WNT ligand biogenesis and trafficking

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06/04/2021
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 76

This document contains 1 pathway and 12 reactions (see Table of Contents)
19 WNT proteins have been identified in human cells. The WNTs are members of a conserved metazoan family of secreted morphogens that activate several signaling pathways in the responding cell: the canonical (beta-catenin) WNT signaling cascade and several non-canonical pathways, including the planar cell polarity (PCP), the regulation of intracellular calcium signaling and activation of JNK kinases. WNT proteins exist in a gradient outside the secreting cell and are able to act over both short and long ranges to promote proliferation, changes in cell migration and polarity and tissue homeostasis, among others (reviewed in Saito-Diaz et al, 2012; Willert and Nusse, 2012).

The WNTs are ~40kDa proteins with 23 conserved cysteine residues in the N-terminal that may form intramolecular disulphide bonds. They also contain an N-terminal signal sequence and a number of N-linked glycosylation sites (Janda et al, 2012). In addition to being glycosylated, WNTs are also lipid-modified in the endoplasmic reticulum by a WNT-specific O-acyl-transferase, Porcupine (PORCN), contributing to their characteristic hydrophobicity. PORCN-dependent palmitoylation is required for the secretion of WNT as well as its signaling activity, as either depletion of PORCN or mutation of the conserved serine acylation site results in the intracellular accumulation of WNT ligand (Takada et al, 2006; Barrott et al, 2011; Biechele et al, 2011; reviewed in Willert and Nusse, 2012).

Secretion of WNT requires a number of other dedicated factors including the sorting receptor Wntless (WLS) (also knowns Evi, Sprinter, and GPR177), which binds WNT and escorts it to the cell surface (Banziger et al, 2006; Bartscherer et al, 2006; Goodman et al, 2006). A WNT-specific retromer containing SNX3 is subsequently required for the recycling of WLS back to the Golgi (reviewed in Herr et al, 2012; Johannes and Wunder, 2011). Once at the cell surface, WNT makes extensive contacts with components of the extracellular matrix such as heparan sulphate proteoglycans (HSPGs) and may be bound by any of a number of regulatory proteins, including WIFs and SFRPs. The diffusion of the WNT ligand may be aided by its packing either into WNT multimers, exosomes or onto lipoprotein particles to shield the hy-

**Literature references**


**Editions**

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N-linked glycosylation of WNTs

Location: WNT ligand biogenesis and trafficking

Stable identifier: R-HSA-3238691

Type: omitted

Compartments: endoplasmic reticulum lumen

Inferred from: Glycosylation of mouse WNTs (Mus musculus)

All WNT ligands are predicted to be highly glycosylated. By similarity with WNT ligands in mouse, human WNTs are believed to undergo N-linked glycosylation at multiple asparagine residues and this glycosylation is critical for their secretion (Smolich et al, 1993; Willert et al, 2003, Komekado et al, 2006; Kurayoshi et al, 2007). The mechanism of N-linked glycosylation is not shown here. For a more detailed description, please refer to pathway "Asparagine N-linked glycosylation".

Followed by: PORCN palmitoleoylates N-glycosyl WNTs

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PORCN palmitoleoylates N-glycosyl WNTs

