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Review

The Ror receptor tyrosine kinase family

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Abstract. Receptor tyrosine kinases (RTKs) participate in numerous developmental decisions. Ror RTKs are a family of orphan receptors that are related to muscle specific kinase (MuSK) and Trk neurotrophin receptors. MuSK assembles acetylcholine receptors at the neuromuscular junction [1, 2], and Trk receptors function in the developing nervous system (reviewed in [3–5]). Rors have been identified in nematodes, insects and mammals. Recent studies have begun to shed light on Ror function during development. In most species, Rors are expressed in many tissue types during development. Analyses of mutants that are defective in the single nematode Ror demonstrate a role in cell migration and in orienting cell polarity. Mice lacking one of the two Ror gene products display defects in bone and heart formation. Similarly, two different human bone development disorders, dominant brachydactyly B and recessive Robinow syndrome, result from mutations in one of the human Ror genes.

Key words. Cell migration; cell polarity; brachydactyly B; Robinow syndrome; bone development.

Introduction

Extensive intercellular signaling directs many metazoan developmental events. A common form of cell-cell signaling utilizes extracellular ligands that bind membrane spanning receptors at the cell surface. One such class of cell surface proteins is the receptor tyrosine kinase (RTK) superfamily. RTKs are a large family of glycoproteins that regulate cell proliferation, polarity, differentiation, migration, metabolism and survival.

RTKs are composed of an extracellular region that typically contains recognizable structural motifs, a single membrane-spanning region and an intracellular region that contains a conserved tyrosine kinase domain. The general mechanism of activation is thought to be through receptor oligomerization (reviewed in [6, 7]). In this model of activation, ligand binding to the extracellular domain stimulates dimerization of receptor. The current model for activation is that dimerization juxtaposes catalytic domains, resulting in phosphorylation of target tyrosine residues within the autoactivation loop of the partner leading to increased kinase activity. RTK autophosphorylation may lead to increased phosphorylation of target proteins and to recruitment of downstream signaling proteins that bind specific phosphotyrosines, often via SH2 or phosphotyrosine binding domains. Recruitment of signaling proteins in turn activates an intracellular cascade of downstream effectors such as the phospholipase C γ or MAP (mitogen-activated protein) kinase pathways.

The RTK superfamily is divided further into families based both on conserved primary sequence and on domain structure. In many cases, ligands for receptor family members are known. In other cases, however, the ligands are not known. One such family of orphan receptors, the Ror RTKs, is the subject of this review. Note that the Rors in this review are distinct from the ROR family of nuclear receptors.

The first Ror family members, two human Ror RTK-encoding genes, *hRor1* and *hRor2*, and two rat partial com-

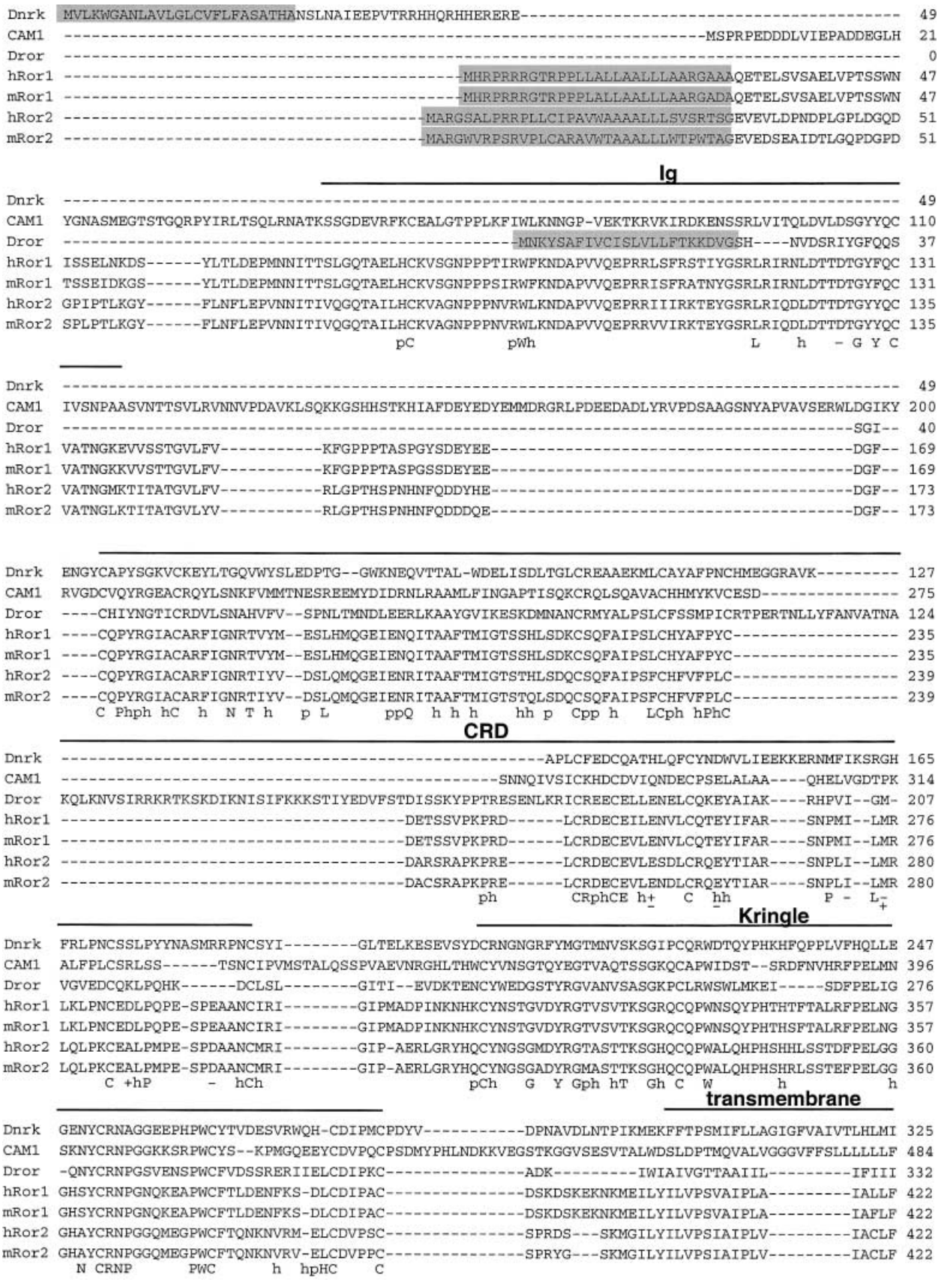
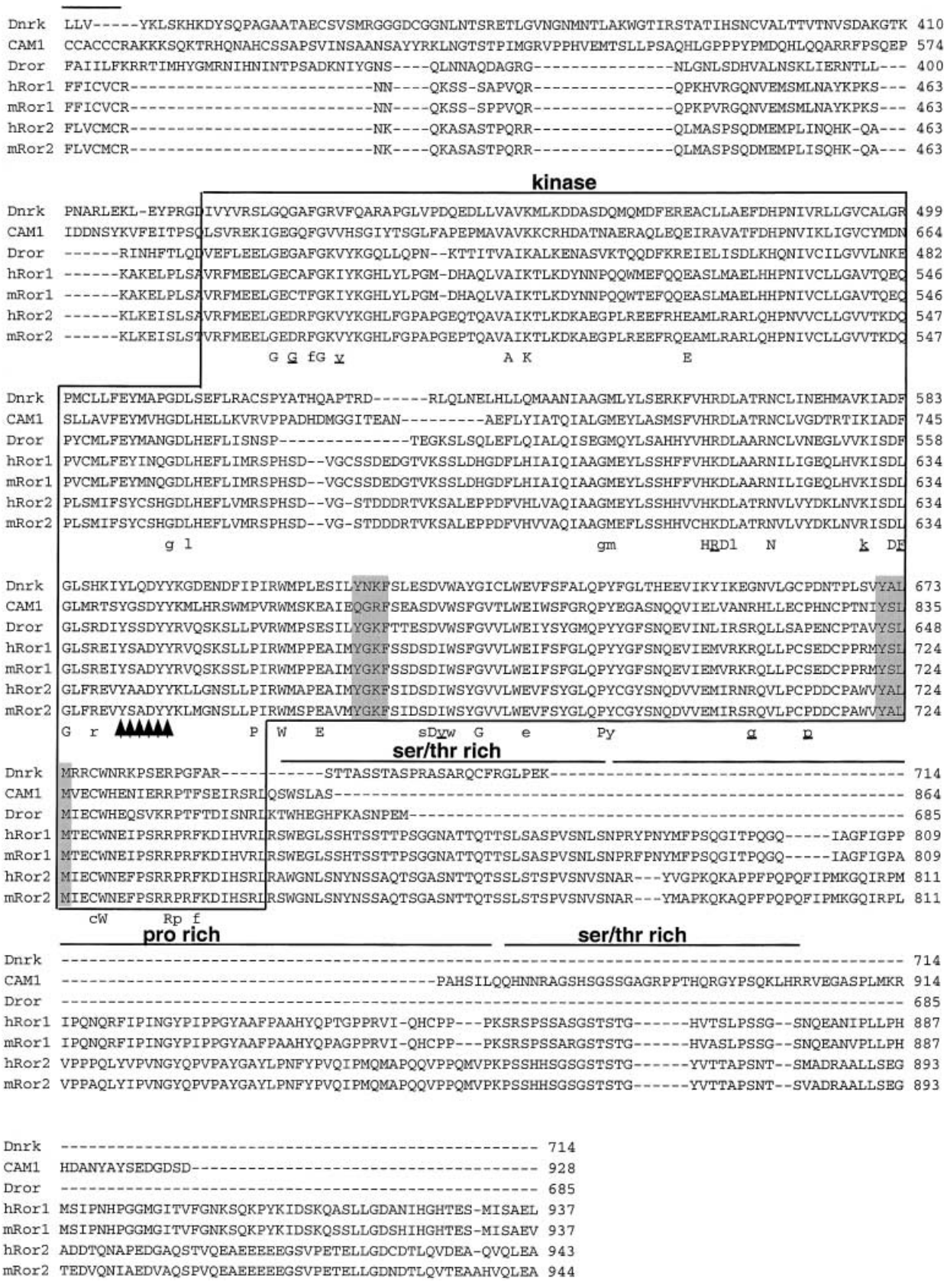


