Chapter 2
Regulation of Wnt Secretion and Distribution

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Abstract Wnts are a family of signaling glycoproteins which play essential roles in many developmental processes and adult homeostasis. Misregulated Wnt signaling has been implicated in a variety of human diseases including cancers. The tight regulation of Wnt signaling is not only reflected by the diverse responses triggered in the Wnt-receiving cells, but also, as more recent work suggested, by the complex control on Wnt processing, secretion, and subsequent distribution. The characterization of the nature and roles of posttranslational modifications of Wnt molecules and the discovery of Wntless (Wls) and the retromer complex as novel and indispensable players in Wnt release have led to closer inspection of the Wnt secretory routes. Moreover, dissection of the functions of lipoproteins and heparan sulfate proteoglycans (HSPGs) in Wnt diffusion shed new light on the mechanism of morphogen distribution. In this chapter, we will summarize the recent advances in the studies of Wnt processing, secretion, and spreading and discuss how these components are integrated into the regulating network of Wnt pathway.

Keywords Wnt processing • Wnt secretion • Wingless (Wg) • Wntless (Wls) • The retromer complex • Lipoprotein • Heparan sulfate proteoglycan (HSPG)

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2.1 Introduction

The Wnt family of secreted cysteine-rich glycoproteins is evolutionarily conserved in metazoans. As one of the best-studied morphogen families, Wnt molecules provide cells in the morphogenetic field with positional information and trigger cellular responses in a concentration-dependent manner. Although a great deal is known about Wnt perception and signal transduction in the Wnt-receiving cells, relatively limited information is available about the events occurring in Wnt-producing cells and the extracellular space. In this chapter, we will review the recent studies on the molecules and mechanisms involved in these events and discuss how the players interact with each other and integrate into the dedicated Wnt secretion route(s).

2.2 Wnts Are Posttranslationally Modified in the ER

As a common structural characteristic, Wnts contain several charged residues and a relatively high number of conserved cysteines (23–25 on average), which might be involved in the establishment of intra- and intermolecular disulfide bonds and thus be important for the proper folding and multimerization of Wnt proteins (Miller 2002; Coudreuse and Korswagen 2007) (Fig. 2.1). In contrast to predictions made based on their primary amino-acid sequences, Wnt proteins are more hydrophobic and insoluble. These characteristics have long hampered the isolation of active Wnts. The first successful attempt in Wnt purification was made by the Nusse group in 2003 (Willert et al. 2003). From their work, they isolated active products of mouse *Wnt3a* and *Drosophila Wnt8* genes. Moreover, they found that

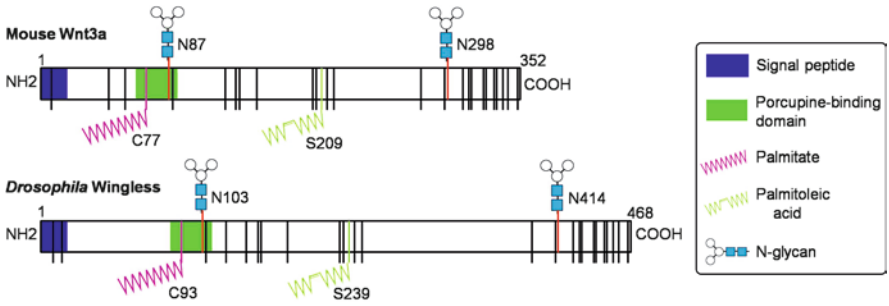

Fig. 2.1 Schematic overview of mouse Wnt3a and *Drosophila* Wingless proteins, showing the approximate positions of cysteine residues (*black vertical lines*). Both Wnts are acylated probably by Porcupine which binds Wnts at the corresponding region. Two lipids are appended to Wnts: one palmitate to the N-terminal cysteine and one palmitoleic acid to the internal serine. Wnts also harbor several potential N-glycosylation sites as indicated. The signal sequence is represented by the boxed area in the N-terminus

