

Plant Wnt: deciphering a novel signalling pathway in plants

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Wnt proteins represents highly conserved family of signalling biomolecules regulating vital developmental processes including embryo axis patterning, regeneration potential, cell fate specificity and determination in animals. Existence of Wnt protein mediated signalling in plants is still unexplored. Homologs of proteins like glycogen-synthase-kinase-3, casein-kinase-1 and Adenomatous-polyposis coli which together act as a negative regulator of metazoan Wnt signalling, have been traced in plants. Interestingly, genome-wide survey of cotton could reveal the presence of Wnt protein and its receptor, Frizzled up in *Gossypium arboreum*. Moreover, publicly available annotated sequence data have unravelled the existence of certain other proteins like Dishevelled homologs of beta-catenin in plants. These proteins orchestrate the initiation of Wnt signalling pathway in animals. Manifestation of homologs of such Wnt signalling factors in plants, indicates a possible mimicry of similar signalling pathways. We have attempted to hypothesize a novel signalling network involving Wnt protein and other associated proteins in plants, considering animal systems as a model.

Keywords: *Gossypium*, plant Wnt, Wnt signalling.

Wnt genes encode a class of protein family that represents highly conserved signalling molecules involved in synchronizing multitude of cell biological and developmental processes. *Wnt* gene was first reported as *int-1* gene by Nusse and Varmus¹ in mouse genome while working with an oncogenic retrovirus known as mouse mammary tumour virus. Later, a homolog of *int-1* was identified in *Drosophila* as *Wingless* gene (*wg*), both of which encoded for similar glycoproteins² and hence the gene was named as '*Wnt*' (*Wg* + *int-1*)³. Several *Wnt* genes have since been isolated from different metazoan species and are known to be associated with scores of biological functions including embryogenesis, cell migration, cell fate specification and cell proliferation^{4–8}. Wnt signalling also plays an active role in embryonic development and body axis patterning in animals^{9,10}. Though existence of active Wnt or Wnt-like signalling in plants is yet unknown, whole genome sequence analyses and evolutionary studies have traced the presence of related metazoan Wnt sig-

nalling transcriptional effectors in plants. For instance, Armadillo repeat proteins (ARM), similar to β -catenin, the principal transcriptional modulator of canonical Wnt signalling in metazoa, are also found conserved in plants like *Arabidopsis*¹¹, rice¹², cotton¹³ and *Physcomitrella patens*¹⁴ playing significant roles in diverse developmental and stress signalling pathways.

Interestingly, the annotated genome of *Gossypium arboreum* has revealed some key mediators of metazoan Wnt signalling like Wnt-3a (NCBI GenBank: KHF99765.1, putatively uncharacterized), Frizzled (NCBI GenBank: KHG22418.1, putatively uncharacterized) and Dishevelled (NCBI GenBank: KHG01632.1 putatively uncharacterized). Presence of such proteins in plant systems, which are similar to those that mediate Wnt signalling in animals, raises several potential questions regarding the existence of a novel signalling system that may mimic metazoan Wnt signalling. Here, we review the possibility of Wnt-like signalling pathways in plant systems that may regulate similar developmental processes as characterized in animal systems.

Wnt proteins

Nineteen *Wnt*-family genes which have been identified in the animal system are found to be highly conserved across several multicellular animals of metazoan kingdom. These genes share considerable amount of sequence similarities, each of which plays a crucial role in several developmental programmes in animals^{15,16}. Wnt proteins are structurally made up of 350–400 amino acids and possess 23–24 cysteine residues that form disulphide bridges shaping the globular secondary structure of the protein^{16,17}. Wnts, which are basically synthesized as glycoproteins, encounter several post-translational modifications preceding its transport and release into the extra-cellular environment. Two of the most important post-translational modifications of Wnt proteins include acylation and glycosylation which may lead to proper folding and functioning of the protein¹⁸. Because of lipid modifications, Wnts tend to linger firmly with the extracellular matrix or cell membrane, thus making it difficult to purify¹⁹. Wnt proteins get palmitoylated at a conserved cysteine residue which makes this protein insoluble in nature. However, palmitoylation has been

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consistently found to be present in all Wnt proteins which may be essential for their signalling functions^{5,20}.