Figure 1. Alignment of nematode (CAM-1), *Drosophila* (Dnrk and Dror), human (hRor1 and hRor2) and mouse (mRor1 and mRor2) Ror proteins. The seven Ror proteins were aligned using a multiple alignment program [66]. Numbers at the end of each line indicate the position of the final amino acid in each line with the presumed initiator methionine equal to position 1. Predicted signal peptides are shaded. Immunoglobulin (Ig), cysteine-rich (CRD), Kringle, transmembrane, serine- and threonine-rich (ser/thr rich) and proline-rich (pro rich) domains are overlined. The kinase domain is boxed. Conserved amino acids within Ig, CRD and kringle domains are indicated in upper



case. p, polar amino acid; h, hydrophobic amino acid; +, positively charged; –, negatively charged and ±, either positively or negatively charged amino acid. The 40 amino acids conserved among tyrosine kinases are indicated by letters below. Invariant amino acids are shown in uppercase and those that vary in three or fewer tyrosine kinases are shown in lowercase. Conserved amino acids that are altered in one or more Rors are underlined. The YXXDYY that corresponds to the site of phosphorylation within the TrkB autoactivation domain is indicated by small arrows. Potential SH2 binding sites within the kinase domain are shaded. CRD consensuses from [19]. Ig and kringle consensuses from [67, 68]. Forty conserved amino acids within the kinase domain from [34, 35].

plementary DNAs (cDNAs), *rRor1* and *rRor2*, were identified nearly 10 years ago [8]. Subsequently, Ror family members have been isolated from *Caenorhabditis elegans* [9, 10], *Drosophila melanogaster* [11, 12] and mouse [13, 14]. Many tissue types, including the nervous system, express Ror RTKs during development. In the last 2 years, the analysis of phenotypes resulting from mutations in *C. elegans*, mouse and human *Ror* genes has begun to shed light on the function of these proteins during development.

Identification of Ror RTKs

Most Ror family members were isolated by polymerase chain reaction (PCR)-based screens for tyrosine kinase-encoding genes. In fact, the first Ror-encoding genes, human *Ror1* and *Ror2*, were isolated in a PCR-based screen for RTKs similar to the Trk neurotrophin receptors [8]. Similar PCR screens yielded *Ror* genes from the fruitfly *D. melanogaster* [11, 12], the mouse *Mus musculus* [13, 14] and the rat [8]. The *C. elegans Ror* gene, *cam-1* (also called *kin-8*), emerged from a genetic screen for mutations that disrupted directed cell migration [9]. *cam-1* also was identified using the *v-ros* viral oncogene as a hybridization probe to identify tyrosine kinases [10]. A gene that encodes a protein with similarity to Rors was isolated from the electric ray *Torpedo californica* by PCR [15].

In the nomenclature of the vertebrate Rors, the genus is indicated with a small letter preceding the gene name. Thus, the human Ror genes are *hRor1* and *hRor2*, the mouse genes are *mRor1* and *mRor2* and so on. Although Oishii et al. have suggested that Dror and Dnrk be renamed dRor1 and dRor2, respectively [13], I have retained the original *Drosophila* Ror nomenclature in this review.

Most species appear to contain two Ror genes. For example, *Drosophila*, mouse, rat and human genomes all contain two Ror genes. In contrast, only a single Ror-encoding gene has been found in *C. elegans*.

Protein sequence conservation among Ror family members

Ror proteins share a conserved domain structure. In general, the extracellular regions are predicted to contain immunoglobulin (Ig), cysteine-rich (CRD) and kringle domains, motifs that all are thought to mediate protein-protein interactions. Typically, the intracellular region of Rors is predicted to contain a tyrosine kinase domain, two regions rich in serine and threonine separated by a region rich in prolines. Ror structure and deviations from the consensus are discussed below.

The two Ror proteins Ror1 and Ror2 from each species are highly homologous to one another (fig. 1). For example, hRor1 and hRor2 share 58% amino acid identity overall, or 68% amino acid identity within the kinase domains. However, the degree of sequence conservation is higher within the *Ror1* and *Ror2* subgroups. For example, mouse and human Ror1 share 97% amino acid identity overall, and mouse and human Ror2 share 92% amino acid identity overall. hRor1 and hRor2 consist of 937 amino acids (914 after cleavage of the signal peptide) and 943 amino acids (916 after signal peptide cleavage), respectively. Murine Rors are the same length as human, except that mRor2 is one amino acid longer than hRor2. In addition to primary sequence homology and similarity in size, mammalian Ror proteins have the same domain structure; they contain extracellular Ig, CRD and kringle domains, and intracellular tyrosine kinase, proline rich and two serine-threonine rich domains (fig. 2).

