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Insights into the Role of the Canonical Wnt Pathway in Embryonic Development

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Abstract

Wnts are a family of proteins that control gene expression and cell differentiation, particularly in embryos. They serve a variety of roles in cell and tissue development, directing the creation and proliferation of embryonic cells and tissue layers, specifying the ectomesoderm and being instrumental in determining muscle cells. Wnt performs these functions through canonical and non-canonical pathways. The canonical Wnt pathway relies on beta-catenin to activate transcription factors that lead to the transcription of genes needed for cell and tissue differentiation. There are two non-canonical Wnt pathways, both independent of Beta-catenin: the planar cell polarity (PCP) pathway, which regulates cell orientation, and the Wnt/Ca^{2+} pathways, which involve the release of intracellular calcium. Wnts were initially thought to be long-range signalers, but a recent experiment with *C. elegans* used in-vitro signaling to suggest that Wnt also can spread locally. Wnt5 is a Wnt protein essential in border endoderm (BE) specification and embryonic tissue formation, making it critical in early embryonic development. A Wnt-endosome complex utilizes Wnt proteins to fine-tune Wnt signaling, controlling cell and tissue differentiation and

development. Future research into Wnt may lead to developing therapies for cancers and degenerative diseases.

Introduction

The Wnts are a family of secreted signaling glycolipoproteins that control gene expression and cell proliferation, and tissue morphogenesis in various biological contexts, including embryonic development, cancer cell regulation, and tissue formation. The Wnt signaling pathways are a set of signal transduction pathways activated by secreted Wnt proteins bound on the cell surface, through which Wnts activate distinct transcription programs that determine body axes, cell fate, and organ formation during development (Hikasa, 2013). They are activated by ligands binding to cell surface proteins during embryonic development. In embryonic development, the dysregulation of the Wnt pathway causes severe birth defects and often embryonic death (Guo, 2021).

Wnt pathways include both canonical and non-canonical pathways. The canonical pathway depends on beta-catenin, while non-canonical pathways are independent of beta-catenin. In both pathways, Wnt uses endocrine, paracrine, and/or autocrine signaling in affecting embryonic development, but paracrine signaling has been more extensively studied (McIntyre, 2013). Furthermore, recent technological advancements allowed direct visualization of Wnt secretion. Historically, Wnts were considered to be long range signaling molecules, however, these recent studies suggest that it may be spread both long-range and locally (Pani, 2018). This review summarizes these recent findings in the research field of the canonical and non-canonical Wnt pathways, which appears to be more versatile in function and flexible in its secretion patterns than originally thought. This paper will also discuss

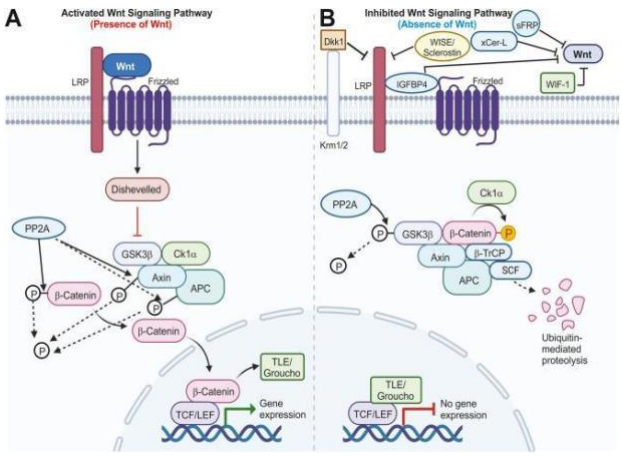
future directions and technological advancements necessary for further investigations of this important signaling pathway.

Wnt Pathways

In the canonical Wnt pathway, Wnt activation catalyzes the inactivation of the Beta-catenin destruction complex (Figure 1a). When Wnt binds to the Frizzled receptor (Fz) and LDL-receptor-related proteins (LRP) 5 and 6, Dishevelled protein (Dvl) is transported to the cell membrane. This leads to phosphorylation of the cytoplasmic tails of LRP 5/6, allowing it to bind to Axin. LRP binding to Axin removes it and inactivates the Beta-catenin destruction complex, causing Beta-catenin to accumulate and concentrate in the nucleus. Afterwards, Beta-catenin attaches to TCF transcription factors, driving downstream target gene transcription necessary for germ layer differentiation.

