Another critically important finding of this study is that c-IAP1 was shown to function in oncogenesis through a mechanism which does not appear to involve direct apoptotic inhibition or direct interaction of the IAP with caspases. It is possible that while c-IAP1 might not function as a caspase inhibitor in the classical, biochemical sense, it might be able to target caspases for polyubiquitination, which would ultimately achieve the same effect. Additionally, recent reports have shown that c-IAP1 can ubiquitinate other IAPs, and can also function as a modulator of the signaling pathways controlling the NF-κB transcription factor pathways by directing the ubiquitination of key signaling intermediates such as NIK (Vince et al., 2007; Varfolomeev et al., 2007), RIP (Petersen et al., 2007) and NEMO/IKKγ (Tang et al., 2003), and so collectively these studies underscore the biological importance of IAPs in diverse cellular processes.

While these and other reports are beginning to shed light on the types of substrates that can be recognized and targeted by the RING-containing IAPs, we still know very little about how and when the ligase interacts with the substrate. The key finding from over a decade ago that c-IAP1 is recruited to the type 2 tumor necrosis factor through its interaction with TRAF proteins (Rothe et al., 1995) strongly suggests a predominantly cytosolic role as a signal transduction intermediate, yet the study by Xu and coworkers reveals both a nuclear and cytosolic distribution of c-IAP1 and suggests that the nuclear pool is likely responsible for the targeting of MAD1. So we have much to learn about how the IAPs traffic intracellularly, how they meet their targets, and how their ubiquitin ligase activities are triggered. Nevertheless, the identification of the targets of these E3 ligases is an essential first step in understanding the regulatory roles of the IAPs normally, as well as in disease states in which the activities of these enigmatic proteins are deregulated.

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Retromer Retrieves Wntless

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Wntless is a sorting receptor required for Wnt secretion. Wntless is retrieved from endosomes to the Golgi by retromer, permitting Wntless reutilization in Wnt transport. In the absence of retromer, Wntless is degraded in lysosomes and Wnt secretion is impaired.

The Wnt family of secreted signaling proteins patterns a huge variety of developing tissues throughout metazoa. In this issue, and in an upcoming issue of *Nature Cell Biology*, work from five different labs addresses the mechanism by which Wntless and the retromer complex work together to promote secretion of Wnt family proteins (Belenkaya et al., 2008; Franch-Marro et al., 2008; Pan et al., 2008; Port et al., 2008; Yang et al., 2008).

The conserved requirement for the multiple pass transmembrane protein Wntless in Wnt secretion was first reported in 2006 by multiple groups working with *Drosophila melanogaster, Caenorhabditis elegans*, and human tissue culture cells (reviewed in Hausmann et al., 2007). Wntless has been variously called Evenness Interrupted (Evi) and Sprinter in *Drosophila* and MOM-3/MIG-14 in *C. elegans*; here, I will refer to it as Wntless for the sake of clarity. In Wntless mutants, Wnts accumulate inside the cells that make them and both surface delivery and secretion are impaired. Wnt secretion appears to be the only nonredundant function for Wntless; *Drosophila wntless* mutants, which do not secrete normal levels of the *Drosophila* Wnt Wingless (Wg), can be rescued to viability by *wntless* expression in Wingless-producing cells.

Retromer was first identified in yeast as being important for efficient trafficking of lysosomal hydrolases to the vacuole.

Its substituents, Vps35, Vps29, Vps26, Vps5, and Vps17, form a multiprotein complex that retrieves the sorting receptor Vps10 from endosomes back to the Golgi. This allows reuse of Vps10 for multiple rounds of lysosomal hydrolase delivery. Retromer function is conserved in mammalian cells, where it mediates endosome-to-Golgi retrieval of the mannose-6-phosphage receptor (reviewed in Seaman, 2005). Despite the importance of retromer in such a basic aspect of cell biology, manipulations of Vps35 or Vps26 were reported to produce rather restricted phenotypes that suggested loss of Wnt signaling (reviewed in Hausmann et al., 2007).

