surface ruffle, a motion visible as numerous temporary cortical invaginations. The ruffle is driven by a surface contraction that involves the actomyosin network, including F actin and non-muscle myosin II (Munro, 2004). This symmetric cortical ruffling rapidly ceases in the presumptive posterior site, where the sperm centrosome localizes close to the cortex. Then, there is a cytoplasmic flow towards the posterior site and a cortical flow towards the anterior site, resulting in anterior accumulation of cortical proteins, such as PAR-3, PAR-6 and the atypical protein kinase C, PKC-3. The anterior PAR-3/PAR-6/PKC-3 complex prevents accumulation of PAR-1 and PAR-2 through phosphorylation by PKC-3 (Benton, 2003; Hao, 2006). This results in the establishment of AP cell polarity with anterior concentrated PAR-3/PAR-6/PKC-3 and posterior concentrated PAR-1/PAR-2. The cue that triggers this polarity has not been exclusively pointed to the centrosome until recently, because of the difficulty to distinguish the PCM from microtubules it concentrates. Mutants of PCM genes, *spd-2*, *spd-5* and *air-1* inhibit the establishment of polarity (Hamill, 2002; O'Connell, 2000; Wallenfang, 2000). Furthermore, genetic or laser ablation of the centrosome prevents the establishment of AP polarity (Munro, 2004; Cowan, 2004); while knock down of β-tubulin by RNAi does not (Cowan 2004; Sonneville, 2004). These data suggest that the polarity is triggered by the

centrosomal PCM instead of the microtubules. However in a recent proposed model, the microtubule aster might function downstream of the centrosome to transmit a polarity signal to the zygote cortex (Munro, 2009). Nevertheless, the exact mechanism requires further investigation.

Asymmetric segregation of cell fate determinants

The cell fate determinants in *C. elegans* zygotes include P granules (germ line specific ribonucleoprotein complex), and CCCH-finger protein PIE-1 (restricted to germ line precursors) (Gönczy, 2005). Polarized PAR proteins and downstream mediators control asymmetric segregation of these determinants into the two daughters of P_0 by at least two mechanisms. First, direct transportation: the P granules move towards the posterior with the cytoplasmic flow (Strome, 1883; Hird, 1996) and concentrate in the posterior daughters. Second, local degradation by E3 ligase (DeRenzo, 2003): the P granules and PIE-1 that specify the germ line fate are diminished in the anterior daughters, and thus accumulate in posterior daughters.

Asymmetric division of Drosophila neuroblasts

Drosophila neuroblasts that underlie the neuroectoderm undergo repeated asymmetric cell divisions and generate all the neurons and glial cells in the central nervous system (CNS). Each asymmetric division produces an apically large cell that retains the neuroblast fate, and a basally small ganglion mother cell (GMC), which further divides into two terminal differentiated neurons. Similar to *C. elegans,* the generation of neuroblast polarity involves PAR proteins. The conserved PAR-3, PAR-6 and PKC-3 form a complex, and asymmetrically localize to the apical cortex. The initial apical localization of PAR-3/PAR-6/PKC-3 is inherited from epithelial cells in the

neuroectoderm (Wodarz, 1999 and 2000; Schober, 1999). The apical PAR complex further controls apical location of adaptor protein Inscuteable, heterotrimeric G protein αi-subunit(Gαi), the adaptor protein Partner of Inscuteable (PINS) and dynein-binding protein MUD (Schaefer, 2000 and 2001; Yu, 2000; Siller, 2006; Izumi, 2006; Bowman, 2006). Eventually cell fate determinants, Numb (that inhibits Notch-Delta signaling), BRAT (translation inhibitor of Brain tumor) and Prospero (transcription factor) are concentrated on the basal side (Berdnik, 2002 and Sonoda, 2001). This leads to the asymmetric inheritance of cell fate determinants, which differ the fates of apical and basal cells.

The role of centrosomes in cell polarity of Drosophila neuroblasts and vertebrates

While the cell polarity of the first *Drosophila* neuroblast division is inherited from the epithelial cells, during subsequent divisions the centrosomes are thought to maintain the polarity (Rebollo, 2007 and 2009). After the first division, the newly generated daughter centriole, absent of PCM, always moves to the basal side of neuroblast, and thus is inherited by the basal daughter cell. The mother centrosome remains close to apical side, and thus is inherited by the apical cell. The similar observation is also obtained during asymmetric cell divisions of radial glial cells in the mouse brain, where the mother centrosome is preferentially inherited by one of the daughter cells (Wang, 2009). Furthermore, the positioning of apical proteins in *Drosophila* neuroblasts can also be mediated by centrosomes (Januschke, 2010). All these observations indicate that the orientation of centrosomes might control the axis of polarity and asymmetric inheritance

of mother and daughter centrosomes might determine the different cell fates during stem cell divisions (Spradling, 2007).

WNT signaling pathway

The Wnt signaling pathway is one of the most important developmental pathways and is evolutionally conserved among animals from Hydra to humans (Eisenmann, 2005). Numerous processes that are governed by the Wnt pathway include cell polarity, differentiation, proliferation, migration and axon outgrowth (Eisenmann, 2005; Veeman, 2003). The function of the Wnt pathway is generally mediated through the nuclear β-catenin, a member of the Armadillo (Arm) family, which contains a central 12 Arm repeat domain (Sharma, 2012). β-catenin was first identified to form a complex with cadherins to regulate cell adhesion and motility at adherens junctions in the epithelial cortex. Later the nuclear level of β-catenin was found to be a hallmark of Wnt signaling pathway (Willert, 1998). Wnt signaling can be divided into two sub-pathways, canonical and non-canonical. Canonical Wnt pathways are well studied and are conserved in the animal kingdom; whereas non-canonical Wnt pathways, which are not as well conserved, include Wnt/Calcium and Wnt/JNK pathways in vertebrates, and Wnt/planar cell polarity pathway (PCP) in flies (Veeman, 2003; Nelson, 2004).

Canonical WNT

After the discovery of the first mammalian wnt gene *Int1* (Nusse, 1982), the function of the canonical Wnt pathway was intensively studied in a wide spectrum of key biological areas, such as cellular proliferation, differentiation, migration, and apoptosis outgrowth (Eisenmann, 2005; Veeman, 2003). Disruption of these pathway cascades might cause developmental defects and diseases, such as cancer (Reya, 2005; Nusse,

2012). The canonical Wnt pathway (Figure 1.3) manipulates the level of β-catenin, which functions as a transcriptional coactivator for TCF (T-cell factor). TCF represses target genes through a direct association with co-repressors such as Groucho. In the absence of Wnts (glycoprotein ligands), β-catenin is maintained at a low level by a destruction complex, composed of two key scaffolding proteins: adenomatous polyposis coli (APC), axin, and two key kinases: casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK-3) (Nelson, 2004; Reya, 2005; Grigoryan, 2008). APC recruits cytoplasmic β-catenin to the destruction complex, where it is phosphorylated at N-terminal serine and threonine residues by CK1 and GSK-3. The phosphorylated β-catenin then undergoes ubiquitination and subsequent proteasomal degradation. Binding of Wnt ligands to the cognate receptor complex, consisting of Frizzled transmembrane receptors and LRP (a member of the LDL receptor family), inhibits the formation of destruction complex through phosphorylation of LRP by GSK3 and CK1, binding of Axin to LRP or/and or the actions of an axin binding molecule, Dishevelled. The resulting stabilized β-catenin enters the nucleus and switches TCF from a transcriptional repressor to an activator by replacing co-repressors, such as Groucho. Although most our knowledge of canonical Wnt pathway came from studies in vertebrates and *Drosophila*. It is also conserved in *C. elegans*, where for instance it functions in the migration of Q neuroblasts (Eisenmann, 2005).

Wnt pathway in C. elegans

As in other species, both canonical and noncanonical Wnt pathways function in *C. elegans*. The *C. elegans* genome encodes five Wnt ligands (*lin-44*, *egl-20*, *mom-2*, *cwn-1* and cwn-2) (Herman, 1995; Eisenmann, 2005), four Frizzled receptors (*lin-17*, *mom-5*,

mig-1 and *cfz-2)* and three Disheveled proteins (*mig-5*, *dsh-1*, *dsh-2*; Eisenmann, 2005). These Wnt components, which function in both canonical and noncanonical Wnt pathways, may act differently in each pathway (Herman, 2003 and 2004). Unlike in mammals and *Drosophila* where one β-catenin controls both adhesion and Wnt signaling (Schneider, 2003 and Bienz, 2005), *C. elegans* has four β-catenin homologs (*bar-1*, *wrm-1*, *sys-1*, *hmp-2*) and thus each performs distinct functions (Korswagen, 2007). Specifically, HMP-2 regulates cell adhesion; BAR-1, WRM-1 and SYS-1 regulate Wnt signaling pathways.

Canonical Wnt pathway in C. elegans

BAR-1 functions in a canonical Wnt pathway consistent with the β-catenin function in other organisms. For instance, migration of Q neuroblast progeny is regulated by this canonical Wnt pathway in *C. elegans*. The two Q neuroblasts are located at left (QL) and right (QR) side at the same position along the anterior-posterior (AP) axis. The lineages of QL and QR are similar, while their descendants (QL.d or QR.d) migrate in opposite directions along the AP axis. The migration relies on the expression of Hox gene *mab-5*, which is a downstream target of the canonical Wnt pathway. MAB-5-positive QL.d migrates posteriorly, whereas MAB-5-negative QR.d moves anteriorly. The Wnt components involved in this process are EGL-20/Wnt, LIN-17/Fz, MIG-1/Fz, MIG-5/Dsh, POP-1/TCF, and PRY-1/Axin and GSK-3/GSK3β, in addition to BAR-1 (Harris, 1996; Maloof, 1999; Eisenmann, 2000; Korswagen, 2000 and 2002; Herman, 2001).

Non-canonical Wnt pathways in C. elegans

Unlike vertebrates, where the non-canonical Wnt pathway does not utilize transcription factors β-catenin and TCF, in *C. elegans* non-canonical Wnt pathways share

players with the vertebrate canonical Wnt pathway, such as *wrm-1/β-cat*, *sys-1/β-cat*, *pop-1/TCF* and Nemo-like kinase LIT-1/Nlk. These pathways regulate the asymmetric division of the EMS blastomere, seam cells, the B cell in males and the Z1/Z4 cells that generate the somatic gonadal primordium (Eisenmann, 2005; Mizumoto, 2007b; Wu, 2006). To lead to a successful asymmetric cell division, the non-canonical Wnt must establishes the polarity of the mother cell before dividing, and then ensures the generation of two daughters with different fates. Instead of forming a destruction complex, the non-canonical Wnt components localize asymmetrically, ultimately regulating unequal segregation of transcription factors SYS-1 and POP-1. Therefore, the pathway was name the 'Wnt/β-catenin asymmetry pathway' (Mizumoto, 2007b) to differentiate from other non-canonical Wnt pathways (Figure 1.4).

Seam cells are regulated by Wnt/β-catenin asymmetry pathway

Seam cells are lateral epidermal cells (called hypodermal cells in nematodes) aligned along the anterior-posterior axis along the *C. elegans* body (Sulston, 1977; Hall, 2008; Herman, 2006). At hatching, there are 10 seam cells each on the left and right side of the hermaphrodite: H0-2, V1-6, and T from head to tail. Through four larval stages, seam cells undergo several stem cell-like self-renewing asymmetric divisions, producing seam cells, other hypodermal cells and neurons (Figure 1.5). The anterior daughters of most seam cell divisions generate hypodermal cells that fuse with the hypodermal syncytium, hyp7; while posterior daughters retain the seam cell fate. In addition, the V1-6 cells each undergo a symmetric proliferative division at the second larval stage, resulting in 16 terminal seam cells.

The Wnt/β-catenin asymmetry pathway regulates asymmetric divisions of the T and V5 cells (Herman, 1994 and 2001; Goldstein, 2006; Mizumoto, 2007a; Takeshita, 2005); in addition, recent studies suggest it has a prominent role in all seam divisions (Gleason, 2010; Yamamoto, 2011). The most upstream components of this signaling pathway are the Wnt ligands, Frizzled receptors and the Dsh proteins. The function of LIN-44/Wnt and LIN-17/Frizzled has been intensively studied in the T cell (Herman, 1994 and 1995), where LIN-17 accumulates at the posterior cortex and its asymmetric localization depends on LIN-44 that is expressed by epidermal cells posterior to T (Goldstein, 2006, Wu, 2006). Similar to LIN-17, Dsh proteins (DSH-2 and MIG-5) also locate to the posterior side of T (Mizumoto, 2007a). EGL-20/Wnt has also been observed to locate on the posterior cortex of V5.p (Mizumoto, 2007a, Takeshita, 2005). It has been proposed that asymmetric accumulation of these upstream Wnt pathway regulators initially polarizes the seam cell, leading to a series of asymmetric distributions of Wnt components, and eventually induces the two different daughter fates (Mizumoto, 2007b). The localization of other Wnt ligands and receptors may not be as clear in other seam cells as in the T and V5.p cells, however recent studies have demonstrated their functional redundancy in regulating cell fates in all the V1-6 cells (Yamamoto, 2011).

Downstream of Fz and Dsh are four proteins: APR-1/APC, PRY-1/Axin, LIT-1/NLK and WRM-1/β-catenin. In contrast to Fz and Dsh, these four proteins locate asymmetrically to the anterior cortex of all V and T cells, with exception of PRY-1 that is symmetrically localized in the T cell cortex (Takeshita, 2005; Mizumoto, 2007a; Korswagen, 2002). The anterior localization of WRM-1 and APR-1 on the T cell cortex depends on the posteriorly localized LIN-17. In addition, the anterior localization of all

these four proteins in V5.p relies on EGL-20/Wnt. This phenomenon suggests that APR-1, PRY-1, LIT-1 and WRM-1 on the posterior cortex are repelled by the Wnt receptor, resulting in the anterior abundance.

Following the cortical asymmetry is the nuclear asymmetry of Wnt components. WRM-1 and LIT-1 concentrate in the nuclei of all the posterior V and T daughters; POP-1 concentrates in nuclei of all the anterior V and T daughters; SYS-1 concentrates in the nucleus of the posterior T daughter (Takeshita, 2005; Phillips, 2007). Recently it has been shown that the Wnt/β-catenin asymmetry pathway eventually regulates all seam divisions through manipulating the nuclear level of POP-1/TCF, which functions as a transcriptional repressor just as in the canonical Wnt pathway (Gleason, 2010). In this study, the nuclear level of POP-1 was directly related to the fates of seam descendants. Specifically, the high nuclear POP-1 level leads to the hypodermal fate, while the low nuclear POP-1 level maintains to the seam cell fate.