Figure 1a

The canonical Wnt signaling pathway in the presence and absence of Wnt

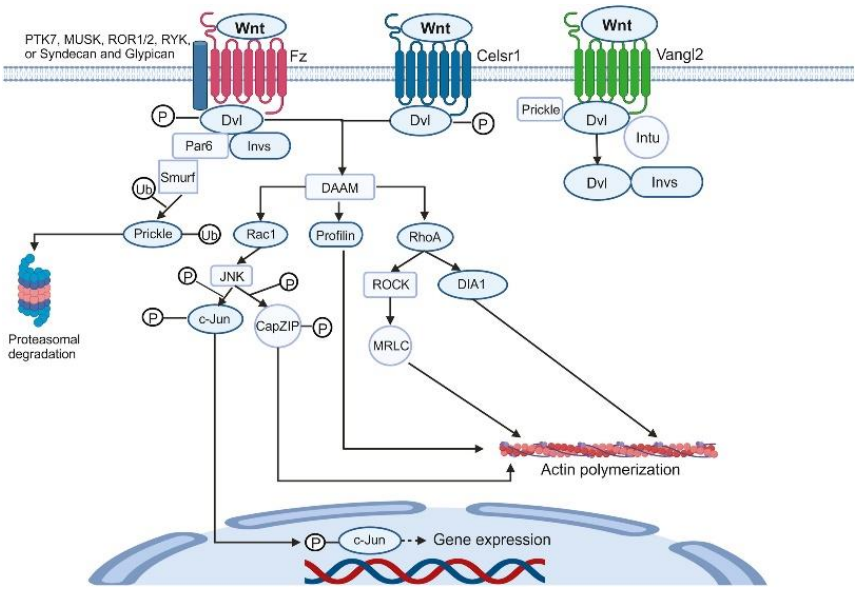


Source: The Wnt Signaling Pathway: The Foundation of Cell Growth, Development, and Potential Therapeutics, K. Qin, 2023

Unlike the canonical Wnt pathway, the non-canonical Wnt pathway is Beta-catenin independent. Non-canonical Wnt signaling can follow the Wnt/planar cell polarity (PCP) pathway or the Wnt/calcium (Ca^{2+}) pathway. The Wnt/PCP pathway primarily uses Fz receptors to bind Wnt, which bind Wnt ligands and phosphorylate Dvl protein, much like in the canonical Wnt pathway (Figure 1b). This leads to the recruitment of Inversin, Par6 protein, and Smad ubiquitination regulatory factor (Smurf). Smurf ubiquitinates the Wnt inhibitor Prickle, targeting it for destruction by proteosomes. With the inhibition of Prickle, Dvl is able to associate with the Dvl-associated activator of morphogenesis, which activates proteins that contribute to actin polymerization, which in turn establishes cell polarity and enables cell migration.

Figure 1b

The non-canonical Wnt/planar cell polarity (PCP) signaling pathway

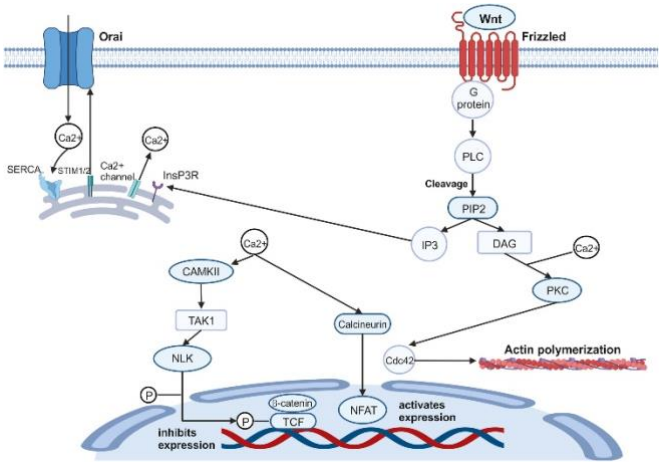


Source: The Wnt Signaling Pathway: The Foundation of Cell Growth, Development, and Potential Therapeutics, K. Qin, 2023

