

Chapter 2

Evolution of Receptor Tyrosine Kinases

Manfred Schartl, Jean-Nicolas Volff, and Frederic Brunet

2.1 Introduction

Receptor tyrosine kinases (RTKs) are important molecules for communication of cells in multicellular animals. It is therefore not surprising that the basal metazoans, like *Hydra* and sponges, already have a well-developed repertoire of RTKs. Even the choanoflagellate *Monosiga brevicollis*, a unicellular eukaryote from a group of protozoa that are thought to represent the ancestors of metazoa or be at least closely related to those, has up to seven genes coding for proteins that present clear features of receptor tyrosine kinases: an extracellular domain composed of EGF and Ig domains, a transmembrane domain, and an intracellular tyrosine kinase domain with a long carboxy-terminus that has several tyrosine residues which may act as substrate docking site after transphosphorylation [1]. It is unclear whether these genes are representing indeed the common ancestors of all RTKs in metazoa, and biochemical studies on signaling properties of the encoded proteins are still missing. The basal metazoans like sponges or *Hydra* already have a well-developed repertoire of RTKs [2–5]. Several of the *Hydra* genes show sequence similarity to the RTKs of *Drosophila* and vertebrates and might represent ancestors of the RTK families that we know today. These include insulin receptors; MUSK, ROR, PTK7, and FGF receptors; and VEGF/PDGF-receptor-like RTKs [3]. How the progenitors of the RTKs may have evolved in the first place is unknown and probably difficult to infer from the genomes of the today existing organism. It can be hypothesized that in the metazoan ancestors,

M. Schartl, Ph.D. (✉)

Department of Physiological Chemistry, Biocenter, University of Würzburg,
Am Hubland, 97074 Würzburg, Germany
e-mail: phchl@biozentrum.uni-wuerzburg.de

J.-N. Volff • F. Brunet

Institut de Génomique Fonctionnelle, UMR5242 CNRS/INRA/University
of Lyon I/Ecole Normale Supérieure, 46 allée d'Italie, Lyon, France
e-mail: Jean-Nicolas.Volff@ens-lyon.fr; frederic.brunet@ens-lyon.fr

preexisting cytoplasmic tyrosine kinases were fused by domain shuffling to surface-associated extracellular proteins that functioned in cell-cell interactions or binding of soluble extracellular proteins [6].

With the increase in complexity of cellular interactions and their regulations, RTKs became more numerous and diversified. This has reached a highest level in vertebrates and created the extensive RTK inventory of the human genome.

A major driving force for the evolution of gene diversity is gene duplication. This can happen on the single gene level and is then called local or tandem gene duplication. It can also happen that the whole genome is duplicated (whole genome duplication (WGD)). Such ideas go back to several visionary evolutionary biologists, most importantly S. Ohno, who has pointed out, already at times where only scarce genomic information was available, the importance of gene duplications because of the creation of new gene loci with *primarily* nonexistent functions. As even more advantageous, he considered complete genome duplications because they increase gene number without upsetting gene dosage and regulatory interactions [7]. Today, we know that such WGD events have indeed happened several times during evolution [8]. In particular, the evolution of vertebrate genomes has been shaped by such events. There were two rounds of such WGDs (called the 1R and 2R event) during early vertebrate evolution when the ancestors of the extant fish groups evolved (the “head-bearing vertebrates,” Craniata) but before the fish lineages that are existing today and land-living vertebrates diverged. In the lineage leading to the modern bony fishes, the teleosts, another WGD (3R) occurred, the fish-specific whole genome duplication (FSGD).

The evolutionary significance of gene duplications becomes evident when one considers the possible consequences. Gene duplications create a situation where two identical copies exist for a function that was previously sufficiently fulfilled by a single one. Consequently, in the first place, only one of the copies is necessary and the second one is dispensable for this function. Now one of the redundant genes can take one of several evolutionary routes. Firstly, it can acquire mutations that will impair its function, and the gene will degenerate and eventually disappear without leaving any detectable trace in the genome. This is the fate of most genes, and in general, the majority of gene duplicates are rapidly lost. Secondly, the dispensable copy can evolve a new somehow related or even unrelated function (neofunctionalization) and will be maintained as a paralogue of the original gene. For instance, a duplicated RTK could be expressed in a different cell type, change its ligand interaction, or can signal through a related pathway or adaptor molecule. Such neofunctionalizations have happened for the vertebrate RTK duplicates from the 1R/2R WGDs. The third possibility is that both duplicates share the functions of the common ancestral gene (gene function partition or subfunctionalization). For example, if an RTK progenitor gene has functioned in the central and the peripheral nervous system, one of the duplicates can exert the CNS function and the other duplicate can take over the function in the periphery. Subfunctionalization appears to be a fate that is very common for the RTK duplicates from the 3R WGD in fish.

Paralogous RTK genes, due to their common origin, share a mostly very obvious sequence similarity and are thus grouped into gene families. The families are named

(often arbitrarily) after their prototypic member, e.g., the EGFR/ERBB family, the insulin receptor family, or the PDGF receptor family. Due to the strong biochemical constraints, the kinase domains show the highest sequence similarity within and between families, and basically, two types of kinase domains are found throughout all RTKs: the uninterrupted and the interrupted enzymatic domains. The extracellular ligand-binding domains are much more divergent in sequence, but within an RTK family, they are a diagnostic common feature. While some families share related domains, like Ig domains or fibronectin type III domains, in general, it is the ligand-binding domain difference that separates the RTK families.

The evolutionary relationships of the RTK families to each other are with few exceptions difficult to resolve, and some might have arisen independently by fusing (even the same) kinase domains to different extracellular domains. To decipher the evolutionary relationships and trajectories inside the RTK gene families, one has to look into the genomes of related organisms, in particular those which diverged in parallel from a common ancestor. In such studies, it is often difficult to identify which is the corresponding family member in the other organism that derived from the same ancestral gene (the true orthologue) and what are paralogous genes that were already separated in the common ancestor. These doubts can be solved by looking at the genomic neighborhood. When a WGD has occurred, whole chromosomes were duplicated and at least some neighboring genes should also be represented on the same chromosome where the duplicated RTK copy is found. This phenomenon is called conserved synteny and is a strong evolutionary tracefinder tool. Gene synteny is reasonably well conserved throughout the vertebrate genomes and has even allowed the reconstruction of the genomic organization of the vertebrate ancestor that has lived more than 500 million years ago [9–12].

