The Role of Ryk and Ror Receptor Tyrosine Kinases in Wnt Signal Transduction

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Receptor tyrosine kinases of the Ryk and Ror families were initially classified as orphan receptors because their ligands were unknown. They are now known to contain functional extracellular Wnt-binding domains and are implicated in Wnt-signal transduction in multiple species. Although their signaling mechanisms still remain to be resolved in detail, both Ryk and Ror control important developmental processes in different tissues. However, whereas many other Wnt-signaling responses affect cell proliferation and differentiation, Ryk and Ror are mostly associated with controlling processes that rely on the polarized migration of cells. Here we discuss what is currently known about the involvement of this exciting class of receptors in development and disease.

The role of the receptor tyrosine kinases Ror and Ryk in Wnt signaling should be considered within the larger group of Wnt signaling receptors. Historically, the Frizzled (FZD) and LRP5/6 molecules were the first proteins implicated as receptors for Wnt ligands (Clevers and Nusse 2012). FZD proteins consist of a seven-pass transmembrane portion and an extracellular cysteine-rich domain (CRD) (Bhanot et al. 1996). Wnt proteins bind with high affinity to the CRD in a fairly promiscuous way: One Wnt will bind to multiple FZDs and conversely, single FZDs can interact with multiple Wnts (Hsieh et al. 1999b; Carmon and Loose 2010). This lack of a high degree of specificity is also borne out by the structure of the Wnt-CRD complex, as recently established for the *Xenopus* Wnt8 protein in a complex with the Frizzled8 CRD. Of the two domains on Wnt that interact with the CRD, one contains a palmitoleic acid modification, presumably present on multiple Wnt proteins, projecting into a pocket in the Frizzled CRD (Janda et al. 2012).

FZD molecules work together with two transmembrane LRP family members, LRP5 and LRP6 in vertebrates (Pinson et al. 2000; Tamai et al. 2000), both homologs of the *Drosophila* Arrow protein (Wehrli et al. 2000). The current model is that a Wnt protein binds to

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FZD and LRP at the same time, forming a dimeric/multimeric structure. This may result in a conformational change in the receptor molecules, which leads to phosphorylation of the LRP cytoplasmic domain by associated protein kinases. Phosphorylation of the LRP tail (He et al. 2004) takes place on several clusters of serines and threonines, each containing a PPPSP motif. The protein kinases involved include GSK3 and CK1γ. GSK3 targets the PPPSP motif and phosphorylates a serine residue in that motif (Zeng et al. 2005). Residues adjacent to the PPPSP motif are phosphorylated by CK1γ, a CK1 family member with a membrane anchor in the form of a palmitoylation domain (Davidson et al. 2005). The phosphorylation of LRP leads to binding of the Axin protein to the cytoplasmic tail of LRP6 (Mao et al. 2001), an event that increases cytoplasmic levels of the signal transducer β-catenin, which subsequently translocates to the nucleus and induces gene expression in complex with TCF/LEF transcription factors. On the cytoplasmic side, Frizzled interacts with Dishevelled (Dsh) (Chen et al. 2003; Tauriello et al. 2012), which in turn may promote interactions with Axin through the DIX domain that these two proteins have in common (Schwarz-Romond et al. 2007; Fiedler et al. 2011). Although signaling via the FZD and LRP5/6 receptors has occupied our attention for many years, the identification of receptor tyrosine kinases (RTKs) as additional Wnt receptors has opened the door to new and exciting discoveries of Wnt signaling in development and disease.

Ror1 AND Ror2

Ror1 and Ror2 were first identified in PCR-based screens for molecules with resemblance to tyrosine kinases of the Trk family (Masiakowski and Carroll 1992). Indeed, although Ror1 and Ror2 occupy a separate corner in the RTK dendrogram, they are more closely related to Trk and Musk RTKs than to other RTK proteins. However, this conservation is largely restricted to their intracellular tyrosine kinase domains; their divergent extracellular domains suggested early on that Ror1 and Ror2 might interact with a distinct set of extracellular ligands.

