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RESEARCH ARTICLE

The Wnt signal pathways

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Abstract

Introduction

Much effort has been placed on tracing the signaling pathways and molecular mechanisms of the cells that control the development of an organism. In the modern researcher this effort is deeply engrained and is singly directed to notion that understanding the mechanisms that control normal development can exponentially increase our hopes to prevent and treat the pleiotropic pathologies that arise when these mechanisms go awry or uncontrolled. One of the critical pathways that have much effort in molecular medicine is the Wnt signal transduction pathway [1].

In 1982, Harold Vamus infected mice which, they identified a new mouse proto-oncogene that they named **int1** (integration 1) [2,3], which has a high degree of conservation across several species, including humans and these finding led researchers to discover in 1987 that the int1 gene in *Drosophila* was actually the already known and characterized *Drosophila* gene known as Wingless (*Wg*). Since all those genes had not been identified in the same manner as int1.Thus, the Int/Wingless family was renamed the Wnt family and int1 became Wnt1. The name Wnt was chosen because it is a combination of int1 and Wg and stands for Wingless-related integration site [3].

Wnt proteins regulate an array of cellular processes including cell fate determination, motility, polarity, primary axis formation and organogenesis. As the signaling pathways that play crucial role during embryogenesis are tightly regulated, expression of the Wnt proteins and Wnt antagonists are exquisitely restricted both temporally and spatially during development [4].

Deregulated Wnt signaling has catastrophic consequences for the developing embryo and it is now well appreciated that defective Wnt signaling is a causative factor for a number of pleiotropic human pathologies. Most notably, these pathologies include cancers of the breast, colon and skin, skeletal defects and human birth defect disorders [5].

Wnt proteins are diverse family of lipid-modified signal secreted glycoprotein .The type of lipid modification that occurs on these proteins is palmitoylation of cystein[6]. Palmitoylation is necessary because it initiates targeting of the Wnt protein to the plasma membrane for secretion and it allows the Wnt protein to bind its receptor due to the covalent attachment of fatty acids. Wnt proteins also undergo glycosylation, which attaches a carbohydrate in order to insure proper secretion [7]. In Wnt signaling, these proteins act as ligands to activate the different Wnt pathways via paracrine and autocrine routes that bind to the N-terminal extra-cellular cysteine-rich

domain of the Frizzled (Fz) receptor family of which there is ten Fz in humans. The Fz protein is a seven-trans-membrane-span protein with topological homology to G-protein coupled receptors [8].

In addition, to the interaction between Wnt and Fz, co-receptors are also required for mediating Wnt signaling. For example the low-density-lipoprotein-related protein5/6 (LRP5/6) is required to mediate the canonical Wnt signal [9].

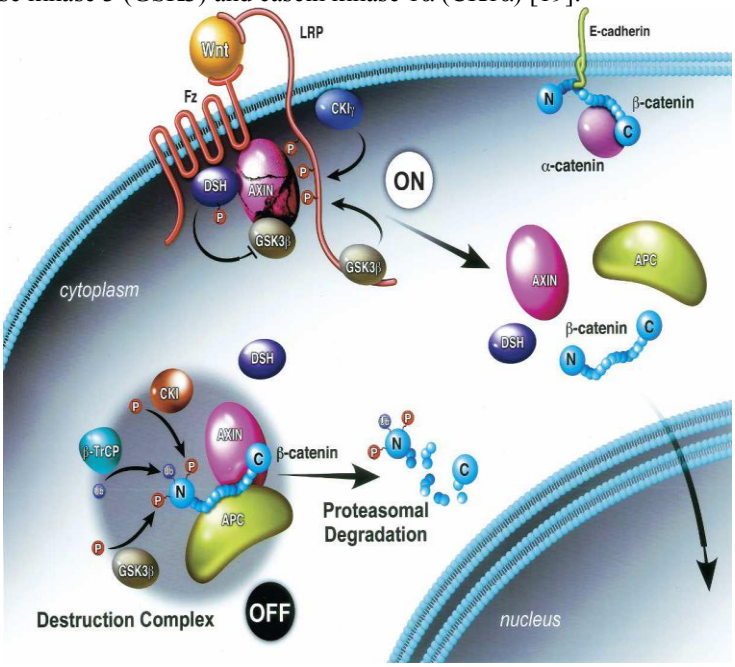
After binding of Wnt to the receptor complex, the signal is transduced to cytoplasmic phosphoprotein Dishevelled (Dsh/Dvl), and studies have uncovered that Dsh can directly interact with Fz. At the level of Dsh, the Wnt signal branches into at least three major cascades, canonical, Planar Cell Polarity and Wnt/Ca2+[10]. Dsh is an important downstream component of this transduction pathway and is the first cytoplasmic protein that is pivotally involved in all three major branches of Wnt signaling. We will focus in this review on understanding of the molecular components of these major signaling branches, the various levels of regulation of signaling, and the certain biological outcomes that are achieved.

