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Post-translational modifications and secretion of Wnt proteins

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Abstract

Wnts are secreted lipid-modified glycoproteins in most mammalian genomes. Wnt ligands are the essential components of Wnt signaling pathways. Before binding to specific receptors, Wnt proteins experienced post-translational modification of acylation and N-glycosylation mediated by acyltransferase porcupine. Mature Wnts are transported from the Golgi to plasma membrane under association with Wntless. Retromer complex, P24 and other molecules are all involved in the secretion and release of Wnts. In this review, we provide an update of the post-translational modification, secretion and release of Wnts.

Keywords: Wnts; Acylation; N glycosylation; Wntless; Retromer

Introduction

Wnt proteins are a family of cysteine-rich secreted glycoproteins of approximately 350-400 amino acids in length [1]. Averagely, Wnts contains several charged residues and 23 -25 cysteines, some of them are associated with the formation of inter- and intra-molecular disulfide bonds that stabilize proper Wnt folding and multimerization [2,3]. Till now, 19 mammalian Wnt proteins have been identified to fall into 12 conserved Wnt subfamilies. By binding to 10 different Frizzled receptor and other co-receptors, Wnt starts its cellular signal transduction. Wnt signaling transduction is essential in embryonic development, cell proliferation, cell migration, cell fate specification, and axis patterning. In the past decades, great achievement has been made in the Wnt and Wnt signaling transduction, initial from mouse and Drosophila model study. We review the progress of post-translational modifications of Wnts in the endoplasmic reticulum (ER) and Golgi apparatus, and further secretion of Wnt proteins.

Post-Translational Modifications of Wnt Proteins

Wnt proteins secreted from cells are post-translationally modified before transporting into the extracellular space. Amino-terminal signal peptide guides Wnt proteins to the ER, where Wnts undergo a series of post-transcriptional modifications in the secretory pathway. Post-translational acylation and N-glycosylation is observed in all Wnt proteins, with exception of Drosophila WntD [4]. They are independent modification and N-glycosylation precedes palmitoylation [5,6]. Both acylation and N-glycosylation of Wnt proteins are mediated by acyltransferase porcupine, which is localized at ER [4,7,8].

Acylation modification is reported to be vital for the secretion of Wnt proteins and for Wnt binding to the co-receptor Frizzled, the chaperone Wntless (Wls) and signaling activity [3,7,9,10]. Initially, two conserved residues of fatty acylation are identified. One is cysteine residue 77(murine Wnt3a), where palmitate is attached through a thioester linkage. The other was serine residue 209 (murine Wnt3a), which is modified by a monounsaturated fatty acid, palmitoleic acid [7,11,12]. Wnt3a mutant with serine to alanine at residue of 209 is retained in the ER and secretion is blocked [11]. XWnt8 structures support that only conserved serine 229 (murine Wnt3a) is acylated. Cys77 participate in the formation of disulfide bond with a second conserved cysteine residue [3]. Mono-acylation is further proved by the lack of Cys77 palmitoylation study [13,14]. Palmitoyleolation process of Wnt proteins is reversible and it can be removed by the serine hydrolase of NOTUM specifically [15,16].

The role of N-glycosylation is unclear, usually, it influences secretion of Wnt proteins, but not folding and structure [4]. There

are no conserved N-glycosylation sites in different Wnt members [17]. Wg protein has two known N-glycosylation sites of Asn103 and Asn414, Wg mutant can also activate downstream signaling in both autocrine and paracrine signaling, though secretion ability is reduced [17]. Meanwhile, Loss of N-glycosylation of Wnt1 impairs paracrine signaling. For Wnt3a and Wnt5a, N-glycosylation is required for secretion, but not for their activities [6,18].

There are also other post-translational modifications identified in Wnt proteins. Tyrosine sulfation of Wnt5a and Wnt11 is essential for the formation of Wnt5a/Wnt11 complexes, which induce the efficient signaling in the initiation of Xenopus axis formation [19]. Wnt1 are attached to glycosylphosphatidylinositol (GPI) anchor on the leaflet of the plasma membrane (PM) by the glycolipid tail. PGAP1 gene participate this modification by creating a hydrophobic Wnt1 that is retained in the ER [20].

Secretion and Release of Wnt Proteins

Mature Wnt proteins are transported from the Golgi to the PM for secretion by the conserved multi-pass transmembranse Wls receptor (GPR177 in mammals) after post-translational modification [21]. Wnt secretion is Wls-dependent; it cannot proceed with the absence of Wls [22]; While other signaling proteins are not influenced by the removal of Wls [23-25]. Wls has a carboxy-terminal ER-targeting signal, which guiding Wls localizing predominantly in the ER, where it binds with acylated Wnt proteins [9,26]. Then, Wnts-Wls complex transports from ER to PM in the presence of COPII vesicles. Wnt is released and binds to lipoprotein particles or heparin sulpate proteoglycans (HSPGs) once Wnts-Wls complex arriving at the PM [27,28]. However, other theory supports that Wnts-Wls complex keep together and internalized at PM and dissociate from each other in endosomes. Then, Wnts are released through a recycling endosomal pathway and Wls is transported back to trans-Golgi network (TGN) though a retromer-dependent pathway [28-30]. Endocytosed Wls progresses to multivesicular bodies (MVBs) and lysosomes for degradation [31].

Retromer is a complex, consisting of Vps35, Vps29, Vps26, Vps10, Vps5, and Vps17 in yeast [32-34]. Vps35, Vps29 and Vps26 subcomplex serve as cargo recognition and retrieve Vps10 from endosomes to the Golgi [33]. Vps5 and Vps17 are membrane-bound subcomplexs, they are sorting nexins (SNX) with a phosphoinositide-binding SNX-phox homology (SNX-PX) domain [35]. Nexins SNX1, SNX2, SNX5 and SNX6 are SNX-BAR coat complex interacting with cargo-selective Vps35-Vps29-Vps26 complex. They are needed for most of the retromer cargo proteins [35,36]. SNX3 co-interact with Wls and Vps26 on early endosomes and help the association of the cargo-selective complex to Wls. Wls recycling relies on SNX3 specifically [36,37]. Wls recycled in Golgi is further retrograde transport to ER in a COPI- dependent way and require ER-Golgi intermediate compartment ERGIC2 [26,38].

The mechanisms of Wnt secretion and release are complex, more and more molecules are discovered to be related with them. P24 protein family, which acts as cargo receptor for Wnt in the early secretory pathway, is important for proper export of Wg from the ER [39-41]. Sec22 is associated with both Wg and p24 during the early secretory phase of Wg [41]. MiR-307a inhibits Wg secretion through targeting Wls, its overexpression induces ER stress specifically in the Wg-expressing cells [38]. Wnts are classic morphogens. They are vital for tissue patterning by activating their target genes in a concentration-dependent manner [13,42]. Secreted Wnts associate to specific receptors on target cells to activate canonical Wnt/β-catenin pathway, or Wnt/Ca2+, Wnt/ planar cell polarity and other non-canonical pathways.

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