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WNKs in Wnt/β-catenin signaling

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Wnt growth factors signal through either the canonical Wnt (Wg)-Frizzled (Fz)/β-catenin-dependent pathway or through non-canonical Wnt pathways, such as the Wnt/Fz-planar cellular polarity (PCP) pathway. These 2 pathways are evolutionarily highly conserved from invertebrates to humans. Canonical Wnt/β-catenin signaling is essential for many aspects of development. In vertebrates, it controls the specification of the embryonic dorsal–ventral (D–V) axis, cell proliferation, maintenance of stem cells, and vascularization. Aberrant canonical Wnt signaling causes detrimental developmental defects and a variety of cancers.[1,2] Thus, precise regulation of the canonical Wnt/β-catenin signaling components is crucial for development and tissue homeostasis.

During (canonical) Wnt signaling, a Wnt (Wingless, Wg in *Drosophila*) signals to a Frizzled (Fz) receptor and co-receptors LRP5/6 (Arrow in *Drosophila*), which leads to the inhibition of the degradation complex composed of the scaffolding protein Axin, the tumor suppressor *adenomatous polyposis coli* gene product (APC), and GSK3β, and thus allows β-catenin to enter the nucleus and activate transcription. Upon Wnt pathway activation, Dsh (Dvl in vertebrates), a scaffold protein downstream of Fz, is transiently recruited to the membrane and becomes hyperphosphorylated. Utilizing Dsh phosphorylation as a Wnt signaling readout, we identified the *Drosophila* Wnk (with no Lysine [K]) kinase homolog, as a positive regulator of Wnt signaling.

Wnk kinases are known for their role in the regulation of the major sodium (NCC, Na–Cl cotransporter; SLC12A) and potassium (ROMK, renal outer medullary K^+ channel) transporters in the distal nephron of the kidney. Mutations in WNK1 and WNK4 cause Gordon syndrome (a.k.a., familial hyperkalemic hypertension [FHHt], or pseudohypoaldosteronism type II), characterized by acidosis, hyperkalemia, and hypertension.[3] More recently WNK1 was linked to hereditary sensory and autonomous neuropathy type II (HSANII; ref. 4). Developmental functions of Wnks are, however, only emerging.

We recently showed that Wnk is required for peak levels of Wnt signaling during wing development in *Drosophila*.[5] Depletion of Wnk by RNAi or in homozygous mutant tissue led to canonical Wnt signaling-like phenotypes such as wing margin and margin bristle defects. Consistenly, we also established that expression of the high-threshold, direct Wnt target Senseless was reduced or lost in tissue lacking *wnk*. Furthermore, reduction of *wnk* activity suppresses cell death induced by overactivation of Wnt signaling (i.e., *sevenless*-driven Dsh overexpression) in the eye. Similarly, extra wing bristles induced by overactivation of canonical Wnt signaling in the wing (via overexpression of dFz2 [*dpp* > *dFz2*]) are suppressed by reducing *wnk* activity. Conversely, concomitant overexpression of dFz2 and Wnk led to a significant increase in the number of ectopic margin bristles.

We were able to identify a similar function of Wnks in cultured human cells. siRNA-mediated knockdown of WNK1 or WNK2 significantly reduced the activity of a TOPFlash Wnt-signaling reporter as well as the levels of stabilized β-catenin in HEK293T cells. On the other hand, transfection of WNK2 stimulated Wnt3a activation of TOPFlash in a dose- and kinase activity-dependent manner. Taken together with the *Drosophila* in vivo and epistasis experiments, these findings imply that WNKs play a conserved positive role in regulating canonical Wnt/β-catenin signaling.

How does Wnk affect Wnt signaling? Our epistasis data suggest that Wnk acts downstream of the Wg ligand, but upstream or at the level of Dsh. In mammals, Wnks are known to phosphorylate ion channels directly or via the intermediate kinases SPAK and OSR1 (STE20/SPS1-related proline–alanine-rich and oxidative stress-responsive protein type 1 kinase) in order to regulate ion homeostasis. Indeed, we and others showed that constitutively active *Drosophila* Wnk is able to phosphorylate Fray, the *Drosophila* OSR1/SPAK homolog, in vitro.[5,6] Knockdown of *fray*, in turn, also resulted in a reduction of Sens expression and in loss of wing margin bristles in vivo, suggesting that Wnk may exert its regulatory function via Fray (**Fig. 1**). This effect of Fray on canonical signaling appears to be conserved in humans as siRNA-mediated knockdown of SPAK and OSR1 also reduced Wnt reporter activity in cell culture.[5]

