Trends in Developmental Biology
Vol. 9, 2016

Review

Emerging roles of lipids in Wnt signaling during development

Hyeyoon Lee, Tabinda Sidrat and Jin-Kwan Han*

Department of Life Sciences, Pohang University of Science and Technology, San31, Hyoja Dong, Pohang, Kyungbuk, 790-784, Republic of Korea.

ABSTRACT

Signaling by Wnt glycoprotein regulates various developmental processes. Recent studies revealed that several lipids and their elaborate regulation have crucial functions in Wnt signal activation during development. Lipids and lipid-related proteins are involved in (1) modification of Wnt glycoprotein ligands for its secretion and activation, (2) selective activation of Wnt signaling branches, (3) endocytosis of Wnt protein complexes for signal activation, and (4) formation and activation of Wnt signalosome. Dysregulation of these processes causes defects in embryogenesis and in numerous developmental processes. Here we review recent efforts to elucidate the functions of lipid in Wnt signaling during development, and highlight remaining questions that need to be addressed in the future studies.

KEYWORDS: Wnt signaling pathway, lipids, embryogenesis, development

1. Overview of Wnt signaling

Signaling by the Wnt family of glycoproteins is important throughout development [1]. Wnt genes encode secreted glycolipoproteins (38-43 kDa) that act as ligands in a wide range of species including zebrafish, *Drosophila*, *Xenopus*, mouse and humans [1-3]. Wnt ligand binds to a seven-transmembrane-domain receptor Frizzled (Fz) and mediates subsequent activation of multiple intracellular signaling cascades which in turn regulate cell fate, differentiation, proliferation, apoptosis, survival, migration and polarity in the developing and adult organisms [1, 4]. Mutations or dysregulated expression of Wnt signaling components are generally linked to birth defects, cancer and other critical diseases [2, 3, 5]. Wnt has two major signaling branches, namely β-catenin-dependent 'canonical' and β-catenin-independent 'non-canonical' pathways, which differ in their ability to mediate β-catenin-dependent transcriptional activation of target genes [1].

The β-catenin-dependent 'canonical' Wnt pathway

In a classical point of view, for the canonical Wnt signaling mechanisms, stabilization of cytosolic β-catenin by regulating its phosphorylation and ubiquitin-mediated degradation is the core of the pathway activation (Figure 1). Without Wnt stimulation, cytosolic β-catenin protein undergoes sequential phosphorylation *via* the β-catenin-destruction-complex machinery that include Axin, glycogen synthase kinase 3 (GSK3), casein kinase 1 (CK 1) and adenomatous polyposis coli (APC), and then the phosphorylated β-catenin can be recognized by E3 ubiquitin ligase β-Trcp for its subsequent ubiquitination and proteasome-dependent degradation. As a result, the level of β-catenin is kept low in the cytoplasm [5, 6] (Figure 1). Upon stimulation of Wnt ligands, binding of Wnt ligand with Fz receptor and the low-density lipoprotein receptor-related protein family (LRP) 6 co-receptor transduces the signal to downstream effector Dishevelled (Dvl). Dvl is a multifunctional scaffolding protein and a mediator of Wnt signaling in cytoplasm, and is recruited with Axin to the Fz-Wnt-LRP6 complex (the so-called 'receptor

*Corresponding author: jkh@postech.ac.kr

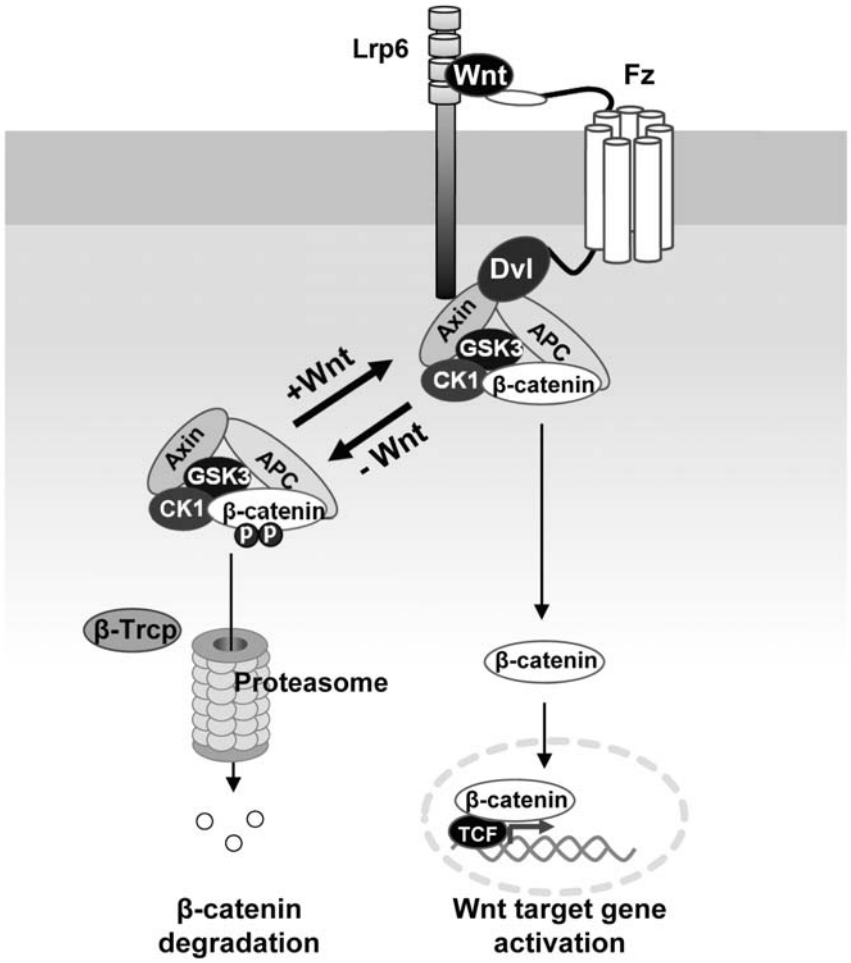

Figure 1. The canonical Wnt signaling pathway. Without Wnt signal stimulation, β-catenin binds with the destruction complex and is phosphorylated for subsequent proteasomal degradation. Wnt ligand binding to the Fz receptor and LRP6 co-receptor induces Dvl-mediated sequestration of the destruction complex to the receptor complex, thereby β-catenin is accumulated and translocalized to the nucleus for the TCF-mediated gene transcription.

complex') at the membrane [7]. With the aid of Dvl, the receptor complex is aggregated and multimerized to form a 'signalosome' [7, 8]. Formation of signalosomes leads to phosphorylation and endosomal vesicular trafficking of LRP6 that are necessary for initiation and amplification of Wnt signaling [8, 9] (Figure 1). These events cause disassembly of the β-catenin destruction complex, which release β-catenin from targeted phosphorylation, ubiquitination and degradation. Subsequently, β-catenin can accumulate in the nucleus and gain access to the transcription factors T cell factor (TCF) and lymphoid enhancer factor (LEF) to mediate transcription of the Wnt target gene [5, 10, 11] (Figure 1).