C. elegans cam-1 encodes a protein containing most of the motifs present in other Rors. However, CAM-1 lacks the intracellular proline rich domain and one of the serine-threonine-rich regions seen in vertebrate Rors [9, 10]. CAM-1 is not obviously more similar to Ror1 or Ror2; for example, the kinase domain is 44% identical to that of both hRor1 and hRor2.

Dror is predicted to contain 685 amino acids (661 after cleavage of the signal peptide) with many, but not all, of the domains present in vertebrate Rors [11]. Specifically, Dror lacks the extracellular Ig domain. In addition, the protein terminates a few amino acids past the kinase domain, and therefore lacks the serine-threonine- and proline-rich regions seen in vertebrate Rors. Dror may be more closely related to hRor1 than to hRor2 [13]. For example, the kinase domain is 58% identical in amino acid sequence to hRor1, whereas it is only 53% identical to hRor2.

The second *Drosophila* Ror, Dnrk, is predicted to contain 689 amino acids (664 after cleavage of the signal peptide), with many of the domains present in vertebrate Rors [12]. However, Dnrk lacks the Ig, proline-rich and one of the serine-threonine-rich domains. Dnrk may be more closely related to hRor2 than to hRor1 [13]. Overall, Dnrk is 45% identical to hRor2 and 34% identical to hRor1.

An unusual Ror-related RTK isolated from the electric ray *T. californica* shares some, but not all, of the domains with Ror family members [15]. Notably, Ror-related RTK is the only reported non-Ror RTK that contains an extracellular kringle domain. Ror-related RTK also has a CRD within the extracellular part of the protein and a kinase domain that is 48% identical at the amino acid level to *hRor1*. These features might suggest that Ror-related RTK represents a Ror family member. However, Ror-related RTK also has three, rather than one, extracellular Ig domains. This arrangement is more like that present in MuSK than Rors. In addition, Ror-related

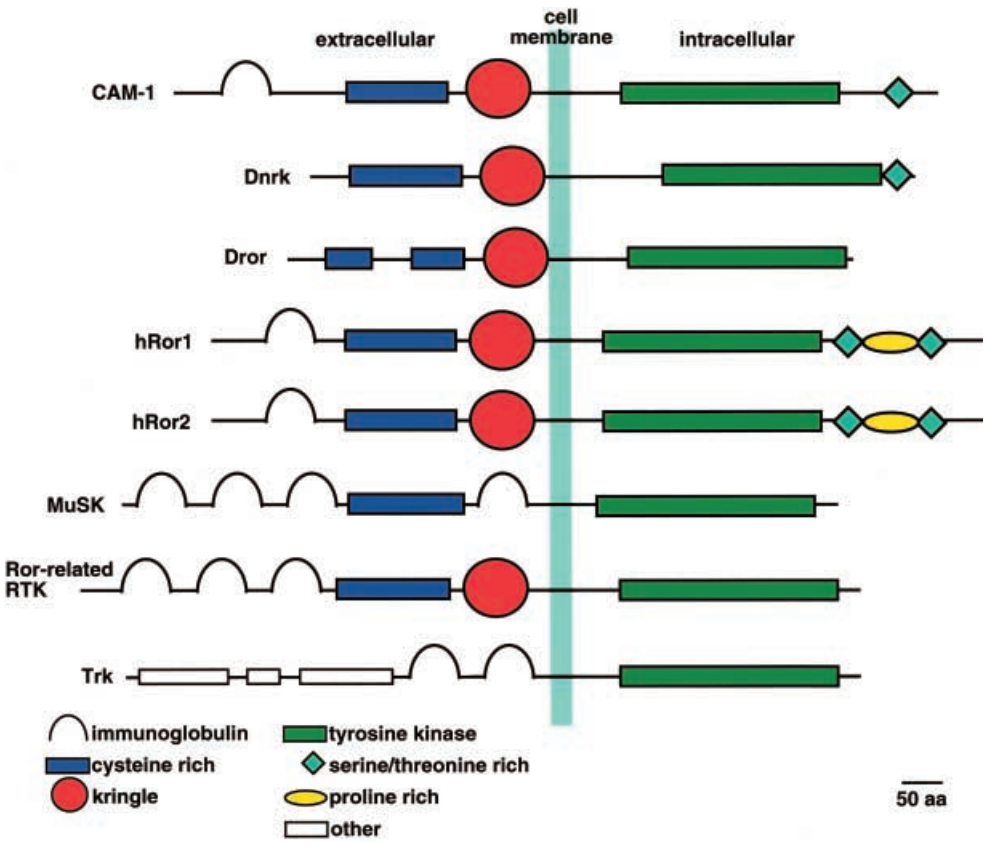


Figure 2. Predicted domain structure of Ror proteins. MuSK, *Torpedo* Ror-related and TrkB RTKs are shown for comparison. Conserved domains are indicated. N-termini are to the left. Murine Rors are not shown because they are nearly identical in sequence to human Rors.

RTK is 60% identical overall to human MuSK and 82% identical within the kinase domain. Ror-related RTK lacks the intracellular serine-threonine-rich and proline-rich regions present in other vertebrate Rors. Therefore, Ror-related RTK is more closely related to the MuSK family of RTKs, and will not be considered further in this review.

Alternate forms of Ror proteins

Many *C. elegans* messenger RNAs (mRNAs) undergo trans-splicing, a modification which results in one of two possible small RNA oligonucleotides, either SL1 or SL2, being spliced onto the 5′ end of the mRNA (reviewed in [16]). Trans-splicing of SL1 onto the 5′ end of the *cam-1* mRNA in one of two locations generates two mRNAs that encode proteins differing by 26 amino acids – 902 amino acids versus 928 amino acids – at their N-termini [10]. The significance of the two forms of CAM-1 is unclear.

An alternatively spliced form of *hRor1*, called truncated *hRor1*, has been described [17]. Translation of truncated *hRor1* mRNA is predicted to produce a protein that completely lacks the extracellular and membrane-spanning regions of hRor1. Truncated *hRor1* mRNA is expressed in fetal and adult central nervous system, in human leukemia and lymphoma cell lines, and in a variety of neuroectodermal cancers. Because no function has been ascribed to truncated hRor1, its significance remains unclear.

Ror protein structure

Signal peptide

As expected for an integral membrane protein, most ROR family members contain a predicted short signal sequence at their N-termini that directs their insertion into the cell membrane (fig. 1). Surprisingly, *cam-1* does not encode an obvious signal peptide, and yet presumably the protein is inserted into the membrane. Consistent with this, a fusion of *cam-1*-coding sequence to the green fluorescent protein (*gfp*) gene results in fluorescence at the periphery of the cell, suggesting that the protein is membrane associated [9, 10]. Presumably, sequences within CAM-1 provide signal peptide function even though they do not display the normal characteristics of one.

Immunoglobulin domain

Vertebrate and *C. elegans* Ror proteins contain an Ig domain within the extracellular part of the protein. Ig domains are found in a large number of secreted and membrane-associated proteins, where they are thought to mediate protein-protein interactions. The two *Drosophila* Rors, Dror and Dnrk, are unique among the Rors because they lack an Ig domain. The significance of this is unclear because the precise function of the Ig domain is not known. One possibility is that the Ig domain mediates or stabilizes an interaction between Ror and some other factor. In this view, fly Rors are able to stably interact with the factor, or perhaps that factor is no longer required for fly Ror function.

