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Nuclear positioning in the gonadal distal tip cells of *C. elegans*

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Morphogenesis of the hermaphrodite gonad of *Caenorhabditis elegans* is directed by the U-shaped migration of the gonadal leader cells, which are called distal tip cells (DTCs). The nuclei of migrating DTCs are always positioned at the leading edge of the cells, even as these cells turn dorsally to contact the hypodermis and intestine. When the DTCs turn dorsally, VAB-10B1/spectraplakin acts in nuclear translocation by regulating the polarized growth of microtubules. The function of spectraplakin in nuclear positioning may be evolutionarily conserved. Here we discuss the possible reason for leading-edge positioning of the DTC nucleus.

Keywords: spectraplakin, cytoskeleton, VAB-10B1, nuclear migration, cell migration

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DTCs Change Their Morphology During Dorsal Migration

The gonadal distal tip cells (DTCs) of *C. elegans* migrate in a U-shaped pattern during larval development and lead the morphogenesis of the U-shaped gonad arms. The DTC migration offers a simple model for the tip-cell-dependent morphogenesis of epithelial tubes. Morphogenesis by extension and branching of epithelial tubes, which is called "branching morphogenesis," is one of the major strategies found in organ formation. Recently, the migration of epithelial tubes was shown to be often controlled by a cell or a group of cells at the tip of the growing tubes.[1] Although the *C. elegans* gonad arms do not branch, the DTCs migrate along a complex trajectory comprising three linear phases punctuated by two orthogonal turns. Each DTC forms a close-fitting cap over the 6–10 germ cells and had been thought to maintain this morphology during the course of its migration.[2] Unexpectedly, however, DTCs were found to change their shape dramatically at the first turn.[3] DTCs that expressed GFP-moesin, which labels cortical actin filaments, were analyzed by confocal microscopy and shown to extend a single large lamellipodium dorsally when they make the first turn (**Fig. 1**). The DTCs then migrate dorsally by invading the contact site of the intestine and the lateral epidermis.

VAB-10B1/Spectraplakin Acts in Nuclear Positioning

It is interesting to note that the DTCs consistently position their nuclei at the leading edge throughout their migration. When the DTCs turn dorsally, their nuclei are relocated to the dorsal side of the DTCs (**Fig. 1**). The DTCs extend lamellipodia at the onset of this turn and thus place their nuclei near the leading edge. This nuclear translocation requires the action of VAB-10B1, one of the spectraplakin isoforms in *C. elegans*.[3] Spectraplakins are large cytoplasmic proteins that regulate the cytoskeleton.[4-6] Nuclear translocation in DTCs is suppressed in mutants lacking VAB-10B1. Although the network of F-actin is mostly normal in these animals, the microtubule (MT) network is severely disrupted.[3] VAB-10B1 has an N-terminal actin-binding domain (ABD), which is followed by a plakin domain, spectrin repeats, and an MT-binding domain (MTBD). Spectraplakins are known as the linker protein that bridges actin and MT filaments, because mini-genes containing only the ABD and MTBD domains can rescue the defects in the MT cytoskeleton in cultured cells,[7,8] and in axonal growth in Drosophila[9] that occur in the absence of functional endogenous spectraplakins.