A distinguishing feature in the extracellular portion of the receptors is the presence of a CRD domain that bears close homology with the Wnt-binding domain found in Frizzled transmembrane receptors as well as in secreted Wnt inhibitors of the SFRP family (Saldanha et al. 1998), signifying that Wnt proteins might be the elusive ligands for this class of receptors (Fig. 1). It was not until much later, however, that this was definitively shown to be the case (Oishi et al. 2003; Mikels and Nusse 2006). In addition to the CRD, Ror proteins are further characterized by extracellular Kringle and immunoglobulin domains, whose functions remain enigmatic to this day, and by intracellular proline-rich and serine-threonine-rich domains (Masiakowski and Carroll 1992).

Ror1 and Ror2 in Development

Ror receptors are evolutionarily conserved across vertebrate and invertebrate species. The genomes of most species are reported to harbor two homologs.

Flies, Worms, and Frogs

Two Ror homologs, named *Dror* and *Dnrk*, have been described in *Drosophila*, but apart from their restricted expression pattern in the developing nervous system, no functional role for these receptors has been reported (Wilson et al. 1993; Makino et al. 1997). *CAM-1*, the sole *Caenorhabditis elegans* Ror homolog, is widely expressed in the nervous system, where it functions to regulate neuronal migration, positioning, and neurite outgrowth (Forrester et al. 1999; Zinovyeva et al. 2008; Hayashi et al. 2009; Kennerdell et al. 2009; Song et al. 2010) as well as synaptic transmission (Francis et al. 2005). CAM-1 also regulates the polarity of epithelial cells and stem cells (Green et al. 2008a; Yamamoto et al. 2011). Functions in cell polarity and cell migration are conserved in *Xenopus*, where *XRor2* regulates polarized cell migrations known as convergent extension (Hikasa et al. 2002; Schambony and Wedlich 2007).

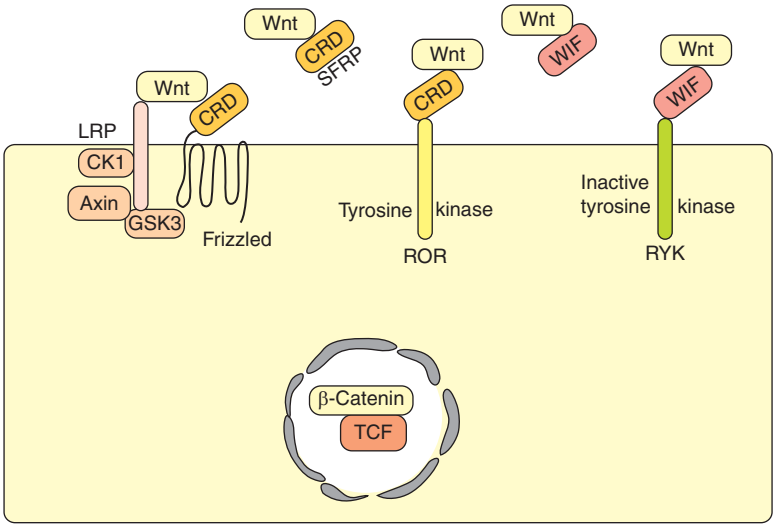

Figure 1. Schematic depicting the use of CRD and WIF domains in Wnt signal transduction. The mammalian genome encodes 19 different Wnts, which can mediate their signaling effects through 10 different FZDs that act in concert with the LRP5 and LRP6 coreceptors. The binding site for Wnt on Fzd is formed by the CRD. This motif is also used as the Wnt-binding site in members of the SFRP family of extracellular Wnt antagonists and in Ror1 and Ror2, both of which are members of the RTK family. A second Wnt-binding module, the so-called WIF domain, is used by extracellular Wnt antagonists of the WIF family, as well as by Ryk, another RTK family member. In spite of possessing functional Wnt-binding domains it remains unclear how Ror and Ryk receptors function in Wnt signal transduction. They have been proposed to function as stand-alone receptors, but also as coreceptors together with Fzd. In addition, they have been implicated in β-catenin-dependent and -independent signaling responses. At present, the molecular mechanisms used by these RTKs to transmit the Wnt-signal are ill understood. Only Ror2 has been shown to have a functional kinase domain, but both Ror1 and Ryk have been proposed to function as pseudokinases. See text for details.