Whether Fray/OSR1/SPAK can directly phosphorylate Wnt pathway components or whether the regulation occurs via changes in ion transport remains to be determined (**Fig. 1**). Although NKCC and KCC channels function in a charge-neutral way, proton pumps have been shown to affect Wnt signaling. Alternatively, Wnks have been shown to alter the localization of some of their targets. For instance, it has been reported that NCC trafficking to the plasma membrane is affected

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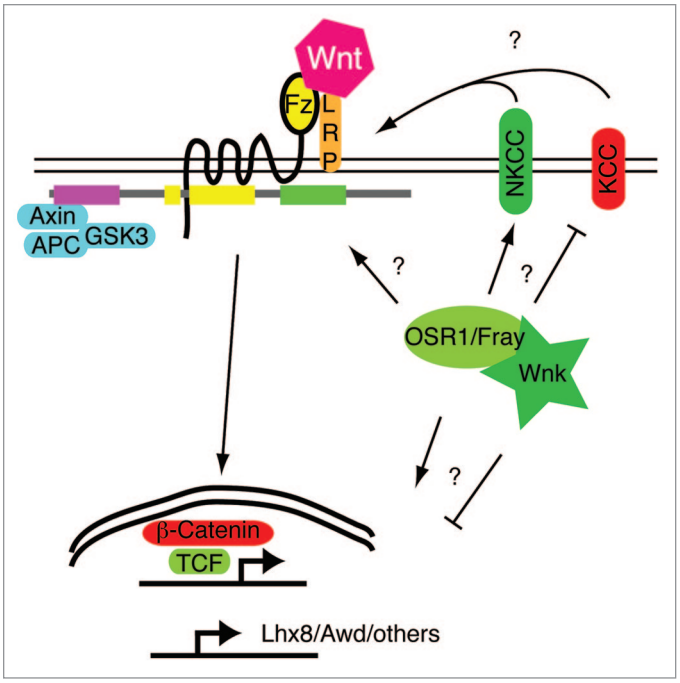

Figure 1. Models of Wnk regulating canonical Wnt signaling. Wnk, via Fray/OSR1/SPAK, could either lead to phosphorylation of Wnt signaling components such as Fz, Lrp, or Dsh or alter their localization/transport. Alternatively, the effect of Wnk on Wnt signaling could be meditated via the regulation of ion channels such as NKCC or KCC. See text for details.

when WNK4 diverts it for lysosomal degradation.[7] Although this process does not involve SPAK and OSR1, we cannot exclude that OSR1/SPAK/Fray could affect the stability or localization of Wnt signaling components.

Recently, Wnks have also been shown to regulate transcription of target genes. In both fly and mouse neural development, Wnks, together with OSR1, regulate the expression of the LIM-Homeobox transcription factor Arrowhead/Lhx8[6] (**Fig. 1**), whereas during zebrafish lateral line development, Wnk1b represses the KCC2 transporter.[8] Together, these findings raise interesting questions regarding the role of Wnk kinases during development, and future work will have to elucidate the exact mechanism by which Wnk modulates Wnt signaling and possibly other pathways.

References

1. Clevers H. Cell 2006; 127:469-80; PMID:17081971; http://dx.doi.org/10.1016/j.cell.2006.10.018
2. Swarup S, et al. Cold Spring Harb Perspect Biol 2012; 4; http://dx.doi.org/10.1101/cshperspect.a007930
3. Kahle KT, et al. Annu Rev Physiol 2008; 70:329-55; PMID:17961084; http://dx.doi.org/10.1146/annurev.physiol.70.113006.100651
4. Shekarabi M, et al. J Clin Invest 2008; 118:2496-505; PMID:18521183
5. Serysheva E, et al. EMBO Rep 2013; 14:718-25; PMID:23797875; http://dx.doi.org/10.1038/embor.2013.88
6. Sato A, et al. PLoS One 2013; 8:e55301; PMID:23383144; http://dx.doi.org/10.1371/journal.pone.0055301
7. Zhou B, et al. J Am Soc Nephrol 2010; 21:82-92; PMID:19875813; http://dx.doi.org/10.1681/ASN.2008121275
8. Bercier V, et al. PLoS Genet 2013; 9:e1003124; PMID:23300475; http://dx.doi.org/10.1371/journal.pgen.1003124