both molecules were palmitoylated at a conserved cysteine residue (C77 in Wnt3a) and this modification was essential for Wnt signaling activity. Subsequent work revealed that *Drosophila* Wingless (Wg), murine Wnt5a, as well as chick Wnt1 and Wnt3a are all palmitoylated at the corresponding cysteine residues (Willert et al. 2003; Miura and Treisman 2006; Galli et al. 2007; Kurayoshi et al. 2007). More recently, Takada et al. reported that mouse Wnt3a was also lipid-modified by palmitoleic acid at a conserved serine residue (S239) (Takada et al. 2006). Therefore, Wnts are potentially acylated at two conserved sites: one palmitate at an N-terminal cysteine and one palmitoleic acid at an internal serine (Fig. 2.1). The only exception so far is WntD, a recently characterized *Drosophila* Wnt family member (Ganguly et al. 2005; Gordon et al. 2005; Ching et al. 2008). WntD does not undergo lipid modification and it takes a secretion route different from other Wnts which will be discussed later (Ching et al. 2008).

Many lines of evidence suggest that lipid modifications play essential roles in Wnt secretion and signaling. In vertebrates, it is generally believed that palmitate at cysteine is required for Wnt action, whereas palmitoleic acid at serine is necessary for Wnt secretion. Point mutations of the palmitoylated cysteine in Wnt3a, Wnt1, and Wnt5a do not interfere with their secretion, but strongly decrease their signaling activity in cell-based assays (Willert et al. 2003; Galli et al. 2007; Kurayoshi et al. 2007). Takada et al. demonstrated that Wnt3a defective in serine palmitoleoylation is not secreted from cells, but is rather retained in the endoplasmic reticulum (ER) (Takada et al. 2006), suggesting a possible role of this residue in protein folding and intracellular transport. However, the recent report using *Drosophila* Wg disagreed on the exact roles of two lipid adducts (Franch-Marro et al. 2008a). In this study, it is found that removal of palmitate moiety at the conserved cysteine residue (C93) causes inefficient exit of Wg from ER in the wing imaginal discs, although this mutant can be readily secreted in cultured *Drosophila* S2 cells. On the other hand, mutation of the equivalent serine site (S209) causes no major defect in secretion and membrane association, but results in poor signaling activities. Nevertheless, all of the mutations in this work were made on Wg with an internal HA tag, so it will be important to repeat all experiments on native Wg to confirm the results. How is lipid modification involved in Wnt secretion and signaling activity? While it is still unclear on the aspect of Wnt secretion, current data argue that acylation contributes to Wnt signaling activity by facilitating its interaction with the Frizzled receptor (Willert et al. 2003; Cong et al. 2004; Komekado et al. 2007; Kurayoshi et al. 2007).

Another posttranslational modification of Wnts is N-glycosylation, in which N-linked oligosaccharide chains are attached to the peptide backbone (Fig. 2.1). Unlike lipid modification, the number and position of glycosylation sites seem to be flexible and its function is poorly understood. In the early studies, it was demonstrated that replacement of all four asparagine-linked glycosylation sites did not affect Wnt1-induced antocrine or paracrine signaling in tissue culture system, indicating that glycosylation was not essential for either secretion or signaling of Wnts (Mason et al. 1992). However, the Kikuchi group recently argued that in the case of Wnt3a and Wnt5a, glycosylation precedes lipid modification and is important

for Wnt secretion, but not for their actions (Komekado et al. 2007; Kurayoshi et al. 2007). As most of the published studies were based on in vitro assays, further in vivo studies on more Wnt members are required to fully unravel the functions of Wnt posttranslational modifications.