This chapter is designed to give an overview of what we know about the phylogenetic history of the RTK gene superfamily and how the repertoire of RTKs in the human genome evolved. A glance at the evolution of some ligands—often occurring also in corresponding families to their receptors—will also be taken, although the evolutionary relationships of this extraordinarily large group of genes are much less clear. We will use the HUGO gene nomenclature of the annotated human genome primarily but will mention the most common and sometimes even better known synonyms.

2.2 The EGF Receptor Family

The EGF receptor (or ERBB) family is represented in the human genome by four members: the prototypic *EGF receptor*, *ERBB2* (*HER2/NEU*), *ERBB3*, and *ERBB4* genes. This situation is a clear result of the WGDs in the vertebrate lineage. In the well-studied invertebrate model species, there is a single bona fide homologue, the *Drosophila* Egf receptor (DER) (also known as the torpedo gene product [13]), and in *C. elegans*, the Let-23 receptor [14]. The sea squirt as the protochordate out-group has two *egfr* genes, which are derived from a lineage-specific duplication of

the common ancestral gene. In the vertebrate lineage, the 1R event has created two genes, one of which generated by the 2R WGD, the *EGF receptor* and *erbb2*, and the other one, the progenitor of *erbb3* and *erbb4*.

While these four family members appear to represent the default situation for all tetrapods, teleost fish have generally seven *erbb*'s as a result of the FSGD (3R), with two copies of *egfr*, *erbb3* and *erbb4*. *Erb2* does not show any duplication in the fish genomes analyzed so far; hence, the second copy may have been lost again very early on. From all the RTK families, the EGFR family has retained the most of its FSGD duplicates.

The unifying characteristic feature of this family is the structure of the extracellular domain, being composed of two cysteine-rich and two L domains. This is reflected in the common, exclusively “receptor-mediated” [15] activation mechanism, where first the bivalent ligand monomer contacts two distinct sites within a single receptor molecule. The resulting conformational change allows then the dimerization arm of domain II to interact with a second ligand-bound receptor molecule.

Interestingly, two of the three amino acid substitutions (H740Q and D815N) in the *Erb3* catalytic domain that are made responsible for the loss of tyrosine kinase activity of this RTK in mammals are also seen in the fish homologues [16]. Thus, these mutations would also have happened early on in the evolutionary history of this gene family and have shaped the functional interactions of *Erb3* with the other family members.

An enigmatic situation is found in a certain lineage of fish, the platyfishes and swordtails. Here a tandem duplication of one of the fish *egfr* genes produced another version, so that these fish have three EGF receptors [17]. However, this third gene, which became known as *xmrk* (*Xiphophorus* melanoma receptor kinase), acquired two mutations in the extracellular domain. These allow the formation of intermolecular cysteine bridges. Hence, the receptor is present as constitutively active dimer and leads to ligand-independent signaling [18], a mechanism of oncogenic RTK activation that has also been found in several human cancers. The expression of the *xmrk* oncogene is normally repressed, so that the increased *Egfr* signal becomes apparent only by the development of large pigment spots. In hybrid crosses, this suppression is lost and the pigment spots grow out to highly malignant melanoma [19] (Fig. 2.1).

2.3 The Insulin Receptor Family

There are three different genes found in the insulin receptor family: the insulin receptor (*insr*) itself, insulin receptor-related protein (*insrr*), and insulin-like growth factor 1 receptor (*igf1r*). The phylogenetic analyses of these three proteins revealed that they can be rooted by one single sequence found in *Ciona* or

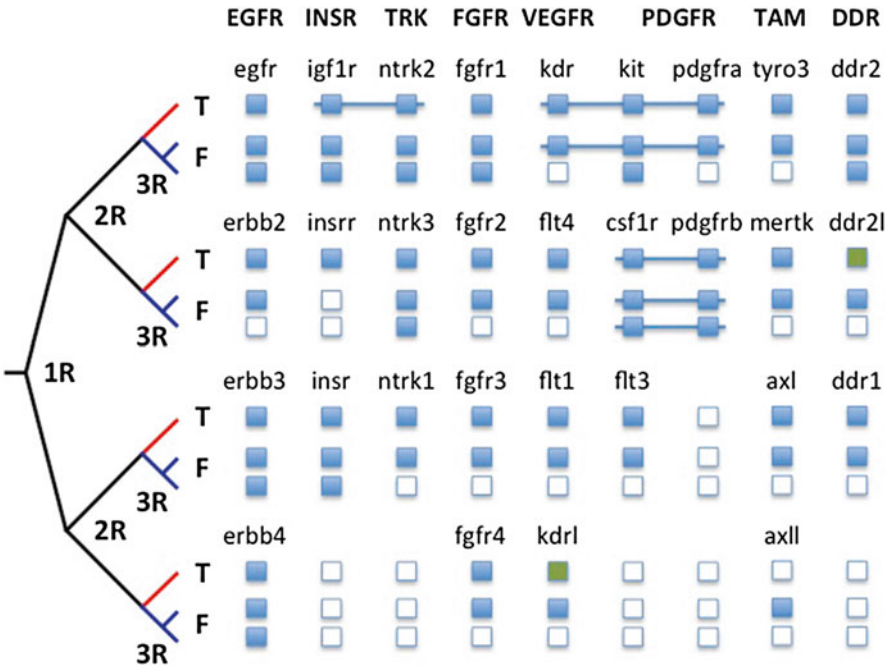


Fig. 2.1 Evolution of the vertebrate EGFR, insulin receptor, FGF receptor, VEGF receptor, PDGF receptor, TRK, TAM, and DDR families, which show a clear 1R/2R/3R pattern. *Filled boxes* represent the occurrence of an RTK gene, in *blue* whenever a gene is found in all main lineages of tetrapods (T) or fish (F) and in *green* for those that have not been found in mammals. *Ddr2l* is also not found in therian mammals and *kdrl* in eutherians. A *white box* indicates that no RTK of this paralogy type has been found among the group of species considered. The *blue lines* indicate genes that are linked on the same chromosome