Cysteine rich domain

Ror proteins contain an extracellular cysteine-rich domain CRD that is defined by the presence of 10 conserved cysteines and by several additional conserved amino acids (fig. 1). A similar CRD is found in the seven-pass transmembrane protein Frizzled [18–21]. Frizzled proteins appear to act as receptors for Wnt extracellular signaling molecules [22]. Additionally, a large class of secreted frizzled related proteins (sFRPs) that contain the CRD have been identified [23–29]. Several reports implicate the CRD of Frizzled and sFRPs in binding to Wnts [19, 22, 30]. This raises the intriguing possibility that Wnt proteins may bind to Ror proteins, thereby regulating their activity. However, data supporting this notion have not been reported. Alternatively, the CRD may act as a general module that functions to mediate protein-protein binding. Note that the conserved CRD also is found in Smoothened, in the α1 chain of type XVIII collagen and in carboxypeptidase Z [18–21]. These proteins have not been reported to bind Wnt. Interestingly, the Dror CRD contains a 55-amino acid insertion between the between the fifth and sixth cysteines. The significance of these additional amino acids is not known.

Kringle domain

The kringle domain present in the extracellular portion of Ror proteins is most commonly seen in proteases of the blood-clotting cascade (reviewed in [31, 32]). Kringle domains are thought to mediate protein-protein interactions. The presence of this domain in a signaling protein is unusual, although not unprecedented; a kringle domain is present in hepatocyte growth factor [33] and is seen in the Ror-related *Torpedo* RTK [15]. Because Ror is the only RTK family reported to contain the kringle domain with the exception of *Torpedo* MuSK, the presence of a kringle domain is a nearly diagnostic characteristic of Ror family members.

Kinase domain

The kinase domain of Ror is most similar to that of the Trk family of neurotrophin receptors and of the muscle-specific kinases, or MuSKs. Tyrosine kinases contain 40 amino acids within the kinase domain that are either invariant or nearly invariant [34, 35]. Few tyrosine kinases contain more than one or two deviations among the conserved 40 amino acids, and only one has as many as five. Of the forty consensus amino acids, 21 are absolutely conserved among all tyrosine kinases that had been examined [35]. The Rors contain between one and seven alterations among the 40 conserved amino acids. These deviations from consensus are not present in Trk or MuSK RTKs (fig. 3). The changes in conserved amino acids are most striking in vertebrate Rors, where 7 amino acids in hRor1 and mRor1, and either 5 amino acids in hRor2 or 6 in mRor2 are altered (fig. 1, table 1). In the vertebrate Rors, 3 of the amino acids that are invariant among all other RTKs are changed. In contrast to the vertebrate Rors, only a single conserved amino acid is altered in each of the invertebrate Rors, and that amino acid is never one of the 21 invariant amino acids (fig. 1, table 1).

All Ror family members contain the YXXDYY sequence, which also is present in a number of RTKs, including the Trk and MuSK RTKs (fig. 3, [36]). This sequence is required for normal activation of TrkA [37]. Within the ki-

Table 1. Deviations from consensus within Ror kinase domains.

Protein	Deviation from consensus	Amino acid position	Kinase domain affected
hRor1	G to C*	482	I
	V to I	488	I
	R to K*	614	VI
	F to L*	634	VII
	V to I	675	IX
	G to R	709	X
	P to S	715	X
hRor2	G to D*	482	I
	R to K*	614	VI
	F to L*	634	VII
	V to I	675	IX
	G to R	709	X
mRor2	K to R	630	VII
Dnrk	R to H	587	VII
Dror	G to R	819	X
CAM-1	G to R	632	X

Change in highly conserved amino acid sequence from the amino acid present in the consensus to that found in Ror is presented. The second column lists the amino acid deviation found in Rors. An asterisk indicates that the change is to an amino acid that is invariant among non-Ror tyrosine kinases [34, 35]. Identical changes to those in hRor1 and hRor2 are present in mRor1 and mRor2, respectively. In addition to changes shared between mRor2 and hRor2, mRor2 contains the additional change shown. Position of the amino acid is relative to the presumed initiator methionine, which equals positon 1. Kinase domain affected utilizes domain numbering of Hanks et al. [34].

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 InsR    -ITLLRELGQGSFGMVYEGNARDIIKGEAETRVAVKTVNESASLRERIEFLNEASVMKGFTCHHVVRLLGVVSKGQPTLVVMELMAHGDL 1111
hRor2    AVRFMEELGEDRFGKVYKGHLFGPAPGEQTQAVAIKTLKDKAEGPLREEFRHEAMLRARLQHPNVVCLLGVVTKDQPLSMIFSYCSHGDL  561
hRor1    AVRFMEELGECAFGKIYKGHLYLPGM-DHAQLVAIKTLKDYNNPQQWMEFQQEASLMAELHHPNIVCLLGAVTQEQPVCMLFEYINQGDL  560
 TrkB    -IVLKRELGEGAFGKVFLAECYNLCPEQDKILVAVKTLKD-ASDNARKDFHREAELLTNLQHEHIVKFYGVCVEGDPLIMVFEYMKHGDL  625
 MuSK    -IEYVRDIGEGAFGRVFQARAPGLLPYEPFTMVAVKMLKEEASADMQADFQREAALMAEFDNPNIVKLLGVCAVGKPMCLLFEYMAYGDL  663
                 G G fG v                A K               E                                  g l

 InsR    KSYLRSLRPEAE--------------NNPGRPPPTLQ--EMIQMAAEIADGMAYLNAKKFVHRDLAARNCMVAHDFTVKIGDFGMTRDIY 1185
hRor2    HEFLVMRSPHSDVG-STDD---------DRTVKSALEPPDFVHLVAQIAAGMEYLSSHHVVHKDLATRNVLVYDKLNVKISDLGLFREVY  641
hRor1    HEFLIMRSPHSDVGCSSDE---------DGTVKSSLDHGDFLHIAIQIAAGMEYLSSHFFVHKDLAARNILIGEQLHVKISDLGLSREIY  641
 TrkB    NKFLRAHGPDAV-------------LMAEGNPPTELTQSQMLHIAQQIAAGMVYLASQHFVHRDLATRNCLVGENLLVKIGDFGMSRDVY  702
 MuSK    NEFLRSMSPHTVCSLSHSDLSMRAQVSSPGPPP--LSCAEQLCIARQVAAGMAYLSERKFVHRDLATRNCLVGENMVVKIADFGLSRNIY  751
                                                  gm         HRDl   N         k  DFG  r

 InsR    ETDYYRKGGKGLLPVRWMAPESLKDGVFTTSSDMWSFGVVLWEITSLAEQPYQGLSNEQVLKFVMDGGYLDQPDNCPERVTDLMRMCWQF 1275
hRor2    AADYYKLLGNSLLPIRWMAPEAIMYGKFSIDSDIWSYGVVLWEVFSYGLQPYCGYSNQDVVEMIRNRQVLPCPDDCPAWVYALMIECWNE  731
hRor1    SADYYRVQSKSLLPIRWMPPEAIMYGKFSSDSDIWSFGVVLWEIFSFGLQPYYGFSNQEVIEMVRKRQLLPCSEDCPPRMYSLMTECWNE  731
 TrkB    STDYYRVGGHTMLPIRWMPPESIMYRKFTTESDVWSLGVVLWEIFTYGKQPWYQLSNNEVIECITQGRVLQRPRTCPQEVYELMLGCWQR  792
 MuSK    SADYYKANENDAIPIRWMPPESIFYNRYTTESDVWAYGVVLWEIFSYGLQPYYGMAHEEVIYYVRDGNILSCPENCPVELYNLMRLCWSK  841
         ▲▲▲▲▲         P  W   E          sDvw  G    e       Py              g     p             cW