**Location:** WNT ligand biogenesis and trafficking

**Stable identifier:** R-HSA-3238694

**Type:** transition

**Compartments:** endoplasmic reticulum lumen, endoplasmic reticulum membrane

**Inferred from:** PORCN palmitoleoylates WNT3a (Mus musculus)

All WNT proteins except Drosophila WntD are lipid modified. Lipid modifications contribute to the hydrophobicity and poor solubility of all known WNT ligands with the exception of Drosophila WntD. Acylation is required for their secretion from the cell and their ability to bind to FRZ receptors (reviewed in MacDonald et al, 2009; Takada et al, 2006; Janda et al, 2012; Herr and Basler, 2012; Ching et al, 2008). Although an initial study suggested that conserved Cys77 in mouse Wnt3a was palmitoylated (Willert et al, 2003), further work showed that mutation of this residue had minimal effect on WNT secretion (Komekado et al, 2007). In contrast, addition of palmitoleic acid to mWnt3a Ser209 is essential for WNT secretion, and mutant S209A is largely retained in the ER (Takada et al, 2006; Galli and Burrus, 2011). This serine residue is conserved at this position in all known WNTs with the exception of Drosophila WntD (Ching et al, 2008; Herr and Basler, 2012). A recent crystal structure of Xenopus WNT8 in complex with a Frizzled cysteine-rich-domain shows a single lipid modification on the conserved serine residue, while the conserved cysteine participates in a disulphide bond (Janda et al, 2012). In addition to being required for secretion, the lipid at S209 also makes direct contact with a groove in the Frizzled receptor and is thus essential for binding (Janda et al, 2012).

Porcupine is a conserved multi-pass transmembrane ER protein that has an O-acyl-transferase domain (van den Heuvel et al, 1993; Kadowaki et al, 1996; Hofmann, 2000). First identified in Drosophila, Porcupine is a WNT-specific modulator that is required for Wingless processing and secretion (Kadowaki et al, 1996). In porcn-deficient cells, Wg and WNT3A have decreased palmitoylation at S209 and accumulate in the ER (Takada et al, 2006), and mutations in PORCN eliminate all WNT signalling and cause embryonic lethality in mice (Barrott et al, 2011; Biechele et al, 2011). Recent studies show that PORCN is required for activity of all human WNT ligands (Proffitt et al, 2012; Najdi et al, 2012).

**Preceded by:** N-linked glycosylation of WNTs

**Followed by:** WNT ligands traffic to the Golgi
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WNT ligands traffic to the Golgi

**Location:** WNT ligand biogenesis and trafficking

**Stable identifier:** R-HSA-3247837

**Type:** omitted

**Compartments:** Golgi lumen, endoplasmic reticulum lumen

This black box event represents the non-WNT-specific ER-to-Golgi trafficking step of protein secretion (reviewed in Dancourt and Barlowe, 2010; Lippincott Schwartz et al, 2000; for more details, please refer to the pathway "ER to Golgi transport"). Two recent screens in Drosophila have identified members of the p24 family as WNT-specific regulators of ER-to-Golgi transport, although the details have not been elucidated (Port et al, 2011; Buechling et al, 2011; reviewed in Strating and Martens, 2009). Depletion of the Drosophila p24 protein Opossum causes accumulation of WNT ligand in the ER, suggesting a role for Opm in ER-to-Golgi transport of WNTs. WNT-dependent reporter activity was reduced in HEK293 cells that were depleted for the human p24 homologue TMED5, supporting a conserved role for these proteins in WNT signaling (Buechling et al, 2011; reviewed in Palmer et al, 2012).

**Preceded by:** PORCN palmitoleoylates N-glycosyl WNTs

**Followed by:** WLS binds WNT ligands in the Golgi

**Literature references**


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WLS binds WNT ligands in the Golgi

**Location:** WNT ligand biogenesis and trafficking

**Stable identifier:** R-HSA-3247840

**Type:** binding

**Compartments:** Golgi membrane, Golgi lumen

Wntless (WLS) (Evi/Sprinter/GPR177) is a conserved transmembrane protein that is required for the secretion of WNT ligands from the cell (Bänziger et al, 2006; Bartscherer et al, 2006; Goodman et al, 2006). Notably, WLS is not required for the secretion of Hedgehog, another acylated signaling molecule, and wls-mutants phenocopy wg/wnt mutants (Bänziger et al, 2006; Bartscherer et al, 2006; Goodman et al, 2006), supporting the notion that WLS is a dedicated WNT pathway member. WLS binds directly to WNT ligands in the Golgi in a WNT-acylation dependent manner, as the interaction is abrogated by mutation of either PORCN or the conserved Ser209 residue (Coombs et al, 2010; Herr and Basler, 2012). WLS is thought to contain a lipocalin-family fold (Coombs et al, 2010), a lipid-interacting domain, which may play a role in binding to the lipid adduct on WNT.