The reversed asymmetry of WRM-1 and LIT-1 in nuclei compared to cortexes made it difficult to explain the mechanism. The nuclear level of WRM-1 and LIT-1 seems to be regulated by nuclear export (Takeshita, 2005; Nakamura, 2005). Interestingly the nuclear level of WRM-1 is inhibited by cortical WRM-1 in a manner that depends on APR-1 (Mizumoto, 2007a). Thus it is possible that APR-1 exports WRM-1 out of nuclei. In turn, the cortical localization of APR-1 depends on cortical WRM-1, which indicates that cortical APR-1 functions downstream of cortical WRM-1, but upstream of nuclear WRM-1. How exactly APR-1/APC regulates nuclear WRM-1 is not clear. However, in other organisms, the cortical APC binds to the plus end of microtubules, where it may

stabilize them (Bienz, 2002). Thus it is possible that the anterior cortical APR-1 stabilizes microtubules, where the nuclear WRM-1 moves towards the cell cortex.

LIT-1, a MAP kinase-related NLK, stably interacts and colocalizes with WRM-1 in both the cortex and nuclei. How does WRM-1/LIT-1 complex regulate nuclear POP-1 asymmetry? In embryos, the WRM-1/LIT-1 complex phosphorylates POP-1, and the mutations of LIT-1-phosphyorylation sites in POP-1 inhibit POP-1 asymmetry (Rocheleau, 1999 and Lo, 2004). Consistently, in seam cells, WRM-1 and LIT-1 concentrate in the posterior nuclei; POP-1 concentrates in the anterior nuclei. In addition, a recent study has shown that *wrm-1* and *pop-1* mutants exhibited opposite phenotypic defects in all seam cells (Gleason, 2010). Taken all together, the data suggested that the nuclear POP-1 asymmetry might directly depend on phosphorylation by the LIT-1/WRM-1 complex and subsequent nuclear export.

The other β-catenin SYS-1 functions as a transcriptional coactivator of POP-1

SYS-1 only contains three Arm repeats instead of twelve like other β-catenins, but it can bind and activate POP-1 (Kidd, 2005). *sys-1* was first identified as a regulator of asymmetric divisions of somatic gonadal precursor cells (SGPs) in *C. elegans*. SGPs originate from Z1 and Z4 cells, which generate the anterior and posterior gonad arm respectively. In wild-type hermaphrodites, Z1 and Z4 divide to generate the distal DTC (distal tip cells), which leads gonad elongation, and a proximal anchor cell (AC). In *sys-1* mutants, both daughters of Z1 or Z4 adopt AC fates, which results in the unelongated and misshapened gonad, called the Sys gonad phenotype (Siegfried, 2004). The asymmetric divisions of SGPs are regulated by Wnt/β-catenin asymmetry pathway components, POP-1 and WRM-1. In addition, SYS-1 functions as a traditional β-catenin, which

transcriptionally activates POP-1 in SGPs (Lam, 2006; Huang, 2007; Siegfried, 2004; Phillips, 2007). Although SYS-1 function in the seam cells still requires further investigation, the facts that *sys-1* mutants show a T cell fate defect (Kidd, 2005; Huang, 2007) and SYS-1 is asymmetrically localized in T daughters, suggest that SYS-1 might function as a transcriptional coactivator of POP-1 in the T cell. How the nuclear asymmetry of SYS-1 is regulated remains unknown. Although some evidence suggests it is regulated by protein degradation (Huang, 2007).

Taken together, the Wnt/β-catenin asymmetry pathway regulates transcriptional factors predominantly through controlling asymmetric localization of its components. However, the mechanisms under the dynamic movements of its components are largely unclear. One possible mechanism is the actomyosin related surface ruffles that distribute proteins in zygotes. However, it is obvious that postembryonic seam cells are not able to undergo surface ruffles as easy as the one cell zygote. Recent studies of the EMS cell in *C. elegans* embryos showed that the nuclear asymmetry of WRM-1 and POP-1 requires microtubule asymmetry along the body axis. The microtubule asymmetry in turn is also regulated by the cortical APR-1/APC (Sugioka, 2011). The interaction of Wnt and microtubules indicates that the possible mechanism to asymmetrically localize Wnt components is transportation along microtubules, which is centered by centrosomes. Therefore, centrosomal components, which can recruit mitotic spindles, such as PCM (SPD-5, γ-tubulin) may be keys to regulate the asymmetric localization of Wnt pathway components.

Figures and Tables

Figure 1.1 Schematic of a centrosome in the cell

The centrosome consists of a pair of centrioles and pericentriolar material (PCM), indicated as the green spot. Microtubules are shown as purple lines.

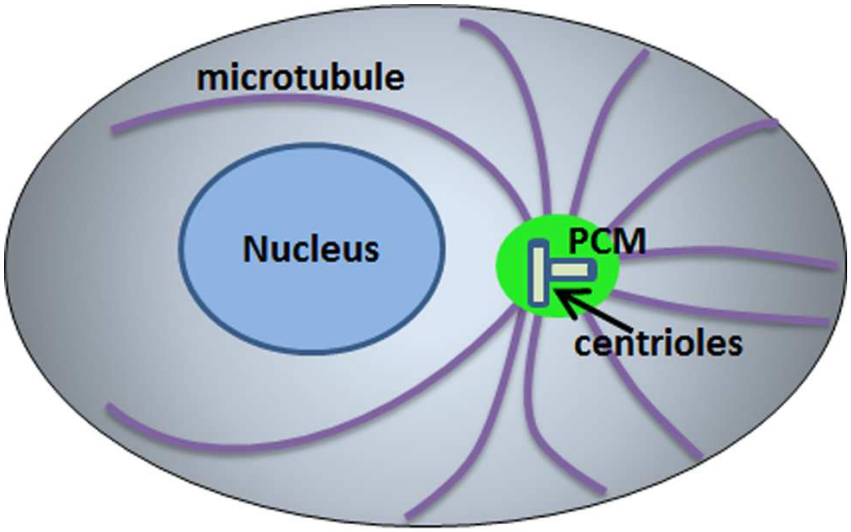


Figure 1.2 The centrosome triggers polarity of the *C. elegans* zygote

A) Right after the entry of sperm centrosome (indicated by the green spot), the entire cortex undergoes a surface ruffle. B) The ruffle ceases in a local manner when the centrosome close associates with the posterior site (right) of the cortex. Then a cortical flow towards the anterior polarizes the localization of PAR proteins PAR-3 and -6 (yellow spots) and a cytoplasmic flow polarizes the localization of the cell fate determinants, P granules (blue spots). C) Eventually, the different sized daughter cells receive different amount of PAR proteins and P granules that differ cell fates.

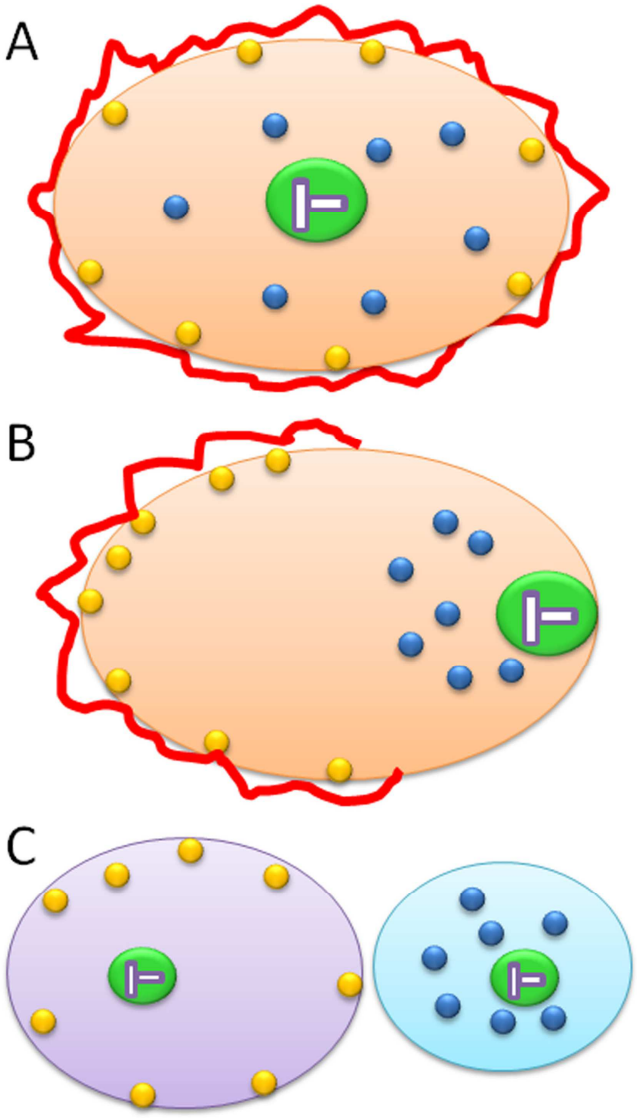
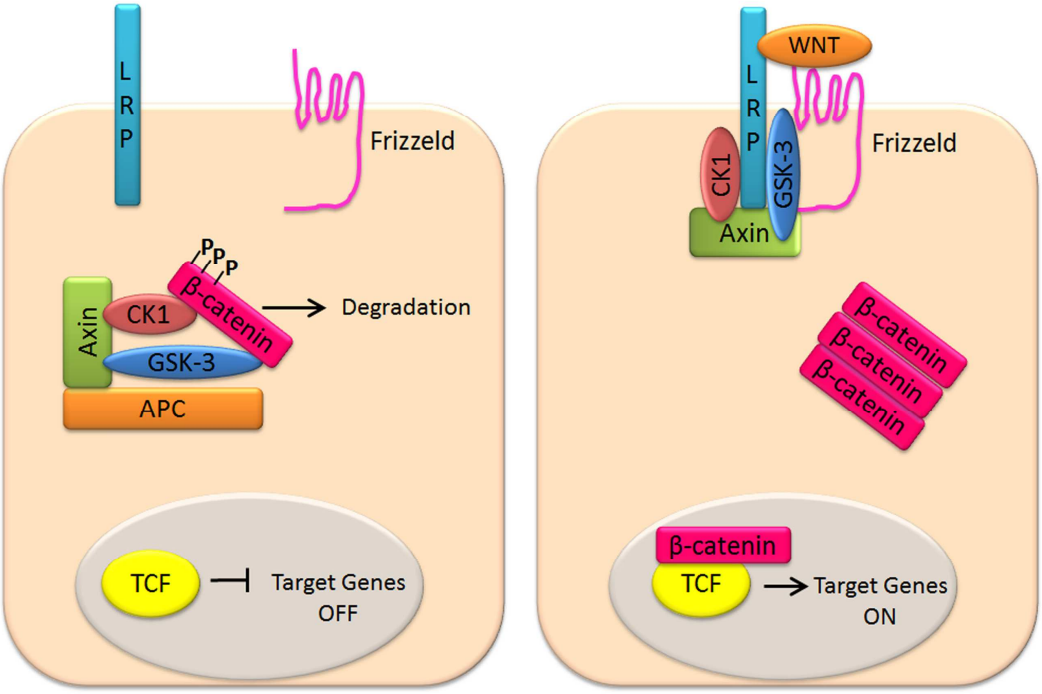


Figure 1.3 The canonical Wnt signaling pathway

The cell in the absence of Wnt ligand is shown on the left, while the cell in the presence of Wnt ligand is shown on the right.



Wnt (Wnt ligands)

Frizzled receptor

LRP (LDL receptor-like protein)

APC (adenomatosis polyposis coli)

GSK-3 (Glycogen synthase kinase 3)

CK1 (Casein kinase 1)

TCF/LEF family transcription factors

Figure 1.4 The Wnt/β-catenin asymmetry pathway in *C. elegans*

A model shows a dividing cell with Wnt ligand binding to the Frizzled receptor on the posterior side (right). Left is anterior and right is posterior. The binding inhibits the posterior localization of APR-1/APC, PRY-1/Axin, WRM-1/β-catenins and LIT-1/MAPK on the cortex. The resulting anterior APR-1/APC facilitates the nuclear export of WRM-1/β-catenins and LIT-1/MAPK. The nuclear levels of WRM-1 and LIT-1 are higher in the posterior that ensures the low POP-1/Tcf level by phosphorylation and nuclear export. The high level of POP-1 in the anterior nucleus suppresses the target gene expression, while low level of POP-1 and high level of SYS-1/β-catenins in the posterior nucleus activates the target gene expression.

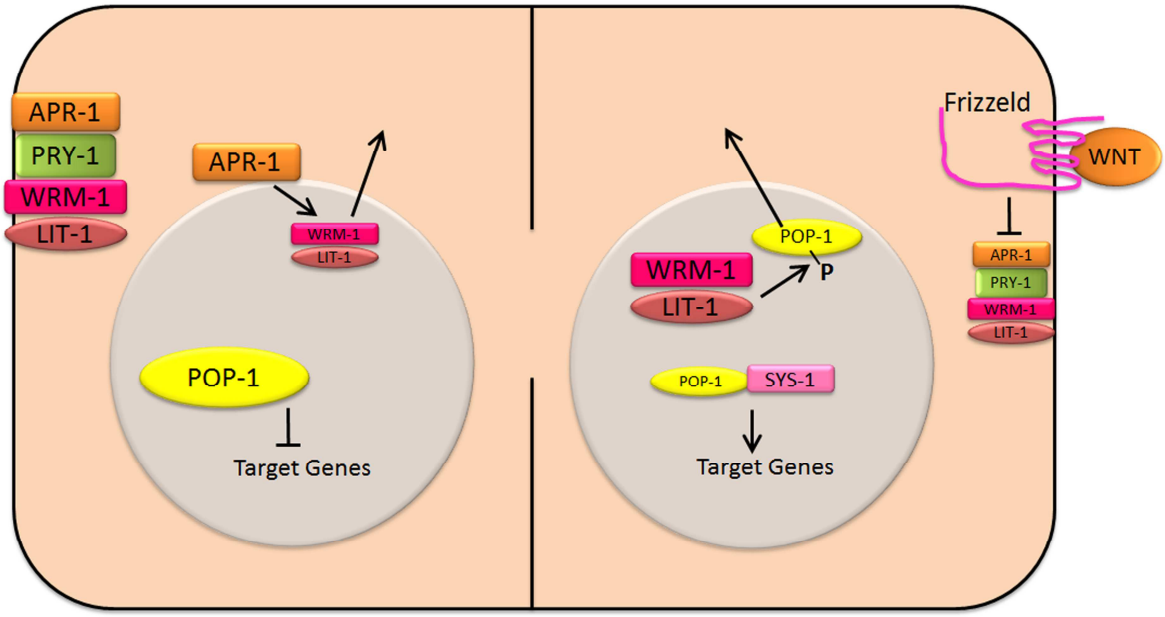
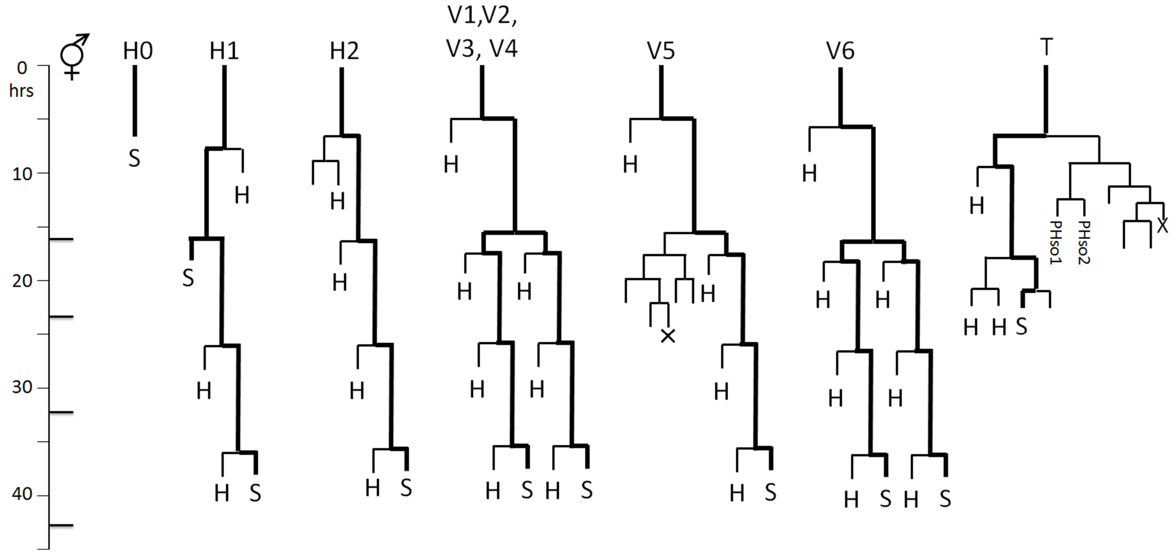


Figure 1.5 Seam cell lineages

The entire post-embryonic seam cell lineages of hermaphrodites are shown. The time after hatching is on the left. The cell fates are shown as 'H' for hypodermal, 'S' for seam and 'PHso1 and 2' for phasmid socket cells. '×' indicates apoptosis. Left indicates the anterior side, while right is the posterior side. Darker lines indicate seam cell fate.