 InsR    NPKMRPTFLEIVNLL-------- 1290
hRor2    FPSRRPRFKDIHSRLR-------  747
hRor1    IPSRRPRFKDIHVRLRSWEG---  751
 TrkB    EPHMRKNIKGIHTLL--------  807
 MuSK    LPADRPSFTSIHRILERMCERAE 1042
             Rp f
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Figure 3. Alignment of human Ror1 and Ror2, TrkB and MuSK kinase domains. Human insulin receptor, another RTK, is included for comparison. The 40 amino acids conserved among tyrosine kinases are indicated by letters below. Invariant amino acids are shown in uppercase, and those that vary in three or fewer tyrosine kinases are shown in lowercase. Amino acids that are identical in all five kinase domains are shown in uppercase, and those that differ only in a single kinase are shown in lowercase. Arrows indicate the YXXDYY sequence that corresponds to the site of phosphorylation with the TrkB autoactivation domain.

nase domain is a conserved sequence (YALM in mammalian Rors and fly Dnrk, YSLM in Dror) that has the potential to bind the SH2 region of *shc*, *csk* or the p85 subunit of phosphotidyl inositol 3 kinase [38]. A second sequence conserved in all Rors except CAM-1, YXXF, provides a reasonable match to the consensus for interaction with the SH2 domain of tensin [38].

C-terminal domains

C-terminal to the kinase domain the Ror family members diverge somewhat (fig. 2). The two fly Rors terminate shortly after the kinase domain, whereas mammalian Rors extend nearly 200 amino acids beyond the kinase domain. Mammalian Rors contain within the sequences C-terminal to the kinase domain two regions rich in serine and threonine residues. For example, in mammalian Ror1, the first such region contains 17 of 30 amino acids that are serine or threonine. Fly Dnrk has a short region where 8 of 13 amino acids are serine or threonine and nematode CAM-1 has a region in which 8 of 26 amino acids are serine or threonine. Perhaps CAM-1 and Dnrk serine-threonine-rich regions provide a function similar to those of mammalian Rors. Mammalian Rors also possess a region rich in proline residues. Invertebrate Rors do not possess a distinct proline-rich region.

Overall, the Ror RTKs are most similar to Trk and MuSK families of RTKs. Within the kinase domains, hRor1 is approximately 45% identical to both TrkB and to MuSK (fig. 3). TRK proteins are RTKs that mediate responses to binding of neurotrophins in nervous system development and maintenance. The Trk extracellular domain structure differs from that of Rors (fig. 2). Trks contain two cysteine-rich clusters separated by a leucine-rich motif, and two Ig domains. Given the differences in extracellular domain structure, it seems unlikely that Trks and Rors bind the same ligands.

MuSK RTKs play critical roles in organizing the synapse during development of the neuromuscular junction. The MuSK ligand appears to be a neuronally secreted protein called agrin [39, 40]. The MuSK extracellular region resembles Rors, in that both contain the CRD (fig. 2). MuSK contains four Ig domains, whereas Ror contains only one, and MuSK proteins, with the exception of *Torpedo* RTK, do not contain the kringle domain. The similarities between MuSK and Ror extracellular domains raise the possibility that Ror and MuSK might bind the same ligand(s). Identification of Ror ligand(s) will resolve this issue.

Ror kinase activity

Kinase activity has been demonstrated for at least two Ror family members. This observation is not surprising given the sequence similarity with known RTKs, but the numerous changes to highly conserved amino acids within mammalian Ror kinase domains raised the possibility that kinase activity may have been lost. Therefore, it was important to demonstrate that mammalian Rors retained kinase ability. Masiakowski and Carroll showed that at least one of the two human Rors, hRor2, retains kinase activity [8]. Similarly, Dnrk also possesses tyrosine kinase activity [12].

Experiments with CAM-1 suggest that some Ror function does not require kinase activity. One of the *cam-1* mutants, *cam-1(gm105)*, is predicted to introduce a stop codon 74 amino acids downstream of the membrane-spanning region and 25 amino acids upstream of the kinase domain (fig. 5). The protein that would be produced in *cam-1(gm105)*-mutant animals is predicted to completely lack the kinase domain, and yet *cam-1(gm105)* clearly is not a null mutation [9, 41]. This observation raises the possibility that at least some CAM-1 function does not require kinase activity.

A more direct test suggests that kinase activity is dispensable for the cell migration function of CAM-1. A conserved lysine and an adjacent lysine within the kinase domain were changed to arginines, an alteration that is predicted to abolish kinase activity by analogy to other tyrosine kinases [34]. The resulting transgene was able to rescue the cell migration, but not the cell polarity defects of a *cam-1* mutant [9]. Similarly, a form of CAM-1 that is predicted to lack kinase activity rescues the dauer constitutive phenotype (see below) of a *cam-1* mutant [10].

Ror expression

One clue to function comes from looking at where and when during development Ror proteins are expressed. *C. elegans* CAM-1 is expressed in many cells during development. Two *cam-1* cDNAs, called type 1 and type 2, have been detected that are identical except that type 1 encodes an additional 24 amino acids at the N-terminus of the protein [10]. Transcription of the two forms may be under the control of different promoter sequences. Expression of GFP under the control of either of the two hypothesized promoter regions results in a similar, widespread pattern of fluorescence where most cells of the animal express GFP [10]. To examine CAM-1 expression further, a plasmid that contains all of the genomic region of *cam-1* corresponding to the type 2 message including approximately 3 kb of upstream DNA was fused to *gfp*. This fusion construct rescues *cam-1*-mutant phenotypes ([9], see below). Therefore, *cam-1::gfp* must be expressed in those cells that require CAM-1 function. Expression appears in most cells at the 200-cell stage of embryogenesis, and GFP is detected throughout the remainder of development. During development, cells that require CAM-1 for proper development appear to express CAM-1::GFP, as do many other cells that are not obviously affected by mutations in *cam-1*. During larval development, CAM-1::GFP is detected throughout much of the nervous system, as well as in intestinal, hypodermal and body-wall muscle cells and in parts of the pharynx. In neurons, CAM-1::GFP is detected predominantly in axons and dendrites. Consistent with its expected membrane localization, the protein appears to associate with the plasma membrane.

Because CAM-1 is expressed in so many cell types, the functional requirement for CAM-1 during CAN cell migration was assessed by mosaic analysis [9]. In wild-type animals, the CANs migrate posteriorly from the head to the center of the embryo [42]. In *cam-1* mutants, the CANs usually fail to migrate and are displaced anteriorly [43]. To test whether *cam-1* functions cell autonomously in the CANs, mosaic animals in which some cells were wild type for *cam-1* and other cells were mutant were analyzed. The results demonstrate that *cam-1* acts cell autonomously for CAN migration.