Amphioxus. The insulin signaling pathway is well conserved in *Drosophila* and *C. elegans*, and consequently, an insulin (or insulin-like) receptor is found there [20]. Even in sponges [2] and *Hydra* [3], insulin receptor-like RTKs are found. The WGD events have generated the *igf1r* and *insrr* paralogues from 2R, while the corresponding paralogue of the *insr* has obviously been lost very early on. Surprisingly, the *insrr* gene has also been lost in the fish lineage. Nevertheless, fish have in general four members of the insulin receptor family, because the FSGD duplicates of *insr* and *igf1r* have been retained.

The insulin receptor family is, besides its sequence similarity, also characterized by a unique quaternary structure. The precursor polypeptide of the receptor monomer is cleaved into an α -fragment and a β -fragment, which are covalently linked in the extracellular part. The mature receptor monomer is further on bound to another monomer. Therefore, the paradigm of RTK receptor proteins existing as monomers in the inactive state is a feature absent from all members of this family.

2.4 The FGF Receptor Family

Alike the EGFR family, the mammalian FGF receptor family is composed of four members, *fgfr1–4*. In the fruit fly, two FGF receptors have been characterized, Btl (breathless) and Htl (heartless), referring to their important developmental function [21, 22]. Heartless, a *Drosophila* FGF receptor homologue, is essential for cell migration and establishment of several mesodermal lineages. They are the result of a lineage-specific duplication, like in planarians, which also have two FGFRs. But, although these invertebrate genes are sometimes named *fgfr1* and *fgfr2* like the mammalian ones, they are clearly not orthologues of the vertebrate receptors, but the result of a lineage-specific local gene duplication. Other invertebrates, like *C. elegans*, have only one FGF receptor [23]. *Fgfr*-like RTKs were also found in *Hydra* [3]. There is a single gene in the protochordate out-group. During the 1R WGD, the progenitors of *fgfr1/2* and *fgfr3/4* were generated, which then during 2R produced the four receptor paralogues that we know today. In fish, the family is less expanded than the EGFR family. Only the 3R duplicate of *fgfr1* has been retained.

The members of this family are united by the split tyrosine kinase domain and three Ig domains in the extracellular ligand-binding part with a small acid box between the first and second Ig domains.

2.5 The VEGF Receptor Family

In placental mammals, the family of receptors for the vascular endothelial growth factors consists of three members: *flt1/vegfr1*, *kdr/vegfr2*, and *flt4/vegfr3*. In marsupials, platypus, chicken, and several fish genomes, a fourth member is found, which is called *kdrl* (for kinase insert domain receptor-like) or consequently *vegfr4*. The family thus shows a clear 1R/2R pattern (Brunet et al. in preparation), which generated at first four duplicates, of which obviously the *kdrl/vegfr4* gene has been lost in the placental mammals. *Vegfr2* and *vegfr3* are the product of the second WGD, and obviously, *vegfr4* arose together with *vegfr1*. Why one member of this family after hundreds of million years of existence became dispensable in mammals is unclear. Either it was accidentally lost and its function was taken over by another RTK or its function is related to features that are not present anymore in the higher mammals. Interestingly, all four fish-specific duplicates of the FSGD have been lost. The common vertebrate ancestor had a single *vegfr* gene. The situation in invertebrates is intriguing as there are receptors, which bind a ligand that is obviously the common precursor of the PDGFs and the VEGFs. In fact, this receptor by sequence similarity could be the ancestor of both receptor families. In *Drosophila*, a single Pdgf/Vegf receptor (PVR) is present, while in *Hydra*, three of these RTKs are found [3].

The structural features of the VEGF receptor family are the interrupted kinase domain and seven Ig domains that form the ligand-binding domain. This part of the receptors is most similar to the PGDF receptor family, which has five instead of seven Ig domains. Such structural similarity hints to a common origin. Indeed, this has been proposed on the basis of the common exon/intron structure [24] and is further supported by the molecular phylogeny on the basis of the kinase domains (Brunet et al., in preparation). Considering also a common origin of VEGFR and PDGFR ligands, it becomes reasonable that these receptors have a common origin. The evolutionary scenario for the origin of these receptors therefore is that in the protochordate ancestor, a single receptor gene was first duplicated to produce the ancestors of the VEGFR family and of the PDGFR family. The latter one duplicated once more to give the progenitors of the PDGFR (*sensu stricto*) subfamily and the KIT/CSF1R/FLT3 subfamily (see below). Then during the vertebrate WGDs, the whole repertoire of this group of RTKs was generated (Fig. 2.2).