Chapter 2 - Interaction of centrosomal component SPD-5 with Wnt signals in the control of cell polarity in *Caenorhabditis elegans*

Abstract

Multicellular organisms consist of a variety of cell types. One of the mechanisms to generate this cellular diversity is the asymmetric cell division, which requires the establishment of cell polarity. In *Caenorhabditis elegans,* 807 of 949 somatic cell divisions are asymmetric. Centrosomes and the Wnt signaling pathway have been known to regulate the polarities of different cells and subsequent asymmetric divisions. However, it is not known whether or not they cooperate to do so. Here, we demonstrate that a centrosomal component SPD-5, which was originally found to be critical for embryonic development, also plays a role during certain post-embryonic cell divisions in *C. elegans*. Particularly, the polarities of seam cells, whose asymmetric divisions are known to be regulated by the Wnt signaling pathway, also required SPD-5 function. Thus the stem-cell like divisions of seam cells are an intriguing system to study the interaction of the centrosomes and the Wnt pathway. It has been shown that the establishment of seam cell polarity relies on asymmetric localization of Wnt pathway components. We showed that SPD-5 was required for a successful cell division, similar to other centrosomal components and also the proper localization of several Wnt components in a way that was independent of a key microtubule-organizing center (MTOC) member γ-tubulin. In addition, SPD-5 genetically interacted with APR-1/APC and POP-1/Tcf, which confirmed the interaction of SPD-5 with the Wnt signaling pathway. Therefore,

our results suggest a novel role of SPD-5 in cooperating with the Wnt signaling pathway to regulate asymmetric cell divisions, in addition to its function as a centrosomal component.

Introduction

All multicellular organisms contain diverse cell types. One of the ways to generate this cellular diversity is through the asymmetric cell division, which produces two daughter cells with different fates. Asymmetric divisions usually involve the establishment of cell polarity, which directs the unequal segregation of cell fate determinants into two daughter cells and eventually partitions their fates. However, the mechanisms that regulate the cell polarity are not well understood. During the development of the transparent and short-living *C. elegans* hermaphrodite, 807 of 949 somatic cell divisions are asymmetric (Horvitz, 1992), which provides many cells in which to study cell polarity and subsequent asymmetric divisions in living animals.

The centrosome controls cell polarity and thus asymmetric cell divisions in many systems, including the *C. elegans* zygote, *Drosophila melanogaster* neuroblasts and mouse brain stem cells (Rebollo, 2007 and 2009; Wang, 2009). The polarity of the *C. elegans* zygote is established once the sperm centrosome closely localizes to the presumptive posterior cortex (Gönczy, 2005). Subsequently, the cell fate determinants are asymmetrically segregated by the polarized surface ruffle that is generated by the actomyosin network (Munro, 2004). The centrosome consists of a pair of perpendicularly oriented cylindrical centrioles and a mass of centrosome proteins, referred to as pericentriolar material (PCM). Mutants of PCM genes, *spd-2 (SPindle Defective), spd-5* and *air-1* inhibit the formation of the centrosome and the establishment of polarity (Hamill, 2002; O'Connell, 2000; Wallenfang, 2000). SPD-5 contains 1198 amino acids and consists of 11 coiled-coil domains (Hamill, 2002). SPD-5 is an essential PCM

component and required for the duplication and maturation of the centrosome in the zygote. Specifically, SPD-5 genetically interacts with SPD-2 and DHC-1 (dynein heavy chain) to control centrosome maturation, and is required for the centrosomal localization of γ-tubulin encoded by *tbg-1*, AIR-1 (Aurora A kinase) and ZYG-9, a centrosomal MT-stabilizing protein related to XMAP-215 in *Xenopus* (Matthews, 1998). The *spd-5* mutants are maternal lethal (Hamill, 2002). However, the function of SPD-5 in the post-embryonic development is not clear.

The Wnt signaling pathway is one of the most important signaling pathways that control animal development (Reya, 2005; Horvitz, 1992; Clevers, 2006; Grigoryan, 2008). The canonical Wnt pathway has been shown to control cellular proliferation, differentiation and migration in various model organisms, including hydra, worms, flies, fish, mice, and humans (Grigoryan, 2008). The canonical Wnt pathway manipulates the level of β-catenin, which functions as a transcription coactivator for TCF (T-cell factor). In the absence of Wnt (glycoprotein ligands), β-catenin is phosphorylated by the destruction complex, which includes APC (adenomatous polyposis coli), axin, CK1 (casein kinase 1) and GSK-3(glycogen synthase kinase 3), and targeted for ubiquitination and subsequent proteasomal degradation. Binding of Wnt to a Frizzled/ LPR (a member of the LDL receptor family) coreceptor complex, dissembles the destruction complex, thereby stabilizes β-catenin.

Non-canonical Wnt pathways, which are not as well conserved, include Wnt/Calcium and Wnt/JNK pathways in vertebrates, and Wnt/planar cell polarity pathway (PCP) in flies and vertebrates (Veeman, 2003; Nelson, 2004). In *C. elegans*, one of the non-canonical Wnt pathways, called the Wnt/β-catenin asymmetry pathway,

regulates the polarity of the EMS blastomere and the seam cells, as well as Z1 and Z4 somatic gonadal precursor cells (SGPs) (Eisenmann, 2005; Mizumoto, 2007b; Wu, 2006). This Wnt/β-catenin asymmetry pathway shares components with canonical Wnt pathways, including WRM-1/β-catenin, SYS-1/β-catenin, POP-1/TCF, LIN-17/Fz, LIN-44/Wnt, EGL-20/Wnt, MIG-5/Dsh, DSH-2/Dsh, PRY-1/Axin, APR-1/APC and in addition a Nemo-like kinase LIT-1/Nlk. However, unlike the canonical Wnt pathway that forms the destruction complex, the components of Wnt/β-catenin asymmetry pathway localize asymmetrically to regulate cell polarities.

The 10 seam cells (H0-H2, V1-V6 and T) are epidermal cells (called hypodermal cells in nematodes), which undergo stem-cell like self-renewing asymmetric divisions during four larval stages, resulting in 16 seam cells and 49 hypodermal cells (Sulston, 1977). The anterior daughters of seam cells become hypodermal cells that fuse with the hypodermal syncytium, hyp7; while the posterior daughters retain the seam cell fate. Components of Wnt/β-catenin asymmetry pathway localize asymmetrically during seam cell divisions, ultimately regulating the unequal localization of transcription factors SYS-1 and POP-1 in the nuclei, which differentiate daughter cell fates. The polarity of T cell is initiated by binding of LIN-44 with LIN-17 on the posterior cortex (Goldstein, 2006), which guides accumulation of DSH-2 and MIG-5 on the posterior cortex (Herman, 1995; Mizumoto, 2007b). By contrast, APR-1, PRY-1, LIT-1 and WRM-1 localize asymmetrically to the anterior cortex of all V and T cells, with exception of the symmetrically localized PRY-1 in the T cell (Takeshita, 2005; Mizumoto, 2007a; Korswagen, 2002). In addition, the anterior localization of all these four proteins depends on the posteriorly localized EGL-20 in V5.p, while the anterior WRM-1 and

APR-1 depends on the posterior LIN-17 in T (Takeshita, 2005; Mizumoto, 2007a). This suggests that they are repelled by Wnt receptors on the cortex. Subsequently, the WRM-1/LIT-1 complex concentrates in the nuclei of all the posterior V and T daughters by the asymmetric nuclear export that depends on APR-1 and WRM-1 that are concentrated in the anterior cortex (Mizumoto, 2007a). The low posterior level of nuclear POP-1 is likely to be regulated by nuclear export, which involves the phosphorylation by WRM-1/LIT-1 complex (Rocheleau, 1999; Lo, 2004; Gleason, 2010). Recently it has been shown that Wnt/β-catenin asymmetry pathway eventually regulates all seam divisions through manipulating the nuclear level of POP-1/TCF, which functions as a transcriptional repressor just as in the canonical Wnt pathway (Gleason, 2010). Particularly, the high nuclear POP-1 level leads to the hypodermal fate, while the low nuclear POP-1 level leads to the seam fate. SYS-1 functions as a conventional β-catenin in the canonical Wnt pathway, which transcriptionally activates POP-1 in somatic gonadal precursor cells (SGPs) (Lam, 2006; Huang, 2007; Siegfried, 2004; Phillips, 2007). Although it is not clear how SYS-1 localizes to the nuclei, the facts that *sys-1* mutants show T cell fate defects (Kidd, 2005; Huang, 2007) and SYS-1 is asymmetrically localized in T daughters (Phillips, 2007), suggest that SYS-1 might be a functional β-catenin in the T cell too.

Taken together, although both the centrosome and the Wnt signaling pathway regulate cell polarity and asymmetric cell division in various model systems, many questions have not been answered. First, although it is clear the centrosome regulates the *C. elegans* zygotic polarity, whether the centrosome or centrosomal component regulates the polarity of post-embryonic cells is largely unknown. Second, even if the centrosome controls the polarity of post-embryonic cells, whether its function depends on MTOC or

not is unknown. Third, whether the centrosome cooperates with the Wnt pathway to regulate certain asymmetric division is not clear. Although a recent study of *C. elegans* embryos showed that the nuclear asymmetry of WRM-1 and POP-1 requires microtubule asymmetry along the body axis, again it is not clear whether the centrosome is involved. To try to answer these questions, here we examined the function of PCM protein SPD-5 during post-embryonic development, especially certain asymmetric divisions that are regulated by Wnt signaling pathway. Furthermore, we aimed to explore the mechanism behind the control of cell polarity by centrosomes, the PCM in particular, and to see whether the Wnt pathway is also involved.

Materials and Methods

Strains

Caenorhabditis elegans strains were cultured using standard procedures (Brenner, 1974). The Bristol N2 strain was used as wild-type. The mutants and transgenes that were used in this study were: *spd-5(or213) I, rrf-3(pk1426) II, sid-1(qt2) V, sid-1(qt2) V; unc-119(e2498) III, mhEx265 [unc-119(+); pSCM::gfp]* was obtained by injecting *pSCM::gfp (pMF1)* into *unc-119 (e2498) III; arIs99 [dpy-7p::2Xnls::YFP], him-5(e1490) V; qIs74[POP-1::GFP], lin-17(n671) I; unc-119(e2498) III; him-5(e1490) V; mhIs9[unc-119(+); lin-17::GFP], unc-76(e911) V;osEx211[apr-1::GFP + unc-76(+)], osIs5 II [scm::wrm-1::venus], sys-1(q544) I; qIs95 III [pSYS-1::VENUS::SYS-1 + pttx-3::dsRed]*.

Strains were obtained from the *C. elegans* Genetics Center (University of Minnesota), unless otherwise noted.

Transgene expression

To generate *pSCM::gfp::spd-5,* a 8kb SCM (seam cell marker) promoter (gift from H. Sawa) and *spd-5* genomic DNA with or without its 3' UTR were cloned into L3781 (from A. Fire, Stanford University, CA), which includes *gfp* and *let-858* 3' UTR. The successful *pSCM::gfp::spd-5,* consisting of either *spd*-5 or *let-858* 3' UTR (pSHH1 and pSHH2 respectively), were confirmed by sequencing. Both were microinjected at 20 ng/µl concentration with the co-injection marker pPDMM0166 [unc-119(+)] (Maduro, 1995) at 40 ng/µl concentration, into *unc-119(e2498).* No variance in expression was observed based on at least two independent lines of each.

RNA interference

Systemic RNAi was performed by feeding with the *E. coli* HT115 strain containing gene fragments cloned into the IPTG inducible L4440 plasmid to target individual genes (Timmons, 2001). Bacteria containing the empty L4440 vector were used as a negative control. Feeding RNAi constructs were obtained from the Ahringer library (Kamath, 2003) except for those that targeted *spd-5, wrm-1, lit-1, lin-17, and spd-2* which were built by standard methods. To obtain animals with partial knock down of *spd-5,* RNAi bacteria were seeded on the nematode growth medium (NGM) plates containing 0.1 or 1 mM IPTG and allowed to grow overnight. Either wild-type N2 or *rrf-3* mutants were transferred onto these plates and the progeny were analyzed (Timmons, 2001). *rrf-3* encodes an RNA-directed RNA polymerase (RdRP) homolog that inhibits somatic RNAi, thus the *rrf-3(pk1426)* mutants are hypersensitive to somatic RNAi. To examine seam and hypodermal numbers, in order to bypass embryonic effects of target genes, eggs of *unc-119(e2498) III, mhEx265 [unc-119(+); pSCM::gfp]* or *arIs99 [dpy-*

7p::2Xnls::YFP], him-5(e1490) V were isolated by bleaching, placed onto plates with the appropriate HT115 stain and 1 mM IPTG, and allowed to develop at 20°C till being analyzed at young adult stage. Growing L4 in 0.1mM IPTG RNAi plates and scoring the adults of progeny were also used to examine seam and hypodermal cell numbers and indicated as ^ in Figure 2.4, Table 2.2 and 2.3. *arIs99 [dpy-7p::2Xnls::YFP]* expresses in almost all hypodermal cells including seam cell descendants, ventral hypodermal cells and hypodermal cells in head and tail (Gleason, 2010). The hypodermal cells generated by seam cells are differentiated from the ventral hypodermal cells by their left or right location on the worm body. However, the hypodermal cells in head and tail are close to the ones generated by seam cells, thus it's difficult to differentiate them. Therefore, consistent with a previous study (Gleason, 2010) the average hypodermal cell number in wild-type (54) is slightly larger than the actual number (49).

