Comment

Where Notch and Wnt Signaling Meet: The Presenilin Hub

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The presenilins (PSs)[1] are part of the molecular machinery responsible for cleaving proteins like the β-amyloid precursor protein (APP) and Notch in the plane of the membrane (Annaert and De Strooper, 1999). Mutations in PS1 are one of the major causes of familiar Alzheimer's disease. PSs are also involved in regulating the Wnt/β-catenin signaling pathway, but how exactly remains a highly controversial issue (for full discussion see www.alzforum.org/members/forums/journal/catenin/index.html). In this issue, Soriano et al. (2001) provide results to tip the scales definitively in favor of the concept that PS1 operates as a negative regulator of the Wnt/β-catenin signaling pathway.

β-catenin, neural plakophilin related armadillo protein (NPRAP, also called δ-catenin), and p0071 previously were known to bind to the large cytoplasmic loop domain of PS1 (see www.alzforum.org/members/forums/journal/catenin/index.html). These proteins are all characterized by repeats of the armadillo motif, a 42 amino acid sequence involved in protein–protein interactions, however, only the function of β-catenin has been fairly well established. One pool of β-catenin is bound at the cell membrane to the cell adhesion molecule E-cadherin and provides a link to the actin cytoskeleton. A second pool is located in the cytoplasm in complex with axin, adenomatous polyposis coli (APC), and glycogen-synthase-kinase-3β (GSK-3β). Phosphorylation of β-catenin by GSK-3β promotes its binding to the β-transducing repeat-containing protein (β-TrCP), an F-box protein, and part of the E2/E3 ubiquitin ligase complex (Maniatis, 1999). Upon ubiquitination, β-catenin becomes rapidly degraded by the proteasome (Fig. 1). Binding of the soluble ligand Wnt to the membrane receptor frizzled inhibits GSK-3β via a pathway involving casein kinases and the protein dishevelled. This results in the accumulation of unphosphorylated β-catenin in the cytoplasm and its subsequent translocation to the cell nucleus. Here, β-catenin binds to members of the LEF/TCF (T cell-specific transcription factor 1) family of transcriptional regulators and activates Wnt target genes like cyclin D1, c-myc, and metalloproteases. Thus, a finely tuned balance between the three β-catenin pools at the cell membrane, in the cytoplasm, and in the nucleus, determines the final outcome of the Wnt signaling pathway (Fig. 1, for a detailed overview see www.stanford.edu/~rnusse/wntwindow.html).

However, when the functional relevance of the PS-β-catenin interaction was investigated, contradictory results were obtained. PS1 appeared to either stabilize (Zhang et al., 1998) or destabilize β-catenin (Kang et al., 1999). Remarkably, clinical PS mutations that cause Alzheimer's disease also affected β-catenin stability positively or negatively. Given these contradictory results it remains unclear to what extent these observations contribute to our understanding of the disease (see www.alzforum.org/members/forums/journal/catenin/index.html). In an attempt to avoid methodological confusion, Soriano et al. (2001) have been careful not to overexpress proteins, to use primary cultures (and not large T-transformed cultures) of PS1 knockout fibroblasts, to apply rigorous controls, and to use several functional assays to make their point. They convincingly demonstrate that β-catenin accumulates in the cytoplasmic pool and LEF/TCF gene transcription becomes activated in the absence of PS1. Their conclusion that presenilin is a negative regulator of the Wnt/β-catenin pathway gains considerable support from recent in vivo studies in *Drosophila melanogaster*, showing that an accumulation of armadillo (the *Drosophila* homologue of β-catenin) was observed in PS-deficient blastoderm embryos (Noll et al., 2000). A genetic screen for suppressors of the armadillo phenotype yielded the *Drosophila* PS (Cox et al., 2000).

An important question is how PS1 affects β-catenin stability. Since PS1 can bind GSK-3β, it is tempting to speculate that it provides a scaffold for the phosphorylation of β-catenin (Kang et al., 1999). Interestingly, the β-catenin interaction site on PS1 contains a consensus sequence for phosphorylation by GSK-3β (Kirchenbaum et al., 2000), suggesting that this interaction is regulated as is the interaction of β-catenin with the axin/APC/GSK-3β complex. However, from the data of Soriano et al. (2001), it appears that PS1 is not needed for the phosphorylation of β-cate-

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[1]*Abbreviations used in this paper:* β-TRCP, β-transducing repeats containing protein; APC, adenomatous polyposis coli; APP, β-amyloid precursor protein; GSK, glycogen synthase kinase; NICD, Notch intracellular domain; PS, presenilin; TCF, T cell-specific transcription factor 1.