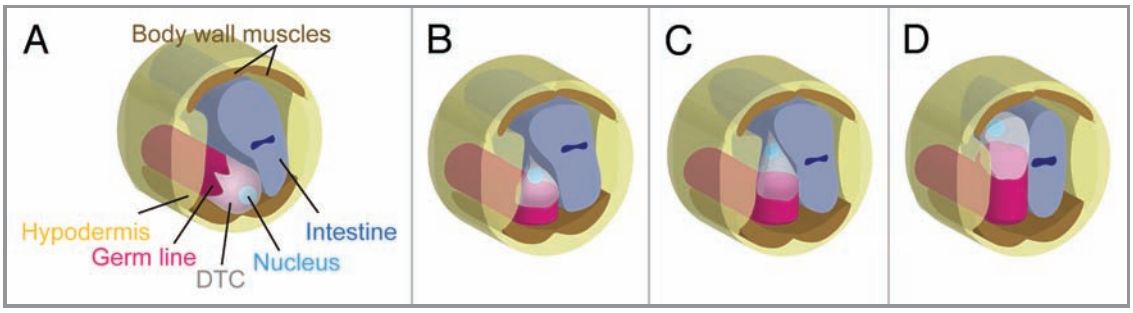

Figure 1. Schematic presentation of DTCs and their nuclear migration during gonadogenesis. DTCs migrating on the ventral muscles (A), initiating dorsal lamellipodial extension into the contact site between the hypodermis and intestine (B), moving dorsally between the hypodermis and intestine (C), and reaching the dorsal muscle (D) are shown. Note that the DTC nucleus is always present at the leading edge of the cell.

DTC-specific expression of the *vab-10B1* mini-gene also rescued defective nuclear translocation in *vab-10B1* mutant larvae, suggesting a similar linker activity for VAB-10B1.[3]

F-actin and MTs are often aligned along the migratory axis of DTCs, and MTs grow toward the nucleus in the leading edge of the migrating DTCs. This polarized outgrowth of MTs is compromised in *vab-10B1* mutants. Disruption of kinesin, a plus-end motor of MTs, also results in nuclear translocation defects in DTCs (Kim and Nishiwaki, unpublished data). These results suggest that, via its linker activity, VAB-10B1 functions in polarized outgrowth of MTs along the actin filaments, which are arrayed in parallel to the migratory axis of DTCs, and that the DTC nuclei are carried over the MT fibers toward the migratory leading edge in a kinesin-dependent manner (**Fig. 2**). Spectraplakin is reported to regulate MT outgrowth along actin bundles in mammalian cultured cells.[7]

Is the Function of Spectraplakin in Nuclear Positioning Evolutionarily Conserved?

The functions of spectraplakins in F-actin and MT regulation (localization, alignment, polarity and outgrowth) have been studied mainly in cell migration and axon extension.[7-9] Kim et al. (2011) for the first time showed that spectraplakin is actively engaged in nuclear translocation via its function in MT regulation. The function of spectraplakin in nuclear positioning was, however, noticed early on in naturally occurring mutant mice. In mice with the neurodegenerative disorder *dystonia musculorum*, the causative gene was found to encode the spectraplakin Bpag1.[10,11] Among the characteristic pathological features detected in *dystonia* mice is the eccentricity of neuronal nuclei.[12-14] The