The β-catenin-independent 'non-canonical' Wnt pathway

The β-catenin-independent 'non-canonical' pathway can be further categorized into two discrete branches: the planar cell polarity (PCP) and the Wnt/Ca^{2+} pathways [12] (Figure 2). Upon Wnt ligand binding of the Fz receptor, this ligand-receptor association transduces signals to the downstream mediator Dvl, which in turn activates several small GTPases and kinases such as Rho, Rac, Cdc42 and C-Jun-N-terminal kinase (JNK), all of which have been implicated in vertebrate PCP signaling [13, 14]. Wnt/PCP pathway favors the receptor tyrosine kinase-like orphan receptor 2

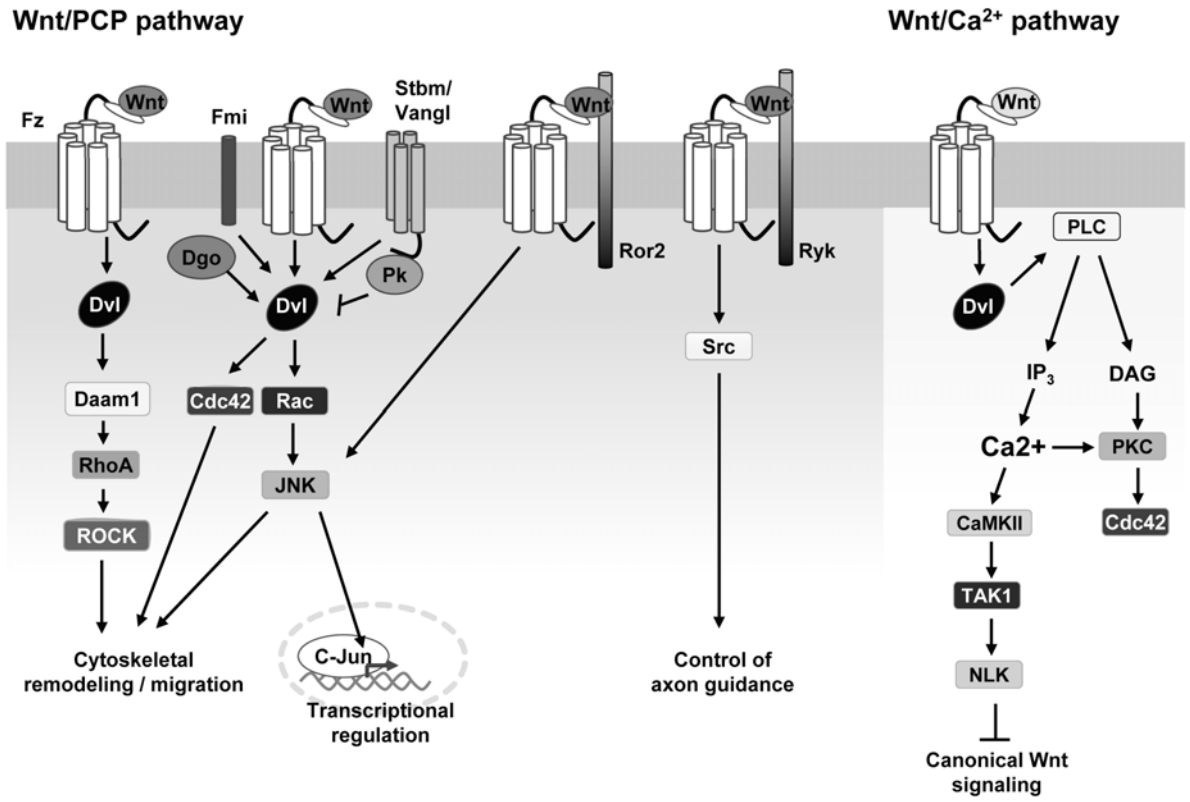

Figure 2. The non-canonical Wnt signaling pathway. The non-canonical Wnt signaling is branched off to the Wnt/PCP pathway and Wnt/Ca^{2+} pathway. Detailed mechanisms of each pathway are described in the text.

(Ror2) or receptor-like tyrosine kinase (Ryk) as coreceptor [15]; this is different from the canonical Wnt pathway. Activated Dvl can relay the signal through either RhoA or Rac. For the RhoA pathway, Dvl transduces the PCP signal along with Dishevelled-associated activator of morphogenesis 1 (Daam1) to activate RhoA-associated kinase (ROCK). The Rac pathway does not require Daam1 but phosphorylates JNK [15-18] (Figure 2). Other Wnt/PCP signaling components such as Strabismus (STBM, also known as VANG), Flamingo (FMI, also known as STAN), Diego (DGO) and Prickle (PK) have been shown to be involved in modulating the PCP of epithelial cells [17, 19, 20] (Figure 2).

In the non-canonical Wnt/Ca^{2+} signaling pathway, Wnt ligand interaction with Fz triggers the release of intracellular calcium from the endoplasmic reticulum by regulating a lipid enzyme phospholipase C (PLC) to activate several calcium sensitive proteins such as PKC (protein kinase C) and calcium/calmodulin-dependent kinase II (CamKII) [21-24]. Diacylglycerol (DAG), the second messenger generated by PLC leads to the activation of PKC and CDC42 [23, 25]. In parallel, the other second messenger Inositol trisphosphate 3 (IP3) from PLC mediates CamKII activation, which can in turn activate TGFβ-activated kinase (TAK1) and Nemo-like kinase (NLK) to inhibit the canonical Wnt transcription [12] (Figure 2).

2. Roles of lipids in Wnt signaling

Although Wnt ligand and its signaling pathway have been well identified and studied, most efforts have focused on protein components and their molecular regulatory mechanisms. Study of the precise functions of lipids in the signaling is a recently emerging field, and many questions on the details of lipid-mediated regulation of the signaling still remain. In this review, we will discuss four emerging functions that lipids and lipid-related proteins are involved in, namely

modification of Wnt glycoprotein ligands for its secretion and activation (1), selective activation of Wnt signaling branches (2), endocytosis of Wnt protein complexes for signal activation (3), and the formation and activation of Wnt signalosome during embryogenesis and developmental processes *via* the involvement of the membrane lipid PIP2 (4).