Mammals

Based on the fact that *Ror1* and *Ror2* were first cloned from a human neuroblastoma cell line and the fact that expression of the *Drosophila* homologs appeared to be largely restricted to the developing nervous system, the initial characterization of the expression pattern of the mammalian Ror proteins was also performed in neural tissues (Oishi et al. 1999). Indeed, both Ror1 and Ror2 play a role in maintaining neural progenitor cell fate in the developing mouse brain (Endo et al. 2012). However, *Ror1* and *Ror2* are expressed with partially overlapping expression patterns in a broad range of tissues during mouse embryonic development, including the skeletal system and internal organs (Al-Shawi et al. 2001; Matsuda et al. 2001). The expression of *Ror2* appears to drop right before birth. In contrast, *Ror1* expression could be detected at the RNA level in a limited number of postnatal tissues, although its expression too drops significantly in the adult (Oishi et al. 1999).

Mice deficient for Ror2 are born with craniofacial abnormalities as well as significantly shortened limbs and tail (DeChiara et al. 2000; Takeuchi et al. 2000), suggesting an important role in skeletal development. The gross appearance of Ror2-deficient and Wnt5a-deficient mice is quite similar, although loss of Wnt5a results in more severe defects than Ror2 (Yamaguchi et al. 1999; Ho et al. 2012). It was in fact this observation that first prompted scientists to investigate a role for Wnt5a in Ror2 signaling (see below) (Oishi et al. 2003).

In humans, mutations in *Ror2* are associated with brachydactyly type B and recessive Rob-

inow syndrome, both of which result in limb malformations (Afzal et al. 2000; Oldridge et al. 2000; Afzal and Jeffery 2003). More recently, mutations in Wnt5a were also discovered in patients with autosomal-dominant Robinow syndrome (Person et al. 2010), suggesting that this signaling axis is disrupted in these patients.

In addition to skeletal malformations, Ror2-null mice have heart and lung defects (Takeuchi et al. 2000), a shortened intestine (Yamada et al. 2010), and aberrant inner ear hair cell orientation (Yamamoto et al. 2008). It should be noted, however, that some of these phenotypes might vary in penetrance and/or might be less pronounced in different mouse strains and/or on a different genetic background, as we have observed for the lung and inner ear defects in *Ror2*-knockout mice (RvA and RN).

The phenotype of mice deficient for *Ror1* is less severe than that of mice lacking *Ror2*. One study reported that these mice died within 24 hours after birth as a result of respiratory failure (Nomi et al. 2001), whereas another study reported more than half of these mice surviving until weaning, with a fair number of them reaching adulthood (Lyashenko et al. 2010). The additional loss of *Ror1* exacerbates the *Ror2*-mutant phenotype, as mice lacking both *Ror* homologs show enhanced skeletal and cardiac abnormalities (Nomi et al. 2001). Of note, it was recently reported that these initial *Ror1*-knockout mice might not represent true null mutants, as a trunctated Ror1 protein was still expressed from the targeted allele. Still, newly generated conditional *Ror1*- and *Ror2*-knockout mice largely recapitulate the previously described mutants and *Ror1*/*Ror2* double-knockout mice largely recapitulate the *Wnt5a*-null phenotype (Ho et al. 2012).

Ror1 and Ror2 in Cancer

Both Ror1 and Ror2 have been ascribed oncogenic functions in cancer cells. In particular, siRNA mediated knockdown of Ror1 was shown to induce apoptosis in Hela cells, showing its importance for cell survival (MacKeigan et al. 2005). Ror1 was subsequently found to be overexpressed in B-cell chronic lymphocytic leukemia (CLL), possibly as a result of enhanced Stat3 signaling (Baskar et al. 2008; Daneshmanesh et al. 2008; Li et al. 2010). It enhances cell viability and might therefore be an attractive candidate for targeted therapy (Fukuda et al. 2008; Hudecek et al. 2010; Yang et al. 2011; Baskar et al. 2012; Daneshmanesh et al. 2012). Its role might extend to other B-cell lymphomas, as a reduction in Ror1 expression affects viability of CLL as well as lymphoblastic leukemia (ALL) cells (Tyner et al. 2009; Choudhury et al. 2010; Dave et al. 2012). Elevated expression of Ror1 was also detected in mantle cell lymphomas (MCLs), marginal zone lymphoma (MZL), and nonhematopoietic malignancies (Barna et al. 2011; Gentile et al. 2011; Zhang et al. 2012).