The enzyme most likely to be responsible for Wnt lipid modification is the ER protein Porcupine (Porc). *Porc* was first identified in *Drosophila* as a segment polarity gene (van den Heuvel et al. 1993), which encodes a conserved multiple-pass transmembrane protein in the family of membrane-bound O-acyltransferases (MBOATs) (Hofmann 2000). Despite the lack of direct evidence, the role of Porc as the lipid-modifying enzyme of Wnt is supported by three lines of evidence. First, Porc controls the hydrophobicity levels of Wnts: in the absence of Porc, Wg becomes less hydrophobic (Zhai et al. 2004), while when Porc is overexpressed, Wnt1 and Wnt3a are more hydrophobic (Galli et al. 2007). Second, the Porc-binding domain in Wnt sequence contains the conserved palmitoylated cysteine residue (Fig. 2.1) (Tanaka et al. 2002). Third, Porc loss-of-function mutations phenocopy mutations of Wnt acylation and show similar disrupted secretion of Wg and Wnt3a (van den Heuvel et al. 1993; Takada et al. 2006). Although the link of Wnt acylation and Porc is well established, we cannot rule out the possibility that Porc is involved in lipid-modifying other sites besides the conserved cysteine and serine. Also, Porc may control Wnt signaling via roles more than acylation, but such roles could not be exhibited in Porc mutant due to its dominating secretion defect. Previously, Porc was also shown to be required for Wg glycosylation and its enzymatic activity correlated with the glycosylation levels of Wg (Tanaka et al. 2002). Inconsistently, the Takada group demonstrated that the glycosylation of Wnt3a was not perturbed when Porc expression was knocked down by RNAi (Takada et al. 2006). One explanation for this discrepancy is that the role of Porc in glycosylation may not be conserved among different Wnt members.

2.3 Wls and the Retromer Complex Are Involved in the Intracellular Trafficking of Wnts

In addition to Porc, Wls (also known as Evenness interrupted or Sprinter) is another key regulator for Wnt secretion (Banziger et al. 2006; Bartscherer et al. 2006; Goodman et al. 2006). The initial identification of Wls was made in *Drosophila* by three independent groups (Banziger et al. 2006; Bartscherer et al. 2006; Goodman et al. 2006). As a multipass transmembrane protein, Wls has been shown to localize in components of the secretory pathway, including the Golgi apparatus, plasma membrane, and endosomes, suggesting a role of Wls in Wnt trafficking downstream of Porc (Banziger et al. 2006; Bartscherer et al. 2006; Belenkaya et al. 2008; Franch-Marro et al. 2008b; Port et al. 2008; Yang et al. 2008). Indeed, mutations of Wls result in cell-autonomous accumulation of Wg and therefore failure in target gene activation in embryos and wing imaginal discs (Banziger et al. 2006;

Bartscherer et al. 2006; Goodman et al. 2006). Particularly, in the absence of Wls, Wg is accumulated in the Golgi and it can no longer reach the cell surface (Banziger et al. 2006). Furthermore, co-immunoprecipitation experiments confirm that Wls physically interacts with Wnt proteins (Banziger et al. 2006). All of the data lead to a model that Wls helps Wnt transport from Golgi to the plasma membrane for secretion, but the underlying mechanism is still unknown. Several possibilities have been raised: Wls could function as a modifying enzyme or chaperone which assists in the proper folding and maturation of Wnt before it is admitted to the public transportation; or, Wls could act as a cargo receptor or router which carries or sorts Wnt into the dedicated secretion track. On this point, one related question needs to be answered, i.e., whether Wls is associated with Wnt during or after the passage of Wnt to the cell surface? One intriguing hypothesis is that Wls can also function in the endocytosis and rerouting of membrane-bound Wnt molecules. As an evolutionarily conserved protein family, Wls and its homologs in *C. elegans*, planarian, mouse, and *Xenopus* have been shown to regulate the secretion of various Wnt ligands both in vitro and in vivo (Banziger et al. 2006; Adell et al. 2009; Fu et al. 2009; Kim et al. 2009). Interestingly, recent work uncovered that mouse Wls is a direct target of the canonical Wnt pathway during embryonic axis formation (Fu et al. 2009), suggesting a feedback mechanism underlying the reciprocal regulation of Wls and Wnt. As mentioned before, *Drosophila* WntD is the only lipid-unmodified Wnt member. Actually, it is also the only Wnt member which can be efficiently secreted without Wls action (Ching et al. 2008). The secretion of WntD, however, does maintain the requirement of Rab1, which is an ER-to-Golgi trafficking component (Ching et al. 2008). This observation strongly argues that the unpalmitoylated WntD takes a different secretion mode subsequent to its Golgi entrance. In other words, lipid modification renders Wnt dependent on a dedicated mechanism for secretion in which Wls is required.