Wnt signalling

Wnt proteins functionally act as ligands to carry out a myriad of cellular signalling activities. Three distinct Wnt signalling pathways have been identified including canonical and non-canonical pathways, of which the latter can again be bifurcated into two sub-branches namely planar cell polarity pathway (PCP pathway) and the Wnt/Ca²⁺ pathway⁶. All the three Wnt signalling pathways are triggered when Wnt ligand interacts with its receptor protein, termed as Frizzled (Fz)²¹. Frizzled proteins are primary receptors for Wnt ligands and characterized as seven pass transmembrane receptors belonging to G-Protein Coupled Receptor family^{22,23}. An extracellular cysteine-rich domain (CRD) present at the amino terminal part of Fz receptor enables its interaction with Wnts to carry out downstream intracellular signalling²⁴⁻²⁶.

Canonical Wnt signalling

Canonical pathway, also known as β -catenin dependent pathway, is initiated once the Wnt ligand binds to Fz receptor along with another co-receptor named low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6, that gives rise to a tertiary complex^{22,24,27}. This event leads to the participation of another cytoplasmic protein known as Dishevelled (Dsh) which interacts with the activated Fz in the cell membrane and gets phosphorylated²⁸⁻³¹. Subsequently, LRP 5/6 also gets phosphorylated and interacts with another component of Wnt signalling called axin^{32,33}. This molecular cascade leads to the inhibition of a kinase protein named glycogen synthase kinase 3 (GSK3) and restricts the formation of a 'destruction complex' comprising three molecules, namely, adenomatous polyposis coli (APC), axin and casein kinase I α (CKI α), which leads to the degradation of β -catenin (an Armadillo repeat containing protein)^{34,35}. In presence of Wnt protein, β -catenin gets accumulated in the cytoplasm and then proceeds towards the nucleus, where it interacts with transcription factors like T-cell factor (TCF) and lymphoid enhancer-binding factor (LEF), which instruct the transcription of target genes^{36,37}. On the other hand, absence of Wnt ligand results in the formation of ' β -catenin destruction complex' mediated by axin that leads to the degradation of β -catenin followed by the repression of the target genes down the line^{35,38}. Canonical pathway is well understood and characterized as compared to the other Wnt signalling pathways and is found to be associated with the regulation of cell differentiation and proliferation^{39,40}.

Non-canonical Wnt signalling

Both PCP pathway and Wnt/Ca²⁺ pathway of non-canonical pathway of Wnt signalling are mediated by interaction between Wnt ligand and Fz receptor like the canonical version but without the aid of LRP5/6 co-receptor^{6,33}. However, unlike canonical Wnt signalling cascade, the non-canonical pathway does not bank on β -catenin to carry out downstream signalling, hence is known as β -catenin independent Wnt signalling. PCP signalling is involved mainly in the regulation of cell polarity during morphogenesis by activating JNK-dependent transcription factors via small GTPases RAC1 and RhoA along with JUN-N-terminal kinase (JNK) and activator protein-1⁴¹⁻⁴³. PCP and β -catenin-dependent signalling pathways may act antagonistic to each other depending upon the ligand-receptor interaction, suggesting a crosstalk between two types of Wnt signalling where inhibition of one can trigger the upregulation of the other and vice versa^{8,40,44}. Notably, *Wnt3a* and *Wnt5a* are known to promote β -catenin dependent and Wnt/PCP signalling respectively by competitively binding to their common Fz receptor^{40,45}. This also reveals the importance of different forms of Wnt proteins as well as Fz and other Wnt co-receptors for regulation of both canonical and non-canonical Wnt signalling.