High levels of Ror2 have been detected in osteosarcoma, melanoma, and renal cell carcinoma cell lines and a reduction in Ror2 expression was shown to decrease cell invasion and motility and extracellular matrix remodeling (Enomoto et al. 2009; Morioka et al. 2009; Wright et al. 2009; O'Connell et al. 2010). Furthermore, Ror2 was recently shown to be a novel prognostic biomarker and potential therapeutic target in patients with leiomyosarcomas or gastrointestinal stromal tumors (Edris et al. 2012). Whereas Ror2 is primarily implicated in cell polarity and migration, it has also been associated with cell survival and proliferation. For example, restoration of *Ror2* expression inhibited tumor cell proliferation in colon cancer, where *Ror2* gene expression is reportedly frequently silenced (Lara et al. 2010). Thus, Ror2 may have tumor-promoting as well as tumor-inhibiting activities depending on the cellular context.

Unfortunately, the biochemical activities of these receptors remain ill defined. As such, there is a large knowledge gap in our understanding of the processes that are controlled by Ror in both healthy and diseased tissues and the molecular mechanisms that underlie them.

Signaling Mechanisms and Effectors

Ror2 has been shown to have an active tyrosine kinase domain and to function as a genuine

RTK under at least some conditions (Masiakowski and Carroll 1992; Mikels et al. 2009). Like other RTKs, forced dimerization induces Ror2 tyrosine phosphorylation, whereas ligand binding can induce either tyrosine or serine/threonine phosphorylation (Liu et al. 2007; Yamamoto et al. 2007; Akbarzadeh et al. 2008; Liu et al. 2008; Mikels et al. 2009; Grumolato et al. 2010). In contrast, Ror1 was found to lack tyrosine kinase activity and most likely functions as a pseudokinase (Masiakowski and Carroll 1992; Gentile et al. 2011).

An alternative splice variant of Ror1, lacking both the extracellular and transmembrane domains, was identified in neural tissues and cancers (Reddy et al. 1996). This is interesting in light of a recent description that, similar to Ryk (see below), the Ror1 receptor might undergo cleavage resulting in a signaling portion that is directed to the nucleus by a signal located in the juxtamembrane domain (Tseng et al. 2010). The biological function of these truncated Ror proteins is unknown, although they have been reported to play a role in cell migration and remodeling of the actin cytoskeleton (Tseng et al. 2011).

It is widely appreciated that Wnt5A is a ligand for Ror2 (reviewed by Green et al. 2008b). At least one potential mechanism underlying Ror2 activation by Wnt5A was proposed recently by the finding that Wnt5A induces the formation of a complex between Ror2 and Frizzled, resulting in Ser/Thr phosphorylation of Ror2 and the recruitment of Dvl, Axin, and GSK3B, the same machinery that mediates the Wnt3A-induced phosphorylation of Lrp5/6 as discussed in the beginning of this article (Yamamoto et al. 2007; Grumolato et al. 2010). According to this model, Wnt3A and Wnt5A compete for binding to Frizzled. The identity of the Wnt ligand determines whether the Frizzled coreceptor will be Lrp5/6 or Ror2, thus dictating whether β-catenin-dependent or -independent signaling will be activated, respectively (Grumolato et al. 2010). In support of this model, Ror proteins have also been reported to bind to Frizzled receptors in *C. elegans*, *Xenopus*, and mammals (Oishi et al. 2003; Li et al. 2008; Nishita et al. 2010; Song et al. 2010). Accessory proteins may promote the use of one type of coreceptor over another. For example, the collagen triple-helix repeat containing protein 1 (Cthrc1) enhances the formation of a complex containing Frizzled and Ror2 (Yamamoto et al. 2008). It remains to be determined, however, whether Ror2 always functions as a coreceptor as proposed by this model.