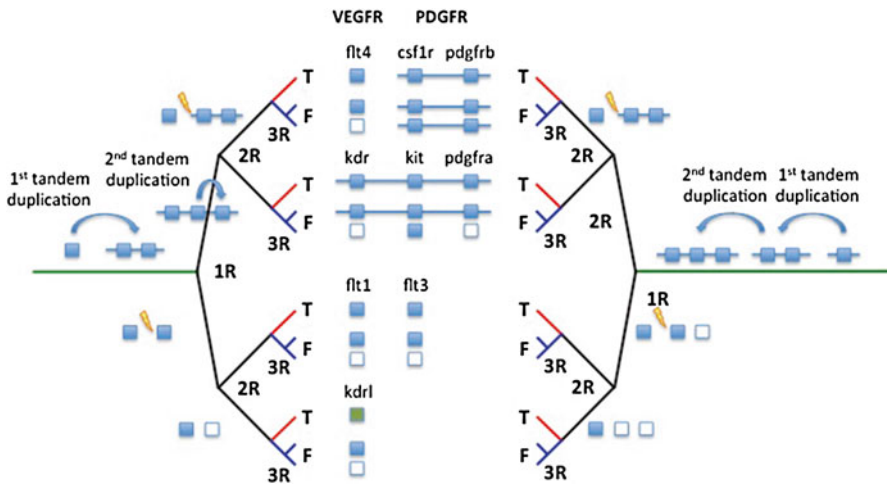


Fig. 2.2 Hypothetical scenarios for the evolution of the PDGF and VEGF receptor families. Due to the fact that *pdgfra*, *kit*, and *kdr* are found in tandem, as well as *pdgfrb* and *csf1r* in tetrapods (T) and in fish (F), two evolutionary scenarios can be proposed that explain this feature by considering two tandem duplications, either with a tandem duplication that generated the ancestors of the VEGFR and PDGFR families before the 1R WGD (*right*) or a tandem duplication between the 1R and 2R events (*left*). The *left* scenario is more parsimonious because it requires one event less of gene loss to fit with the actual distribution of the two RTK families. Synteny disruptions are shown with yellow lightnings. Filled boxes represent the occurrence of an RTK gene, in blue whenever a gene is found in all main lineages of tetrapods (T) or fish (F). *Krdl* is in green because it is not found in eutherians. White boxes indicate that no RTK is found among the group of species considered

2.6 The PDGF Receptor Family

The platelet-derived growth factor family is composed of five members in the human genome: *PDGFRA* and *b*, *KIT/SCFR*, *FMS/CSF1R*, and *FLT3*. Phylogenetically, they are placed in two subfamilies, the PDGFRs and the remaining three RTKs. *PDGFRA* and *b* originate from the 2R event (Brunet et al. in preparation). There is a third PDGF receptor in the teleost fishes, which is called *pdgfrb2* to distinguish it from the *pdgfrb1*. Both are the result of the FSGD of *pdgfrb*, while the duplicate of *pdgfra* was lost early on during the evolution of the teleost fishes.

The KIT/FMS/FLT3 subfamily is also again the product of the early WGDs. *Kit* and *csflr* were generated in the 2R event from a common precursor, while the 2R paralogue of *flt3* was obviously lost. *Kit* and *csflr* in fish have their FSGD paralogues, but not *flt3*. Thus, the whole PDGFR superfamily is represented by eight members in the teleosts compared to only five in mammals.

Intriguingly, *kit* and *pdgfra* as well as *csflr* and *pdgfrb* are closely located side by side on the same chromosomes in tetrapod and some fish genomes. Thus, it can be inferred with fidelity that before the 2R event, a local gene duplication generated the precursors of the genes that then gave rise to *kit* and *csflr*, on the one hand, and *pdgfra* and *b*, on the other hand (Fig. 2.2) (Brunet et al. in preparation).

Moreover, *kdr* is linked to *kit* and *pdgfra*, suggesting another evolutionary relationship within the RTKs with Ig domain containing ligand-binding domains. In birds and lizards, *flt4* is also only 2–4 genes away from the *csflr/pdgfrb* gene pair. Thus, a scenario becomes likely that a single RTK gene in the protochordate ancestor with seven Ig domains was first locally duplicated on one chromosome to become the progenitor of the seven Ig and five Ig domain RTKs. One of the genes became the progenitor of the VEGFR family, which has seven Ig domains. The other one lost two Ig domains and then underwent another local gene duplication. These two genes then constituted the progenitors of the five Ig domain RTK gene families, the PDGFR, and FMS/CSF1R/FLT3 subfamilies. The resulting cluster of three RTK genes then was expanded through the 1R/2R WGDs with subsequent loss of some genes [23] (Fig. 2.2).

2.7 The NTRK Family

The human gene family is composed of *NTRK1/TRKA*, *NTRK2/TRKB*, and *NTRK3/TRKC*. The phylogenetic tree of this RTK family again proposes an evolutionary history from the 1R/2R WGDs, indicating that one of the four genes has been lost early on, most probably the duplicate of *ntrk1*. *Ntrk2* and *3* group together by sequence similarity but also by a common genomic structure (large size of the gene), which is different in *ntrk1* [25]. In fish, *ntrk2* and *3* have 3R WGD duplicates, but an NTRK1 duplicate is not present. In the vertebrate ancestors, a gene homologous to the NTRKs has been found in *Amphioxus* and sea urchin, but not in the sea

squirt [26, 27]. *Drosophila* and *C. elegans* do not have *ntrk*'s, but the pond snail *Lymnaea stagnalis* has a Trk-like RTK [28]. Hence, it is possible that this RTK family might also have its origin in a common ancestor of the deuterostomes and the protostomes, which is supported by a similar evolutionary scenario for the neurotrophin ligands [25].

NTRKs share with many other RTK families the uninterrupted kinase domain and extracellular Ig domains but uniquely have an N-terminal solenoid-like leucine-rich domain. Moreover, all three NTRKs function together with a common neurotrophin receptor, the p75NTR. This co-receptor is structurally and functionally unrelated to the NTRKs but apparently has coevolved with this RTK family. p75NTRs are present in all vertebrates and in the protochordates.

A possible relationship of NTRKs with other RTK families could be inferred from the fact that in tetrapods, *ntrk2* is located side by side with *igf1r*. However, the total different structure of the extracellular domains of both families makes a common origin from an ancestral gene duplication difficult to explain without involving a domain shuffling event. On the other hand, *ntrk2* and *ror2* are only 25 genes away (~5 Mb) on human chromosome 9 and linked on chrZ in birds and chr2 in the lizard as well. Sequence similarity also indicates a closer relationship and thus possibly a common origin of the NTRK and ROR RTK families. We hypothesize that the progenitors of both families were generated by a local gene duplication of an RTK gene with affiliation to the *Drosophila* sevenless receptor in the chordate ancestors before the 1R WGD.