**Preceded by:** WNT ligands traffic to the Golgi

**Followed by:** WNT ligands traffic to the plasma membrane

**Literature references**


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https://reactome.org
WNT ligands traffic to the plasma membrane

Location: WNT ligand biogenesis and trafficking

Stable identifier: R-HSA-3247844

Type: omitted

Compartments: Golgi membrane, plasma membrane

WLS accompanies WNT through the secretory pathway to the cell surface, where the ligand is released into the extracellular space (Bänziger et al, 2006; Bartscherer et al, 2006; Goodman et al, 2006).

Preceded by: WLS binds WNT ligands in the Golgi

Followed by: WLS:WNT complex is internalized for maturation into exosomes, secretion of WNT ligands

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Fractionation of extracellular WNT activity shows that between 12-40% of secreted WNT ligand is present on exosomal vesicles (Gross et al, 2012; Beckett et al, 2013). Exosomes are 40 - 100 nm microvesicles of endocytic origin with established roles in cell-cell communication. They are produced by multivesicular bodies (MVBs) and directed to the plasma membrane for secretion (reviewed in Simons and Raposo, 2009). WNT secretion in the exosomal fraction is dependent on WLS/EVI/SPR in both human and Drosophila cells (Gross et al, 2012; Beckett et al, 2013). While exosomes have been shown to be required for presynaptic release of EVI and Wg at Drosophila neuromuscular junctions, there is conflicting evidence about whether they play a role in the formation of a Wg gradient at the Drosophila imaginal disc (Korkut et al, 2009; Gross et al, 2012; Beckett et al, 2013). Exosomal WNT fractions co-purify with TSG101 and other components of the ESCRT machinery, and knockdown of ESCRT 0 components reduces the levels of WNT3A and the signaling activity of the exosomal fractions (Gross et al, 2012; Beckett et al, 2013).

**Preceded by:** WNT ligands traffic to the plasma membrane

**Followed by:** WNTs are secreted in exosomes

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https://reactome.org
Exosomes have been shown to be involved in secretion of WNTs from HEK293 cells as well as from the human colon cancer cell line Caco2 (Gross et al, 2012). In addition, exosomes from cancer-associated fibroblasts have been shown to promote autocrine PCP signaling and protrusive activity and motility in breast cancer cells (Korkut et al, 2009).

**Preceded by:** WLS:WNT complex is internalized for maturation into exosomes

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Vacuolar acidification is required but not sufficient for the release of WNT ligands from WLS at the cell surface. V-ATPase inhibitors cause the accumulation of WLS-WNT complexes both within the cell and at the plasma membrane (Coombs et al, 2010).

Once in the extracellular space, the lipid-modified WNT ligand must be shielded to allow the morphogen to diffuse away from the plasma membrane. Possible mechanisms include interaction with HSPGs, exosomes, multimerization or incorporation into lipoprotein particles (reviewed in Eaton, 2006; Port and Basler, 2010).

**Preceded by:** WNT ligands traffic to the plasma membrane

**Followed by:** WLS is endocytosed to the early endosome

**Literature references**


WLS is endocytosed to the early endosome

Location: WNT ligand biogenesis and trafficking

Stable identifier: R-HSA-3247847

Type: omitted

Compartments: early endosome membrane, plasma membrane

WLS endocytosis is a clathrin-dependent process. In Drosophila cells, internalization of WLS has been shown to depend on clathrin, AP-2, dynamin, Rab5 and HRS (Belenkaya et al, 2006; Port et al, 2008), while in HeLa cells, WLS colocalizes with endogenous AP-2, and depletion of AP-2 increases WLS levels at the cell surface (Yang et al, 2008). A recent study identified a conserved YEGL endocytosis motif in the third intracellular loop of WLS that is required for its clathrin- and dynamin-dependent internalization (Gasnereau et al, 2011).