It is well established that Wnt5a, unlike Wnt3a, has the capacity to inhibit β-catenin-dependent signaling. However, the mechanism underlying this inhibition remains controversial. Proposed explanations include competition with other Wnts for binding to Frizzled (Grumolato et al. 2010; Sato et al. 2010), down-regulation of β-catenin via the E3 ubiquitin ligase Siah2 (Topol et al. 2003), or inhibition downstream of β-catenin (Ishitani et al. 2003; Mikels and Nusse 2006; Verkaar et al. 2010). It has been reported that Wnt5A inhibits Wnt3A-induced β-catenin signaling via Ror2 (Mikels and Nusse 2006; Li et al. 2008); however, others report that Ror2 is not absolutely required for this inhibition (Grumolato et al. 2010; Sato et al. 2010; Ho et al. 2012). Elucidation of the relationship between Ror proteins and canonical Wnt signaling is further complicated by the ability of Ror proteins to modulate Wnt signaling independently of Wnt5A. In *C. elegans*, expression of the Ror extracellular domain (ECD) is sufficient to interfere with Wnt signaling by binding and sequestering Wnt ligands (Forrester et al. 2004; Green et al. 2007). Although a similar sequestration function has not been clearly shown for vertebrate Rors, ectopic expression of a membrane-tethered ECD modulates Wnt signaling in various vertebrate contexts (Hikasa et al. 2002; Nishita et al. 2010). Reports that Ror2 can potentiate canonical Wnt signaling further cloud the issue, although these reports are thus far limited to overexpression studies (Billiard et al. 2005; Li et al. 2008).

In contrast to Ror2, the function of Ror1 in mediating Wnt signals has been explored in very limited detail. Moreover, although Wnt5A has been reported to bind to Ror1 in vitro (Fukuda et al. 2008), Ror1 has also been implicated in various non-Wnt responses. For instance, Ror1 was shown to interact with and be inhibited by

Resistin, a cysteine-rich protein produced by mature adipocytes (Sanchez-Solana et al. 2012). As indicated, Ror1 is generally presumed to be a pseudokinase, its tyrosine phosphorylation either undetectable (Bicocca et al. 2012) or because of transphosphorylation by other kinases (Gentile et al. 2011).

The downstream effector mechanisms of Ror1 remain ill defined. For instance, in B-cell malignancies, cross talk between Ror1 and the pre-B-cell receptor complex has been reported, with Ror1 activating small GTPases to promote survival signaling (Bicocca et al. 2012). In contrast, a dual function for Ror1 was shown in lung adenocarcinoma (Yamaguchi et al. 2012). Here, Ror1 was required to sustain EGFR/ERBB3 signaling in a presumably kinase-independent manner. At the same time, Ror1 was responsible for Src phosphorylation and activation of PI3K/AKT signaling, suggesting that kinase-dependent functions for Ror1 should not be ruled out completely.

Summarizing, at present it remains incompletely understood just how promiscuous the interaction between the Ror receptors and their ligands, both Wnts and others, really is. Similar to the situation observed for Wnts and Fzds, it is unlikely that the Ror CRD will interact with only a single, specific ligand, but just how specificity in ligand/receptor interactions is achieved, particularly in the context of a complex multicellular organism, remains one of the outstanding questions in the Wnt field.

In both *C. elegans* and vertebrates, Ror proteins contribute to the orientation of cells within a tissue, a process known as planar cell polarity (PCP) (Green et al. 2008a; Yamamoto et al. 2008). In the murine limb bud, Wnt5A induces a complex between Ror2 and Vangl2, a core component of the PCP pathway, in a concentration-dependent manner. Thus, Ror proteins transmit directional information provided by a Wnt gradient to the PCP machinery in developing tissues. Likewise, *Xenopus XRor2* regulates convergent extension, a PCP-related process (Hikasa et al. 2002; Schambony and Wedlich 2007).