The Wnt ligands

Wnt ligands are secreted glycoproteins that are heavily modified prior to transport and release into the extra-cellular milieu. Studies have revealed that the Wnt proteins are glycosylated in the endoplasmic reticulum and also are palmitolated [11].The *porcupine* protein has been shown to play an important role in the palmitolation of the Wnt proteins [12]. Wnt proteins in the extra-cellular matrix may be bound to and stabilized by heparan sulfate proteoglycans including Dally and glypican3 [13]. In addition to activated protein, there are a number of secreted proteins that bind to Wnts and prevent their interaction with either Fz or LRP5/6 to antagonize Wnt signaling including Dickkopf (Dkk) proteins, Wnt-inhibitor protein (WIF), soluble Frizzled-related proteins (SFRP) [14], Cerebrus, Frzb and the context dependent Wnt inhibitor [15]. Each of these secreted inhibitors are tightly regulated during embryogenesis and serve to limit or likely create a gradient of Wnt signaling for pattern formation [16]. An interesting recent finding is the identification of Sclerostin protein that in humans is encoded by the *SOST* gene can also bind to LRP5/6 where it can antagonize Wnt signaling [17].

The Canonical Wnt Pathway

The hallmark of the canonical Wnt pathway is the accumulation and translocation of the adherent junction associated-protein β-catenin into the nucleus [Fig-1] [18]. Without Wnt signaling, cytoplasmic β-catenin is degraded by a β-catenin destruction complex, which includes Axin, adenomatosis polyposis coli (APC), protein phosphatase 2A (PP2A), glycogen synthase kinase 3 (GSK3) and casein kinase 1α (CK1α) [19].



[Fig -1] The Canonical Wnt signal transduction Pathway (Willert and Jones (2006).

Phosphorylation of β-catenin within this complex by Casein Kinase and GSK3 targets it for ubiquitination and subsequent proteolytic destruction by the proteosome. Binding of Wnt to its receptor complex composed of the Fz and the LRP5/6 triggers a series of events that disrupts the APC/Axin/GSK3 complex that is required for the targeted destruction of β-catenin. The binding of Wnt to the Fz/ LRP5/6 complex induces the membrane translocation of a key negative regulator of signaling Axin, which binds to a conserved sequence in the cytoplasmic tail of LRP5/6[20]. Upon membrane translocation of Axin, its binding to LRP5/6 is catalyzed by the phosphorylation of LRP5/6, mediated by either CK1γ or GSK3.

An important point to realize is that CK1 and GSK3 appears to play distinct roles at two levels of canonical signaling; at the level of LRP5/6 their influence is positive, whereas at the level of β-catenin is negative. The binding of Axin has been proposed to remove the negative activity of Axin on canonical Wnt signaling somehow leading to the activation of the phosphoprotein Dsh [21]. Dsh is phosphorylated by a number of kinases including Casein Kinase 1, Casein Kinase 2, Metastasis Associated Kinase and Protein Kinase C [22].

It is likely that this phosphorylation event regulates both the subcellular localization of Dsh and its ability to interact with effectors for the many branches of Wnt signaling. Dsh itself is a modular protein that contains three distinct domains, a DIX, a PDZ and a DEP domain and for canonical signaling, the DIX and PDZ domain appear to be central for mediating signaling [23] .Once Dsh is activated, it inhibits the activity of the GSK3 enzyme, and activates a complex series of events that lead to the prevention of degradation of β-catenin and its consequent stabilization and accumulation in the cytoplasm [24].

For export of β-catenin, studies have identified two mechanisms for this process, first an involvement of the Ran-binding protein3 (RanBP3) along with the APC protein and another that is Ran-independent directly engaging proteins within the nuclear pore complex [25]. In the nucleus, β-catenin exerts its effect on gene transcription by functioning as a transcriptional co-activator. A large number of binding partners for β-catenin in the nucleus are involved and the best characterized are the members of the LEF/TCF DNA-binding transcription factors [26]. This complex binds to the promoter of target genes. The target genes include those required for organizer formation during embryogenesis such as *Siamois* and *Twin* and genes involved in oncogenesis such as Myc and CyclinD1 during cancer formation [27]. Other binding partners of β-catenin also include Legless and Pygopus that influence the nuclear retention and transactivating ability of β-catenin for transcriptional regulation of target genes [28].