The widespread expression of CAM-1 is in contrast to the specific expression in *Drosophila* nervous system of Dror and Dnrk. Dror and Dnrk expression were examined by in situ hybridization, using the corresponding cDNA as a probe [11, 12]. Dror and Dnrk expression appears by the time of germ-band extension. mRNA levels rise gradually during the next few hours after initial expression, and then gradually decay through the rest of embryogenesis. Dror and Dnrk expression is not detected outside the nervous system. Unlike Dror, however, Dnrk expression persists throughout embryonic, larval, pupal and adult stages, although at decreased levels after embryogenesis.

Murine Ror expression also has been examined by in situ hybridization. *mRor1* is detected clearly at stage E9, reaches a maximum at stages E11–13 and gradually declines after that. *mRor1* is expressed in heart, lung, kidney, thymus and brain [13]. mRor2 is detected at stage E8, reaches a maximum at E11–13 and declines to barely detectable levels by postnatal day 23. The distribution of *mRor2* message is similar, but more widespread than *mRor1* in neural tissue and is detected in telencephalon, heart and dermis [13, 14]. Analysis of *mRor2-lacZ* in which *lacZ* has replaced much of the *mRor2* coding sequence confirmed the expression seen by in situ hybridization, and also showed additional sites of expression [14, 44]. mRor2-lacZ was expressed in developing axial skeleton and digits. β-gal was detected in chondrocytes of the developing anlagen of all bones that develop by endochondral ossification, such as limb and rib. Expression was not seen in bone that develops by in-

tramembranous ossification, where cartilaginous anlagen are not formed. β-gal also was detected in the perichondrial region and dermis.

In nematodes and mice, Rors are expressed in many tissue types, whereas in insects both Rors are expressed specifically in the nervous system. The reasons for these differences are unclear. One possibility is that Rors function in the nervous system and in other tissues in nematodes and in mammals. In the insect lineage, perhaps Ror function in tissues other than the nervous system is not required, and only the nervous system retains the requirement for Ror function. The identification of mutations that disrupt fly Ror genes will be important for assessing their roles during development.

Ror function

Important insights into Ror function have come from analyses of mutant animals defective in *Ror* genes. Such analyses in *C. elegans* and mice have demonstrated multiple roles for Rors during development. In addition, the discovery that two distinct human disorders result from mutations in *hRor2* have further increased our understanding of Ror function.

C. elegans CAM-1

Analysis of *cam-1* mutants has shown that CAM-1 functions to direct migrating cells to their proper positions and to properly orient cell polarity [9, 43]. In *cam-1* mutants, several embryonic cell migrations are disrupted. Posteriorly migrating CAN, ALM and ccM cells terminate prematurely along their migratory routes (fig. 4A). Also, the posterior CAN axon often terminates prematurely, suggesting that there are defects in the posteriorly directed migration of the CAN axon. In contrast, the anterior CAN axon reaches its normal target. Unlike posteriorly directed migrations, the anteriorly migrating HSN and BDU neurons continue beyond their normal destinations. The general pattern for embryonically migrating cells is that they migrate to more anterior positions. This observation has led to the model that CAM-1 is required to direct migrating cells to their proper positions.

Postembryonic migrations do not conform to the general pattern seen with embryonically migrating cells [43]. The Q cells and their descendants migrate during the first larval stage (fig. 4A, [45]). Here we refer to the Q cells and their descendants collectively as the Q descendants. The Q descendants on the right side migrate anteriorly. In *cam-1* mutants, the anteriorly migrating QR descendants stop short along their migratory routes some of the time. This difference may reflect a different role for CAM-1 in embryonic and postembryonic cell migrations. Alternatively, the role of CAM-1 in cell migration may be complex.

In addition to directing migrating cells to their proper position, *cam-1* mutants also display defects in asymmetric cell divisions and axon outgrowth [9]. Normally, six V cells on each side of first-stage larvae divide asymmetrically to produce an anterior daughter that joins an epithelial syncytium and a posterior blast cell (fig. 4B, [45, 46]). In *cam-1*-mutant animals, 12% of V1 cell divisions are reversed, with the anterior daughter adopting the blast-cell fate and the posterior daughter joining the syncytium.

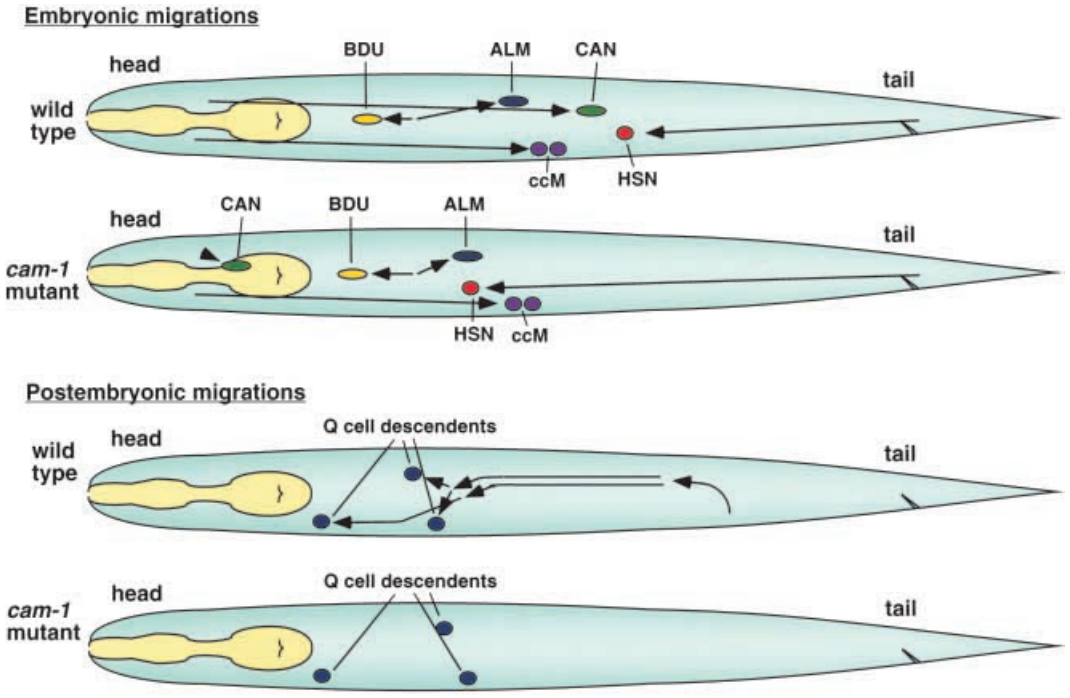
Like V cells, six neuroblasts divide asymmetrically in first-larval-stage males to generate two different daughter neurons (Fig. 4B, [47]). The posterior daughters, the CP neurons, express high levels of the neurotransmitter serotonin and extend an axon posteriorly to the tail. The anterior daughters, the CA neurons, appear to accumulate low levels of serotonin [48]. Instead of extending its axon posteriorly, the most anterior CP neurons often extended their axons anteriorly to the head in *cam-1* mutants. Occasionally, a weakly staining serotonergic neuron was observed just posterior to the most anterior CP neuron, suggesting that polarity of the precursor division was reversed.