In case of Wnt/calcium pathway, Wnt binds to Fz and recruits Dsh, which in turn activates calcium-binding proteins, including protein kinase C (PKC) and calmodulin-dependent kinase II (CamKII). This results in the release of calcium from the endoplasmic reticulum followed by activation of transcription factor NFAT (nuclear factor of activated T cells) regulating the downstream target gene expression^{46,47} (Figure 1). NFAT has been found to compete with β -catenin to interact with Dsh and thus, negatively regulates β -catenin dependent canonical Wnt signalling⁴⁸. Several other components of Wnt/calcium pathway like CamkII and PKC, have also been reported to be involved in inhibiting canonical Wnt signalling possibly by targeting β -catenin^{40,49-51}, thus indicating a crosstalk between canonical and Wnt/calcium noncanonical signalling.

ARMs and Wnts in plants

Armadillo repeat protein (ARP), the homolog of metazoan β -catenin, was first identified in *Drosophila*, featuring as a component of signal transduction through Wingless/Wnt⁵²⁻⁵⁴. These proteins are conserved across eukaryotes containing a repeat of 42 amino acid residues (Armadillo motifs) with three alpha helices that form hairpin structures as observed in the core region of β -catenin^{55,56}. In animals, ARPs function in intracellular signal transduction and cytoskeleton regulation. However, ARPs which have also been identified in plants, are

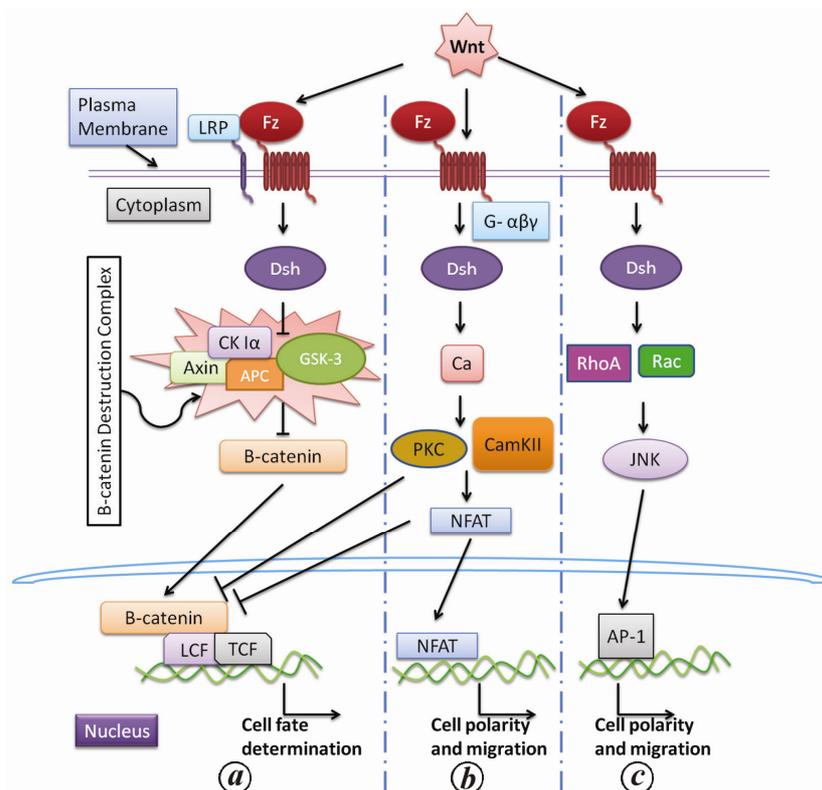


Figure 1. A diagrammatic representation of three different forms of activated Wnt signaling. *a*, Canonical/B-catenin dependent pathway; *b*, Wnt-calcium pathway; *c*, Planar cell polarity pathway. Fz, Frizzled; Dsh, Dishevelled; GSK-3, Glycogen synthase kinase-3; LRP, Low density lipoprotein receptor; APC, Adenomatous polyposis coli; CK1 α , Casein kinase 1 α ; TCF, T-cell factor; LEF, lymphoid enhancer-binding factor; G- $\alpha\beta\gamma$, G-protein α , β and γ ; Ca, Calcium; PKC, Protein kinase C; CamKII, Calmodulin-dependent kinase II; NFAT, Nuclear factor of activated T cells; RhoA, Ras homolog gene family, member A; RAC, Ras related GTPase; JNK, c-JUN-N-terminal kinase; AP-1, Activator protein-1.