Wnt ligand lipidation

To gain insight into the regulatory mechanism of the long-range function of Wnt signaling pathway during development, the steps involved in processing, sorting and secretion of Wnt protein must be understood, because they have enormous effects on the modulation of its signaling activities [26]. Several crucial genes related to Wnt ligand lipidation and secretion are reported in figure 3.

Porcupine (*porcp*) is a segment polarity gene that belongs to the membrane-bound O-acyl transferase (MBOT) family that serves as a key enzyme involved in lipid remodeling of Wnt protein, and is responsible for catalyzing the palmitate moiety on conserved cysteine and serine residues of Wnt protein to enable Wnt secretion and trafficking [26, 27] (Figure 3). Porcupine gene is conserved in all species and is exclusively required for endoplasmic reticulum (ER)-endowed acylation of majority of Wnt ligands [28]. Porcupine-mediated acylation activity delivers the protein into a specialized microdomain structure of plasma membrane and sorts the protein into secretory vesicles where it is recognized by secretory machinery composed of multi-pass transmembrane protein Wntless (*Wls*) (Figure 3). Thus secretion of Wnt and its

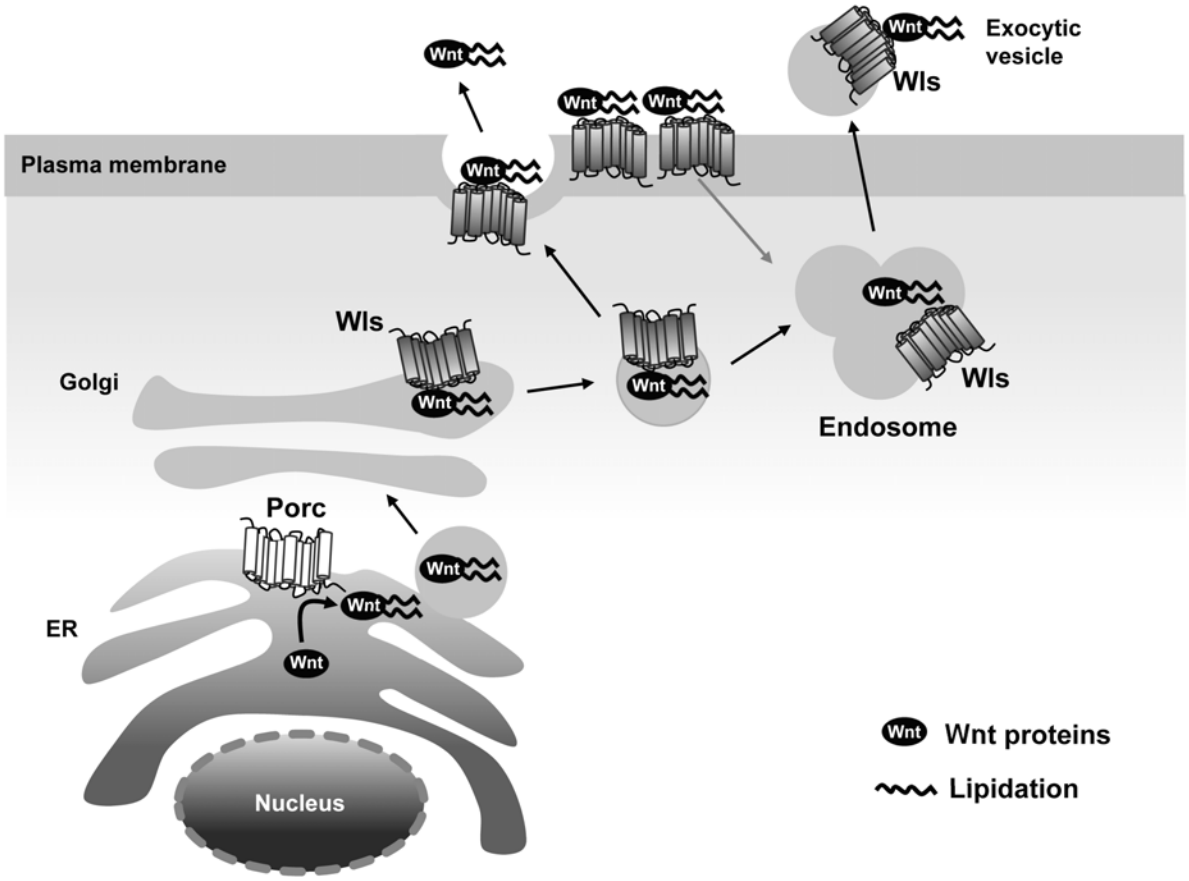

Figure 3. Wnt ligand lipidation and secretion. Newly synthesized Wnt proteins (black ovals) undergo lipidation such as palmitate modification by Porcupine (Porc) in the endoplasmic reticulum (ER). Wntless (Wls) interacts with lipidated Wnt proteins in Golgi and transfer them for secretion to the extracellular space. Related developmental processes are described in the text.

recognition by Wls depend on the porcupine-dependent lipidation status of the protein [28, 29]. Mouse embryos with mutated *porcp* fail to gastrulate due to failure of differentiation of endoderm and mesoderm; therefore such embryos remain in an epiblast-like state compared to the wild type ones [30].

A study of chick neural tube development also implicated porcupine in the formation of a Wnt gradient. The study showed that porcupine-mediated lipidation of Wnt1 and Wnt3 ligands are required for proliferation of the dorsal to ventral Wnt gradient in a chick embryo. The suggested detailed mechanism of gradient formation was that porcupine promotes Wnt hydrophobicity that influences the Wnt protein to accumulate at the site of synthesis and make it diffuse slowly to a distant site, thus establishing the gradient that guides neural tube development in vertebrates [31].

Several studies also reported that porcupine-mediated lipidation is required for activating non-canonical Wnt signaling pathway during development. Study in zebrafish embryos provided the two evidence suggesting that blockade of porcupine gene function leads to the convergent extension defects as well as genetic interaction occurs between porcupine and non-canonical Wnt ligands [32].