2.8 The TAM Receptor Family

The three TAM family members *TYRO3*, *AXL/UFO*, and *MERTK* are the results of the WGDs. *TYRO3* and *MERTK* evolved from a common ancestor during the 2R event. The corresponding 2R duplicate of *AXL/UFO* is most likely represented by the *axl-like* gene in the teleost fishes, but it has been lost in the tetrapod lineage. Strikingly, in the bird genomes, no homologue of *axl/ufo* gene is found.

The origin of this family is obscure. There are homologues in the sea squirts, which are now considered to be more closely related to the vertebrates. No homologue was found in sea urchin or *Drosophila*.

2.9 The DDR Family

The two *DDR* RTK genes in humans originated during the early WGDs (Brunet et al. in preparation). The nonmammalian vertebrates have a third family member, *ddr2-like*, which is clearly the 2R ohnolog of *ddr2*. Thus, the corresponding 2R duplicate of *ddr1* was lost without leaving a trace in any of the known vertebrate genomes. While *ddr1* was lost in reptiles and birds but kept in the therian mammals,

the opposite happened for *ddr2-like*, which was lost in mammals but kept in reptiles and birds. In the *Xenopus* genome, all three DDRs can be found. Fish have a 3R duplicate in the *ddr2* branch; consequently, this group has four family members compared to only two in mammals. *Ddr* is present as a single gene in the protochordates. In the *Drosophila* genome, also a clear homologue of *ddr* is present. A *ddr* gene was also identified in *Hydra* [3]. The DDRs are characterized by their unique extracellular domain, which consists of two discoidin domains.

2.10 The EPH Receptor Family

The ephrin receptors are the largest family of RTKs. In humans, 15 members can be identified: *EPHa1–8*, *10*, and *EPHb1–6*. EPHA and EPHB receptors are members of two phylogenetically distinct clades and are distinguished also by their different ligands: A-type receptors bind GPI-linked ephrin-A ligands (ephrin-A1–6) and B-type receptors bind to ephrin-B ligands (ephrin-B1–3), with the exception of EPHA4 and EPHB2, which can bind a ligand of the other type in addition. *Drosophila* and sea urchin have a single ephrin receptor, but in the sea squirts, a lineage-specific expansion of the ephrin receptors occurred. In *Hydra magnipapillata*, three different ephrin receptors were identified [3], which also were generated specifically during *Hydra* evolution. Ephrin receptors are also present in sponges [29]. It has been proposed that until the appearance of the protochordates, a single EPH receptor was present, which was a common receptor for the ancestral ephrin-A and ephrin-B ligands [30]. How the components of the two subfamilies were generated during vertebrate evolution by local gene duplications, WGDs, and gene losses is unclear at present and remains a challenging task for the future. In fish, clearly, the 3R WGD has generated additional genes for *epha2*, *4*, *6*, and *ephb1–5* (Brunet et al. in preparation). The EPHA4-like receptor is only found in fish and *Xenopus* and appears to be lost in the “higher” vertebrates. Conversely, *epha1* is absent from these organisms but present in reptiles, birds, and mammals. *Eph5* exists only in chicken, where it appears to be lineage-specific duplicate of *eph6* (Fig. 2.3).

2.11 The ROR, TIE, MET, and ALK Receptor Families

The ROR, TIE, MET, and ALK RTK families have only two members each (*ror1* and *2*; *tie1* and *2*; *met* and *mst1r*; *alk* and *ltk*) in the mammalian genomes and all other vertebrates, except for the *mst1* receptor, which in fish is represented by two paralogues resulting from the 3R WGDs. All go back to a single protochordate ancestor; however, it is unclear so far how the two family members were generated during the early vertebrate WGDs. In *Drosophila*, RTK homologues of *alk* and *tie*’s from the vertebrate families are present. *Met* and *ron* appear to be absent from insects and other protostomes, but *ror* has a homologue in *Drosophila*, at least what

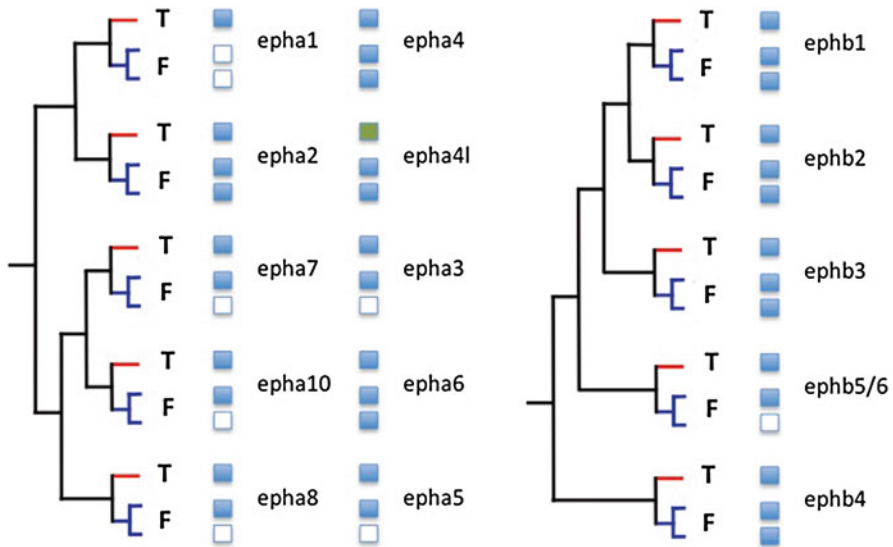


Fig. 2.3 Phylogenetic distribution and evolutionary relationship of the *epha* and *ephb* genes. The nature of the duplication events (WGDs and local gene duplications) that occurred among these genes could not be traced down by molecular phylogenetic analyses and conserved synteny. Filled boxes represent the occurrence of an RTK gene, in blue whenever a gene is found in all main lineages of tetrapods (T) or fish (F). *Epha4l* is in green because it is only found in *Xenopus*. White boxes indicate that no RTK is found among the group of species considered

can be said from the sequence similarity of the kinase domain, while the other portions are less obviously related. In *Hydra*, a Ror1-like RTK was identified [3].