Double RNAi feeding experiments were carried out using 1:1 mixture of each RNAi bacteria on 1 mM IPTG plates. 1:1 mixture of bacteria with RNAi construct and empty L4440 was used as a reference for the respective single RNAi.

To specifically knock down *spd-5* in the seam cells, we constructed a plasmid for transgenic expression of *spd-5* hairpin dsRNA (Timmons, 2003; Tavernarakis, 2000). The transgenic construct contains two inverted copies of a 188-bp fragment from the second exon of *spd-5*, and separated by the 47-bp second intron of *spd-5*. These three fragments were amplified (primer sequences available upon request) and cloned into pEL597 that contains 1 kb SCM promoter (a gift from Erik A. Lundquist) to generate the plasmid pSHH3. To obtain transgenic worms, 20 ng/µl of the pSHH3 with 20 ng/µl *pSCM::gfp* were microinjected into *sid-1(qt2)* hermaphrodites and the resulting lines

contain *mhEx267*; 100 ng/µl of the pSHH3 with 20 ng/µl *pSCM::gfp* and 40 ng/µl pPDMM0166 [unc-119 (+)] (Maduro, 1995) were microinjected into *sid-1(qt2); unc-119(e2498)* hermaphrodites and the resulting lines contain *mhEx266*. *sid-1* encodes a dsRNA channel that enables passive (ATP-independent) uptake of double-stranded RNA (dsRNA), thus *sid-1(qt2)* restricted RNAi effect to seam cells. The pSHH3 and *pSCM::gfp* both in 20 ng/µl concentration were microinjected into *unc-119(e2498)* strain to demonstrate the embryonic lethality of *spd-5(RNAi),* with 0 hatched eggs from 32 non-Unc F1s.

Microscopy

Living animals were observed using a Zeiss Axioplan microscope equipped with Nomarski optics and epifluorescence; cell nomenclature and cell lineage analysis were as previously described (Sulston, 1977). Digital images were captured by QImaging Retiga EXI CCD camera and processed by Adobe PhotoShop. The seam and hypodermal number were scored manually; the dye-filling phasmids were scored as previous described (Herman, 1994). Briefly, when hermaphrodites are soaked in green fluorescent 3,3'-dioctadecyloxacarbocynine (DiO), the phasmid neurons that are DiO filled on each side of the worm body are scored. The percentage of sides that contain DiO filled phasmid neurons are determined as dye filling (%).

Quantitative Reverse transcribed PCR

The strain containing *mhEx265* was used for qRT-PCR to test the RNAi efficiency. For each RNAi condition, RNA of three biological replicates, consisting of 25 young adults, was extracted using Trizol with PureLink RNA Mini Kit (Invitrogen) as described by the manufacturer. The reverse transcriptions of each biological replicate

included two control reactions containing either no reverse transcriptase or no RNA to determine genomic DNA contamination. The resulting cDNA was diluted 1/5 in water and 1 µl was used in 20 µl qPCR using iQ SYBR Green Supermix (Bio-Rad). Each set of qPCR, consisting of three technical replicates, were performed on a Bio-Rad iCycler. Primers used to amplify *spd-5* and *tbg-1* span exon-exon junctions to prevent amplification of genomic DNA. The sequences of *ama-1* primers are ccatcctcgccgacatacaatcc and cggcgtgtagctaggcgac; *spd-5* primers are tgctgttcgaaatcagcaacaatttgcc and aggaaccttcaagggtgtcattatggt; *tbg*-1 primers are tgctgagcaaaacgttgatttcgc and tggccgaacatatggagagagtgg. Cycling conditions were: 1× [3 min 95°C] and 40 × [15 s 95°C, 30 s 57°C, 15 s 72°C]. Relative expression levels (ΔΔCt values) were determined according to (Pfaffl, 2001), using the housekeeping gene *ama-1* as a standard.

Results

SPD-5 controls T cell divisions

To determine whether *spd-5* interacts with Wnt signaling pathway during post-embryonic development, we first tested the T cell divisions that are regulated by Wnt pathway. The T cell is the most posterior seam cell on the tail and undergoes a series of asymmetric divisions. Among the posterior descendants of T cell are two phasmid socket cells, which are glial cells that extend out to the surface of the tail and whose presence can be assayed by their ability to allow the phasmid neurons, PHA and PHB, to take up the lipophilic dye DiO (Figure 2.1A). Reduction of Wnt pathway activities caused missing or misplaced phasmid socket cells, resulting in the decreased dye-filling (%) (Herman, 1994; Wu, 2006). We used both a temperature sensitive mutant *spd-5(or213ts)*

and systemic feeding RNAi to disrupt SPD-5 function. Consistent with a previous study (Hamill, 2002), both *spd-5(or213)* animals raised at the restrictive temperature of 25°C and *spd-5(RNAi)* showed the strong embryonic lethality (Table 2.1 and Figure 2.1B). Although *spd-5(or213)* raised at the temperatures between the permissive 15°C to 25°C showed little phasmid dye-filling defects, the IPTG inducible feeding RNAi caused a significant decrease of phasmid dye-filling (%) and a vulval morphology defect both in wild-type N2 and *rrf-3* mutant strain (Figure 2.1B). To determine whether the phasmid dye-filling defect was caused by T cell division defects, we visualized the position of the phasmid socket cells, T.paa and T.pap. Consistently, the percentage of correctly positioned phasmid socket cells in *spd-5(RNAi)* is much lower than the feeding vector control (Figure 2.2). These results suggest that *spd-5* regulates T cell divisions during the post-embryonic development. However, since SPD-5 is a centrosomal component, *spd-5(RNAi)* might cause the mitotic defects, resulting in failures of cell divisions. Thus, it is important to investigate whether the T cell defect came from the failure of general cell division or establishment of the T cell polarity. The most direct method is to examine the T cell lineage. In the wild-type hermaphrodite, T.a divides to generate epidermal fates and T.p generates primarily neural fates (Herman, 2004). About 1 hour after it is formed, T.pp begins to move anteriorly past T.pa, thus resulting switched positions of these two cells (n = 5; Figure 2.3A). However, T.pp and T.pa of *spd-5(RNAi)* did not move before they further divided (Figure 2.3B). This defect in the T cell polarity and thus the asymmetric division yielded misplaced phasmid socket cells, resulting in the low phasmid dye-filling (%). Interestingly, *spd-5(RNAi)* animals also exhibited defects in the cell division, which was presented either as no mitosis (Figure. 2.3B) or the failure of the

division ending with undivided nucleus with more than one nucleolus (Figure. 2.3C). These results suggest that *spd-5* might function both as a centrosomal component to regulate the mitosis or cytokinesis and similar to Wnt pathway components to control the T cell polarity. In addition, we also observed a protruding vulva phenotype, similar to that observed in *bar-1/*β-catenin mutants. Since the RNAi animals we were examining bypassed the embryonic lethality, we were concerned about the efficiency of RNAi to knock down *spd-5* in these survivors. Therefore, we tested the transcriptional level of *spd-5* in these survived *spd-5(RNAi)* animals by qRT-PCR ($\Delta\Delta$Ct = 0.22; Figure 2.4), which confirmed the high efficiency of RNAi. Thus we used RNAi to test the post-embryonic function of *spd-5* hereafter.

SPD-5 controls seam cell divisions

It was previously shown that the asymmetric divisions of the seam cells are regulated by the Wnt/β-catenin asymmetry pathway (Mizumoto, 2007b; Gleason, 2010). Most seam cells undergo self-renewing asymmetric divisions, which generate an anterior hypodermal cell and a posterior seam cell. The Wnt pathway components regulate the seam versus hypodermal cell fates (Gleason, 2010, Yamamoto, 2011). To test whether seam cell divisions are also regulated by *spd-5*, we used RNAi to knock down both Wnt/β-catenin asymmetry pathway and centrosomal components and scored the terminal seam and hypodermal cell number. Since the morphology of seam and hypodermal cells are similar, we used transgenes *pSCM::gfp* and *dpy-7p::2Xnls::YFP* to mark seam and hypodermal nuclei respectively (Fig. 2.5). In wild-type adults, each side of the worm has 16 seam cell and 54 hypodermal cells (Table 2.2). Knock down of different Wnt pathway components had reciprocal effects on seam and hypodermal cell number (Table 2.2).

Specifically, *pop-1(RNAi)* and *apr-1(RNAi)* animals showed increased seam and decreased hypodermal numbers; whereas *wrm-1(RNAi)* and *lit-1(RNAi)* animals showed decreased seam and increased hypodermal numbers. Consistent with a previous study (Gleason, 2010), our results suggest that the Wnt pathway regulates asymmetric divisions thus choice between seam and hypodermal cell fates. On the other hand, we observed that knock down of the centrosomal components *tbg-1 (tubulin gamma),* cytoskeletal components *dhc-1 (dynein heavy chain)* and *tba-1, 2 (tubulin alpha)* decreased both seam and hypodermal number (Table 2.2). This suggests that the reduction of these genes function caused the cell division defects, which led to overall reduction in the number of cells generated. In addition, several genes regulating cytokinesis *zen-4* and *cyk-4* in P and T cells, and *cyk-1* in B cell of males (Wu, 2006; Sugioka, 2011) and other centrosomal components *air-1* and *spd-2* had little effect on seam cell number (Table 2.2). *spd-5(RNAi)* again decreased both seam and hypodermal number, indicates its role as a centrosomal component. Interestingly, unlike any of the centrosomal or cytoskeletal components, in one *spd-5(RNAi)* method, which is growing L4 in 0.1mM IPTG RNAi plates and scoring the adults of progeny, the seam cells began with 10 (Table 2.3) but ended up with 7.4. If *spd-5* only functioned as a centrosomal component, *spd-5(RNAi)* should reduce the number of cells produced, resulting in 10-16 terminal seam cells. But *spd-5(RNAi)* animals had less than 10 terminal seam cells. One explanation for this observation is that *spd-5* regulates the cell fate decisions of seam cells in addition to its function as a centrosomal component. Therefore, *spd-5(RNAi)* caused a switch from seam to hypodermal cell fate, as well as the reduction of the overall hypodermal number was

due to the cell division defects. Moreover, the RNAi efficiency of *spd-5* ($\Delta\Delta$Ct = 0.15) and *tbg*-1($\Delta\Delta$Ct = 0.23) was confirmed by qRT-PCR (Fig. 2.4).

To test whether *spd-5* functions autonomously in seam cells, we generated a transgene pSHH3, which produces a hairpin RNA specifically in seam cells. The transgenic worms exhibited both defects in overall seam cell number and the phasmid dye-filling assay (Figure 2.6). The result demonstrated that knock down of *spd-5* specifically in seam cells caused defects in seam cell divisions.

The function of SPD-5 may be restricted to certain cells during post-embryonic development

Consistent with the defects in seam and hypodermal cell numbers, the seam cell descendants in *spd-5(RNAi)* animals exhibited the abnormal nuclei that contains multi-nucleoli, a sign of cell division failure (Figure 2.3 and 2.7). To determine whether *spd-5* functions in other post-embryonic cells, we looked to see whether the multi-nucleoli defect was apparent in the daughters of Pn.p and intestinal cells. These cells are morphologically similar to the seam cells, and although we observed the multi-nucleoli in these cells, they were not as significant as in the descendants of seam cells (Figure 2.7). As the divisions of these cells are not regulated by Wnt/β-catenin asymmetry pathway, these data suggest that SPD-5 might function specifically in cells that are regulated by the Wnt pathway.

*sys-1/*β-catenin has been shown to regulate the asymmetric divisions of two somatic gonadal precursor cells (SGPs) Z1 and Z4 in *C. elegans*. Z1 and Z4 cells generate anterior and posterior gonad arms, respectively. In wild-type hermaphrodites, both Z1 and Z4 divides to generate a distal DTC (distal tip cell), which leads the gonad

elongation, and a proximal anchor cell (AC). In *sys-1* mutants, both daughters of Z1 or Z4 adopt AC fates, which results in the loss of gonad arms and DTCs, called the Sys gonad phenotype (Siegfried, 2002). Consistently, *spd-5(RNAi)* showed the Sys gonad phenotype (Figure 2.8D, E), which suggests *spd-5* contributes to the control of the asymmetric divisions in the gonad that are also regulated by Wnt pathway components (Siegfried, 2002 and 2004).

In the ventral nerve cord, the original Pn.ps are generated from the migration and subsequent asymmetric divisions of 12 Pns (Sulston, 1977) that have not been documented to be controlled by the Wnt pathway. The number of Pn.p was also affected by *spd-5(RNAi)* (Figure 2.8A, B and C). How *spd-5(RNAi)* caused this defect is unclear. One possible explanation is that *spd-5* regulates the asymmetric divisions of these Pns. Another explanation is that *spd-5* controls cell divisions which generate Pns during the embryonic development. The last possible explanation might be *spd-5* regulates the ventral migration of Pns.

spd-5(RNAi) disrupts the asymmetric localization of Wnt/β-catenin asymmetry pathway components

To further investigate whether *spd-5* interacts with the Wnt/β-catenin asymmetry pathway to regulate the cell polarity, we examined the asymmetric localization of Wnt components in seam cells. In wild-type animals the different daughter cell fates are controlled by SYS-1/β-catenin and POP-1/Tcf, which are reciprocally concentrated in the daughter nuclei: POP-1 levels are high in the anterior nuclei, whereas SYS-1 levels are high in the posterior nuclei (Herman, 2001; Takeshita, 2005; Phillips, 2007; Gleason, 2010). POP-1 functions as a transcriptional repressor, while SYS-1 might function as its

co-activator (Mizumoto, 2007b). The nuclear asymmetry of POP-1 is ensured by APR-1/APC, WRM-1/ β-catenin and LIN-17/Frizzled that localize asymmetrically on the mother cortex. The mechanisms that ensure the nuclear asymmetry of SYS-1 are not clear.