First identified in yeast decades ago, the retromer was recently implicated in the same Wnt secretion pathway as Wls. The retromer is an evolutionarily conserved multisubunit complex, consisting of two smaller complexes, the cargo recognition Vps26-Vps29-Vps35 heterotrimer and a membrane-targeting SNX heterodimer or homodimer (Seaman et al. 1998; Seaman 2005; Verges 2007). It has been shown that the retromer mediates various intracellular transporting processes, including endosome-Golgi trafficking of yeast hydrolase transporter Vps10 and mammalian cation-independent mannose-6-phosphate receptor, and transcytosis of the polymeric immunoglobulin receptor (Seaman et al. 1997; Seaman et al. 1998; Verges et al. 2004; Seaman 2005).The function of the retromer complex in Wnt signaling was first uncovered in *C. elegans* by two independent groups (Coudreuse et al. 2006; Prasad and Clark 2006). In *C. elegans*, mutations in components of the retromer complex, especially Vps35, show disrupted Wnt signaling and, by epistatic assays, both groups established the role of the retromer in Wnt-producing cells (Coudreuse et al. 2006; Prasad and Clark 2006). Of note, Coudreuse and colleagues observed a much stronger impairment in long-range Wnt signaling vs. short-range Wnt signaling and more importantly a loss of Wnt gradient (Coudreuse et al. 2006), arguing a role of the retromer in packaging and/or transporting Wnt for secretion.

The similarity of Wnt signaling defects in the retromer and *wls* mutants suggests that they may act together to facilitate Wnt secretion. This hypothesis is supported by parallel studies from five independent groups. The authors of this chapter as well as others demonstrated that the retromer complex regulates Wls stability by preventing it from degradation in the lysosomes (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Pan et al. 2008; Port et al. 2008; Yang et al. 2008). Wls is internalized from the plasma membrane by a clathrin-dependent endocytosis (Belenkaya et al. 2008; Pan et al. 2008; Port et al. 2008; Yang et al. 2008) and can be recycled back to the trans-Golgi network (TGN) as shown by antibody-uptake assays (Belenkaya et al. 2008; Franch-Marro et al. 2008b). Interference of AP-2, Rab5, and dynamin function causes accumulation of Wls on the cell surface, increase in total Wls levels, and reduced amount of Wls in the Golgi, indicating recycling of Wls following endocytosis from the cell surface (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Pan et al. 2008; Port et al. 2008; Yang et al. 2008). An interaction between Wls and the retromer complex has been proposed based on colocalization in endocytic vesicles and co-immunoprecipitation assays (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Port et al. 2008; Yang et al. 2008). By analogy with the previous reported role of the retromer in selective recycling of cargo receptors, it is proposed that the retromer supports Wnt secretion by retrieving Wls from endosomes to the Golgi after its clathrin-mediated endocytosis. So far, the function of the retromer in Wls recycling is shown to be conserved in *C. elegans* (Pan et al. 2008; Yang et al. 2008), *Drosophila* (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Port et al. 2008), *Xenopus* (Kim et al. 2009), and mammalian cells (Belenkaya et al. 2008; Franch-Marro et al. 2008b; Port et al. 2008).

2.4 Wnts Associate with Lipoproteins in the Dedicated Secretory Route

As mentioned before, Wnt proteins are hydrophobic due to attachment of lipid moieties. In vitro purified Wnt proteins are poorly diffusible and insoluble, which seems inconsistent with the in vivo role of Wnt in tissue patterning. During development, Wnt molecules act both as a short-range inducer and a long-range morphogen which spreads in long distances to activate expression of different target genes at different threshold levels. Therefore, it has been speculated that a dedicated secretion route exists in addition to the unregulated bulk flow pathway. While the later releases poorly mobile molecules close to the source of production, the former produces specifically packed morphogens for efficient spreading and long-range signaling. The existence of such a dedicated pathway has been implicated in the secretion of Hedgehog (Hh) (Gallet et al. 2003). In this study, they found that in the *Drosophila* embryonic epithelium, Hh forms large punctate structures and the movement of these puncta segregates away from a secreted form of GFP. As the morphogen