Another well-identified factor for efficient secretion of Wnt is a Wntless (*Wls*) also known as Evenness Interrupted (*Evi*) or Sprinter (*Srt*). Wls was first identified as a dedicated component of the Wnt signaling pathway. Wls possesses enzymatic function and comprehensively regulates secretory processes of Wnt protein [33-35]. Secretion of Wnt as a result of the influence of Wls involves multiple regulatory steps such as packaging of Wnt protein into secretory vesicles, transportation to Golgi, and finally its secretion into the extracellular milieu near the Fz/LRP6 receptor complex to activate the signaling cascade [36]. Wls regulates the secretion of Wnt indirectly by influencing retromer which is involved in recycling of Wls from cell membrane back to Golgi, and thus maintains the level of Wls protein [37]. Wls also facilitates the stacking of Wnt with lipoprotein particles that function as vehicles to transport acylated Wnt for long-range signaling activities [38]. Several reports have suggested that both Wls and retromer complexes are necessary for Wnt-producing cells to form a Wnt gradient along the anterior-posterior axis during development, as demonstrated in *Drosophila* and *Xenopus* studies in which mutation or knockdown of Vps35 led to intracellular retention of Wnt, and thereby eliminated the Wnt gradient. These malformations of the Wnt gradient induced abnormalities such as defects in the wing disc in *Drosophila*, and phenocopies of Wnt-defective embryos in *Xenopus* [37, 39].

Involvement of Wls for Wnt signaling was further highlighted in the cerebellar development of mice. Wls is required for the normal development of cerebellar rhombic lip by interplaying with cerebellar markers such as Math1 and Pax6. Conditional deletion of Wls in a mouse model caused retinal defects that had phenotypes similar to those produced by either mutation of LRP6 co-receptor or impaired Wnt-dependent retinoic acid signaling [40]; these results indicate that Wls regulates the curvature of the optic cup during early development of the vertebrate eye.

Wls is required during habenular development in the dorsal forebrain of zebrafish. This conclusion is drawn from observations that mutation in the Wls gene reduces the Wnt target gene expression that is necessary to regulate the specification and patterning of habenular development [41]. Critical function of Wls in Wnt secretion was also observed in the patterning of respiratory epithelium during embryonic lung development. Conditional deletion of Wls from respiratory epithelium disrupted Wnt signaling and produced pulmonary vasculature defects [42].

Selective activation of Wnt pathway

One of the least understood features of the signaling mechanism is how each Wnt pathway can be selectively activated upon Wnt ligand stimulation [22]. Interestingly, both canonical and non-canonical pathways share Fz receptors and Dvl for the initial signal activation [5, 7]. Dvl is a scaffold protein that serves as the branch point of the canonical Wnt signaling and non-canonical Wnt signaling pathways [7, 9] (Figures 1 and 2).

Lipid binding of Dvl is a key process that gives selectivity to each pathway [9, 43, 44] (Figure 4). The first clue to the importance of interaction between Dvl and lipid for Wnt signal activation

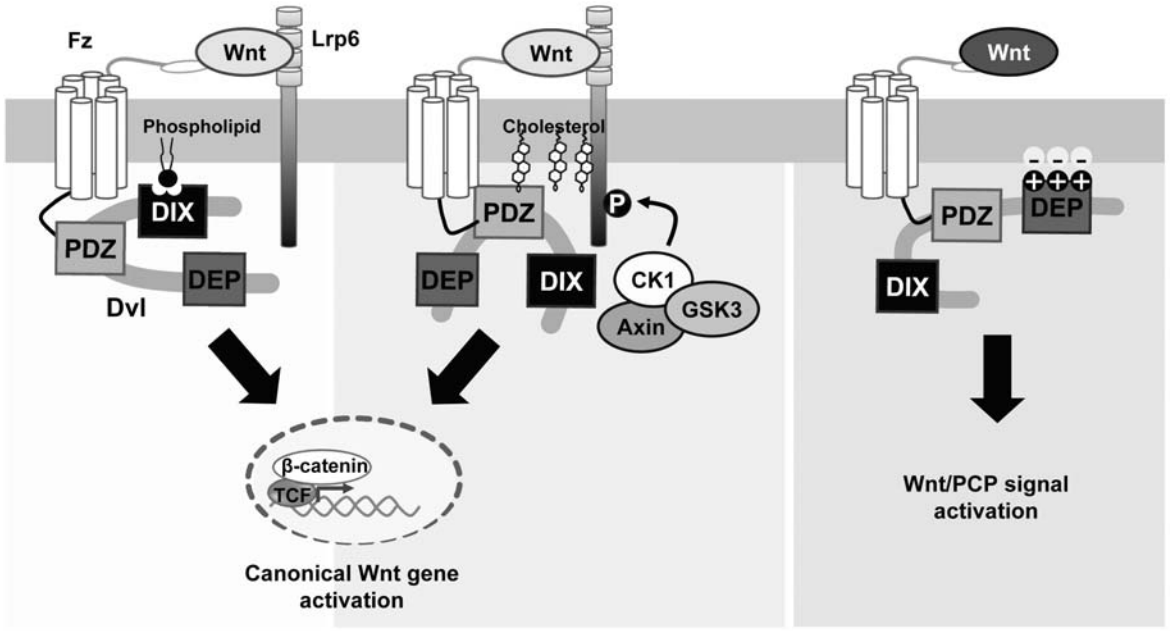

Figure 4. Selective activation of the Wnt signaling pathway. Several lipids bindings with Dishevelled (Dvl) are involved in selective activation of canonical versus Wnt/PCP signaling. Detailed mechanisms and related developmental processes are described in the text.

was the observation that the Dvl DIX domain contains an apparent phospholipid interaction loop [43]. Lys68 and Glu69 residues within the DIX domain were suggested as crucial sites for phospholipid interaction. A Dvl mutant in which these residues are replaced by Ala cannot bind to phospholipids and loses its abilities for vesicular association, phosphorylation, and mediating stabilization of β-catenin for activation of target genes in Chinese hamster ovary K1 (CHO) cells [43] (Figure 4).

The crucial function of phospholipid binding of the DIX domain for Wnt activation during development was further assessed in *Xenopus* embryos. Formation of the *Xenopus* dorsal axis requires activation of β-catenin signaling targeted genes such as *Xnr3* and *Siamois*. Several experimental assessments revealed that ectopic overexpression of Dvl could induce those genes and cause duplication of the dorsal axis. However, evidence showed that Dvl mutant that is defective in binding of phospholipids and vesicle targeting cannot induce *Xnr3* and *Siamois* expression or axis duplication. These results strongly indicate that phospholipid binding ability of Dvl and this binding-mediated vesicular localization are required for canonical Wnt signaling during formation of the vertebrate axis [43] (Figure 4).