involved in intracellular signalling like their animal homologs⁵⁷. Several studies have shown that ARPs play multiple roles in plant system including embryogenesis, endosperm development⁵⁸, stress signalling and protein degradation^{59,60}. ARM proteins have been associated with light signalling, hormonal response and maintenance of self-incompatibility in plants⁵⁷. Despite the active participation of ARM proteins in mediating several crucial biological processes in plant system, any direct connection between ARM proteins and Wnt signalling in plants is yet to be unraveled. However, it is evident that there are some events analogous to Wnt signalling existing within plant system as well. For instance, *in silico* studies revealed that glycogen synthase kinase-3 (GSK-3), casein-kinase1 (CK1) and adenomatous polyposis-coli (APC) which act as the mediator of canonical Wnt signalling in animals, are conserved in plants too⁵⁴. It is also hypothesized that Wnt like signalling exists in plants and is analogous to Brassinosteroid signalling based on sequence homology between plant GSKs and BIN-2 (Brassinosteroid insensitive-2), both involved in Brassinosteroid (Br) signalling^{61,62}. Then again, neither the downstream players of Br signalling like BES-1 and BZR-1, belong to ARP family, nor Wnts and Br-

insensitive-1, which encodes a membrane receptor for Br, are the members of the same receptor protein family^{54,57}. These results are surprising and interesting and lead to the speculation about the cross kingdom network of gene families and if they are working in analogy in both plants and animals. Thus, Wnt or Wnt like proteins, if found in plants, can potentially address a number of unresolved issues concerning signalling network in plants.

Plant GSKs and brassinosteroid signalling

Glycogen-synthase-kinase-3 (GSK3), a key component in Wnt pathway and cell signalling in animals, has been traced in plants. Genome analyses have revealed the presence of a family of ten GSKs in Arabidopsis also known as Arabidopsis SHAGGY like kinases (ASKs). This indicates the involvement of GSKs in plant developmental activities including stress response and signal transduction⁶³⁻⁶⁵. Plant GSKs are associated with Br signalling, wherein Br-insensitive-2 (BIN-2), a GSK-like kinase, acts as a negative regulator for downstream Br signalling^{61,66-68}. Brassinosteroids (BRs) are a class of plant steroid hormones, which in association with their receptor, termed as BRASSINOSTEROID INSENSITIVE1

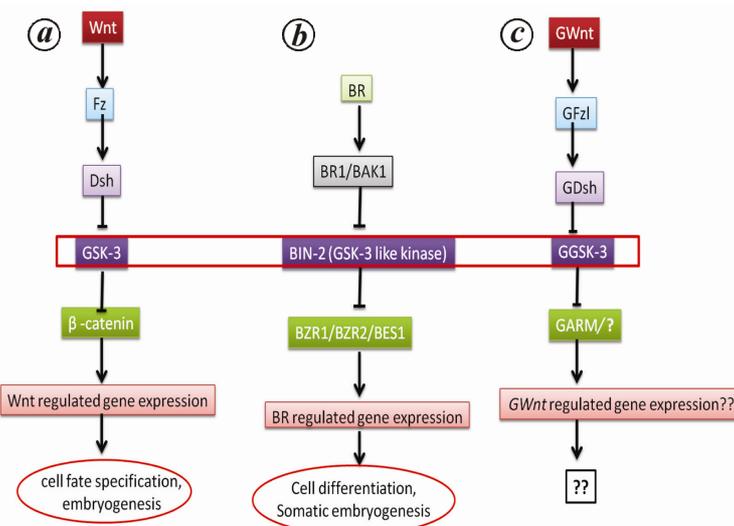


Figure 2. A diagrammatic representation depicting the comparisons and commonalities between Wnt signalling and Brassinosteroid signal transduction pathways where (a) Canonical Wnt signalling as modeled in animal system; (b) Typical pathway for Brassinosteroid signalling in plants; (c) A hypothetical model for Wnt signalling in *Gossypium* sp. with unknown phenotypic response. Fz, Frizzled; Dsh, Dishevelled; GSK-3, glycogen synthase kinase-3; GFz, *Gossypium* Frizzled; GDsh, *Gossypium* Dishevelled; GGSK-3, *Gossypium* glycogen synthase kinase-3 and GARM, *Gossypium* Armadillo protein.