In response to high temperature, high population density and limited food, wild-type animals enter a morphologically distinct alternate third larval stage called dauer [49, 50]. In the presence of low population densities and sufficient food, *cam-1* mutations cause a dauer constitutive phenotype, with approximately 15% of the larvae becoming dauers [10, 41]. Under the same conditions, wild-type animals would not become dauer. One of the chemosensory neurons involved in sensing environmental cues that lead to dauer formation is ASI [51]. The dendrite of ASI normally extends to the amphid, a chemosensory structure containing the chemosensory endings of several neurons [52, 53]. *daf-7* encodes a transforming growth factor-β (TGF-β) like molecule that normally is expressed in ASI and that is required to prevent animals from inappropriately entering dauer [52]. In *cam-1* mutants, ASI is defective; its dendrite fails to reach the amphid pore and it fails to express *daf-7-gfp*. Expression of *cam-1* in ASI from the *daf-7* promoter rescues the ASI morphological defects but fails to rescue the Daf-c phenotype. This result suggests that CAM-1 acts cell autonomously in ASI [10].

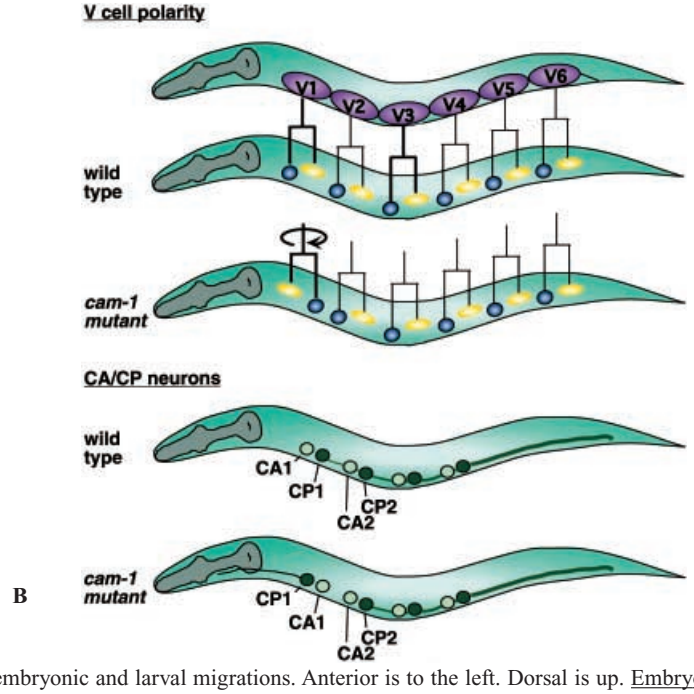
Thus, *C. elegans* CAM-1 functions to direct migrating cells to their proper positions, to orient cell polarity and to direct proper ASI neuron development. Which of these result from defects in primary functions of CAM-1 rather than from secondary consequences of defects in CAM-1's primary function is an issue that needs to be resolved.

mRor2

To examine the role of murine Ror in development, two groups have engineered mice in which the *mRor2* gene



A



B

Figure 4. (*A*) *C. elegans* embryonic and larval migrations. Anterior is to the left. Dorsal is up. Embryonic migrations. Schematic lateral view of the left side of a newly hatched first larval stage hermaphrodite. Final positions of the ALM, BDU, CAN, ccM and HSN cell bodies (ovals and circles), and their migration routes (arrows) in wild type are indicated. Average final positions of ALM, BDU, CAN, ccM and HSN in *cam-1* mutant are indicated. Postembryonic migrations. Schematic lateral view of the right side of a late first larval stage animal. The final positions of the cell bodies of the QR descendants (circles) and their migration routes (arrows) are indicated. Cell deaths that occur among QR descendents are not shown. In *cam-1* mutants, some of the Q cell descendents are misplaced posteriorly a short distance. (*B*) *cam-1* cell-polarity defects. V cells. Six V cells divide to produce two different daughters, an anterior cell that fuses with the epithelial syncytium and a posterior blast cell. In *cam-1* mutants the polarity of the first division is reversed some of the time. CA/CP neurons. CA neurons appear to express low levels of serotonin, whereas CP neurons express high levels of serotonin and extend axons posteriorly to the tail. In *cam-1* mutants, the relative position of cells expressing high levels of serotonin and those expressing low levels are reversed some of the time, suggesting that CA and CP are reversed. In addition, CP axons sometimes extend anteriorly rather than posteriorly.

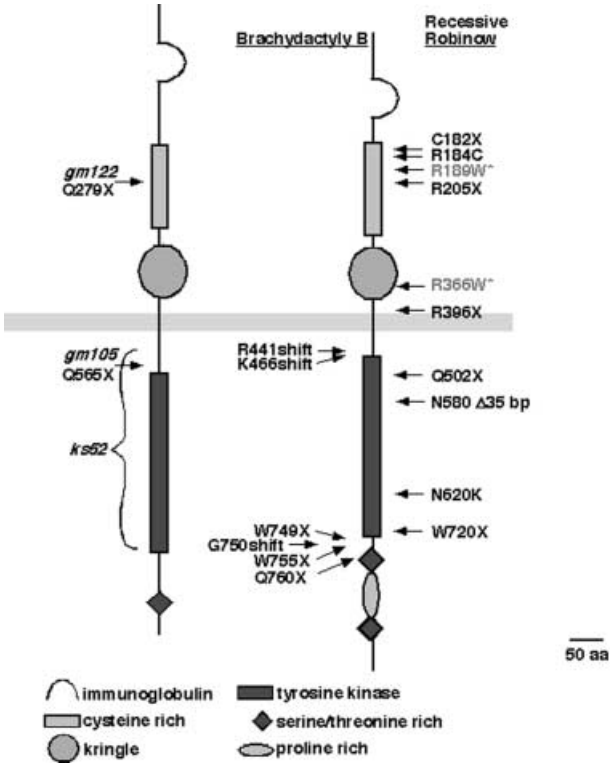


Figure 5. Reported *C. elegans* and human *Ror* mutations. The drawing on the left shows the CAM-1 protein, indicating the domains and the relative location of two point mutations in *cam-1*. The bracket indicates the region deleted by *cam-1(ks52)*. The drawing on the right shows the hRor2 protein, indicating domains and relative location of mutations. Mutations that result in dominant BDB are shown to the left of the drawing, and those that result in recessive Robinow syndrome are shown to the right. The letters show the amino acid present in the wild-type protein, its position within the protein and the substitution. X indicates a change that is predicted to introduce a stop codon. Shift indicates a nucleotide addition or deletion that introduces a frame shift, and Δ indicates a larger deletion. The two changes indicated (*) were present within the gene of a single patient.

has been disrupted. Both groups report similar findings [14, 54]. Mice heterozygous for *mRor2* knockout are phenotypically wild type, indicating that the deletion is recessive. Animals homozygous for the *mRor2* knockout die shortly after birth. *mRor2* homozygotes display shortened snouts, limbs, tails and a cleft palate. Essentially all bones produced by endochondral ossification are shortened or misshapen. Also, the membraneous part of the cardial ventricular septum is defective, whereas the rest of the heart appears normal. In bone production by endochondral ossification, chondrocytes first deposit a cartilaginous anlage (reviewed in [55]). Subsequently, the anlage becomes mineralized to form bone. In *mRor2* mutants, the cartilaginous anlage is smaller than normal, and a reduced number of chondrocytes are present. In addition, the mRor2 knockouts display defects in the differentiation of chondrocytes in growth plate, characterized by reduced numbers of proliferating chondrocytes. These observations demonstrate a role for *mRor2* in endochondral development, possibly regulating the proliferation or differentiation of chondrocytes.