The similarity of the *Wntless* and *Vps35* mutant phenotypes raised the compelling possibility that the proteins they encoded were part of the same machinery for Wnt secretion. With these two pieces of the puzzle available, there has been a race to understand how they are connected. Two of the groups whose work is published this month have focused on Wnt secretion in the nematode worm *C. elegans* (Pan et al., 2008; Yang et al., 2008), and three others have examined secretion of Wingless (Wg), a *Drosophila* Wnt protein, in developing wing discs (Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008). In many cases, human tissue culture cells have also been exploited for the clear visibility of intracellular membrane compartments and accessibility to cell biological manipulations (Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008; Yang et al., 2008).

The work reported in these five papers clearly confirms the importance of retromer function in Wnt secretion and shows that this role is conserved for a wide variety of Wnts in *Drosophila, C. elegans*, and human cells. Each group now conclusively demonstrates that retromer functions primarily to maintain Wntless levels. In all three systems, knockdown or mutation of Vps35 destabilizes Wntless, and Wnt signaling in Vps35 mutants is rescued by Wntless overexpression. To investigate how retromer stabilizes Wntless, all five groups have followed its trafficking in normal cells and in cells in which either the endocytic machinery or retromer function has been perturbed. Taken together, their results show that retromer maintains Wntless protein levels by inhibiting its delivery to the lysosomes and promoting delivery to the Golgi. Wntless is normally found both in the Golgi and in endocytic compartments. Mutation or RNAi-mediated knock-down of Rab5 or dynamin relocalizes Wntless to the plasma membrane (Belenkaya et al., 2008; Pan et al., 2008) and increases Wntless protein levels (Yang et al., 2008), suggesting that a fraction of internalized Wntless is normally degraded after internalization. However, some Wntless escapes this fate and is returned to the Golgi apparatus by a mechanism that depends on Vps35. Two groups have shown that incubation of tissue culture cells with antibodies to tagged Wntless results in delivery of the antibodies to the Golgi apparatus; this Golgi delivery can be blocked by RNAi-mediated knock-down of Vps35 (Belenkaya et al., 2008; Franch-Marro et al., 2008). Loss of Vps35 causes a corresponding increase in Wntless accumulation in lysosomes (Franch-Marro et al., 2008; Pan et al., 2008; Yang et al., 2008). Interestingly, Wntless protein stability is most sensitive to the loss of Vps35 in Wg-expressing cells (Port et al., 2008). This suggests that Wntless cycles through endosomes more frequently in these cells and may indicate that Wntless moves with Wg to the plasma membrane. The finding that Wntless and Vps35 can be coimmunoprecipitated (Belenkaya et al., 2008; Franch-Marro et al., 2008) suggests that Vps35 may act directly to promote incorporation of Wntless into vesicles destined for the Golgi. Thus, the function of retromer in Wntless trafficking is similar to its function in trafficking of sorting receptors for lysosomal hydrolases; it retrieves Wntless from endosomes before they mature into lysosomes and delivers it to the Golgi. In sum, the work of these five groups provides strong support for the idea that retromer promotes Wnt secretion by ensuring the retrieval and reutilization of its sorting receptor, Wntless (Figure 1).

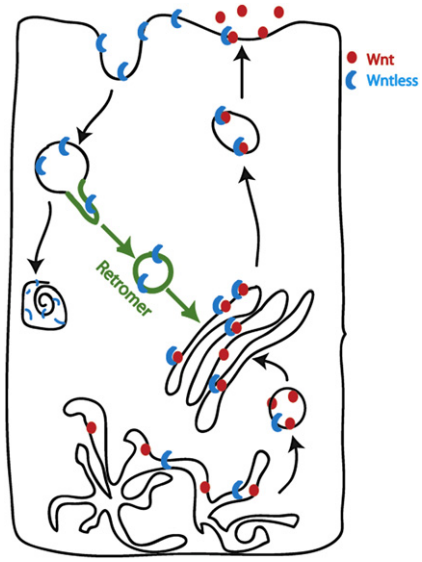

Figure 1. A Model for Wntless and Retromer Action

Wntless (blue) accompanies Wnt proteins (red), promoting their delivery from the Golgi to the cell surface. Wntless is then internalized into endosomes and either degraded in lysosomes or retrieved by the retromer complex (green) for delivery to the Golgi apparatus. Retromer-mediated trafficking of Wntless from endosomes to Golgi allows Wntless to be used many times for Wnt transport.