To determine whether *spd-5* controls the Wnt/β-catenin asymmetry pathway, we first tested whether *spd-5(RNAi)* affects the localization of the downstream players SYS-1 and POP-1. As we expected, the levels of POP-1::GFP in T daughter nuclei were equal in *spd-5(RNAi)* animals (52.4% symmetry, n = 21), while the nuclear levels of POP-1::GFP were higher in the anterior daughters in the *FV control* animals (89.5% anterior asymmetry and 10.5% symmetry, n = 19) (Figure 2.9A-F). However, the nuclear level of SYS-1::GFP was always higher in the posterior daughter both in *spd-5(RNAi)* (n = 20) and *FV control* animals (n = 17) (Figure 2.9G-J). This result not only confirmed that *spd-5* controls the T cell fate, but also suggested that *spd-5* functions through regulating the localization of Wnt pathway component. Furthermore, to examine whether the function of *spd-5* on T cell fate is dependent on the centrosome or cytoskeletal network, we examined the nuclear POP-1::GFP level in *tbg-1(RNAi)* and *dhc-1(RNAi)*. Surprisingly, neither affected the asymmetric localization of POP-1::GFP, (Figure 2.9D-F). This finding indicated a novel function of *spd-5* that is independent of γ-tubulin and thus the MTOC.

The nuclear level of POP-1 was suggested to be related to the cortical level of WRM-1 and APR-1 in seam cells (Rocheleau, 1999; Lo, 2004; Gleason, 2010; Mizumoto, 2007a; Takeshita, 2005; Nakamura, 2005). Thus we next tested whether *spd-5* regulates the localization of WRM-1::GFP and APR-1::GFP. *spd-5(RNAi)* caused the WRM-1::GFP to be symmetrically localized on the seam cortex (67.4%, n = 46), while the

cortical level is higher on the anterior in the *FV control* (97.1%, n = 35) (Figure 2.9K-N). Similarly, the cortical localization of APR-1::GFP in *spd-5(RNAi)* was symmetric (54%) or reversed with posteriorly enrichment (14%, n = 50), while in the *FV control* was concentrated anteriorly (97.4%, n = 39; Figure 2.9O-S).

Finally we examined the effect of *spd-5(RNAi)* on LIN-17/Fz receptor, which accumulates at the posterior cortex of the T cell (Goldstein, 2006, Wu, 2007). The posterior localization of LIN-17 on the cortex relies on the extracellular Wnt cue LIN-44 which is expressed from hypodermal cell that lie posterior to the T cell (Goldstein, 2006; Herman, 1995). The asymmetric localization of LIN-17::GFP in *spd-5(RNAi)* was not different from *FV control* (n = 20, Figure 2.9T-W).

The γ-tubulin and dynein are components of cytoskeletal network and important for the microtubule mediated protein transport. The fact that the γ-tubulin and dynein were not involved in the localization of Wnt components raises a question of how SPD-5 regulates the asymmetric localization of the Wnt pathway components. The subcellular localization of SPD-5 might light on the mechanism. We observed GFP::SPD-5 localized to one or two bright spots close to the nucleus, consistent with being localized to the centrosome (Figure 2.10). Interestingly, GFP::SPD-5 also surrounded the nucleus, and extended out towards the cortex, which suggests that the interaction of SPD-5 and Wnt components in the cytoplasm is possible.

spd-5 genetically interacts with pop-1 and apr-1 to regulate seam versus hypodermal fates

We performed double RNAi experiments to determine the functional order of *spd-5* and the Wnt/β-catenin asymmetry pathway components. However, since *spd-*

5(RNAi) decreased both seam and hypodermal number, we first needed to determine the alteration of cell fates in *spd-5(RNAi)* animals. The observation of the terminal seam number (7.4; Table 2.2) was less than the original number (9.8; Table 2.3) suggested that *spd-5(RNAi)* caused seam to hypodermal transition. Furthermore, the nuclear asymmetry of POP-1::GFP in knock down of *wrm-1* animals has been shown to be equalized in a manner similar to what was observed in *spd-5(RNAi)* animals, in which the posterior nuclear level seems increase (Wu, 2006; Gleason, 2010). Again, the high nuclear POP-1 level is an indicator of the hypodermal fate, while the low nuclear POP-1 leads to the seam fate (Gleason, 2010). Taken together, *spd-5(RNAi)* appears to switch the seam to hypodermal fate through its interaction with the Wnt/β-catenin asymmetry pathway, resulting in decreased seam and increased hypodermal number. Since *spd-5* might also function as a centrosomal component that regulates the cell division, the overall seam and hypodermal number were also decreased.

Conversely, *pop-1(RNAi)* and *apr-1(RNAi)* increased seam cell number and decreased hypodermal number (Table 2.2 and 2.4). The seam cell number of *spd-5(RNAi); pop-1(RNAi)* animals (34.2) was close to that of *pop-1(RNAi)* animals (43.3) and much higher than that of *spd-5(RNAi)* animals (13.7). Similarly the seam cell number of *spd-5(RNAi); apr-1 (RNAi)* (17.4) animals was close to that of *apr-1(RNAi)* (20.5) and higher than that of *spd-5(RNAi)* animals. Combined with the previous data, the seam cell numbers in *spd-5(RNAi); pop-1(RNAi)* and *spd-5(RNAi); apr-1(RNAi)* were higher than 16, which suggest that *spd-5* functions upstream of *apr-1* and *pop-1*. Consistently, the seam cell numbers in both of these double RNAi were not as high as in *apr-1(RNAi)* or

pop-1(RNAi) again indicates *spd-5* functions partially as a centrosomal component to decrease overall cell number.

Discussion

We have shown that the PCM component SPD-5 functions in the T cell, the seam cells and the somatic gonad, whose asymmetric divisions are regulated by components of Wnt/β-catenin asymmetry pathway in *C. elegans.* However, the divisions of Pn.p and the intestinal cells that are not regulated by the Wnt/β-catenin asymmetry pathway were not affected by *spd-5(RNAi).* We also found that knock down of the centrosomal components, cytokinesis proteins and cytoskeletal proteins decreased both terminal seam and hypodermal numbers, which suggest the failure of cell divisions. Interestingly, knock down of *spd-5* also disrupted asymmetric localization of some Wnt/β-catenin asymmetry pathway components, including *pop-1, wrm-1* and *apr-1*. In addition, *spd-5* genetically regulates the asymmetric localization of POP-1 and APR-1 to regulate seam cell divisions. Together, these observations suggest that *spd-5* not only functions as a centrosomal component but also interacts with Wnt/β-catenin asymmetry pathway to regulate seam cell polarities and thus asymmetric divisions.

spd-5 regulates certain cell divisions during post-embryonic development

As microtubule-organizing center (MTOC) the centrosomes are important for the mitosis. However, the essentialness of centrosomes in mitoses was challenged in certain systems, in which after laser ablation or reduction of centrosome function the mitotic spindles can still form and leads to a successful mitosis (Mahoney, 2006; Debec, 1995; Bonaccorsi, 2000; Khodjakov, 2000; Heald, 1997; Megraw, 2001). In addition to a MTOC, the centrosomes were recently recognized as docking sites where multiple cell

activities take place (Kramer, 2004). This suggests that centrosomes might perform diverse functions in different cell types.

Here we showed that knock down of a centrosomal protein SPD-5, which is required for the mitotic spindle assembly in the *C. elegans* zygote (Hamill, 2002), only disrupted certain post-embryonic cell divisions. Although only a few of the animals in the *spd-5(RNAi)* animals survived beyond embryogenesis, almost all of these survivors grew into adults (data not shown). Consistent with the previous study (Hamill, 2002), the *spd-5(RNAi)* caused maternal-effect lethality. Interestingly, the visible phenotypes in the surviving *spd-5(RNAi)* adults included the protruding vulva and the Sys gonad, which were similar to phenotypes observed in the mutants of Wnt pathway components, including *bar-1/*β-catenin and *sys-1/*β-catenin (Eisenmann, 2000; Siegfried, 2004). Furthermore, the abnormal nuclei, which appear to be caused by cell division failure, were observed much more frequently in the seam cells descendants than in the daughters of Pn.p and intestinal cells that are morphologically similar and lie close to the seam cells. While the polarity of the seam cells is regulated by the Wnt/β-catenin asymmetry pathway, the first divisions of the Pn.p and intestine cells are not. Taken together, the function of SPD-5 might be restricted to certain cells during post-embryonic development, possibly only those cell types that are regulated by the Wnt pathway.

SPD-5 functions partially as a centrosomal protein during post-embryonic development

Because the centrosome serves as a MTOC, it performs microtubule-related functions, such as segregating chromosomes during cell division, maintaining cell shape, regulating the cell polarity and motility, and localizing organelles and cargos (Schatten,

2008). In addition, centrosomes have been recently shown to control several cell cycle regulatory events, such as entry into mitosis, cytokinesis, G1/S transition and monitoring DNA damage (Kramer, 2004). In the *C. elegans* zygote, SPD-5 was demonstrated to be a component of PCM and required for mitotic spindle assembly, thus for the success of the cell division (Hamill, 2002). *spd-5* mutants also exhibited maternal-effect lethality. During the post-embryonic T cell divisions, we also observed the failure of cell division too, which is consistent with the role of SPD-5 as a centrosome protein. The result of this failure is an abnormal nucleus with more than one nucleolus, perhaps caused by the disruption of chromosome segregation.

Moreover SPD-5 shares some functions with other centrosomal proteins in seam cells, as shown its effect on the terminal number of seam and hypodermal cells. The numerous asymmetric divisions of seam cells result in 16 seam cells and around 54 hypodermal cells. Reduction of α-, γ-tubulin, dynein and SPD-5 all caused a decrease in both seam and hypodermal cell numbers. The γ-tubulin (*tbg-1)* complex directly anchors microtubules (composed of α- and β-tubulin) into the MTOC. Dynein (*dhc-1*), a motor protein, genetically interacts with SPD-5 in the zygote. *tbg-1(RNAi)* (not shown) and *spd-5(RNAi)* (Figure 2.7) animals both showed the multi-nucleoli phenotype in seam cells. This suggests that decreased seam and hypodermal cell numbers were caused by the failure of cell division. SPD-5 is required for the recruitment of γ-tubulin into the centrosome in the *C. elegans* zygote (Hamill, 2002). Hence, it is possible that in seam cells SPD-5 also functions to accumulate γ-tubulin to the centrosome, and further regulates cell division as a centrosomal component. Taken together, we suggest that SPD-

5 partially functions as a centrosomal protein to regulate certain post-embryonic cell divisions, such as seam cells (Figure 2.11).

SPD-5 interacts with Wnt/β-catenin asymmetry pathway to regulate seam cell polarity

Examination of the T cell lineage (Figure 2.3) demonstrated that *spd-5(RNAi)* caused both cell fate defects and the failure of cell division. Furthermore, the cell fate defects we observed were similar to those observations in *lin-44*/Wnt and *lin-17*/Fz mutants (Herman, 2002). This suggests that SPD-5 might control both the success of cell division and the polarity of T cell that is regulated by the Wnt/β-catenin asymmetry pathway.

For the seam cell divisions, we found that the terminal seam cell number (7.4) was less than that observed in newly hatched animals (10). This phenomenon was not seen in knock down any of centrosomal components, cytokinesis proteins and cytoskeletal proteins. This suggests that SPD-5 might regulate the cell fates of the seam cell descendants, which are also controlled by Wnt/β-catenin asymmetry pathway.

Furthermore, we discovered that SPD-5 is required for the asymmetric localization of several components of Wnt/β-catenin asymmetry pathway, including POP-1, WRM-1 and APR-1, but not for SYS-1 and LIN-17. The function of Wnt/β-catenin asymmetry pathway in controlling seam cell polarity depends on the asymmetric localization of Wnt components (Mizumoto, 2007b). The T cell polarity begins with the localization of LIN-17 to the posterior cortex. Then, the anterior localizations of WRM-1 and APR-1 in all seam cells depend on the asymmetric localization of LIN-17 in T cell and EGL-20 in V5.p. Consistently, the result from the double RNAi experiments

positioned SPD-5 functions before APR-1 in Wnt/β-catenin asymmetry pathway. Thus these results show that SPD-5 interacts with Wnt pathway components to regulate the polarity and thus asymmetric divisions of seam cells.

Then how does SPD-5 cooperate with Wnt/β-catenin asymmetry pathway? One possibility is that SPD-5 acts to assemble MTOC that anchors and stabilizes the microtubules, and thus facilitates the protein transport along the microtubules. If that were the case, the γ-tubulin that directly anchors the microtubules and the microtubule mediated motor protein dynein might probably also regulate the asymmetric localization of Wnt components. However, the results from the *tbg-1(RNAi)* and *dhc-1(RNAi)* animals showed the opposite. This suggests that this novel role of SPD-5 in regulating the cell polarity was independent of MTOC, at least of γ-tubulin. Nevertheless other motor proteins might still be involved. Another prediction came from the subcellular localization of GFP::SPD-5, which not only localizes to the centrosome but also the cytoplasm that surrounds the nucleus and expands toward the cell cortex. Recently the centrosome has been proposed to function as a docking station, where multiple cell activities take place (Kramer, 2004). Generally by anchoring components of cellular pathways, centrosomes help to physically link these molecules and provide spatial control of their interactions. Therefore based on our data, SPD-5 might directly or indirectly anchor Wnt components to regulate their localization. But which Wnt pathway component might interact with SPD-5? The similar subcellular localization with SPD-5 raises the possibility that APR-1 might be the one. APR-1::GFP localized in the cytoplasm that surrounds the nucleus and towards the cell boundary, which is quite similar to GFP::SPD-5 except in the centrosome. Additionally SPD-5 genetically controls

the asymmetry of APR-1::GFP. The anterior abundance of cortical APR-1 regulates the asymmetry of nuclear WRM-1 through the nuclear export in the anterior (Mizumoto, 2007b). However, where and how this cytoplasmic and cortical APR-1 regulates and interacts with the nuclear WRM-1 is not clear. SPD-5 with 11 coiled-coil domains might anchor and acts as a docking station for interaction of APR-1 with WRM-1. Since APC functions as a scaffold protein to recruit β-catenin in canonical Wnt pathway, APR-1/APC might recruit and interact with WRM-1/β-catenin in the Wnt/β-catenin asymmetry pathway. Therefore we can image a model that SPD-5 functions as a platform where anchored APR-1 interacts with WRM-1, thus regulates the nuclear export of WRM-1.