family of Hh shares significant structural (both are lipid-modified) and functional similarities with Wnt (Nusse 2003). Wnt may take a similar route in secretion. Moreover, the discovery of Wls as a specific player in Wnt-producing cells hints again at specific cellular machinery dedicated to controlling Wnt release. Interestingly, the functional equivalent of Wls in Hh secretion is the multipass transmembrane protein Dispatched, which contains a sterol-sensing domain potentially interacting with the cholesterol modification of Hh (Burke et al. 1999). The last evidence resides in the association of Wnt with lipid rafts (Zhai et al. 2004). Lipid rafts are specialized detergent-resistant membrane microdomains which are shown to act as platforms for the sorting and trafficking of particular subgroups of proteins (Rietveld et al. 1999; Schmidt et al. 2001; Le Roy and Wrana 2005). Importantly, the Basler group recently reported that in *Drosophila*, a major component of membrane microdomains, Reggie-1/Flotillin-2, promotes the secretion and spreading of Wnt and Hh especially for long-range signaling (Katanaev et al. 2008). This result consistently supports the view that targeting to lipid rafts may direct Wnt to specialized sorting and secretion routes.

How could Wnt overcome its hydrophobic nature and achieve effective secretion and diffusion in vivo? Generally, two models have been proposed. In the first model, acylated Wnts may form micelle-like multimers with the lipid chains facing the interior. This multimeric complex has recently been suggested by the sucrose-density gradient experiments with secreted Wnts from tissue-cultured cells (Katanaev et al. 2008). Although Wnt oligomerization is ambiguous, the formation of Hh oligomers has been much better demonstrated. In addition to previous biochemical evidence (Zeng et al. 2001; Chen et al. 2004; Gallet et al. 2006), Neha Vyas and colleagues predicted, based on FRET microscopy, that Hh forms nanoscale oligomers which require the electrostatic interaction between Hh molecules (Vyas et al. 2008). Another model for the movement of lipid-modified morphogens involves the association of Wnt with the lipoprotein particles (LPPs), which were demonstrated in *Drosophila* and originally termed "argosomes" (Greco et al. 2001; Panakova et al. 2005). LPPs consist of a hydrophobic core of lipids, surrounded by a hydrophilic monolayer harboring specific apolipoproteins (Rodenburg and Van der Horst 2005). In *Drosophila*, LPPs are derived from fat body and are found in endocytic compartments in the secreting cell and in the extracellular space of wing imaginal discs (Kutty et al. 1996; Panakova et al. 2005). Both Wnt and Hh are shown to associate with LPPs and this association is important for morphogen spreading and activation of long-range signaling. In the *Drosophila* wing epithelium, loss of lipoproteins reduces the range of spreading and signaling of Wg and Hh (Panakova et al. 2005). In the mammalian tissue culture system, it was recently shown that Wnt3a is released on high-density LPPs (Neumann et al. 2009). However, it remains unclear by which manner Wnt proteins distribute on LPPs. One possibility is that cell surface Wnt is "brushed off" by the LPPs in the extracellular space. Alternatively, a more attractive model would be that Wnts are loaded onto LPPs in endocytic compartments of Wnt-producing cells in which process Wls may play a role.

2.5 HSPGs Regulate Extracellular Diffusion and Gradient Formation of Wnts

Heparan sulfate proteoglycan (HSPGs) are cell-surface and extracellular matrix (ECM) macromolecules consisting of a protein core attached by heparin sulfate (HS) glycosaminoglycan (GAG) chains (Bernfield et al. 1999; Esko and Selleck 2002). Over the past decades, studies in *Drosophila* and vertebrates have demonstrated that HSPGs are involved in several signaling pathways, including Wnt, Hh, transforming growth factor-β (TGFβ), and fibroblast growth factor (FGF).