The canonical Wnt signaling plays a pivotal role in cell fate decisions during early embryogenesis, after fertilization, the process of cortical rotation moves a dorsalizing factor to the future dorsal side of the embryo to establish the formation of the signaling center termed the Spemann-Mangold Organizer. Studies have uncovered that cortical rotation moves the Dsh protein and other components of the Wnt pathway leading to stabilization of β-catenin within the future dorsal side and is a critical event in the formation of the dorsal Spemann-Mangold Organizer.

However, a recent study demonstrated that Wnt11 was indeed the Wnt ligand responsible for this action in early dorsal axis formation [29].

Furthermore, in early development of Xenopus, the β-catenin/TCF complex promotes transcription of *Twin* and *Siamois*, which encode homeodomain transcription factors. *Twin* and *Siamois* proteins are critical for the expression of organizer-specific genes [30]. These data together cement the idea that the canonical pathway is required for dorsal axis formation during early development. Wnt signaling also has recently been shown to play important roles in stem cell renewal [27].

There seems to be that the canonical Wnt pathway regulate directly or indirectly the formation of organ system during embryogenesis. This fact give highlights importance and crucial function of the canonical Wnt pathway.

The non-canonical Wnt pathway

The second group of Wnts, which includes Xwnt4, Xwnt5a and Xwnt11, activate the non-canonical Wnt signaling pathway which often referred to as the β-catenin dependent pathway that control morphogenetic cell movements [31]. It was shown in zebrafish that mutations in *Wnt11/silberbrick* and *Wnt5a/pipetail* inhibit normal gastrulation movements [32]. The non-canonical Wnt pathway branched into two cascades. One is the Wnt/JNK pathway, which involves c-Jun N-terminal kinase [33]. The other is the Wnt/Ca2+ pathway [31].

Planar Cell Polarity pathway

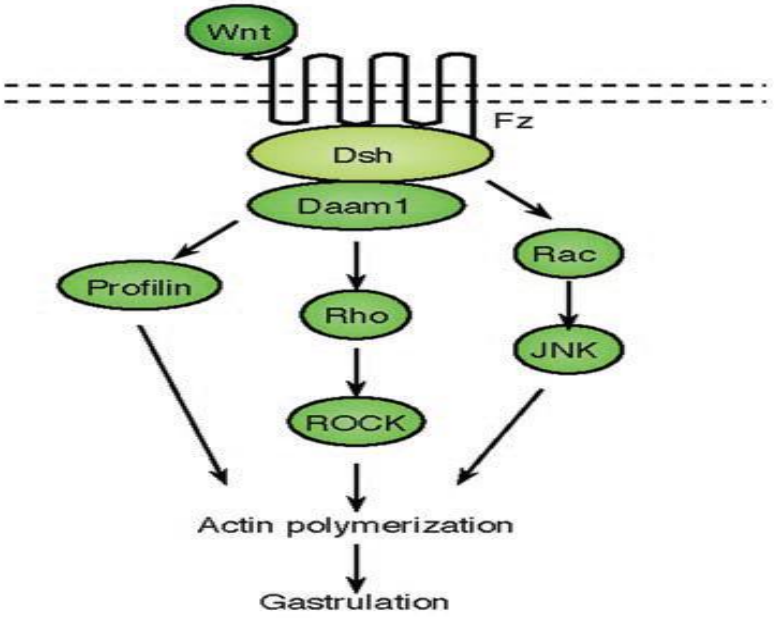
In *Drosophila*, the Wnt/JNK pathway is called the planer cell polarity (PCP) pathway, and it specifies cell polarities in epithelial cells and other types of cells [34].The PCP pathway emerged from genetic studies in

Drosophila in which mutations in Wnt signaling components including Frizzled and Dishevelled were found to randomize the orientation of epithelial structures including cuticle hairs and sensory bristles [35]. Cells in the epithelia are known to possess a defined apical-baso-lateral polarity but in addition they are also polarized along the plane of the epithelial layer. This rigid organization governs the orientation of structures including orientation of hair follicles, sensory bristles and hexagonal array of the ommatidia in the eye [36]. In vertebrates, this organization has been shown to underlie the organization and orientation of stereocilia in the sensory epithelium of the inner ear, the organization of hair follicles, and the morphology and migratory behavior of dorsal mesodermal cells undergoing gastrulation. The defining feature of this pathway is its regulation of the actin cytoskeleton for such polarized organization of structures and directed migration.