In the mouse knockout experiments, two different strategies were used to knock out *mRor2*. In the first, the Ig domain of *mRor2* was replaced with the neomycin resistance gene (*neoR)* [54]. This knockout is predicted to eliminate *mRor2* expression. Consistent with this, no *mRor2* mRNA is detected by in situ hybridization. In the second *mRor2* knockout, the kinase domain was replaced by *lacZ* and *neoR* genes [14]. In light of the observation that Ror proteins may possess a kinase-independent function, it is possible that the second *mRor2* knockout does not eliminate all function. Although both knockouts produce similar gross phenotypes, it will be interesting to learn whether there are subtle differences between the two knockout strains that might suggest a kinase-independent-function for *mRor2*.

Mutations in *mRor1* have not been reported.

hRor2 genetic disorders

Recently, two different human genetic disorders, recessive Robinow syndrome and brachydactyly B (BDB), have been found to result from mutation in *hRor2* [44, 56–58]. Recessive Robinow syndrome is characterized by short-limbed dwarfism and brachydactyly [59–62]. Affected individuals exhibit abnormal morphogenesis of face and external genitalia, defective vertebral segmentation and rib fusions. A second syndrome, brachydactyly B (BDB), is inherited as an autosomal dominant disorder. Affected individuals have shortened fingers and toes. The thumb and big toe may display broadening or partial duplication, or they may be unaffected [63–65].

Molecular lesions in many families displaying Robinow syndrome have been identified (fig. 5, table 2). Interestingly, the recessive mutations all are predicted to reduce or eliminate *hRor2* function (fig. 5, table 2). In contrast, BDB apparently results from gain-of-function mutations in *hRor2*. The *hRor2* mutations that result in BDB cluster in two regions. One region, the proximal cluster, is predicted to truncate hRor2 just C-terminal to the membrane-spanning region, thereby removing the entire kinase domain. The second region, the distal cluster, is predicted to truncate the protein immediately after the kinase domain, deleting the serine-threonine- and proline-rich regions. The distal mutations cause the more severe phenotype. The proximal mutations result in less severe and more variable defects [58]. The BDB mutations are unlikely to result from loss of hRor2 function for two reasons. First, mutations that are predicted to eliminate hRor2 protein are recessive and result in Robinow syndrome [56, 57]. Second, individuals who are heterozygous for a deficiency that completely deletes the gene are unaffected [44]. It is un-

Table 2. Ror2 mutations in humans.

Nucleic acid change	Amino acid change	Effect on protein	Reference
Dominant brachydactyly B			
1321Δ5	R441shift	14 novel amino acids, then stop	58
D3/+19 intron 8		adds 10 nt to mRNA, 67 novel amino acids, then stop	58
1398+1 nt	K466shift	57 amino acids, then stop	58
2246G to A	W749stop	truncated after kinase	44
2249ΔG	G750shift	23 novel amino acids, then stop	44
2265C to A	Y755stop	truncated after kinase	44
2278C to T	Q760stop	truncated after kinase	58
Recessive Robinow syndrome			
545G to A	C182stop	truncated in CRD	57
550C to T	R184C	missense	56
565C to T			
1096C to T	R189W	nonconserved amino acid in CRD conserved amino acid in kringle	56
613C to T	R205stop	truncated in CRD	57
1189C to T	R396stop	truncated before TMD	57
1504C to T	Q502stop	truncate shortly after start of kinase	56
1740Δ35 nt	N580shift	frameshift in kinase, 123 novel amino acids, then stop	57
1860T to A	N620K	changes invariant K in all tyrosine kinases	56
2160G to A	W720stop	truncated near end of kinase	57

Specific *hRor2* mutations found in patients afflicted with either dominant BPB or recessive Robinow syndrome, and the predicted effects on the resulting protein. Mutations 565C to T and 1096C to T both were detected in the same patient.

clear whether the BDB mutations result in a new function, or result in a dominant negative action of the truncated hRor2 protein produced in effected individuals.

To summarize, in *C. elegans*, the Ror family member CAM-1 is required for proper cell migration, to orient cell polarity and for development of ASI. These phenotypes might reflect a single function of CAM-1, for example in orienting cell polarity. In this model, the cell migration defects would result as a secondary consequence of a failure to orient cell polarity. Alternatively, CAM-1 may display two primary functions – to orient cell polarity and to direct migrating cells to their proper positions. This second model is supported by the observation that cell migration may not require CAM-1 kinase activity whereas cell polarity does [9].

Mouse and humans lacking Ror2 function have defects in endochondral bone formation. Cartilaginous anlagen are abnormally small, and the number of chondrocytes is reduced in *mRor2* mutants. A similar defect may underlie the bone defects exhibited by individuals affected by Robinow syndrome and by BDB.

The relationship between the nematode and mammalian defects that result from loss of Ror function are unclear. It is possible that nematode CAM-1 and mammalian Ror2 perform the same cellular function, such as orienting cell polarity or directing cell migrations. In this model, the defects in mammals lacking Ror2 result from failure in one or both of these functions, which ultimately leads to defects in chondrocyte proliferation and differentiation with the physical manifestation being bone defects. Alternatively, the function of CAM-1 and Ror2 may be fundamentally different.

Future directions

Analysis of *C. elegans* and mouse mutants, and human patients defective in *Ror* genes has begun to illuminate the function of this class of RTKs. However, several important questions remain. First, do Ror proteins possess both kinase-dependent and kinase-independent activities, as is suggested by analysis of *C. elegans cam-1* mutants [9, 10]? If so, what is the nature of the kinase-independent role of Rors? It will be interesting to know whether the *mRor2* knockout in which the kinase domain is deleted produces the same phenotypes as a null mutation in *mRor2*. By analogy to other RTKs, the kinase activity of Rors is likely to be regulated by ligand binding. It will be interesting to see whether ligand binding also regulates the kinase-independent function of Rors. And if so, does the same ligand regulate both activities, or are there different ligands that independently regulate kinase-dependent and -independent functions?

Clearly an important impediment to understanding Ror function is the lack of Ror ligand(s). Ror family members contain the CRD similar to that of frizzled and frizzled-related proteins, raising the possibility that a Wnt molecule may act as a Ror ligand. Also, Rors are similar in extracellular domain structure to MuSK, suggesting that perhaps agrin, the MuSK ligand, may be a Ror ligand. However, evidence supporting either of these possibilities has not been reported.

Another remaining question is, What are the downstream molecules that respond to Ror activation? A combination of genetic and biochemical approaches will likely provide answers to this question.

What is the role in the nervous system of Rors? All Rors are expressed in the nervous system. In fact, the two *Drosophila* Rors appear to be expressed exclusively in the developing nervous system. Mutations in nematode *cam-1* result in defects in nervous system development [9, 10]. However, mutations in *mRor2* result in no gross defects in the nervous system [54]. So what, if any, role do Rors play in vertebrate nervous system development? What is the role of vertebrate Ror1? The analysis of *mRor2* knockout animals have provided important insights into Ror function, as have the examination of patients affected by *hRor2* mutations. However, similar analyses of *Ror1* mutants remain to be reported. It seems likely that the next few years will see answers to most of these questions forthcoming.

Accession Numbers. Acession numbers for genes discussed in this paper are *hRor1*; AAA60275; *hRor2*, AAA60276; *mRor1*, BAA75480; *mRor2*, BAA75481; *Dnrk*, AAD02091; *Dror*; AAA28860; *cam-1*, CAC29085 (short form); *cam-1*, CAC29084 (long form); human *MuSK*, AAB63044; *Torpedo* Ror-related, A47299; and human *TrkB*, 1093345.

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