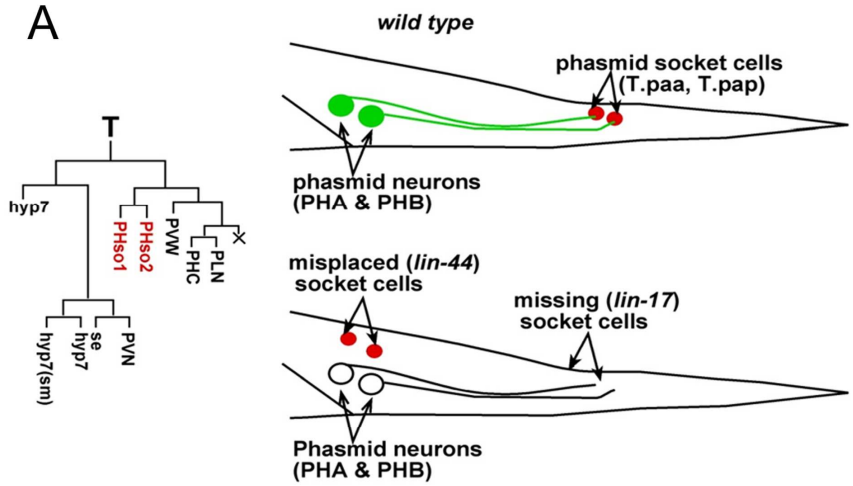
In summary, we have shown that SPD-5 functions in specific cells during post-embryonic development. Particularly in seam cells, SPD-5 not only functions partial as a centrosomal component to control the cell divisions, but also interacts with the components of Wnt/β-catenin asymmetry pathway to regulate cell polarity in a way that is independent of MTOC. However, a number of intriguing questions remain to be answered. For instance, does SPD-5 physically associate with Wnt pathway components, such as APR-1? If not, what molecules mediate the interaction? Does SPD-5 cooperate also with Wnt in the gonad? The further investigation upon the mechanism behind the asymmetric localization of Wnt components by SPD-5 will explain how SPD-5 interacts with Wnt pathway, thus linking cell signaling to cellular components to regulate cell polarity.

Figures and Tables

Figure 2.1 Knock down of *spd-5* affects T cell division by phasmid dye-filling assay

A) T cell lineage (left) and schematic of phasmid socket cell position (right; Van Hoffelen, 2008). The schematic illustration of the positions of the phasmid neurons and the socket cells in wild type (above) as well as lin-44 and lin-17 (below). B) Phasmid dye-dilling assay. The percentage of sides that contain DiO filled phasmid neurons are determined as dye filling (%).

A



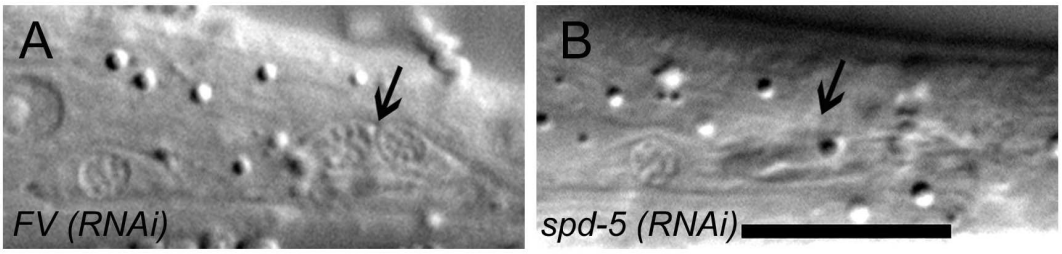
B

Genotype	[IPTG]	N	Dye filling (%)	Protruding vulva[3](%)
N2; *(FV RNAi)*[1]	0.1 mM	78	97	0
N2; *spd-5(RNAi)*		48	70	29
rrf-3; (FV RNAi)[1]		53	99	0
rrf-3; spd-5(RNAi)		53	26	13
N2; *(FV RNAi)*[1]	1mM	63	95	0
N2; *spd-5(RNAi)*		56	78	41
rrf-3; (FV RNAi)[1]		50	98	ND
rrf-3; spd-5(RNAi)		48	21	15
spd-5 (or213)[2] in 25℃		No Survival eggs		
spd-5 (or213)[2] in 15~19 ℃		Too many	98%~99%	

1, *FV RNAi:* L4440 empty vector is used as a control.
2, Temperature sensitive strain *spd-5(or213).*
3, Knockdown of *spd-5* also affects vulva development.
N = number of worms.
ND, not determined.

Figure 2.2 Phasmid socket cell assay

A) and B) The correct position of phasmid socket cells in *FV(RNAi)* and the empty spot in *spd-5(RNAi)* are pointed by the arrow respectively. Scale bar is 10μm. C) The rate of correctly located socket cell in *RNAi* animals.



C

Genotype	[IPTG]	N	correctly located socket cell (%)
N2; *FV (RNAi)*[1]	0.1mM	40	95
N2; *spd-5 (RNAi)*		40	68
N2; *FV (RNAi)*[1]	1mM	54	94
N2; *spd-5 (RNAi)*		48	63

1, *FV(RNAi)*: L4440 empty vector is used as a negative control.
N = number of sides.

Figure 2.3 *spd-5(RNAi)* causes defects in both divisions and polarity of T cell

Lineages of the T cell from three single hermaphrodites: A) *pSCM::gfp* transgenic worms treated with *FV(RNAi)* empty vector control were followed at 20°C; B) and C) *pSCM::gfp* transgenic worms treated with *spd-5(RNAi)* were followed at 20°C. The fates of neural cells (N) can be distinguished from seam (S) and hypodermal (H) cells based on their nuclear morphologies (Sulston, 1977; Herman, 1994). The fate of seam cells was distinguished from hypodermal cells based on GFP expression. Observation began after the seam cells had undergone their first division at L1. Hours of post-hatching is indicated on the left. Curved arrow indicates the time when T.pp and T.pa switched positions. 5/5 wild type animals and 3/6 of *spd-5(RNAi)* animals analyzed had the lineage pattern showed in A); 1/6 *spd-5(RNAi)* animals analyzed had the lineage pattern showed in B); 1/6 *spd-5(RNAi)* animals analyzed had the lineage pattern showed in C). The other *spd-5(RNAi)* animals had cell division defects randomly in T descendants.

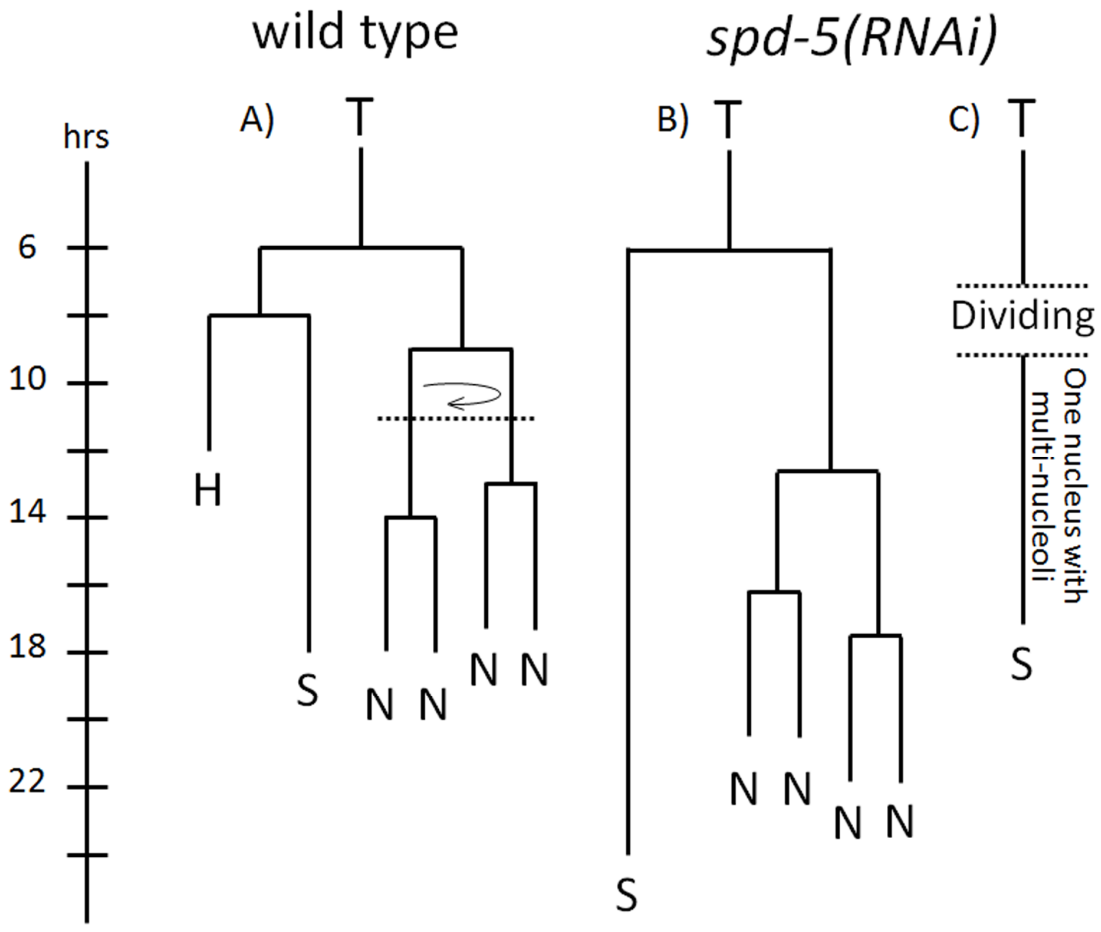


Figure 2.4 qRT-PCR of *spd-5* and *tbg-1* in RNAi

ΔΔCt values of individual RNAi are shown. ΔΔCt values were normalized to control RNAi. The housekeeping gene *ama-1* was used as a standard. ^ indicates growing L4 on 0.1mM IPTG RNAi plates, and scoring the adults of the progeny. The others are scoring the adults that grew on 1mM IPTG RNAi plates from hatching as L1s.

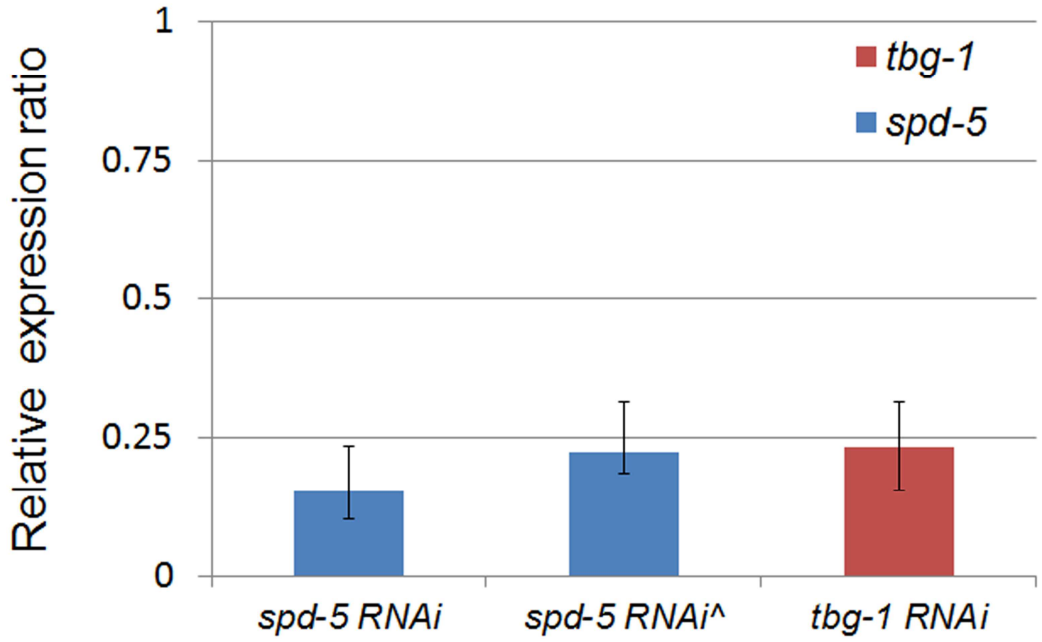


Figure 2.5 Terminal seam and hypodermal number are both reduced by knocking down *spd-5* and *dhc-1*

Representative young adult hermaphrodites expressing GFP or YFP on one side are shown. Worms in A, C and E contain an extrachromosal array that expresses GFP in seam cell nuclei at all development stages. Worms in B, D and F contain an integrated array that expresses YFP in hypodermal cells, including the hypodermal descendant of seam cell divisions. Worms were grown on bacteria that target individual genes as shown. Scale bar is 50μm.

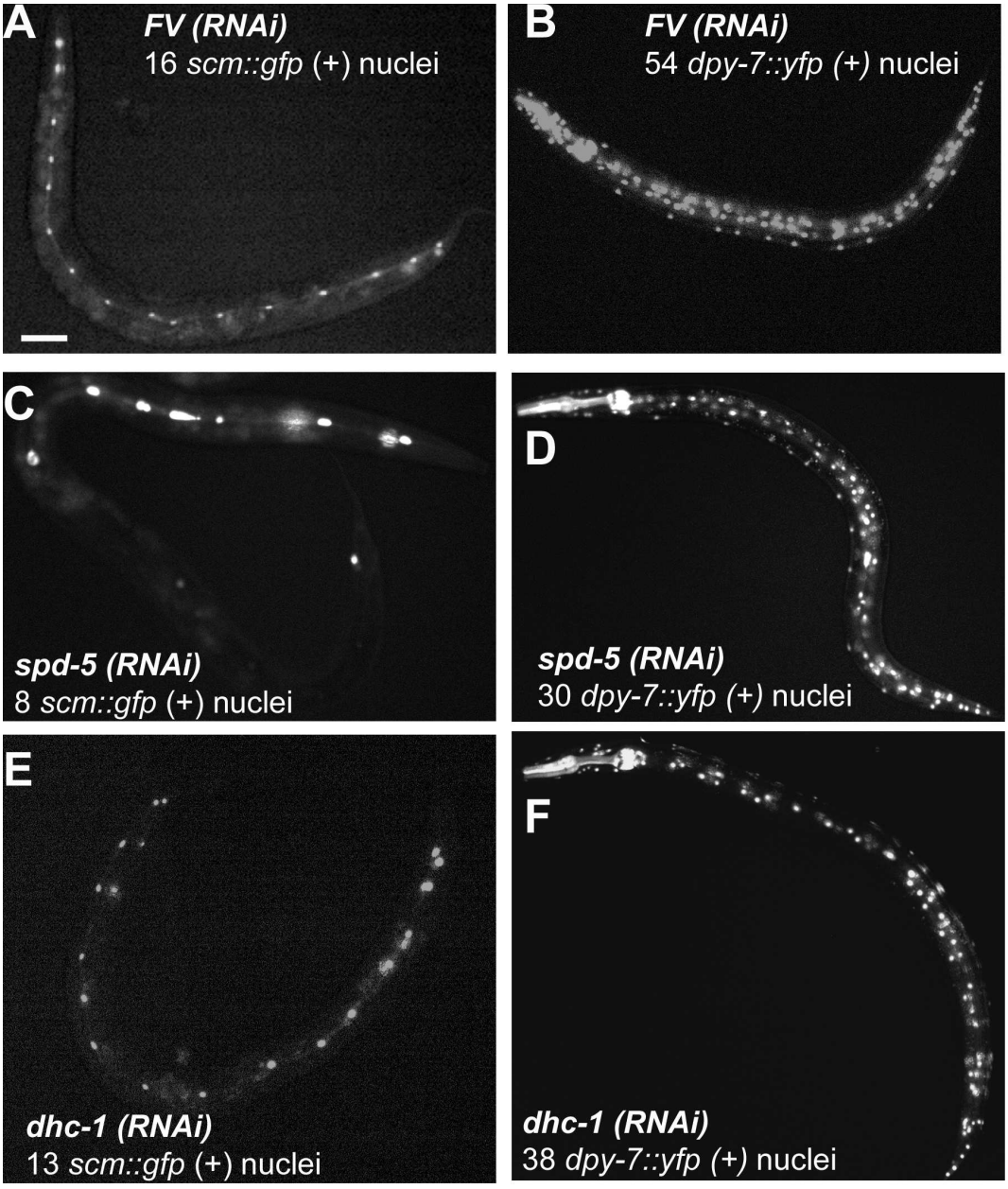
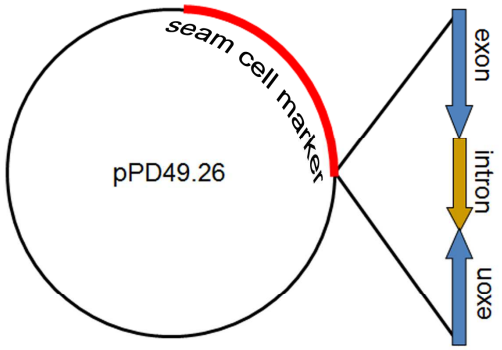