(BRI1), along with BRI1 associated receptor kinase 1 (BAK1) activate a signal transduction cascade in plants⁶⁶. When BR is absent or scanty, BIN-2 comes into action and phosphorylates two homologous transcription factors BRASSINAZOLE RESISTANT1 (BZR1) and BZR2 or BRI1-EMS-SUPPRESSOR1 (BES1), thus restricting them from activating transcription BR related genes. Presence of BR drives the proteasomic degradation of BIN-2 mediated by BRI1-SUPPRESSOR1 (BSU1), leading to BR signal response^{69–71}. The BR signalling therefore, bears a resemblance to Wnt ‘on and off’ modes of signalling. In fact, a few functions such as cell differentiation and division, are performed by both Wnt and BR in animal and plant systems respectively^{72–75}. Although the receptors for both Wnt and BR belong to distinct families respectively⁵⁴, GSKs represent the common factor between the two forms of signalling, a feature that might point to a cross kingdom signalling platform (Figure 2).

Wnt signalling in *Gossypium*!!

Genome-wide survey of *Gossypium arboreum* (commonly known as tree cotton) showed the presence of Wnt3a protein (putatively uncharacterized, unpublished) in *G. arboreum* (GenBank: KHF99765.1) annotated based on sequence similarity with Wnt-3a of *Xenopus laevis* (<http://www.ncbi.nlm.nih.gov/protein/728810636>). In addition, Frizzled-6 (GenBank: KHG22418.1, putatively uncharacterized), Fz CRD domain-like protein (GenBank: KHG28263.1, putatively uncharacterized), segment polarity protein dishevelled homolog DVL-3 (GenBank: KHG01632.1, putatively uncharacterized) and shaggy-

related protein kinase alpha (GenBank: KHG22311.1, putatively uncharacterized) have also been annotated in *G. arboreum* genome. Armadillo repeat proteins (ARMs) which shows similarity with metazoan β -catenin have also been traced in *Gossypium* sp.¹³. Presence of Wnt3a, a prototype of canonical Wnt^{76–79}, may lead to the existence of canonical form of Wnt signalling in *Gossypium arboreum*. However, there are reports which indicate that Wnt3a is capable of triggering non-canonical Wnt signalling too^{80,81}. These can possibly be the indicator of existence of a novel Wnt/Wnt like signalling pathway in plants or at least in *Gossypium* sp. And if so, it will be interesting to know the role of such signalling in plant system which is yet to be unravelled. On the contrary, based on the signalling pattern, Br signalling in plants has been correlated with metazoan Wnt like signalling. It is reported that Br is involved in the process of callusing, somatic embryogenesis⁸² and promotes fibre initiation and elongation in *Gossypium*^{83,84}. This role of Br is rather comparable to that of Wnt’s action in animals where Wnt promotes embryogenesis and cell reprogramming. Although Br signal transduction is not comparable to steroid signalling system of animals, the presence of Wnt signalling ingredients in *Gossypium* may be an indicator of a possible crosstalk between these two forms of cross kingdom signalling.

Conclusion

Evolutionary conserved Wnt signalling mediates several vital functions in animals including regeneration, embryogenesis, cell polarity and cell fate determination. On the contrary, quest for Wnt or Wnt-like signalling in

plants is still in infancy. Massive availability of plant whole genome data has revealed the presence of several transcription effectors resembling key players of metazoan wnt signalling. However, further molecular validation and characterization with functional evidences may pave the way for a new regime of research in plant biology.

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