All four families have an uninterrupted kinase domain. The ALK family is most peculiar because the extracellular domain is composed of two Mam domains and one LdlA domain, which are not found in any other RTK. The three other families share with many other RTK families the presence of Ig domains. Ror1 and 2 are somehow similar to MuSK by the additional Fz domain but uniquely have a kringle domain. Tie1 and Tie2/Tek show similarity to the AXL family having three Ig and fibronectin domains instead of two. They are unique by the presence of three EGF domains. Met and Ron have in front of their four Ig domains a Sema/Psi domain which is absent from all other RTKs [15] (Fig. 2.4).

2.12 MUSK, PTK7, RET, ROS, and RYK

This group of RTKs is peculiar, because they present themselves as “sibling-less” without a clear paralogue in the genomes of vertebrates.

Musk has in its extracellular portion a Fz domain like the Ror’s and an Ig domain, which is present in Ror’s and many other RTKs, but the phylogenetic

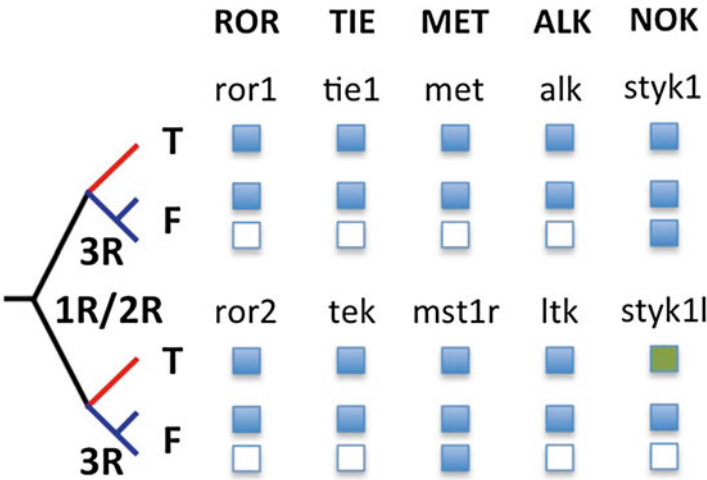


Fig. 2.4 Schematic view of the ROR, TIE, MET, and ALK RTK families that expanded either in the 1R or 2R whole genome duplications, where, however, the exact history cannot be inferred. The gene duplication of *mst1r* and *styk1l* resulting from the 3R is also shown by two boxes in fish. *Filled boxes* represent the occurrence of an RTK gene, in *blue* whenever a gene is found in all main lineages of tetrapods (T) or fish (F). A *white box* indicates that no RTK has been found among the group of species considered. *Styk1l* is in *green* because it is only found in *Xenopus* within the tetrapods

analyses did not group it together with any of the others. The “earliest” homologue of *musk* was found in the ray *Torpedo californica* from the basal vertebrate group of cartilaginous fish. Neither in fish nor in any other vertebrates a paralogue could be identified. On the other hand, a Musk-like RTK has been reported from sea urchin [27] and from *Hydra* [3]. Thus, its evolutionary origin remains obscure. From sequence similarities, a relationship to the ROR family can be drawn.

For *ptk7*, *ret*, *ros*, and *ryk* each a distantly related but clear protochordate out-group orthologue could be identified in *Ciona*, *Branchiostoma* (Brunet et al. in preparation), and sea urchin [27]. In *Hydra*, a putative orthologue of *ptk7* was described [3]. No remnants of the three WGDs were detected in any of the vertebrate genomes. The “orphan” character of *ryk* is reflected by the unique presence of a WIF extracellular domain. Besides an ephrin-binding domain that is shared with the many members of the EPH receptor family, Ret harbors uniquely a WIF domain. Similarly, Ros has besides the widespread fibronectin type III domains exclusively the YWTD propeller domain, which supports the status of singularity. Ptk7, also known as Cck4, has seven Ig domains and no other type as the extracellular part alike the VEGFR family members (and five or three for the PDGFR and FGFR families, respectively) and an uninterrupted kinase domain as many other RTKs (Fig. 2.5).

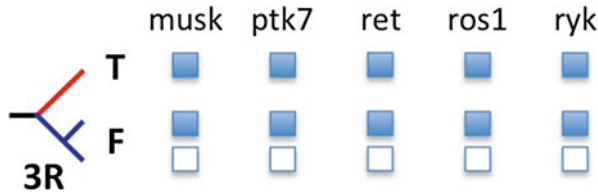


Fig. 2.5 Common origin of the *musk*, *ptk7*, *ret*, *ros*, and *ryk* RTKs in tetrapods (T) and fish (F). These RTKs have not build up a gene family even though the ancestral gene surely went through the two whole 1R and 2R genome duplications. Also none of the FSGD genes has been retained

2.13 The NOK Family

The NOK family is represented in vertebrates by *stykl1*, which is found in all vertebrates. In teleost fishes, two genes are present, *stykl1a* and *stykl1b*, which are clearly duplicates from the 3R WGD. Interestingly, there is another *stykl1-like* gene (*stykl1l*), which is only present in *Xenopus* and in fishes (without a 3R paralogue). No homologue could be found in protochordates or invertebrates. Styk1 and Styk1l possess no extracellular domain and are therefore here discussed outside the RTK families, but their kinase domains show clear sequence similarity to the kinase domains of the PDGFRs and FGFRs. It appears that *stykl1* emerged in a vertebrate ancestor from a duplicate of an RTK gene of the seven Ig domain/split kinase domain family, which subsequently lost the extracellular part (Fig. 2.6).

2.14 Evolution of RTK Ligands

The evolutionary history of the RTK ligands is much more difficult to elucidate. First, the number of known and potential ligands is very high and exceeds by far the number of RTKs. Second, they are much less conserved on the amino acid level, making interferences from protein sequence comparisons more difficult or often impossible. In many cases, only a few amino acids, which are important for the common three-dimensional structure of a certain growth factor family, are conserved, like cysteines involved in cystin bridges. Only a few studies have approached therefore a comprehensive evolutionary history of groups of ligands, and thus, only some prototypic examples can be discussed here.