Figure 2.6 *spd-5* functions autonomously in seam cells

A) Schematic of the seam cell specific *spd-5* hairpin RNAi construct (pSHH3). B) Terminal seam cell number and phasmid dye-filling (%) of strains which carry extrachromosomal array of *spd-5* hairpin RNAi. *mhEx267 [pSCM::gfp; pSHH3]*, *mhEx26 [pSCM::gfp; unc-119(+);* pSHH3*]*. *sid-1* encodes a dsRNA channel that enables passive (ATP-independent) uptake of double-stranded RNA (dsRNA), thus *sid-1(qt2)* restricts the RNAi effect in seam cells.

A



B

Genotype	Conc. hairpin	Terminal Seam cell			T cell defect	
		N	Ave. #	Range	N	Dye filling (%)
sid-1(qt2); mhEx267	20ng/µl	64	15.5*	11-17	106	96.0
unc-119(e2498); sid-1(qt2); mhEx266	100ng/µl	50	14.0*	10-17	47	95.7

N = number of worms scored.
* P< 0.01 compared to wild type.

Figure 2.7 Abnormal nuclei in different cell types

A) One nucleus with multi-nucleoli is pointed by red arrow, while the normal nuclei are pointed by black arrows. Scale bar is 10μm. B) The frequency of abnormal nuclei in the daughters of Pn.p and intestine cells and descendants of seam cells are shown.

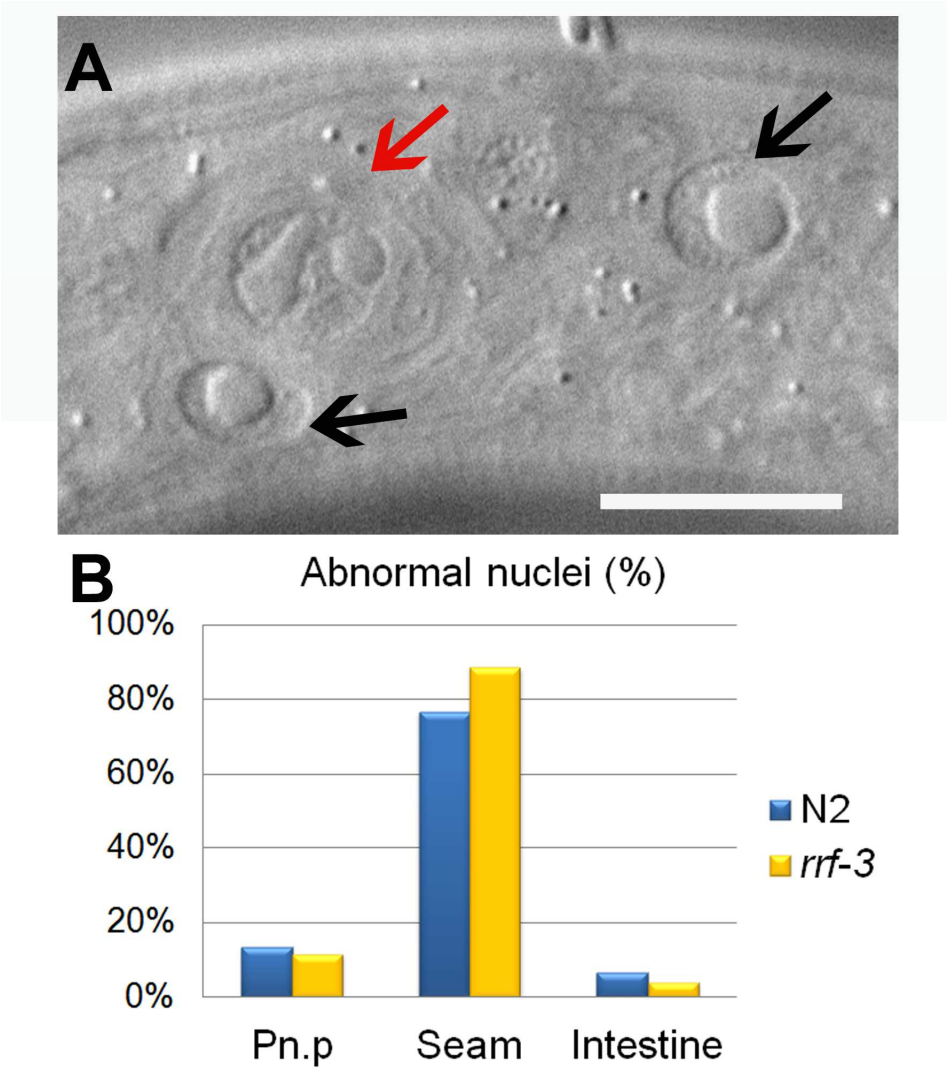
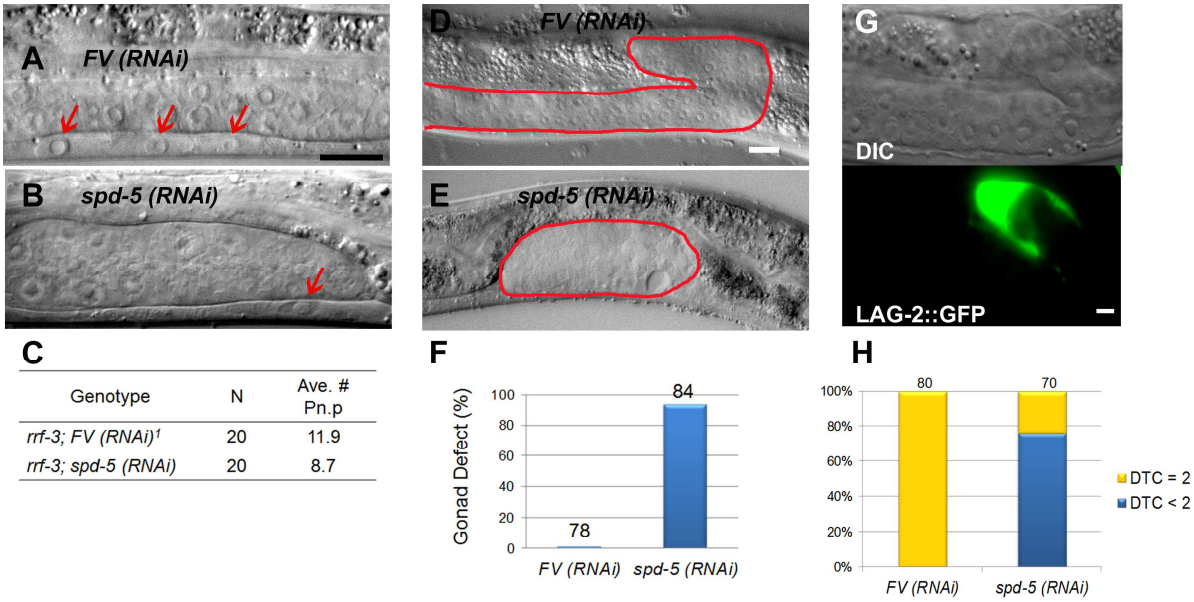


Figure 2.8 SPD-5 regulates Pn.p and gonad development

The Pn.p cells are indicated by red arrows in A and B. The average Pn.p number is shown in C. The wild type gonad and Sys gonad are shown as D and E, respectively. The gonad boundaries are outlined in red. The frequency of gonad defect is shown in F. The GFP labeled DTCs and the rate of worms with decreased DTC are shown in G and H. Scale bar is 10μm.



Genotype	N	Ave. # Pn.p
rrf-3; FV (RNAi)[1]	20	11.9
rrf-3; spd-5 (RNAi)	20	8.7

1, *FV(RNAi):* L4440 empty vector is used as a control.
N = number of worms.

Figure 2.9 *spd-5* regulates localization of the Wnt/β-catenin asymmetry pathway components

The localization of POP-1, SYS-1 and LIN-17 are shown in T cells (A-H and R-U). The localization WRM-1 and APR-1 are shown in V and T cells (I-Q). The relative level of GFP tagged proteins were determined by eye. Scale bar is 10μm.

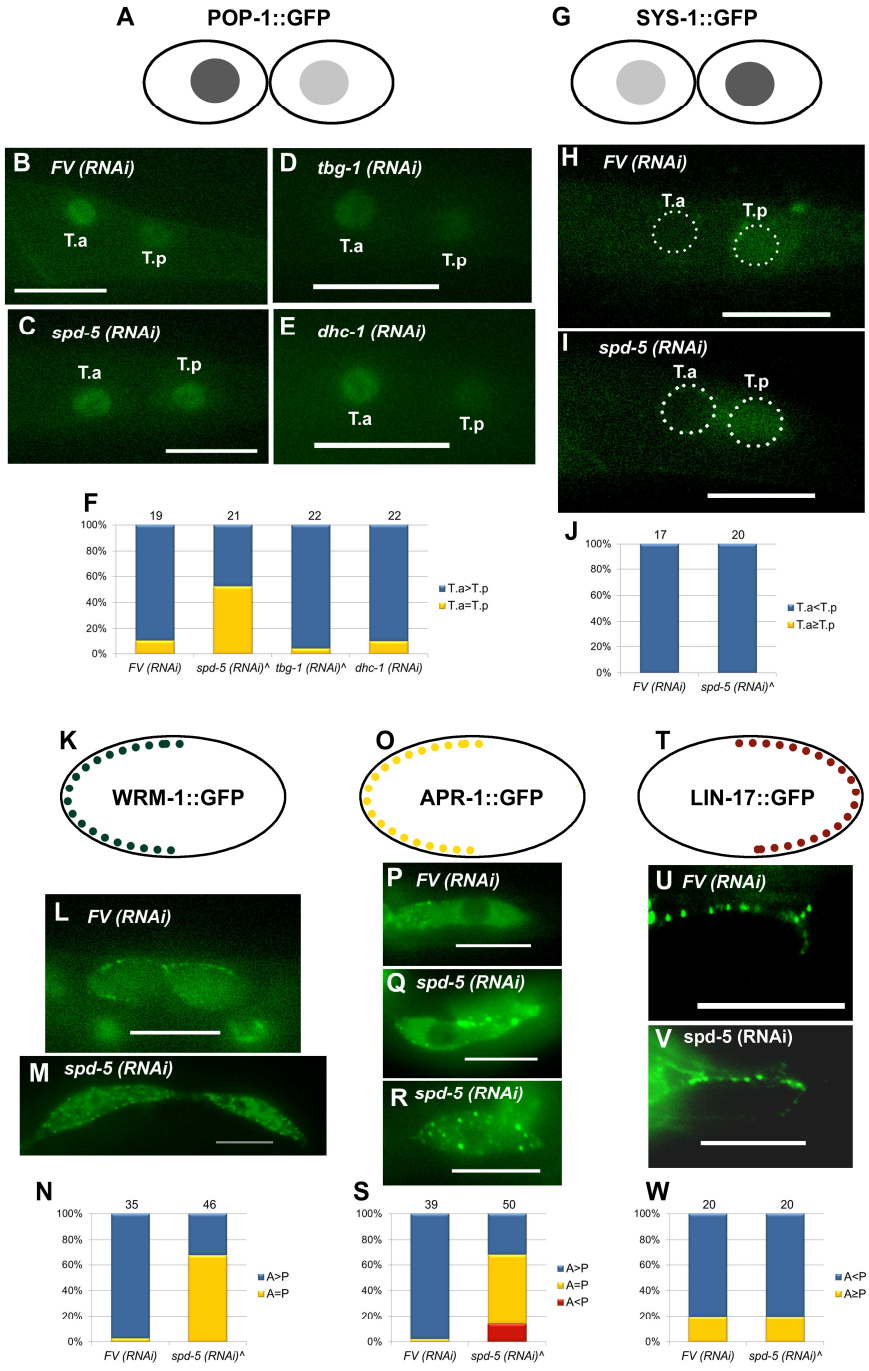


Figure 2.10 *spd-5* expression pattern

The expression of GFP::SPD-5 fusion protein driven by scm promoter in wild type worms. The fluorescence image is on top, the corresponding DIC image is on the bottom. Scale bar is 10μm.

Figure 2.11 Models for SPD-5 function

The role of SPD-5 in the centrosome is shown on the top. The SPD-5 function in controlling cell polarity and asymmetric divisions is through genetically regulating the components of the Wnt/β-catenin asymmetry pathway, as shown on the bottom.

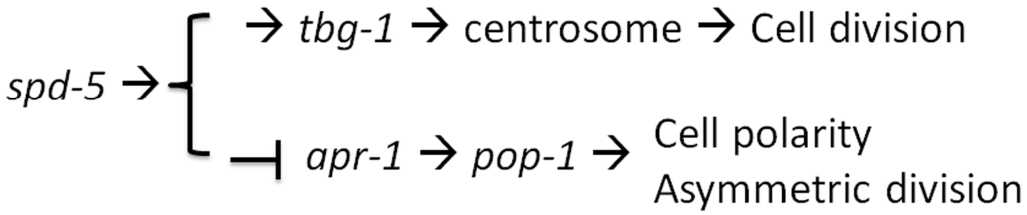


Table 2.1 Knock down of *spd-5* causes embryonic lethality

The rate of RNAi animals that bypass the embryonic effect of reducing genes activities and grow into adults is shown.