Moreover this pathway appears to function independently of transcription. During vertebrate gastrulation, mesodermal and ectodermal cells undergo convergent extension. In this processes, polarized cells intercalate along the mediolateral axis, resulting in mediolateral narrowing (convergent) and anteroposterior elongation (extension) [37].The non-canonical Wnt pathway was shown to regulate both cell polarity and movements of dorsal mesodermal cells during convergent extension and later during neural tube closure [38]. The Wnt4, Wnt5a and Wnt11 ligands have been established to signal via the non-canonical pathway, though recently Wnt11 has been shown to play a crucial role in early axis formation via the canonical pathway [29]. Over-expression of these Wnts disrupted convergent extension in both Xenopus and zebrafish, without dramatically affecting cell fate determination regulated by the canonical pathway.

When the signal transducer from the Fz co-receptors to Dsh, leading to its activation. The PDZ and DEP domains of Dsh are both utilized to activate two parallel pathways that activate the small GTPases Rho and Rac [Fig-2] [6]. For activation of the Rho branch of signaling, Wnt signaling induces a Dsh-Daam1 (Dishevelled associated activator of morphogenesis 1) complex that leads to the activation of Daam1 and consequently activation of the Rho GTPase [39]. Activation of Rho GTPase leads to the activation of the Rho-associated kinase (ROCK) and myosin [40], which leads to modification of the actin cytoskeleton and cytoskeletal rearrangement.

The second branch of signaling requires the DEP domain of Dsh and activates the Rac GTPase. This activation is independent of Daam1 and activated Rac in turn stimulates JNK activity [41].



(Figure .2) - Schematic representation of the Planar Cell Polarity transduction cascade. **(Komiya & Habas (2008).**

The Non-canonical Wnt/Ca2+ Pathway

It is the second branch of the non-canonical Wnt signaling pathway, this pathway share a number of components of the Planar Cell Polarity pathway. This pathway further modulates canonical signaling for dorsal axis formation and Planar Cell Polarity signaling for gastrulation cell movements. It was finding that some Wnts and Fz receptors can stimulate intracellular Ca2+ release from ER and this pathway is dependent on G-proteins [42].Wnt5a, Wnt11 and

rat Fz2 (RFz-2) are capable of intracellular Ca2+ release, without affecting β-catenin stabilization.91 The calcium release and intracellular accumulation activates several Ca2+ sensitive proteins, including protein kinase C (PKC) and calcium/calmodulin-dependent kinase II (CamKII) [43]. CamK11 have been shown to activate the transcription factor NFAT to promote ventral cell fates in the Xenopus embryo [44]. Ca2+ can also activate PKC which regulates the process of tissue separation during gastrulation via activation of the small GTPase [45] The role of the Wnt/Ca2+ pathway during embryogenesis is diverse and includes the negative regulation of dorsal axis formation, promotion of ventral cell fate, regulation of tissue separation and convergent extension movements during gastrulation, and later in heart formation. The Wnt5a and Wnt11 ligands can induce Ca2+ release and activate PKC and CamK11. The Dsh protein via the PDZ and DEP domains can also induce Ca2+ release and activation of PKC and CamK11 in order to regulate heart formation suggesting that it is a crucial component of the Wnt/Ca2+ pathway [46].

Conclusion

Recent evidence cemented the fact that Wnt signaling plays a critical role in pattern formation during embryogenesis. Many studies have identified numerous signaling components that have helped to build a molecular framework for the many branches of the Wnt signal transduction pathway. Furthermore, from these large studies it is still not understanding of many of the biochemical aspects within this signaling framework. Recent studies have demonstrated a strong correlation and at times causative relationships between deregulated Wnt signaling and human diseases. Thus the investigation of Wnt signaling remains an important goal for dually understanding both the basic mechanism of embryonic development and human diseases. Activation of Wnt/beta-catenin signaling has been found to be important for both initiation and progression of cancers of different tissues. Therefore, targeted inhibition of Wnt/beta-catenin signaling is a rational and promising new approach for the therapy of cancers of various origins

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