2.15 EGFR Ligands

In mammals, there are seven recognized ligands that bind and activate Egfr, namely, epidermal growth factor (Egf), transforming growth factor alpha (Tgfa), amphiregulin (Areg), betacellulin (Btc), epiregulin (Ereg), heparin-binding EGF-like growth

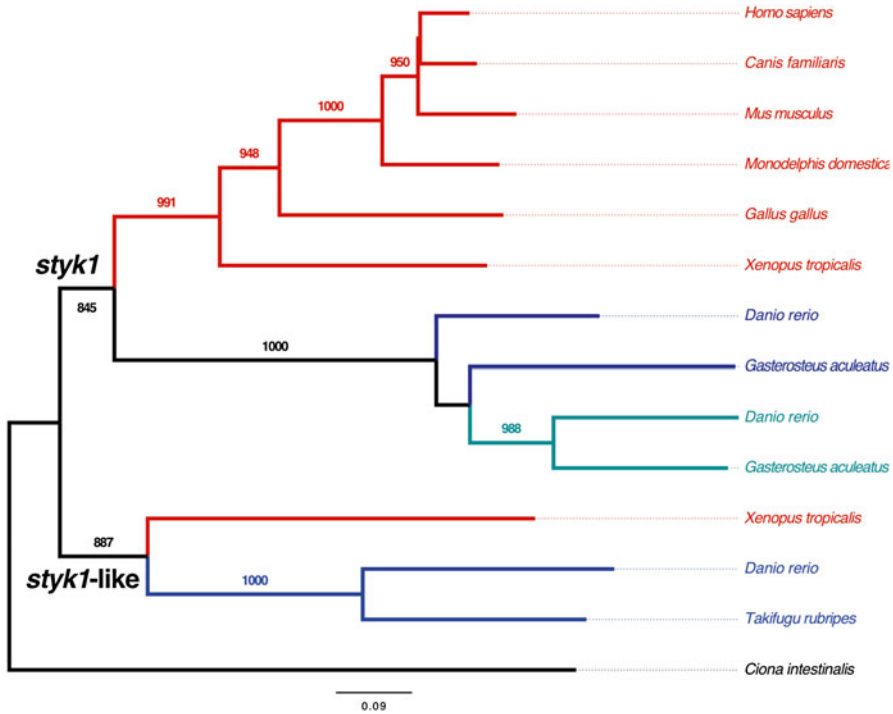


Fig. 2.6 Phylogenetic tree reconstruction of the NOK family using maximum likelihood (PhyML). This family is rooted by the protochordate *Ciona intestinalis* ENSCINP00000025821. *Styk1* is shown for human, *Homo sapiens* ENSP00000075503; dog, *Canis familiaris* ENSCAFP00000019768; mouse, *Mus musculus* ENSMUSP00000044098; opossum, *Monodelphis domestica* ENSMODP00000022763; chicken, *Gallus gallus* ENSGALP00000023762; and *Xenopus tropicalis* ENSXETP00000035460, and in the fish, *styk1a* is shown for zebrafish, *Danio rerio* ENSDARP00000095367, and stickleback, *Gasterosteus aculeatus* ENSGACP00000002580, and *styk1b* for the same species ENSDARP000000101865 and ENSGACP00000013787; *styk1*-like is shown for *Xenopus tropicalis* ENSXETP00000020968; zebrafish, *Danio rerio* ENSDARP000000115394; and fugu, *Takifugu rubripes* ENSTRUP00000012210

factor (Hbegf), and epigen (Epgn). Although a lot of genomic information exists for the other vertebrate lineages, there is for most organisms no clear answer how many ligands the Egfr and any of the other family members would be exposed to. The problem is that only the mature part of the ligands with the Egfr-interacting motif, which covers not more than approximately 140 bases, is suitable for phylogenetic tree reconstruction. This small region gives in most cases not enough phylogenetic information, so only the synteny analyses can give final cues to define true orthology vs. paralogy and to trace the phylogenetic relationships. Such analyses revealed the evolution of the Egfr ligands in vertebrates [31] (Fig. 2.7).

All ligands of the Egf receptor can be traced back to a single ancestral gene on protochordate chromosome C. This gene was duplicated twice during the 1R and 2R whole genome duplication events of early vertebrate evolutionary history in

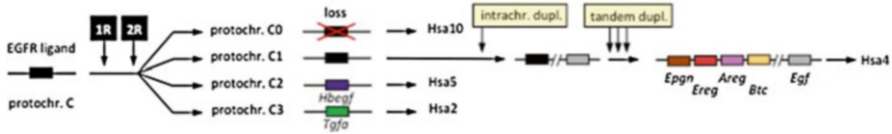


Fig. 2.7 Evolution of the *Egfr* ligands from a single common ancestor. Hsa, human chromosome

organisms that preceded the appearance of sharks and fish. One of the four progenitor genes was lost, one became *tgfa*, and the other one the *hbegf* gene. The remaining gene on the protochordate chromosome C1 underwent a local gene duplication. One of the resulting duplicates evolved into the *egf* gene, while the other one was again duplicated twice giving rise to the *Btc*, *Areg*, *Ereg*, and *Epgn* encoding genes. This cluster is, however, not always fully intact in all vertebrates; for instance, in birds, *btc* has been moved to another chromosome. A similar situation is seen in some fish, and some organisms have obviously even lost one or the other gene family member or the cluster broke further apart.

Of special evolutionary interest is the situation in fish. Here most species have as a result of the 3R FSGD two *egfr* genes (*egfra* and *egfrb*), but there is no indication that there are also more ligands. Obviously, immediately after the FWGD, the second version of each of the ligand genes was lost. Moreover, there is evidence that the two *egfr* genes have partitioned the tasks of the ancestral *egfr* gene by being differentially expressed in separate tissues and organs and they are working in concert with specific subsets of the *Egfr* ligands [31].