Two recent studies presented stronger and more detailed evidence that binding of membrane lipids to Dvl can selectively activate Wnt pathways in development [9, 44]. According to the studies, membrane cholesterol can selectively activate canonical Wnt signaling [9], whereas membrane anionic lipid can selectively activate non-canonical Wnt signaling [44]. By binding to the PDZ domain of Dvl, membrane cholesterol can specifically facilitate the recruitment of Dvl to the membrane and of Dvl's partners that are required for the canonical Wnt activation to regulate Wnt-dependent developmental processes in *Xenopus* [9]. Bioinformatics algorithms and computational modeling revealed that membrane cholesterol can associate with Dvl, and that Phe227 and Met300 residues within PDZ domains are responsible for this interaction. Single-molecule tracking analysis in culture cells showed that a canonical Wnt ligand stimulation markedly increased the wild-type Dvl population that can be co-localized with Fz7 and LRP6 receptors. However, Wnt had no

effect on the co-localization of those receptors with a cholesterol-binding-deficient form of Dvl (Figure 4).

These wholly-new aspects of the regulatory mechanisms of canonical Wnt signaling were further assessed in *Xenopus*. Cholesterol binding of Dvl was specifically required in the canonical Wnt-dependent embryogenic processes but was dispensable in the non-canonical Wnt-dependent processes. In *Xenopus* embryos, precise regulation of canonical Wnt signaling is required for axis induction, early dorsal specification, paraxial mesoderm induction, anteroposterior neural pattering and midbrain-hindbrain boundary and neural crest development. Replacement of wild-type Dvl with the cholesterol-binding-deficient Dvl in *Xenopus* embryos disrupted these processes. However, this exchange did not affect convergent extension movements mediated by the non-canonical Wnt/PCP signal; this result again emphasizes that membrane cholesterol is important for canonical Wnt signaling but not for non-canonical Wnt signaling. These results may suggest a link between intracellular cholesterol levels at the membrane and the balance between canonical and non-canonical Wnt signaling activities during vertebrate development [45] (Figure 4).

A different approach suggested that electrical cues given by anionic membrane lipid can specifically activate non-canonical Wnt/PCP pathway over the canonical pathway for PCP of *Drosophila* eye development [44]. The study also suggested the importance of membrane lipid binding to Dvl, not by PDZ but by a polybasic stretch within the DEP domain of Dvl. This region binds to negatively-charged phospholipids and is essential for PCP signaling *in vivo*. A mutant form of Dvl that cannot bind to anionic lipids caused PCP defects such as chirality inversions, symmetrical cluster formations and rotation defects of ommatidia during *Drosophila* eye development [20] (Figure 4).

Collectively, these results indicate that distinct membrane lipids can promote different Wnt signaling pathways and developmental processes, but remaining questions still exist on the possible involvement of other membrane lipids and their binding partners in controlling the selectivity of Wnt signaling.

Receptor endocytosis is required for the Wnt signaling during development

After the perimembranous events of Wnt stimulation occur, receptor complexes are internalized to the cytosol for subsequent relay of the signal [4]. Two types of receptor endocytic pathways are involved in these processes: Caveolin- and clathrin-mediated endocytosis [4] (Figure 5). The known traditional function of endocytosis is that these two endocytic pathways negatively regulate signaling by desensitizing receptors to the extracellular ligands in the signal-receiving cells [4]. However, interestingly, both pathways can participate in endocytosis of the receptor complexes in the membrane lipid-raft regions, and these are required events for activating Wnt pathways *in vitro*. A lipid-raft is a membrane microdomain that is enriched in cholesterol and sphingolipid [4, 9]. The exact functions of such regions are not fully understood, but some reports hint that it may be actively involved (positively or negatively) in Wnt signaling during development.

Caveolin is located in the lipid rafts, and several lines of evidence have shown that LRP6 is also localized in this region [46]. Caveolin-mediated endocytosis and translocalization of Wnt components is important in development [47]. Endocytic protein Rab8b was identified by functional RNAi screening, and is required for stabilization of Wnt signaling activity. Rab8b transcripts were expressed in regions of the central nerve system (CNS) in the zebrafish embryo that are known as Wnt signaling centers. Depletion of Rab8b in *Xenopus* led to abnormal phenotypes including smaller heads, eyes and tails due to the reduced Wnt activity. Biochemical analyses further demonstrated that Rab8b is required for caveolin-mediated LRP6 endocytosis, suggesting the positive role of caveolar and its related proteins in the Wnt signaling [48] (Figure 5).

Other evidence suggests that caveolin-mediated vesicular transport suppresses signaling events [49, 50]. Canonical Wnt signal activation is crucial for the formation and specification of the dorsal organizer in vertebrate embryogenesis, and dorsal activity of maternal β-catenin is important for this process. During zebrafish development, transcriptional expression of caveolin-1 is suppressed by Wnt and BMP signals after the mid-blastula

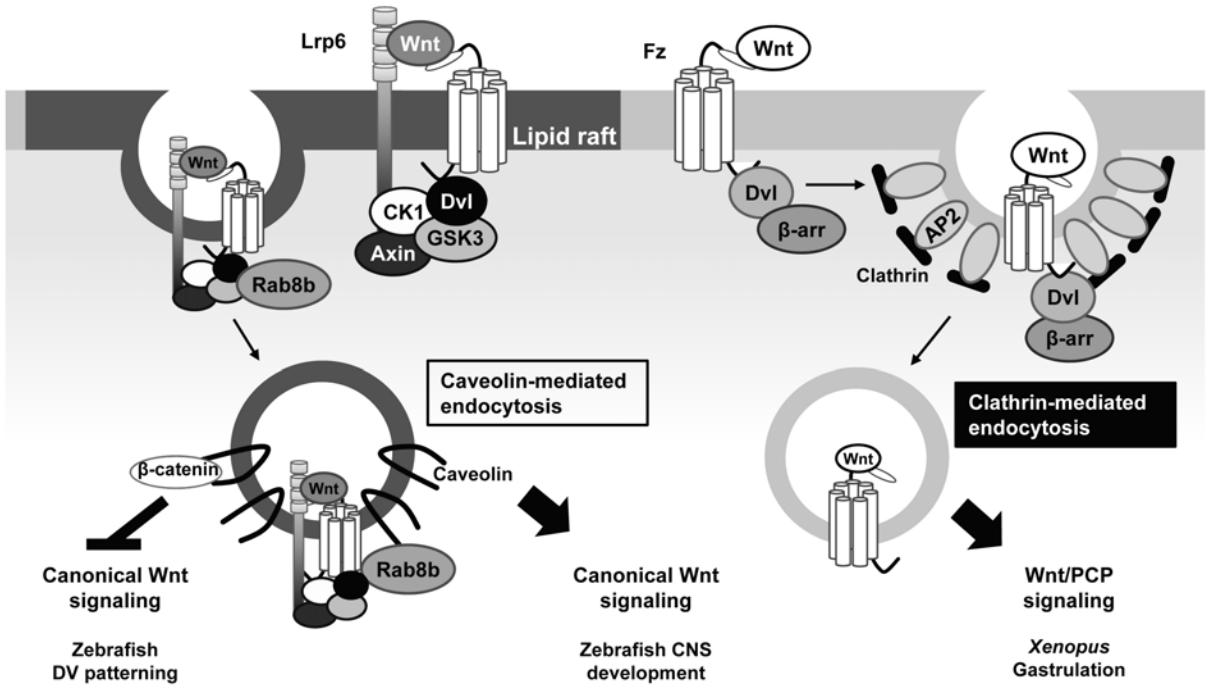

Figure 5. Receptor endocytosis in the Wnt signaling pathway. Wnt receptor complexes can be internalized with caveolin-mediated or clathrin-mediated endocytosis. Detailed mechanisms and related developmental processes are described in the text.