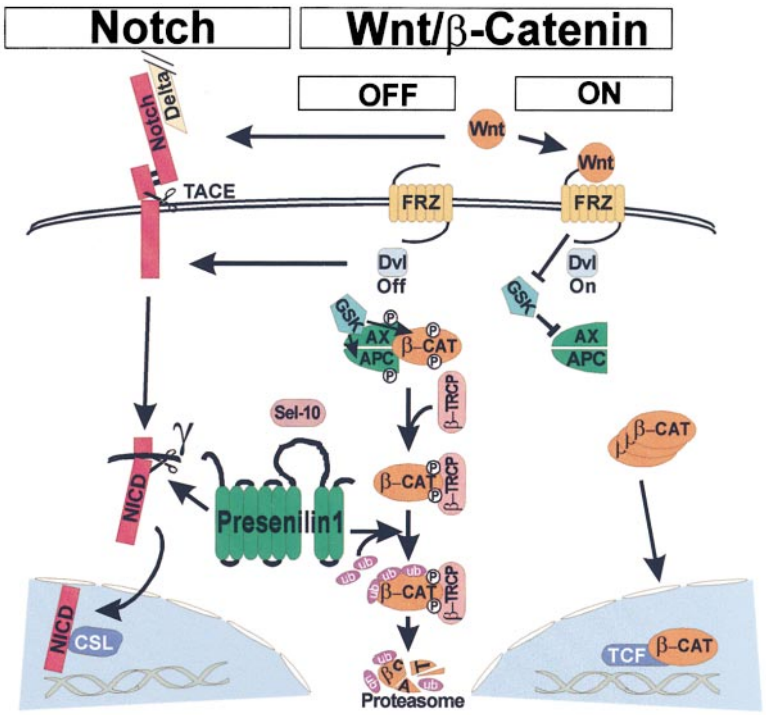

Figure 1. Presenilin in the Notch and Wnt signaling pathways. Schematic outline of both signaling pathways. The horizontal arrows indicate possible (negative) cross-talk. β-CAT, β-catenin; β-TRCP, β-transducing repeats containing protein; APC, adenomatous polyposis coli; AX, axin; CSL, CBF1, Su(H), Lag(1); Dvl, Disheveled; FRZ, frizzled; GSK, glycogen synthase kinase; NICD, Notch intracellular domain; TCF, T cell-specific transcription factor 1; Ub, ubiquitin. For further details see text.

nin since phosphorylated β-catenin species accumulate in PS1 deficient cells. Furthermore, in the presence of the proteasome inhibitor MG132, fewer ubiquitinated β-catenin species are observed in PS1 deficient cells than in wild-type cells (Soriano et al., 2001). Instead, these results suggest that PS1 is needed for the ubiquitination of β-catenin (but see Noll et al., 2000) and raise intriguing questions concerning the relationship of PS1 with the ubiquitin ligase machinery and the proteasome (Fig. 1).

PS1 is also involved in the Notch 1 signaling pathway. Notch 1 belongs to a family of large type I integral membrane receptors involved in cell fate decisions. Upon ligand binding, Notch becomes proteolytically processed. Its intracellular domain translocates to the nucleus where it binds to transcription regulators of the CSL family and induces the expression of Notch target genes (Fig. 1). PS1 functions in the proteolytic complex needed to generate the intramembranous cleavage of Notch, not unlike the cleavage of APP (Kopan and Goate, 2000). The embryonic lethal phenotype of the mice in which the PS1 genes have been inactivated can largely be explained by deficiencies in the Notch signaling system and therefore the physiological relevance of the β-catenin/PS1 connection in mammals remained somewhat speculative. However, as discussed in Soriano et al. (2001), mice that specifically lack expression of PS1 in the skin have been generated (Zheng, H., unpublished results). These mice develop a spectrum of skin tumors, suggestive of increased β-catenin activity and the concept that PS1 may act as a repressor of this pathway in adulthood.

Inhibitory cross-talk between the Notch and the Wnt signaling pathways previously was thought to occur at the level of the Notch extracellular domain binding to Wnt (Wesley, 1999), and at the level of the Notch intracellular domain binding to disheveled (Axelrod et al., 1996). PS1 seems now to provide a third level where such interaction could theoretically occur. Deleting the cytoplasmic loop domain of PS1 annihilates its binding with β-catenin, whereas the proteolytic cleavage of Notch and APP is maintained (Saura et al., 2000; Soriano et al., 2001). The function of PS1 in β-catenin turnover can thus be separated from its function in γ-secretase processing. As in many biological questions, the worm *Caenorhabditis elegans* could shed some light on this matter. Wu et al. (1998) provided evidence that a protein encoded by *sel-10*, a suppressor of the Notch signaling pathway, associates functionally and physically with SEL-12, the worm's homologue of human PS. SEL10 is related to the CDC4 family of F box/WD40 repeat containing proteins. These proteins are part of the E3-enzymes of the ubiquitin-ligation pathway and appear to function as adapters that recruit target proteins to a complex containing the E2–ubiquitin-conjugating enzyme (Maniatis, 1999). More than 30 mammalian homologues including β-TrCP have been identified (Winston et al., 1999). The challenge now is to find out whether PS1 provides a scaffold for β-TrCP or other members of the F-box protein family and ubiquitin-ligase substrates like β-catenin. It also will be important to establish whether the current findings can be extended to other proteins that bind to PS, e.g., NPRAP and p0071. In conclusion, PS1 is involved in at least two proteolytic machines regulating signal transduction cascades: regulated intramembrane proteolysis of Notch and APP on one hand (Brown et al., 2000), and regulated protein degradation of β-catenin in the Wnt signaling pathway on the other (Noll et al., 2000; Soriano et al., 2001).

We apologize to our colleagues for not being able to cite all relevant work because of editorial constraints.

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