Based on the structure of the core protein, HSPGs are classified into three major families. Glypicans and syndecans are cell-surface HSPGs and are linked to the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage or a transmembrane domain, respectively. Perlecans are secreted HSPGs mainly distributed in the ECM. Besides HS chains, syndecans are decorated with chondroitin sulfate. All three HSPG families are evolutionarily conserved from *C. elegans*, *Drosophila* to vertebrates (Esko and Selleck 2002; Nybakken and Perrimon 2002). HS chain biosynthesis is initiated at the GAG attachment site(s) of the core proteins and involves various glycosyltransferases and modification enzymes which are also evolutionarily conserved (Bernfield et al. 1999; Lin and Perrimon 2002; Nybakken and Perrimon 2002). The function of HSPGs in Wnt signaling was first revealed by the characterization of sugarless (sgl) (Binari et al. 1997; Hacker et al. 1997; Haerry et al. 1997) and sulfateless (sfl) (Lin and Perrimon 1999), both of which are important enzymes involved in HS biosynthesis. *sgl* and *sfl* mutants develop Wg-dependent embryonic defects (Hacker et al. 1997; Lin and Perrimon 1999) and loss of Sfl activity results in reduced Wg target gene expression and extracellular Wg levels (Lin and Perrimon 1999; Baeg et al. 2001). In subsequent research, other enzymes, such as the EXT proteins, are also shown to be involved in Wg/Wnt signaling and distribution (Lin 2004). To date, various studies support the idea that HSPGs regulate Wnt signaling and its gradient formation via their attached HS GAG chains.

Existing evidence, especially those from *Drosophila,* suggests that glypican core proteins also play important roles in regulating the gradient formation of morphogens, including Wnt and Hh. The *Drosophila* genome encodes two glypicans (division abnormally delayed [Dally] and Dally-like [Dlp]) (Nakato et al. 1995; Khare and Baumgartner 2000; Baeg et al. 2001). It has been shown that Dally and Dlp play cooperative and distinct roles in regulating Wg signaling and distribution. First, Dally-Dlp double mutants exhibit stronger reduction in Wg signaling and extracellular deposition in the embryos and wing discs than in either alone (Baeg et al. 2001; Han et al. 2005). Second, as opposed to the positive role of Dally in Wg signaling (Lin and Perrimon 1999), Dlp shows biphasic activities, functioning to repress short-range Wg signaling but activate long-range Wg signaling (Baeg et al. 2004; Kirkpatrick et al. 2004; Franch-Marro et al. 2005). One explanation for it resides in the fact that Dlp has stronger affinity for Wg. Both Dally and Dlp can bind Wg in cell culture, but only overexpression of Dlp causes Wg accumulation in the imaginal discs (Baeg et al. 2001; Franch-Marro et al. 2005). Therefore, while

Dally helps present Wg to the signaling receptor dFz2 as a coreceptor (Lin and Perrimon 1999; Franch-Marro et al. 2005), Dlp retains Wg on the cell surface to either compete with dFz2 or facilitate its binding with Wg depending on the ratio of Wg, dFz2, and Dlp (Yan et al. 2009). Concerning the distinct functions of Dally and Dlp, HSPG core proteins may contribute to Wnt signaling much more than being a carrier for HS GAG chains. Indeed, HSPG core proteins can affect the modifications of HS GAG chains (Esko and Zhang 1996; Chen and Lander 2001). Moreover, several studies indicate that HSPG core proteins can be directly involved in Wnt signaling. In vertebrate cells, the nonglycanated glypican-3 (GPC3) core protein can form complexes with Wnts and stimulate Wnt signaling (Capurro et al. 2005). In *Drosophila* wing discs, it was recently demonstrated that Dlp core protein has similar biphasic activity as wild-type Dlp (Yan et al. 2009; Hufnagel et al. 2006).