Preceded by: secretion of WNT ligands

Followed by: Retromer associates with WLS

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Retromer associates with WLS

Location: WNT ligand biogenesis and trafficking

Stable identifier: R-HSA-3247839

Type: binding

Compartments: early endosome membrane, cytosol

Retromer is a conserved multi-protein complex that is required for retrograde transport of transmembrane proteins. It was initially characterized in yeast as a pentameric complex required for the recycling of the transmembrane receptor VPS10 to the trans-Golgi, and was subsequently shown to be conserved in flies, worms and humans. In humans, retromer consists of a cargo-recognition subcomplex made up of VPS35, VPS26 and VPS29 and a membrane-targeting subcomplex containing a heterodimer of SNX proteins (SNX1 or 2 paired with SNX5 or 6). The SNX proteins contain a BAR domain that is believed to promote membrane curvature, and SNX-BAR proteins are thought to aid in the formation of endosomal membrane tubules into which cargo is loaded (reviewed in Pfeffer, 2001; Seaman, 2012).

Retromer is required for the recycling of WLS to the Golgi to allow further rounds of WNT-ligand delivery to the plasma membrane (Coudreuse et al 2006; Belenkaya et al 2008; Port et al, 2008). In the absence of essential retromer component VPS35 or VPS26, WLS is diverted to the MVB and degraded, and WNT ligand accumulates inside the cell; overexpression of WLS is sufficient to rescue the vps35 defect in WNT signaling (Belenkaya et al, 2006; Franch-Marro et al, 2008). WLS and retromer colocalize on endosomal structures and WLS and VPS35 co-precipitate in pull down studies (Belenkaya et al, 2006; Port et al, 2008; Franch-Marro et al, 2008).

Several recent studies have suggested that WLS recycling depends on a WNT-specific retromer in which the SNX-BAR proteins of the classic complex are replaced by SNX3 (Zhang et al, 2011; Harterink et al, 2011; reviewed in Johannes and Wunder, 2011). Unlike SNX1/2/5/6, SNX3 does not contain a BAR domain, and WLS is suggested to accumulate in endocytic vesicles rather than in the tubular structures of the 'classic' retromer (Harterink et al, 2011; Zhang et al, 2011). SNX3 is recruited from the cytosol to the early endosome through the interaction of its PX domain with PIP3 in the membrane. Mutation of critical residues in the PX domain abolish the interaction with PIP3 and ablate endosomal recruitment of SNX3 (Xu et al, 2001; Zhang et al, 2011; Harterink et al, 2011). SNX3 has been shown to co-immunoprecipitate with VPS35 and VPS26, and some studies have also shown a direct interaction between SNX3 and WLS (Zhang et al, 2011; Harterink et al, 2011).
**Preceded by:** WLS is endocytosed to the early endosome

**Followed by:** Retromer recycles WLS to the Golgi

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Retromer recycles WLS to the Golgi

Location: WNT ligand biogenesis and trafficking

Stable identifier: R-HSA-3247836

Type: omitted

Compartments: Golgi membrane, early endosome membrane

Retromer is believed to escort WLS from the early endosome back to the Golgi for subsequent rounds of WNT secretion (reviewed in Johannes and Wunder, 2011; Willert and Nusse, 2012).

Preceded by: Retromer associates with WLS

Followed by: WLS dissociates from retromer

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WLS dissociates from retromer

**Location:** WNT ligand biogenesis and trafficking

**Stable identifier:** R-HSA-3247849

**Type:** omitted

**Compartments:** Golgi membrane

Although the role of retromer in delivering WLS back to the Golgi is reasonably well established (reviewed in Johannes and Wunder, 2011; Willert and Nusse, 2012), the details of how the complex is disassembled at the TGN remain to be determined.

**Preceded by:** Retromer recycles WLS to the Golgi

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