In addition to modulating canonical Wnt signaling and regulating cell polarity, Ror2 is required for Wnt5A to mediate cell migration, a function that has been reported to involve protein kinase C, JNK, and the actin-binding protein Filamin A (Nishita et al. 2006; Yamamoto et al. 2007; Nomachi et al. 2008; O'Connell et al. 2009). Ror2 signaling also contributes to the invasiveness of various human cancers including those of the skin (O'Connell et al. 2010), prostate (Yamamoto et al. 2010), bone (Enomoto et al. 2009; Yamagata et al. 2012), and kidney (Wright et al. 2009). This increased invasiveness has been associated with the increased expression of extracellular matrix remodeling enzymes called MMPs by Ror2-JNK or Ror2-cSrc signaling. Further complicating matters, Wnt5a was recently also shown to promote cancer cell invasion in a presumably Ror-independent manner by disrupting the association between N-cadherin and β-catenin, thereby promoting β-catenin/TCF signaling (Grossmann et al. 2013).

Many other potential Ror1/2 signaling effectors have been reported in various contexts, but further study is required to determine when and how they are used and in what combinations. In addition to Src and Src family kinases (Akbarzadeh et al. 2008; Enomoto et al. 2009), these include Dlxin-1 (Matsuda et al. 2003), casein kinase Iε (Kani et al. 2004), 14-3-3β (Liu et al. 2007), NF-κB (Fukuda et al. 2008), and BMPR1b (Sammar et al. 2004). It also remains to be determined if and how any of these components are connected to the upstream Wnt signaling machinery. This includes the Dishevelled protein, which plays an important role in both β-catenin-dependent and -independent Wnt-signaling events and which can be phosphorylated in response to Wnt5a/Ror signaling (Ho et al. 2012).

DERAILED/Ryk/lin-18

The Derailed/Ryk/lin18 receptors form a family of conserved transmembrane molecules with an extracellular domain resembling the Wnt inhibitory factor (WIF) protein (Patthy 2000; Angers and Moon 2009; Fradkin et al. 2010). Similar to secreted Frizzled related proteins (SFRPs), WIFs are extracellular Wnt antagonists (Hsieh et al. 1999a). Unlike SFRPs, how-

ever, the WIF Wnt-binding domain is different from the classic CRD domain that is observed in both FZD and Ror receptors (Fig. 1). Interestingly, it has been suggested that the WIF domain interacts directly with the lipid modification on the Wnt protein (Malinauskas et al. 2011), similar to the way in which the Fzd CRD domain was recently shown to engage in Wnt binding (Janda et al. 2012). The cytoplasmic portion of Derailed/Ryk/lin-18 has a tyrosine kinase motif, but according to the actual sequence, Derailed and Ryk are not considered to be active tyrosine kinase enzymes. This has been confirmed by functional assays (Yoshikawa et al. 2001; Inoue et al. 2004).

The mammalian Ryk gene was found by homology screens (Stacker et al. 1993), whereas the original identification of *derailed* was based on genetic screens in *Drosophila* for mutants that affect axon path finding in the embryo (Callahan et al. 1995; Bonkowsky et al. 1999). *Derailed* is also required for the specificity of muscle attachment sites (Callahan et al. 1996). A *C. elegans* homolog, *lin-18*, is critical for vulva development, in parallel to the Frizzled family member *lin-17* (Inoue et al. 2004; Wang and Sommer 2011).

Of note, whereas the mammalian genome appears to harbor just a single Ryk gene (in addition to a *Ryk* pseudogene) (Halford et al. 1999), multiple derailed/Ryk/lin-18 family members exist in *Drosophila*. In addition to *Derailed*, these include the *doughnut*, which is itself also involved in muscle attachment formation (Lahaye et al. 2012). Yet another homolog, *Derailed2*, plays a role opposing *Derailed1* during development of the *Drosophila* olfactory system (Sakurai et al. 2009).

A ligand of *Derailed* in these processes was initially not known, but the homology between the cell external domain of *Derailed* with WIF (Patthy 2000) suggested that Wnt proteins could interact with *Derailed*. Indeed, one of the *Drosophila* Wnt family members, *DWnt5* (Fradkin et al. 1995), has genetic interactions with *Derailed* (Yoshikawa et al. 2003; Fradkin et al. 2004). *DWnt5* is expressed in a pattern similar to *Derailed* and the phenotypes of these genes resemble each other as well. In *C. elegans*, the Wnt proteins LIN-44, MOM-2, and CWN-2 redundantly regulate P7.p patterning, suggesting that Ryk might respond to multiple Wnt ligands. As in *Drosophila*, the mammalian Ryk gene is required for axon guidance and neurite outgrowth (Lu et al. 2004a; Keeble and Cooper 2006). More recently, mammalian Ryk was also shown to interact with the core planar cell polarity (PCP) machinery, and Ryk-deficient mice presented with typical PCP defects such as the misorientation of stereocilia in the inner ear (Macheda et al. 2012).