The Wnt/calcium (Ca^{2+}) pathway also results in actin polymerization, although it is through a different process (Figure 1c). The Wnt/Ca^{2+} pathway is activated by the Wnt5a ligand binding to the Fzd2 receptor, which triggers G protein to activate phospholipase C. Subsequently, Phospholipase C cleaves phosphatidylinositol-4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). Both IP3, through the stimulation of intracellular Ca^{2+} production, and DAG activate protein kinase C to stimulate actin polymerization. The increased cytosolic Ca^{2+} also stimulates the production of both calcineurin and CAMKII. Calcineurin activates the nuclear factor of activated T cells, causing gene transcription. On the other hand, CAMKII leads to the activation of Nemo-like kinase (NLK), which inhibits the Beta-catenin/TCF complex, preventing gene transcription.

Figure 1c

The non-canonical Wnt/calcium (Ca^{2+}) signaling pathway

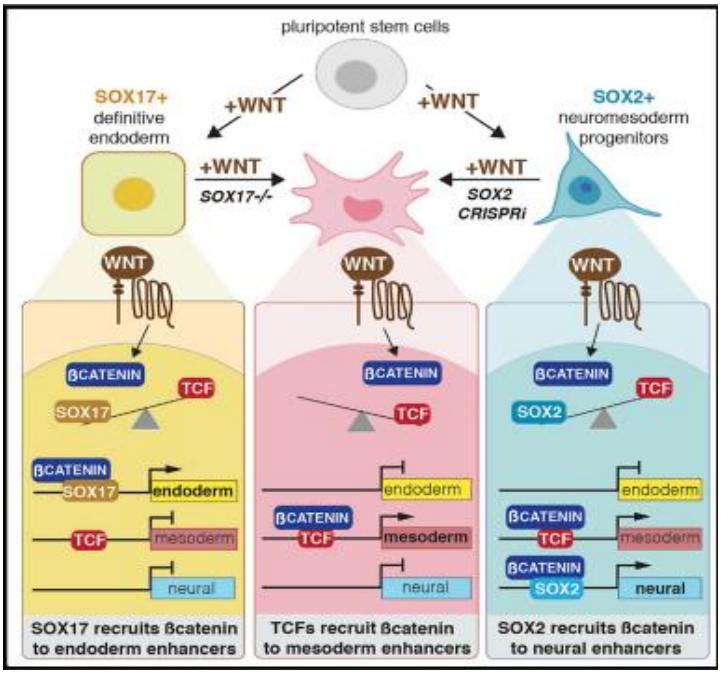


Source: The Wnt Signaling Pathway: The Foundation of Cell Growth, Development, and Potential Therapeutics, K. Qin, 2023

In the canonical and Wnt/Calcium processes, Beta-catenin has been considered to work exclusively with TCF to drive specific downstream transcription. However, recent studies suggest that Beta-catenin partners with other transcription factors, such as SOX proteins, drive different downstream targets for transcription, leading to different germ layer differentiation. Exposure to the SOX2+ protein leads to mesoderm and ectoderm formation, while exposure to the SOX17+ protein results in endoderm formation. TCF can recruit Beta-catenin to exclusively target the mesoderm from either of these pathways (Figure 2).

Figure 2

Beta-catenin recruitment in human pluripotent stem cells



Source: Sox Transcription Factors Compete with TCF for WNT/Beta-catenin Responsive Transcription to Govern Cell Fate in Human Pluripotent Stem Cells, S. Mukherjee, 2022