transition (MBT) [49]. Ectopic expressions of Cav-1 and knockdown of endogenous Cav-1-induced abnormalities during development. Biochemical analysis also revealed that Cav-1 specifically inhibits canonical Wnt signaling indirectly by interaction with β-catenin. This interaction disrupts nuclear translocalization of β-catenin, which is the process required for the canonical Wnt signal activation and for the regulation of zebrafish dorso-ventral patterning [49] (Figure 5).

Another report regarding mammalian intervertebral disc degeneration (IVD) suggested that caveolin suppresses canonical Wnt signaling [50]. Canonical Wnt signaling is known to be critical for maintenance of intervertebral disc structure during mammalian development; over-activation of this signaling leads to deformation of IVD structure [51]. Use of caveolin as a regenerative therapy to downregulate excess canonical Wnt signaling activity has been suggested [50].

The non-canonical Wnt/PCP signaling pathway seems to require endocytosis of receptors *in vitro* and *in vivo* [4]. Fz receptor endocytosis/ internalization may occur during convergent extension movements that are required for the dorsal body axis elongation to activate Wnt/PCP signaling in *Xenopus* [52]. In *Xenopus* dorsal marginal tissues where the endogenous Wnt/PCP signal is highly activated, the Fz7 receptor protein was localized as cytoplasmic puncta [52] (Figure 5). Moreover, the evidence suggested that the endocytosis of receptors required for Wnt/PCP signaling occurs *via* the clathrin endocytosis route. For convergent extension (CE) movements during gastrulation, multifunctional adaptor proteins β-arrestin1 and arrestin2 that are originally known to regulate numerous aspects of G-protein coupled receptors (GPCR) [53, 54], are also involved in the Wnt/PCP signaling pathway activation. Depletion of β-arrestin1 or 2 in *Xenopus* embryos induced defects in convergent extension movements. Classically, β-arrestins have been known for GPCR desensitization and subsequent signal blocking [55, 56], but the recent study found that the functions of β-arrestins are also required for Wnt/PCP signaling to mediate the Fz7 receptor endocytosis using clathrin machinery. Mutation in the basic amino acid residues of β-arrestin2 that are necessary for the formation of clathrin coated pits caused

CE-defective phenotypes [53]. In addition, in *Xenopus*, Dvl is also important for Wnt-mediated endocytosis of Fz receptor by virtue of its scaffolding ability for mediating Fz receptor to make a complex with β-arrestin and adaptor protein-2 (AP-2) which is known as a clathrin-binding protein [57]. Deletion of the AP2-interacting site in Dvl blocks gastrulation in *Xenopus* (Figure 5).

However, the involvement of the clathrin endocytosis route is not definitely and specifically related to the Wnt/PCP pathway, because live confocal imaging analyses of intact zebrafish embryos showed that interaction of AP2μ2 and Dvl at the membrane is highly dynamic and actually required for the Wnt-induced formation of LRP6 signalosomes [58].

Many questions remain regarding the mechanisms of each endocytotic pathway and their specific requirements. One clue is that the structural components of both endocytic pathways (e.g., caveolae and clathrin-coated pits that are formed by phospholipid layer) are involved in both Wnt pathways [59], and that lipids have a significant influence on the modulation of Wnt signaling pathway during various developmental processes.

Membrane PIP2 is required for the Wnt signaling

Phosphatidyl-inositol 4,5-bisphosphate (PIP2) is present in the inner leaflet of the cell membrane and makes up only 1% of the total repertoire of phospholipids in the membrane. This phospholipid is the best-studied lipid that is involved in canonical Wnt signaling [60]. Regulation of Wnt signaling by PIP2 is important in developmental processes and defects (Figure 6).

The effects of PIP2-synthesizing kinases and PIP2 metabolism upon Wnt stimulation highlighted the regulation of spatially-controlled PIP2 production and greatly emphasized its involvement in developmental processes that are mediated by Wnt signaling [59, 60]. The first general evidence that suggested the importance of PIP2 for Wnt signaling showed that PIP kinases PI4KII and PIP5KI are recruited by Dvl to the membrane upon Wnt3a stimulation, and that these kinases mediate LRP6 signalosome formation and its phosphorylation. In *Xenopus* embryos, MO-targeted knockdown of both PI4KII and PIP5KI kinases blocked the formation of the secondary axis by Wnt8 and β-catenin mRNA [61] (Figure 6).