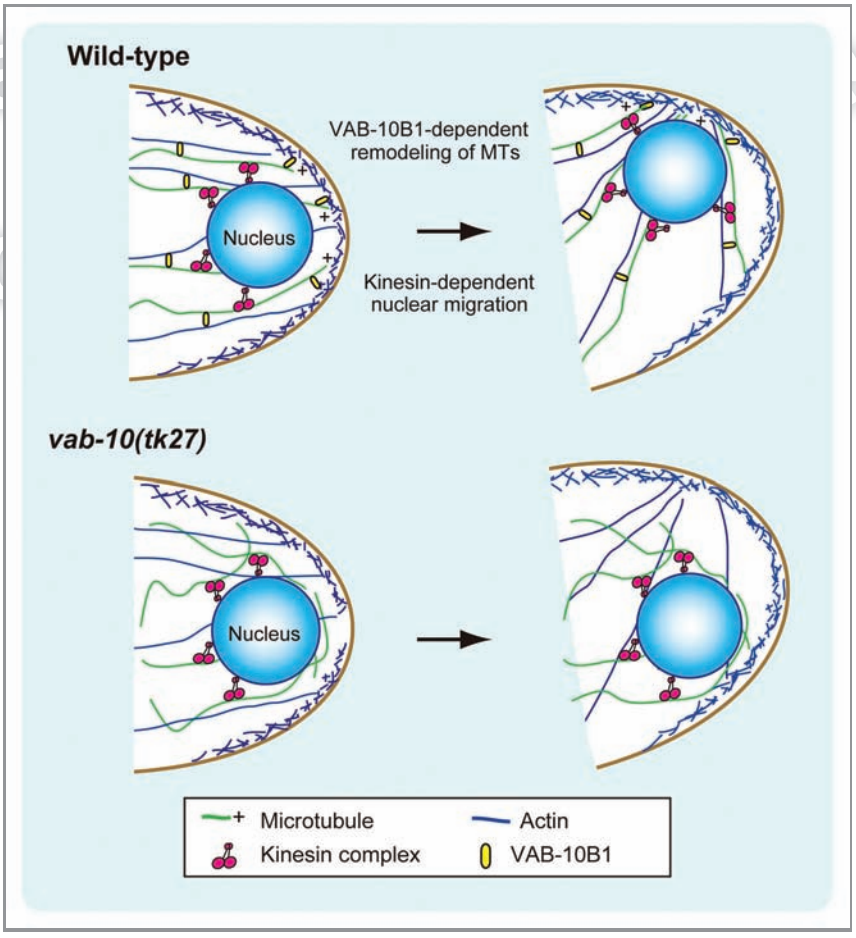

Figure 2. Model for nuclear translocation in DTCs. VAB-10B1 mediates linking between MTs and F-actin, which facilitates the outgrowth of MTs to direct the plus ends toward the migratory leading edge of the cell. As DTCs turn dorsally, it is likely that F-actin is first redirected dorsally. Then the MTs are remodeled to grow dorsally along the F-actin network. The nuclei of the DTCs are translocated to the leading edge of the cells over the MT fibers in a kinesin-dependent manner. In *vab-10B1* mutants, as the polarized outgrowth of MTs is disrupted, the kinesin-dependent nuclear migration fails.

zebrafish mutant magellan, which affects the spectraplakin microtubule-actin cross-linking factor 1 (MACF1), is also defective in nuclear positioning in oocytes.[15] In the zebrafish mutant, although abnormal MT localization is found in the oocytes, it is not known if this MT mislocalization affects nuclear positioning. As there are many studies that have shown an involvement of MT activity in the migration or positioning of cellular nuclei in various developmental contexts,[16,17] it is possible that the role of spectraplakin in directional MT alignment to achieve appropriate positioning of nuclei in cells is conserved evolutionarily.

Why is the Nucleus of the DTC Placed at the Leading Edge of These Migrating Cells?

Nuclear migration occurs during the differentiation and morphogenesis of diverse cell types. For example, the migration of the nucleus into the bud neck in *S. cerevisiae* is important for normal distribution of chromosomes between mother and daughter cells.[18] The interkinetic nuclear migration seen in vertebrate neuroepithelia is correlated with the cell cycle and is thought to regulate the fates of daughter cells.[19] In Drosophila early embryos, the migration of nuclei to the cell cortex is essential for forming the syncytial blastoderm.[20]

In mammalian cultured cells, nuclei are actively moved away from the migratory leading edge.[21] This nuclear replacement is important for positioning the microtubule-organizing center (MTOC) and Golgi apparatus in front of the nucleus, which may facilitate the delivering of membrane precursors and actin regulators to the leading edge.[22,23] In contrast, the nuclei of migrating DTCs are positioned at the leading edge. It is unclear why the nuclei of DTCs are actively translocated to this region of the migrating cell. The absence of UNC-83/KASH, a nuclear membrane protein required for DTC nuclear translocation, also affects DTC cell migration, albeit weakly. These mutant DTCs exhibit weak pathfinding defects during their migration (Kim and Nishiwaki, unpublished data; ref. 24). Thus, it is possible that nuclear positioning at the leading edge may play some role in guiding DTC migration. Because the guidance of DTCs during their dorsal migration depends on UNC-6/netrin signaling,[25,26] it might be possible that the UNC-6 receptors UNC-5 and/or UNC-40 may be expressed in the membrane region at the leading edge of DTCs. If the nucleus was positioned close to the leading edge under these circumstances, this would allow the efficient transduction of the UNC-6 signal to the nucleus. The UNC-6 signal could then activate the expression of genes that are required for polarized extension of the lamellipodium of the DTC. Further analysis of cell and nuclear migration of DTCs is required to determine a detailed understanding of nuclear positioning at the leading edge.

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