How do the Derailed/RYK/lin-18 receptors function molecularly? As mentioned above, all Derailed/RYK/lin-18 are catalytically inactive. Hence, Ryk may act as a coreceptor rather than as a primary signal transducing receptor. As a coreceptor, Derailed/RYK/lin-18 molecules may bind Wnts that are then cotargeted to other Wnt receptors. Indeed, whereas the intracellular kinase domains of Derailed/RYK/lin-18 proteins are largely dispensable for function, the extracellular WIF domains are not (Inoue et al. 2004; Taillebourg et al. 2005). Among the candidates for a receptor working with Derailed/RYK/lin18 are, perhaps not surprisingly, the Frizzleds. In neurite outgrowth in mammalian cells, the Ryk molecule stimulates Frizzled activity, both in vivo and in cell culture assays (Lu et al. 2004a). Moreover, in *Xenopus*, Ryk cooperates with Fzd7 and Wnt11 during gastrulation and convergent extension movements (Kim et al. 2008; Lin et al. 2010), suggesting some form of interaction. On the other hand, although the *lin-18* (Ryk) and *lin-17* (a Frizzled) genes in *C. elegans* both operate in vulva development, they are thought to act independently of each other (Inoue et al. 2004). As has been described for Ror2, Ryk might also couple directly to the core planar cell polarity pathway as it has also been shown to form a complex with and promote the stability of Vangl2 (Andre et al. 2012).

When it comes to the mechanism of action of Derailed/RYK/lin-18 receptors at the cytoplasmic side, there is also relatively little known. Surprisingly, Ryk was shown to undergo cleavage and, in response to Wnt-ligand stimulation, the carboxy-terminal fragment translocated to

the nucleus (Lyu et al. 2008). Cleavage is mediated by γ-secretase and appears to be essential for the neuronal Ryk-mediated signal transduction events (Lyu et al. 2008). The carboxy-terminal fragment undergoes ubiquitination and degradation by the proteasome, in a way that is inhibited by Cdc37, a subunit of the molecular chaperone Hsp90 complex (Lyu et al. 2009). Inhibition may also require the Src protein kinase (Wouda et al. 2008). In the process of ubiquitination, the RYK molecule also interacts with the E3 ubiquitin ligase Mindbomb 1 (Berndt et al. 2011). The latter work also showed that Ryk can activate β-catenin signaling, suggesting yet another level of interactions among the various Wnt pathways (Berndt et al. 2011).

CONCLUDING REMARKS

In summary, much remains to be learned about the mechanism of action of both the Ror and Ryk tyrosine kinase receptor families. The fact that several of the main classes of Wnt receptors, including the Frizzleds, the RORs, and the RYKs, have ligand-binding domains that exist by themselves as secreted Wnt-binding factors, suggests a complex extracellular landscape of Wnts, secreted inhibitors, and receptors that may compete or assist each other in setting signaling levels and boundaries. But just how Ryk and Ror work as (co)receptors is still far from clear. It should also be stressed that additional non-Frizzled (co)receptors might still be out there. An example is the transmembrane protein PTK7/CCK4, which has an extracellular domain containing immunoglobulin repeats as well as an intracellular tyrosine kinase homology domain and which is required for the establishment of planar cell polarity in mammals (Lu et al. 2004b; Yen et al. 2009). Moreover, in spite of the fact that it does not appear to contain a bona fide Wnt-binding domain, PTK7 has been shown to interact with some, but not other, Wnt ligands and to inhibit Wnt/β-catenin signaling (Peradziryi et al. 2011). Given the multitude of experimental systems that can now be used, it is expected that significant progress will be made in understanding the involvement of these RTKs in development and disease.

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