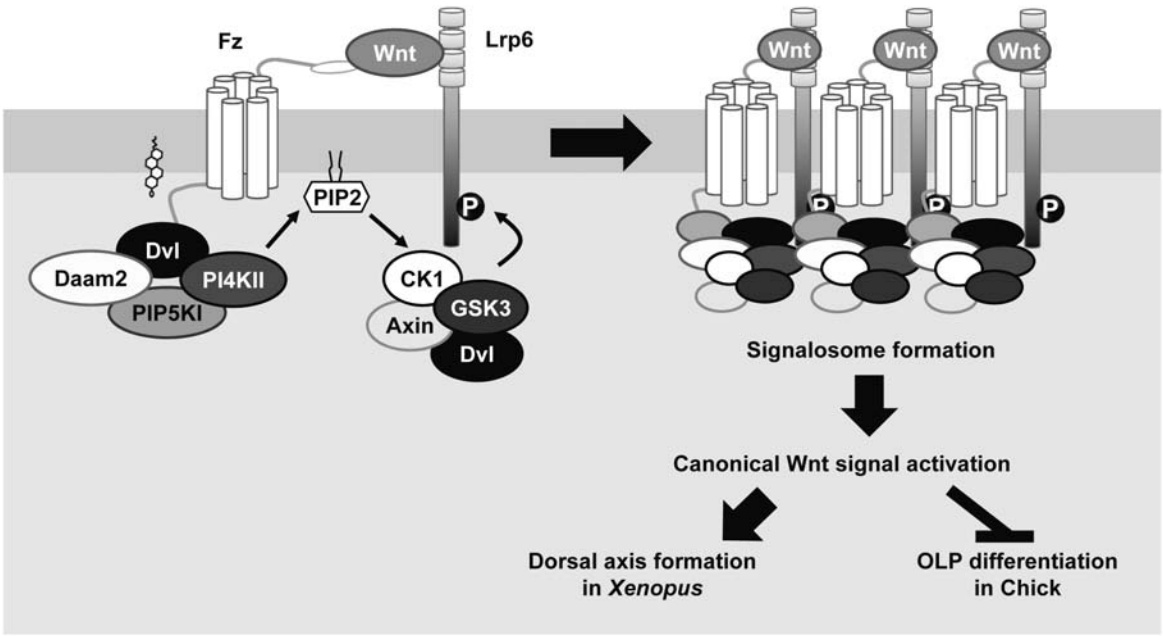

Figure 6. Membrane PIP2 lipid in the Wnt signaling pathway. Upon Wnt stimulation, PI4KII and PIP5KI that are recruited by Dvl generate membrane PIP2. PIP2 mediates LRP6 phosphorylation and signalosome formation for downstream activation of canonical Wnt signaling during various developmental processes.

The necessity of PIP2 for Wnt/β-catenin signaling during vertebrate development is emphasized by the identification of Daam2, which contributes to regulation of the canonical Wnt signaling pathway [62]. The Daam2-PIP5KI-PIP2 axis promotes formation of the signalosome complex and regulates remyelination of the CNS in the developing chick embryo. In chick spinal cord, knockdown of Daam2 caused defects in dorsal patterning and drastically decreased PIP2 production, although these effects cannot be associated directly. Moreover, defects and reduced canonical Wnt signaling that resulted from knockdown of Daam2 could be rescued by PIP5K expression; this result suggests that Daam2 regulates canonical Wnt signaling *via* a PIP5K-PIP2 cascade during dorsal patterning in the chick (Figure 6).

Further assessment for elucidating the detailed regulatory mechanisms for Daam2-PIP5K-PIP2 cascade revealed that Daam2 and PIP5K interacts directly [62]. In addition, during gliogenesis in the developing and post-natal spinal cord, Daam2 is expressed in glial lineages and suppresses OLP differentiation indirectly by disrupting the PIP5K cascade. These results suggested undiscovered mechanisms of Wnt signalosome formation in CNS and the involvement of PIP2 in this process.

3. Conclusion and Perspectives

Lipids have a great influence in the modulation of the Wnt signaling pathway during development. This influence begins with the biogenesis of Wnt, accompanies its secretion, sorting and relay, and continues until the propagation of its downstream signal *via* signalosome formation and regulation of receptor endocytosis. The lipidating character of Wnt critically affects the distribution of Wnt in the extracellular milieu and has an important function in the establishment of the anterior-posterior Wnt gradient, which is crucial for proper development of head and determination of the normal body axis in all vertebrates. Lipids can also affect selective activation of canonical and non-canonical Wnt pathways during *Xenopus* body axis and neural development, and during *Drosophila* eye development, although these observations are based on limited information that focuses on Dvl. Moreover, clathrin and caveolin-mediated endocytosis that occur in the lipid-raft microdomain of the cell membrane are involved in the Wnt signaling pathway during early vertebrate embryogenesis. Lastly, membrane lipid PIP2 is definitely involved in the Wnt signaling cascade during *Xenopus* body axis formation and chick dorsal patterning and CNS development.

However, compared with *in vitro* research that shows the detailed mechanisms of lipid-mediated Wnt signaling and the importance of several lipid-related components, evidence for *in vivo* developmental functions is relatively sparse. Also, many functions of lipids in the Wnt signaling pathway remain to be revealed. Further studies to identify how lipids are involved in Wnt signaling *in vivo* will advance our understanding of the functions of lipids during Wnt signaling in development and disease.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. NRF-2014R1A2A2A01002859).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

1. Logan, C. Y. and Nusse, R. 2004, Annu. Rev. Cell Dev. Biol., 20, 781-810.
2. Clevers, H. 2006, Cell, 127, 469-80.
3. Klaus, A. and Birchmeier, W. 2008, Nat. Rev. Cancer, 8, 387-98.
4. Kikuchi, A., Yamamoto, H. and Sato, A. 2009, Trends Cell Biol., 19, 119-29.
5. MacDonald, B. T., Tamai, K. and He, X. 2009, Dev. Cell, 17, 9-26.
6. Clevers, H. and Nusse, R. 2012, Cell, 149, 1192-205.
7. Gao, C. and Chen, Y. G. 2010, Cell. Signal., 22, 717-27.
8. Bilic, J., Huang, Y. L., Davidson, G., Zimmermann, T., Cruciat, C. M., Bienz, M. and Niehrs, C. 2007, Science, 316, 1619-22.
9. Sheng, R., Kim, H., Lee, H., Xin, Y., Chen, Y., Tian, W., Cui, Y., Choi, J. C., Doh, J., Han, J. K. and Cho, W. 2014, Nat. Commun., 5, 4393.