Is Wntless/retromer-dependent secretion the only mechanism by which Wnt proteins can leave the cell? It was originally suggested that long-range Wnt signaling was more sensitive to loss of retromer function than was short-range signaling, raising the possibility that Wnt proteins might be released by different mechanisms (Coudreuse et al., 2006). However, further studies in *C. elegans* clearly show a role for retromer in short-range Wnt signaling as well (Pan et al., 2008). In fact, in the *Drosophila* wing disc, it is short-range Wg signaling that is most strongly affected by loss of retromer function. Transcription of the long-range target Distalless is not affected by mutation of either Wntless or retromer (Franch-Marro et al., 2008; Port et al., 2008). This might suggest that an alternative pathway exists for secreting Wg with long-range activity. But it is equally plausible that residual, maternally contributed retromer proteins allow secretion of small amounts of Wg that suffice for Distalless transcription. Further experiments will be necessary to resolve this issue.

While these five papers have focused on the role of retromer in Wnt secretion, another recently published study of *Drosophila vps35* has shown that it also regulates Rac1-dependent actin polymerization, promotes endocytosis of a subset of membrane receptors, and downregulates both BMP signaling and signaling through MAP kinases (Korolchuk et al., 2007). Clearly, Vps35 influences cellular functions at many levels. Interestingly, however, there is no evidence that lysosomal degradation is weakened in Vps35

mutant animals. When endosome-to-Golgi retrieval of MPR is blocked by dominant-negative Rab9 expression, cells compensate by elevating the production of lysosomal hydrolases and by internalization of secreted hydrolases (Riederer et al., 1994). Similarly, these robust and redundant mechanisms probably insure that lysosomal function is unaffected by the mutation of retromer components. Trafficking of signaling molecules and their receptors appears to be more sensitive to perturbed retromer function. While no data yet suggests that cells normally modulate retromer-dependent trafficking to control cellular signaling pathways, this is an intriguing subject for future investigations.

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MIT Domainia

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The AAA ATPase Vps4 disassembles the membrane-bound ESCRT-III lattice. Four recent publications show how Vps4 carries out this task in a partnership with another ESCRT-associated protein, Vta1. Vps4 and Vta1 both contain MIT domains, which bind to "MIT-interacting motifs" (MIMs) of ESCRT-III proteins. As new MIT domain proteins are rapidly being identified, these studies will likely have relevance well beyond Vps4.

The ESCRT (endosomal sorting complexes required for transport) complexes and associated proteins constitute a fundamental membrane scission machine (Hurley and Emr, 2006). Conserved throughout eukaryotes, the ESCRT machinery directs membrane budding away from the cytosol. ESCRTs are required for the budding of intralumenal vesicles from the limiting membrane of endosomes to form multivesicular bodies (MVBs), for the budding of many enveloped viruses from the plasma membrane of animal cells, and for the membrane abscission step in cytokinesis. The upstream components of the system contain specific phosphoinositide-binding domains, as well as specific ubiquitin-binding domains, which probably serve to concentrate ubiquitinated cargo. The interaction between lipid- and ubiquitin-binding domains and their partners is a major theme underpinning the ESCRT field and begs the question of whether there are other themes in molecular interactions that the field has missed.

The upstream complexes are not thought to mediate membrane scission on their own. Rather, they recruit subunits of the ESCRT-III complex to the endosomal membrane, where they assemble into an insoluble array of poorly understood stoichiometry. The ESCRT-III array is disassembled by the ESCRT-associated ATPase Vps4, which, like many other AAA ATPases, forms a double hexameric ring. ATP hydrolysis is required for the disassembly, and Vps4 is essential for all identified ESCRT functions. Vps4 contains an N-terminal MIT (microtubule interacting and transport) domain and a C-terminal catalytic domain. ESCRT-III is the only component of the system known to form arrays on the membrane, whereas Vps4 is the only ATP-burning machine in the ESCRT pathway. Although there is almost no hard evidence that these proteins directly catalyze membrane scission, ESCRT-III and Vps4 are clearly at some level pivotal players in driving the membrane scission reaction. Thus an understanding of the interaction between Vps4 and ESCRT-III is a prerequisite for understanding the larger process of membrane scission.

MIT domains were first noted in a study of the sorting nexin SNX15 (Phillips et al., 2001) and subsequently identified in other trafficking proteins (Ciccarelli et al., 2003; Row et al., 2007; Tsang et al., 2006), many connected in some way to the ESCRT pathway. The MIT domain of Vps4 binds directly to a ~20 residue C-terminal