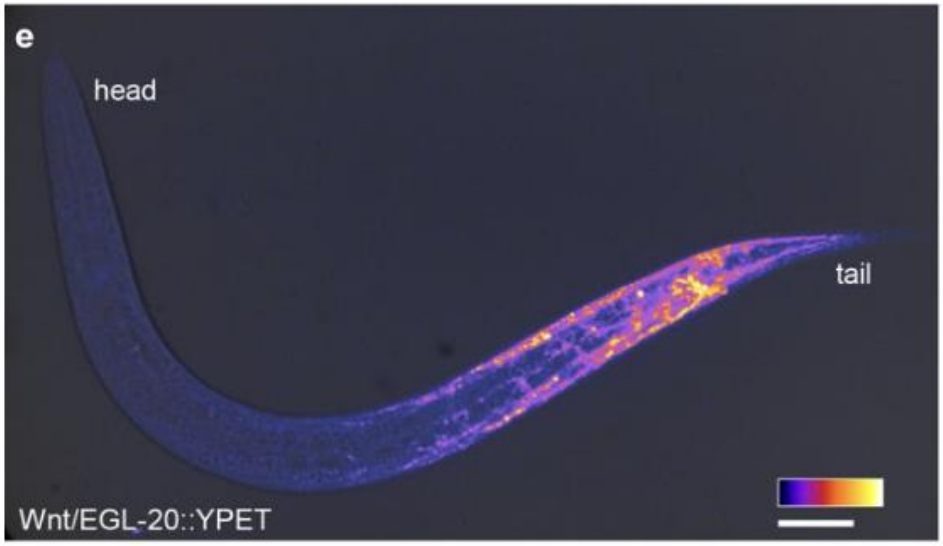
Wnt Signaling

Wnt proteins were traditionally thought to be extracellular, long-range signalers, but recent studies in *C. elegans* suggest that this may not necessarily be the case (Pani, 2018). Historically, it was very difficult to visualize Wnt dispersal *in vivo* in animals due to the complications with fluorescently tagging Wnt proteins, which limited concrete conclusions on the exact mechanisms of Wnt signaling. However, advances in imaging techniques have enabled the direct visualizing of Wnt dispersal *in vivo*, suggesting that Wnt is spread both long-range and locally. These technological developments in visualizing Wnt proteins in vivo have led to the discovery of molecular mechanisms such as a possible molecular oscillator that controls somite formation and, with Fgf8, sets the segment boundary position (Yamaguchi, 2024). This is critical to Wnt proteins during embryogenesis germ layer patterning.

In contrast, another study in *C. elegans* found the Wnt homolog egl-20 to be a long-range signal. Using fluorescently-tagged knock-in Wnt alleles, it was found that Wnt forms a long-range gradient *in vivo* that is strongest near the tail and tapers off towards the head (Figure 3a).

Figure 3a

YPET fluorescent-knock in image of Wnt/EGL-20 in vivo

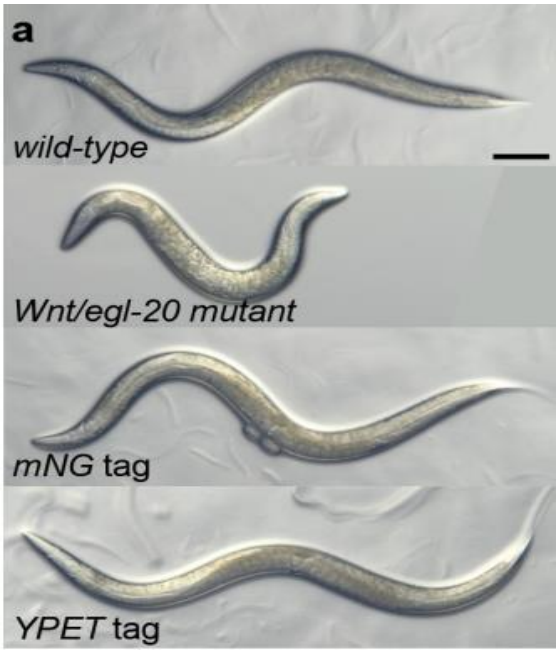


Source: Pani, 2018

That study also found that the egl-20 mutant failed to develop to the level of control *C. elegans* organisms (Figure 3b), indicating that Wnt is integral to the growth and development of all varieties of animal embryos. In addition, neuroblasts failed to properly migrate in mutated *C. elegans*, as evidenced by the mutant organisms' shortened length, further proving Wnt's role in stem cell proliferation.