10. Komiya, Y. and Habas, R. 2008, Organogenesis, 4, 68-75.
11. Nusse, R. 2005, Cell Res., 15, 28-32.
12. Veeman, M. T., Axelrod, J. D. and Moon, R. T. 2003, Dev. Cell, 5, 367-77.
13. Mezzacappa, C., Komiya, Y. and Habas, R. 2012, Methods. Mol. Biol., 839, 119-31.
14. Choi, S. C. and Han, J. K. 2002, Dev. Biol., 244, 342-57.
15. Green, J., Nusse, R. and van Amerongen, R. 2014, Cold Spring Harb. Perspect. Biol., 6.
16. Marlow, F., Topczewski, J., Sepich, D. and Solnica-Krezel, L. 2002, Curr. Biol., 12, 876-84.
17. Seifert, J. R. and Mlodzik, M. 2007, Nat. Rev. Genet., 8, 126-38.
18. Kikuchi, A., Yamamoto, H. and Sato, A. 2009, Tanpakushitsu Kakusan Koso, 54, 1-10.
19. Zallen, J. A. 2007, Cell, 129, 1051-63.
20. Simons, M. and Mlodzik, M. 2008, Annu. Rev. Genet., 42, 517-40.
21. van Amerongen, R. and Nusse, R. 2009, Development, 136, 3205-14.
22. Wallingford, J. B. and Habas, R. 2005, Development, 132, 4421-36.
23. Kuhl, M., Sheldahl, L. C., Park, M., Miller, J. R. and Moon, R. T. 2000, Trends Genet., 16, 279-83.
24. Kuhl, M., Sheldahl, L. C., Malbon, C. C. and Moon, R. T. 2000, J. Biol. Chem., 275, 12701-11.
25. Sheldahl, L. C., Park, M., Malbon, C. C. and Moon, R. T. 1999, Curr. Biol., 9, 695-8.
26. Coudreuse, D. and Korswagen, H. C. 2007, Development, 134, 3-12.
27. Bartscherer, K. and Boutros, M. 2008, EMBO Rep., 9, 977-82.
28. Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R. 3rd. and Nusse, R. 2003, Nature, 423, 448-52.
29. Herr, P. and Basler, K. 2012, Dev. Biol., 361, 392-402.
30. Biechele, S., Cox, B. J. and Rossant, J. 2011, Dev. Biol., 355, 275-85.
31. Galli, L. M., Barnes, T. L., Secrest, S. S., Kadowaki, T. and Burrus, L. W. 2007, Development, 134, 3339-48.
32. Chen, Q., Takada, R. and Takada, S. 2012, J. Cell Sci., 125, 2224-34.
33. Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G. and Basler, K. 2006, Cell, 125, 509-22.
34. Bartscherer, K., Pelte, N., Ingelfinger, D. and Boutros, M. 2006, Cell, 125, 523-33.
35. Goodman, R. M., Thombre, S., Firtina, Z., Gray, D., Betts, D., Roebuck, J., Spana, E. P. and Selva, E. M. 2006, Development, 133, 4901-11.
36. Ching, W. and Nusse, R. 2006, Cell, 125, 432-3.
37. Belenkaya, T. Y., Wu, Y., Tang, X., Zhou, B., Cheng, L., Sharma, Y. V., Yan, D., Selva, E. M. and Lin, X. 2008, Dev. Cell, 14, 120-31.
38. Hausmann, G., Banziger, C. and Basler, K. 2007, Nat. Rev. Mol. Cell Biol., 8, 331-6.
39. Kim, H., Cheong, S. M., Ryu, J., Jung, H. J., Jho, E. H. and Han, J. K. 2009, Mol. Cell Biol., 29, 2118-28.
40. Carpenter, A. C., Smith, A. N., Wagner, H., Cohen-Tayar, Y., Rao, S., Wallace, V., Ashery-Padan, R. and Lang, R. A. 2015, Development, 142, 972-82.
41. Kuan, Y. S., Roberson, S., Akitake, C. M., Fortuno, L., Gamse, J., Moens, C. and Halpern, M. E. 2015, Dev. Biol., 406, 117-28.
42. Cornett, B., Snowball, J., Varisco, B. M., Lang, R., Whitsett, J. and Sinner, D. 2013, Dev. Biol., 379, 38-52.
43. Capelluto, D. G., Kutateladze, T. G., Habas, R., Finkielstein, C. V., He, X. and Overduin, M. 2002, Nature, 419, 726-9.
44. Simons, M., Gault, W. J., Gotthardt, D., Rohatgi, R., Klein, T. J., Shao, Y., Lee, H. J., Wu, A. L., Fang, Y., Satlin, L. M., Dow, J. T., Chen, J., Zheng, J., Boutros, M. and Mlodzik, M. 2009, Nat. Cell. Biol., 11, 286-94.
45. Zhu, X., Zhao, P., Liu, Y., Zhang, X., Fu, J., Ivy, Yu. H. M., Qiu, M., Chen, Y., Hsu, W. and Zhang, Z. 2013, J. Biol. Chem., 288, 12080-9.
46. Yamamoto, H., Komekado, H. and Kikuchi, A. 2006, Dev. Cell, 11, 213-23.
47. Demir, K., Kirsch, N., Beretta, C. A., Erdmann, G., Ingelfinger, D., Moro, E., Argenton, F., Carl, M., Niehrs, C. and Boutros, M. 2013, Cell Rep., 4, 1224-34.

48. Voloshanenko, O., Erdmann, G., Dubash, T. D., Augustin, I., Metzig, M., Moffa, G., Hundsrucker, C., Kerr, G., Sandmann, T., Anchang, B., Demir, K., Boehm, C., Leible, S., Ball, C. R., Glimm, H., Spang, R. and Boutros, M. 2013, Nat. Commun., 4, 2610.
49. Mo, S., Wang, L., Li, Q., Li, J., Li, Y., Thannickal, V. J. and Cui, Z. 2010, Dev. Biol., 344, 210-23.
50. Erwin, M. 2013, Arthritis. Res. Ther., 15, 113.
51. Kondo, N., Yuasa, T., Shimono, K., Tung, W., Okabe, T., Yasuhara, R., Pacifici, M., Zhang, Y., Iwamoto, M. and Enomoto-Iwamoto, M. 2011, Spine (Phila Pa 1976), 36, E513-8.
52. Kim, G. H., Her, J. H. and Han, J. K. 2008, J. Cell Biol., 182, 1073-82.
53. Kim, G. H. and Han, J. K. 2007, EMBO J., 26, 2513-26.
54. Kim, G. H., Park, E. C., Lee, H., Na, H. J., Choi, S. C. and Han, J. K. 2013, Biochem. Biophys. Res. Commun., 435, 182-7.
55. Luttrell, L. M. and Lefkowitz, R. J. 2002, J. Cell Sci., 115, 455-65.
56. Lefkowitz, R. J. and Shenoy, S. K. 2005, Science, 308, 512-7.
57. Yu, A., Rual, J. F., Tamai, K., Harada, Y., Vidal, M., He, X. and Kirchhausen, T. 2007, Dev. Cell, 12, 129-41.
58. Hagemann, A. I., Kurz, J., Kauffeld, S., Chen, Q., Reeves, P. M., Weber, S., Schindler, S., Davidson, G., Kirchhausen, T. and Scholpp, S. 2014, J. Cell Sci., 127, 3970-82.
59. Le Roy, C. and Wrana, J. L. 2005, Nat. Rev. Mol. Cell Biol., 6, 112-26.
60. McLaughlin, S., Wang, J., Gambhir, A. and Murray, D. 2002, Annu. Rev. Biophys. Biomol. Struct., 31, 151-75.
61. Pan, W., Choi, S. C., Wang, H., Qin, Y., Volpicelli-Daley, L., Swan, L., Lucast, L., Khoo, C., Zhang, X., Li, L., Abrams, C. S., Sokol, S. Y. and Wu, D. 2008, Science, 321, 1350-3.
62. Lee, H. K., Chaboub, L. S., Zhu, W., Zollinger, D., Rasband, M. N., Fancy, S. P. and Deneen, B. 2015, Neuron, 85, 1227-43.