The functions of vertebrate HSPGs are not well characterized, but accumulating data suggest that HSPGs are also involved in Wnt signaling during development. Examples include the *zebrafish* glypican knypek in Wnt11 signaling (Topczewski et al. 2001), *Xenopus* EXT1 in Wnt11 signaling and distribution (Tao et al. 2005), as well as mammalian GPC3 in Wnt signaling in cancer cells (Filmus and Capurro 2008; Stigliano et al. 2009). As major regulators in morphogen molecules, HSPGs are under a hierarchical regulation during development. First, the expression of HSPGs and the related enzymes is tightly controlled. Both *Dally* and *Dlp* are transcriptionally regulated by Wg and Hh signaling, forming a feedback loop (Fujise et al. 2001; Han et al. 2005; Gallet et al. 2008). The translation of Ttv (one Drosophila EXT protein) and Sfl is controlled by internal ribosome entry sites (Bornemann et al. 2008). Interestingly, the Hippo pathway that modulates multiple morphogen signaling pathways also regulates the transcriptional control of *Dally* and *Dlp* (Baena-Lopez et al. 2008). Second, the intracellular trafficking of HSPGs is a regulated process. Several studies demonstrated that HSPGs are transported to specific membrane domains after synthesis and are actively endocytosed (Bernfield et al. 1999; Kramer and Yost 2003; Bishop et al. 2007). In a recent study, Dlp, which is apically targeted by the GPI anchor, undergoes internalization and redistribution to the basolateral compartment, and this so-called "transcytosis" of Dlp is required for Wg basolateral spreading (Gallet et al. 2008). Finally, HSPGs can be regulated by various shedding mechanisms. The membrane-tethered sydecans and glypicans can be shed into the ECM by proteolytic cleavage (Kato et al. 1998) and GPI cleavage, respectively. Notum, an extracellular lipase, was shown to be involved in the release of glypicans from the cell surface (Gerlitz and Basler 2002; Giraldez et al. 2002; Kreuger et al. 2004; Traister et al. 2008). Alternatively, the HS GAG chains can be cleaved by extracellular heparanase (Sanderson et al. 2004). Although shedding is proposed to regulate cell surface HSPG levels and affect its activities, the in vivo roles await further investigation.

How do HSPGs modulate Wnt distribution? Current data support roles of HSPGs in controlling the spreading of morphogens along the epithelial cell surface through a "restricted diffusion mechanism" in which the secreted morphogen molecules move while interacting with their receptors and other ECM proteins especially HSPGs (Strigini and Cohen 2000; Baeg et al. 2004; Lin 2004;

Han et al. 2005; Hufnagel et al. 2006). In restricted diffusion, HSPGs may play two roles in regulating Wnt movement. First, Wnt movement may be mediated by transferring between HSPGs, especially HS GAG chains. Alternatively, HSPGs control Wnt stability to ensure it moves across a field of cells without being degraded. The two mechanisms may be coupled in Wnt gradient formation. Particularly, it was recently reported that membrane-associated glypicans recruit LPPs in the wing disc cells (Eugster et al. 2007), adding another level of complexity to Wnt gradient formation. In addition to a role in Wnt planar transportation, HSPGs can also regulate Wnt apical/basolateral distribution in epithelial cells. As mentioned before, the apicobasal trafficking of Dlp was recently shown to be involved in the basolateral redistribution of apically secreted Wg in polarized wing disc cells (Gallet et al. 2008).

2.6 Concluding Remarks

Left aside for a long period, the process of Wnt maturation, sorting, and secretion is attracting the interest of more and more researchers in the Wnt field. Especially in the past decade, many important discoveries have been made, including the characterization of Wnt posttranslational modifications, the involvement of LPPs in Wnt trafficking, and the discovery of Wls and the retromer complex in Wnt secretion (as summarized in Fig. 2.2). However, many questions remain to be answered. Regarding Wnt processing and maturation, systematic analysis is needed to verify the results from cell-based assays and to resolve the inconsistence between published data. Particularly, the role of lipid and glycosyl group in Wnt gradient formation as well as short-range vs. long-range signaling has not been examined yet. Recently, the Nusse group identified WntD, the Wnt member which is not lipid-modified and not dependent on Wls for secretion. This exceptional case links Wnt acylation with the need of specialized accessory proteins. It seems that as acylation renders Wnt hydrophobic, certain dedicated secretion mechanisms are required to enable long-range movement and signaling and Wls as well as LPPs may be part of it. In this dedicated pathway, several molecular mechanisms are still elusive. First, how lipid modification affects Wnt-Wls interaction is unknown. Second, the mode of Wls action in Wnt secretion is not fully understood. Third, mechanisms of Wnt apical/basolateral distribution are unclear. Finally, both working in Wnt secretion, the cooperation of Wls and LPPs has not been characterized.