Figure 3b

C. elegans specimens with Wnt/egl-20 mutant compared to control specimens



Source: Pani, 2018

The above results indicate that endogenous Wnt can freely spread across multiple spread *in vivo*, challenging the assertion that Wnt is primarily a short-range signaler. This, combined with a membrane-tethered Wnt signaling mechanism in *Drosophila*, shows that there are a variety of mechanisms for generating Wnt gradients.

Wnt and B-cat partners

In the sea urchin, Wnt is involved in determining the endoderm from the ectoderm and distinguishing the ectomesoderm. The posterior half of the embryo is classified as endomesoderm, with the anterior cells becoming ectoderm and neural ectoderm, ectoderm being the default germ layer fate in embryos in the absence of any inductive signals. In the

absence of Beta-catenin, embryos are a blastula entirely composed of ectoderm and neural ectoderm and fail to develop further.

Wnt5a is a member of the Wnt protein family, playing a role in regulating and inhibiting both the canonical and non-canonical Wnt pathways. Wnt5a operates by binding to Frizzled receptor, leading to the transmission of signals into the cell. Wnt5a regulates tissue regeneration and embryonic tissue formation, including epithelial-mesenchymal cell migration. Wnt5a also promotes the polarization, elongation, and redirection of primitive germ cells.

Endodermal Wnt5 is necessary for activation of border endoderm (BE) specification. The signal necessary for activation of the BE comes from the endoderm. Wnt5 activates the BE subcircuit in a narrow band of adjacent endodermal cells. BE cells use primary and secondary axis positional information to determine where to form the skeleton, making them essential in embryonic development.

Recent studies put forward the existence of a transcription complex known as the "Wnt-endosome" where Beta-catenin interacts on chromatin with cofactors such as BCL9 (B-cell 9), Pygopus, and the BAF (BRG/BRM-associated factor). Current models suggest that recruitment of Beta-catenin changes the shape of the Wnt-enhanceosome and initiates transcription upon Wnt activation (Kormish, 2010). The Wnt endosome uses Wnt signaling proteins to control Wnt signaling pathways, helping control cell growth and differentiation in embryos.

Beta-catenin also plays a role in the activation of several other signaling cascades involved in animal development, such as the Hedgehog, Notch, and TGFBeta/BMP signaling pathways. When Beta-catenin translocates into the nucleus, it can mediate signaling of transcription factors due to its role as a scaffold molecule. Beta-catenin also localizes at the cortex and functions in maintaining stable adherens junctions. The Beta-catenin protein is composed of 12 imperfect Armadillo repeats that are flanked by the amino terminal domain (NTD) and the carboxyl terminal domain (CTD), both of which are structurally flexible. This flexibility allows Beta-catenin to attach to several proteins, allowing it to act as a transcriptional regulator in the nucleus and scaffold protein at the cortex (Valenta, 2012).

Additionally, Beta-catenin is used to regulate the transcription activity of muscle differentiation through the canonical Wnt signaling pathway (Kim, 2007). Upon Wnt stimulation, activated Dishevelled prevents Beta-catenin from being destroyed, allowing the Beta-catenin to translocate to the nucleus and activate the expression of basic helix-loop-helix (bHLH) regulatory factors, such as Myf5 and MyoD, that initiate the development of muscle. Finally, the Wnt canonical pathway is necessary for the specification of muscle-derived CD45+ stem cells that repair injured muscle, regenerate vertebrate limbs, and differentiate multipotent cells into myogenic cells.

Conclusion and Future Directions

Wnt signaling pathways, particularly the canonical Wnt pathway, are essential to embryonic development and play a variety of roles in various stem cells. The canonical Wnt pathway depends on Beta-catenin, which accumulates in the nucleus, activating TCF transcription factors. They can be used both in the initial determination of cell fate in early

embryos and in repairing injured muscle tissue. Future research into Wnt may be dedicated to developing disease therapies. Wnt could treat cancers through controlling tumor growth and limiting its progression to metastatic stages. Wnt could also treat degenerative diseases by directing the creation of stem cells that restore lost tissue damaged by the disease (Post, 2024).

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