Genotype	N	Survivorship (%)
rrf-3; (FV RNAi)[1]	100	92.0
rrf-3; spd-5(RNAi)	100	2.0
N2; (FV RNAi)[1]	587	86.2
N2; spd-5(RNAi)	584	0.2

1, *FV (RNAi):* L4440 empty vector is used as a negative control.
N = number of worms.

Table 2.2 Centrosomal components and the Wnt/β-catenin asymmetry pathway determine the terminal seam and hypodermal cell numbers and T cell division

The number of average terminal seam and hypodermal cells are shown with phasmid dye-filling (%) in individual RNAi. All strains contain either extra-chromosomal array *mhEx265 [unc-119(+) +pscm::gfp]* to determine seam cell number, or *arIs99* (*dpy-7p::2Xnls::YFP*) to determine hypodermal cell number.

Genotype	Seam cell			Hypodermal cell			T cell defect	
	N	Range	Ave. #	N	Range	Ave #	N	Dye filling (%)
wild-type (FV RNAi)[1]	66	14-17	15.9	69	50-60	54.6	63	98
pop-1(RNAi)	47	28-73	53.4*	61	33-44	39.4*	51	89
apr-1(RNAi)	75	16-31	22.9*	50	36-50	43.7*	90	92
wrm-1(RNAi)	76	6-16	12.1*	72	50-63	57.9*	72	95
lit-1(RNAi)	66	6-18	13.7*	70	53-63	57.9*	73	92
pry-1(RNAi)	74	14-18	16.1	50	50-58	53.9	91	96
lin-17(RNAi)	20	15-17	16.0	52	49-57	53.3	20	100
*spd-5(RNAi)^*	30	2-17	7.4*	50	24-42	28.4*	ND	
spd-5(RNAi)	74	8-17	13.6*	88	37-54	45.1*	64	97
tbg-1(RNAi)	61	10-17	15.0*	55	43-56	49.2*	61	98
*tbg-1(RNAi)^*	67	5-17	10.9*			ND		
spd-2(RNAi)	75	14-17	15.9	60	47-56	55.8	25	98
*spd-2(RNAi)^*	88	13-17	15.9			ND		
air-1(RNAi)	52	11-19	16.0	50	40-57	43.7*	52	89
*air-1(RNAi)^*	56	10-18	15.3			ND		
dhc-1(RNAi)	48	7-17	12.2*	69	38-48	44.0*	90	99
cyk-4(RNAi)	55	12-24	16.0	61	39-66	52.6	55	91
zen-4(RNAi)	50	15-18	16.1	53	48-56	52.6*	50	97
cyk-1(RNAi)	59	14-19	16.6*	50	51-56	53.4*	59	90
tba-1(RNAi)	54	8-17	12.2*	50	32-50	39.5*	54	94
tba-2(RNAi)	56	6-16	11.5*	50	25-51	40.7*	56	98
tbb-1(RNAi)	52	15-27	19.2*	58	46-58	52.3*	52	96
tbb-2(RNAi)	54	14-17	15.8	63	38-56	51.3*	54	96

1, *FV RNAi*: L4440 empty vector is used as control.
N = number of worm sides.
Results from t tests: *p<0.01 compared to wild-type (*FV RNAi*).
^ indicates growing L4 on 0.1mM IPTG RNAi plates, and scoring the adults of the progeny. The others are scoring the adults that grew on 1mM IPTG RNAi plates from hatching as L1s.

Table 2.3 *spd-5* does not regulate the original seam cell number in L1s

The seam cell numbers in L1 stage before the first seam cell division are shown. All strains contain extrachromosomal array *mhEx265[unc-119(+)+pscm::gfp]*,

Genotype	N	Range	Ave. # L1 seam cells
wild-type (FV RNAi)[1^]	41	6-10	9.1
spd-5(RNAi)^	46	8-10	9.8

1, *FV RNAi*: L4440 empty vector is used as control.
N = number of worm sides.
^ indicates growing L4 on 0.1mM IPTG RNAi plates, and scoring the adults of the progeny.

Table 2.4 *spd-5* functions upstream of *apr-1* and *pop-1* to regulate asymmetric seam cell divisions

All strains contain either extra-chromosomal array *mhEx265 [unc-119(+)+pSCM::gfp]* to determine seam cell numbers, or *arIs99* (*dpy-7p::2Xnls::YFP*) to determine hypodermal cell numbers. 1:1 mixture of bacteria with RNAi construct and empty L4440 was used as a reference for the respective single RNAi.

Genotype	Seam cell			Hypodermal cell		
	N	Range	Ave. #	N	Range	Ave #
wild-type(FV RNAi)[1]	66	14-17	15.9	69	50-60	54.6
spd-5 (RNAi)	61	8-16	13.7*	63	38-53	45.5*
pop-1(RNAi)	53	26-67	43.3*	53	28-42	35.5*
spd-5(RNAi); pop-1(RNAi)	60	18-52	34.2**	54	24-42	34.8*
apr-1(RNAi)	54	12-27	20.5*	52	37-50	44.3*
spd-5(RNAi); apr-1(RNAi)	67	14-25	17.4**	50	38-49	44.3*

1, *FV RNAi*: L4440 empty vector is used as control.
N = number of worm sides.
Results from t tests: *p<0.01 compared to wild-type (FV RNAi); **p<0.01 compared to both single RNAi.

Chapter 3 - Conclusion

Cell polarity and the subsequent asymmetric cell division are regulated by centrosomes and Wnt signaling pathway. The centrosome functions as the MTOC and performs microtubule-related functions, such as segregating chromosomes during cell division and localizing organelles and cargos (Schatten, 2008). The centrosome has been shown to regulate cell polarity of the *C. elegans* zygote, but whether the centrosome also functions to control the polarities of post-embryonic cells is unknown. One of the non-canonical Wnt pathways, called Wnt/β-catenin asymmetry pathway, controls the polarities of several post-embryonic cells of *C. elegans*. In this pathway, the Wnt signaling triggers the asymmetric localization of the pathway components, which further establishes the cell polarity and thus controls asymmetric divisions. However, how this extracellular Wnt signaling is translated to the cellular localization of the Wnt pathway components is not very clear. In this study, we aimed to test whether the cellular organelle centrosome can facilitate the Wnt/β-catenin asymmetry pathway to regulate the cell polarity and the subsequent asymmetric division. We showed that a cemtrosomal protein SPD-5 which was originally found to be critical for the embryonic development also played a role during certain post-embryonic cell divisions in *C. elegans*. Particularly the polarity of seam cells that required SPD-5 function was also known to be regulated by the Wnt/β-catenin asymmetry pathway. Consistent with its function in the zygote, SPD-5 acts as a centrosomal component to regulate seam cell divisions. The asymmetric localization of Wnt pathway components is essential for their function upon the establishment of seam cell polarity. Strikingly, we discovered that SPD-5 was required for the proper localization of several Wnt components in a way that was independent of a

key MTOC member γ tubulin. This suggests a novel role of SPD-5 in regulating the protein localization. In addition, SPD-5 genetically interacted with APR-1 and POP-1, which confirmed the interaction of SPD-5 and Wnt signaling pathway. In summary, SPD-5 not only functions partial as a centrosomal component to control the cell divisions, but also interacts with the components of Wnt/β-catenin asymmetry pathway to regulate cell polarity in a way that is independent of MTOC. This finding suggests a novel role of a centrosomal component in regulating polarities of certain post-embryonic cells in *C. elegans*, furthermore indicates that centrosomes might link the extracellular signaling to the cellular events that controls the cell polarity and the asymmetric division.

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Appendix A - List of genes

Table 3.1 Summary of genes, corresponding proteins and their functions

Protein	Organism that protein is in (if not in *C. elegans)*	*C. elegans* gene	Orthologue in other organisms (if any)	Summary of the protein function and feature
AIR-1		*air-1*	Aurora A kinase	Kinase; required for PCM assembly; vertebrates Aurora A phosphorylates and activates Cdc25B.
AMA-1		*ama-1*		Encodes large subunit of RNA polymerase II required for mRNA transcription
APR-1		*apr-1*	APC	Adenomatous polyposis coli; functions both in canonical and non-canonical Wnt pathways; component of destruction complex targeting β-catenin in canonical Wnt pathways.
BAR-1		*bar-1*	β-catenin	β-catenin
BRAT	*Drosophila*			Translation inhibitor of Brain tumor; cell fate determinants in *Drosophila* neuroblasts
Cdc25B			Cdc25B	Phosphatase; activates Cdk1
Cdk1			Cdk1	Cyclin-dependent kinase; entry into mitosis
CeGrip-1		*gip-1*	GCP3	γ-tubulin ring complex

				component, required for assembly of spindles
CeGrip-2		*gip-2*	GCP2	γ-tubulin ring complex component, required for assembly of spindles
CFZ-2		*cfz-2*		Frizzled receptors in Wnt pathway
CK1	Vertebrates		CK1	Casein kinase 1; component of destruction complex targeting β-catenin in canonical Wnt pathways.
CWN-1		*cwn-1*		Wnt ligands
CWN-2		*cwn-2*		Wnt ligands
cyclin B1			cyclin B1	Cyclin B
CYK-1		*cyk-1*	DIAPH1	Regulate cytokinesis in B cell of males
CYK-4		*cyk-4*		Encodes a Rho GAP (Rho guanosine triphosphatase (GTPase) activating protein); regulates anterior-posterior polarity in the one-cell embryo; regulates cytokinesis in T cell
DHC-1		*dhc-1*	dynein heavy chain	Dynein heavy chain 1; microtubule mediated motor protein; functions in centrosome separation, mitotic spindle orientation
DPY-7		*dpy-7*		Encodes a cuticular collagen, expresses in hypodermal cells

DSH-1		*dsh-1*		Disheveled proteins; inhibits the formation of destruction complex in the present of Wnt ligands
DSH-2		*dsh-2*		Disheveled proteins; inhibits the formation of destruction complex in the present of Wnt ligands
EGL-20		*egl-20*		Wnt ligands
GCP4	*Drosophila* and vertebrates			γ-tubulin ring complex component, required for assembly of spindles
GCP5	*Drosophila* and vertebrates			γ-tubulin ring complex component, required for assembly of spindles
GCP6	*Drosophila* and vertebrates			γ-tubulin ring complex component, required for assembly of spindles
GSK-3		*gsk-3*	GSK-3	Glycogen synthase kinase 3; component of destruction complex targeting β-catenin in canonical Wnt pathways.
Gαi	*Drosophila*			Heterotrimeric G protein
HIM-5		*him-5*		Identified in screens for genes that when mutated resulted in an increased frequency of genotypically XO males in self-fertile populations
HMP-2		*hmp-2*	β-catenin	β-catenin
Inscuteable	*Drosophila*			

LET-858		*let-858*		Encodes nucampholin, a highly conserved protein rich in acidic and basic residues; expressed ubiquitously throughout development; is likely to be involved in protein synthesis and/or RNA binding.
LIN-17		*lin-17*		Frizzled receptors in Wnt pathway
LIN-44		*lin-44*		Wnt ligands
LIT-1		*lit-1*	NLK	Nemo-like kinase; functions in non-canonical pathway
LRP			LRP	A member of the LDL receptor family; form the receptor complex with Frizzled transmembrane receptors in canonical Wnt pathway.
MIG-1		*mig-1*		Frizzled receptors in Wnt pathway
MIG-5		*mig-5*		Disheveled proteins; inhibits the formation of destruction complex in the present of Wnt ligands
MOM-2		*mom-2*		Wnt ligands
MOM-5		*mom-5*		Frizzled receptors in Wnt pathway
MOZART1 and 2	vertebrates			γ-tubulin ring complex component, required for assembly of spindles

MUD	*Drosophila*			Dynein-binding protein
Numb	*Drosophila*			Inhibits Notch-Delta signaling; cell fate determinants in *Drosophila* neuroblasts
PAR-1,2,3 and 6		*par-1,2,3 and 6*		Name from Partitioning-defective; required for polarization of the *C. Elegans* zygote along the anterior-posterior (A-P) axis
Pericentrin	mammals			Coiled-coil proteins; required for PCM assembly
PIE-1		*pie-1*		Zinc-finger protein; maternally provided PIE-1 is essential for germline cell fate determination
PINS	*Drosophila*			Partner of Inscuteable
PKC-3				Atypical protein kinase C; required for polarization of the *C. Elegans* zygote along the anterior-posterior (A-P) axis
POP-1		*pop-1*	TCF	Transcription factor; represses target gene expression in the absent of β-catenin in Wnt pathway
PP4	*Drosophila* and *C. elegans*	*pph-4.2*	PP4	Protein phosphatase 4; required for PCM assembly
Prospero	*Drosophila*			Transcription factor; cell fate determinants in *Drosophila*

				neuroblasts
PRY-1		*pry-1*	Axin	Functions both in canonical and non-canonical Wnt pathways; component of destruction complex targeting β-catenin in canonical Wnt pathways.
RRF-1		*rrf-1*		An RNA-directed RNA polymerase (rdrp) homolog that inhibits somatic RNAi, thus mutants are hypersensitive to somatic rnai
SID-1		*sid-1*		Encodes a dsRNA channel that functions to enable passive (ATP-independent) uptake of double-stranded RNA (dsRNA) and is required cell autonomously for systemic RNA interference (RNAi)
SPD-2		*spd-2*	FLJ10352	SPindle Defective 2; coiled-coil protein; required for centrioles duplication and PCM assembly; localizes to centrioles and PCM
SPD-5		*spd-5*		SPindle Defective 5; Coiled-coil proteins; required for assembly of PCM, including γ-tubulin, AIR-1 and ZYG-9; genetically interacts with

				SPD-2 and DHC-1
SYS-1		*sys-1*	β-catenin	β-catenin
SYS-4		*sys-4*	CPAP	Coiled-coil protein; required for centrioles duplication; localizes to centriles
SYS-5		*sys-5*		Coiled-coil protein; required for centrioles duplication; localizes to centriles
SYS-6		*sys-6*	HsSAS-6	Coiled-coil protein; required for centrioles duplication; localizes to centriles
UNC-119		*unc-119*		Is required for proper development of the nervous system, and hence, for normal movement, chemosensation, and feeding
WRM-1		*wrm-1*	β-catenin	β-catenin
ZEN-4		*zen-4*		A kinesin-like protein that is a member of the kinesin-6 subfamily of plus-end-directed microtubule motors; required for completion of cytokinesis after mitosis
ZYG-1		*zyg-1*		Kinase; required for centrioles duplication; localizes to centriles
ZYG-9		*zyg-9*	XMAP-215	PCM component
α-tubulin		*tba-1~9*	α-tubulin	Microtubules components
β-tubulin		*tbb-1,2,4,6*	β-tubulin	Microtubules components
γ-tubulin		*tbg-1*	γ-tubulin	γ-tubulin ring complex

				component, required for assembly of spindles