While intensive studies have illustrated the major functions of HSPGs in Wnt gradient formation (Fig. 2.2), the molecular nature of Wnt-HS GAG or Wnt-HSPG core protein interaction waits to be resolved, which may need combined approaches and advanced technology. Clearly, the characterization of HSPG-binding partners, the investigation of HSPG function in vertebrates, as well as lessons from other morphogenic fields will make important contributions to our understanding of the mechanisms by which HSPGs regulate Wnt distribution.

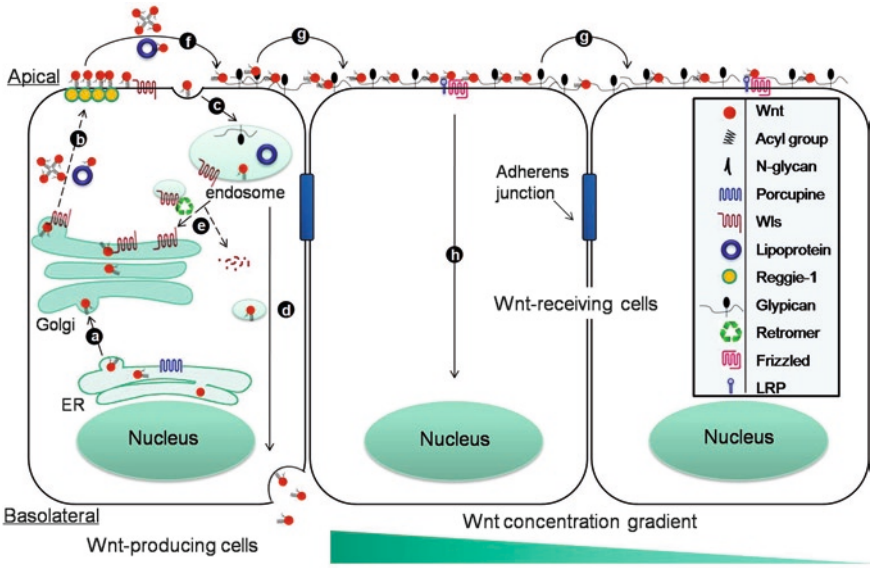

Fig. 2.2 Models of Wnt secretion and distribution. (**a**) Wnt synthesized in the endoplasmic reticulum undergoes N-glycosylation and acylation by the ER enzyme Porcupine. (**b**) In the Golgi apparatus, Wls binds Wnt and helps its delivery to the cell surface by an unknown mechanism. The efficient secretion of Wnt may involve Wnt oligomerization and its association with lipoprotein particles. (**c**) Secreted Wnt is enriched in Reggie-1/Flotillin-2 containing lipid rafts and can be internalized into endocytic vesicles dependent or independent from the endocytosis of Wls, glypican, and lipoprotein. (**d**) Internalized Wnt may be rerouted for basolateral secretion. (**e**) After dissociation with Wnt, Wls, otherwise degraded in the lysosome, is recycled by the retromer complex to the trans-Golgi network. (**f**) Wnt after secretion moves in the extracellular space possibly in the form of Wnt oligomers and/or Wnt–lipoprotein complex. (**g**) The cell surface glypicans facilitate the restricted diffusion and gradient formation of Wnt across a field of cells. (**h**) When captured by Frizzled and the coreceptor LRP, Wnt induces a series of signaling events in the receiving cells in a concentration-dependent manner

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