It is intuitive that other ligand genes with EGF-like domains, i.e., neuregulins, tomoregulins, and others, share an evolutionary history with the seven *Egfr* ligands due to their striking sequence similarity [32, 33]. However, the tomoregulin genes do not show any obvious synteny with the gene regions that harbor the *Egfr* ligands, so their evolutionary history remains unclear for the moment. The neuregulin gene family members, NRG 1, 2, 3, are also located on mammalian chromosomes that are derived from the ancestral vertebrate chromosome C and therefore could have evolved along with the canonical *Egfr* ligands [31].

2.16 The FGF Family

The mammalian FGF family (reviewed in [34]) comprises 22 members (*fgf1–23*). *Fgf15* is not present in humans, while in mouse and rat, *fgf19* is missing. The protein sequence similarity and syntenic relationships define seven subfamilies, which mirrors also the classification by mode of action: the intracrine FGFs (*Fgf11–14*), the paracrine FGFs (subfamilies 1/2/5, 3/4/6, 7/10/22, 8/17/18, 9/16/20), and the endocrine FGFs (*FGF15/19/21/23*). Notably, the intracrine FGF subfamily members are not ligands for the FGFRs but interact with the intracellular domains of voltage-gated sodium channels and with a neuronal MAPK scaffold protein,

islet-brain-2 [35, 36]. The subfamily of endocrine FGFs is also united by their very low affinity to FGFRs and heparin/heparin sulfate.

An evolutionary scenario has been proposed [34] with one heparin binding but secreted signal peptide lacking ancestral gene of the intracrine subfamily. This gene was duplicated in the early metazoan history and a secreted, receptor-binding gene evolved. This further duplicated in the protochordate ancestor to generate the ancestor of the endocrine FGFs. Although in the genome of *Amphioxus* no homologue of the intracrine FGFs was found [37], one of the six FGF genes present in the *Ciona* genome is firmly nested within the *fgf11–14* branch of the phylogenetic tree [38]. The expansion of the family that we see today then was the result of the 1R/2R WGDs. Remarkably, the intracrine and endocrine subfamilies consist of four members, being in line with this hypothesis. All paracrine subfamilies have only three members, which could be interpreted as if one of the four 2R duplicates got lost very early after the WGD event.

2.17 The PDGF/VEGF Family of Growth Factors

Vegfa–d, Plgf, and Pdgfa–d form a family of RTK ligands with a well-conserved growth factor domain, the so-called Pdgf/Vegf homology domain. This domain forms a tight cysteine knot structure and in most cases is involved in (homo- or hetero-) dimerization by disulfide bonding. Pdgfc and d have a CUB domain in addition to the classical Pdgfs (a and b).

On the basis of their amino acid sequence, the evolutionary history of these growth factors is difficult to decipher. *Pdgfa* and *b*, *c*, and *d*, *vegfc* and *d*, and *vegfa* and *plgf* are more closely related to each other, but there is no further clear rooting [39].

In *Drosophila*, three PDGF-/VEGF-like factors (PVFs) have been identified, which act through a single receptor, and a single PVF homologue is present in *C. elegans* [40]. Although a lineage-specific expansion of the corresponding receptors has occurred in the worm, the entire PDGF/VEGF family and all their receptors go back to a single growth factor/receptor pair in the early bilaterian ancestor. The PDGF/VEGF family appears to be related as a superfamily by the cysteine knot feature to the glycoprotein hormone- and mucin-like proteins and more distantly to the transforming growth factor β (TGF β) family [40].

2.18 Conclusions

Tracing the evolutionary history of the whole complement of 58 RTKs in the human genome uncovers that they developed from a set of progenitor RTK genes in the ancestor of vertebrates. In general, the picture emerges that all RTK families and superfamilies go back to a single common ancestral gene in the early metazoan

lineage and in most cases in extant invertebrates only a single representative of each family is found. Representatives of the EGF receptor, insulin receptor, FGF receptor, the PDGFR/VEGFR superfamily, EPH receptor, DDR and ROR families, and MuSK and PTK7 receptors have been found. This basic set of RTKs has diverged and expanded in certain branches of the protostomian invertebrates, but it has been this basic repertoire of RTK that was used again and again for creating evolutionary and developmental diversity.

The set of 9 early metazoan RTKs was expanded to 16 in the protochordate ancestor, and representatives can be found in the genomes of sea urchin, *Ciona*, and *Amphioxus*. They are the ancestors of the mammalian gene families. How these ancestors are related is difficult to say. The sequence similarity in the kinase domain suggests a close relationship of the insulin receptor family to Ptk7 and Ros and also to the DDR family, MUSK with the ALK family, the AXL family with the MET family and RYK, the TIE family with RET and the FLT/CSF1R/KIT subfamily, and the FGFR family with the PDGFR subfamily. The full complement of receptors of the human RTK families is the result of the early vertebrate WGDs. The average retention rate for gene duplicates from the 1R/2R events is 20–30 % [41]. Without considering the EPH receptor family, for which the evolutionary relationships could not be fully solved, the duplicate retention rate for RTKs is much higher: 55–58 %. This high tendency for duplicates to be kept—rather than being lost in the process of reinstalling the normal 2 N gene dosage of the genome—in this class of proteins is easily explained by the important cellular function of these receptors. With the increasing complexity of cell types, cell functions, and the intracrine, autocrine, juxtacrine, paracrine, and endocrine cellular interactions, the RTKs were targets to implement evolutionary innovations that required novel signaling pathways.

It appears that each RTK family is mirrored by a family of corresponding growth factors that also expanded during the evolution of the vertebrates. The need for specific ligand/receptor interactions has the immediate consequence that both partners coevolve. A receptor mutation in an amino acid that is involved in ligand binding and which will change the intermolecular interaction forces will only be fixed if a compensating mutation occurs in the corresponding growth factor. On the other hand, the need for creating evolutionary novel interactions to make an increased complexity possible has led to new ligand/receptor interactions. The ability for receptors and ligand to dimerize as heteromers has probably also driven the retention of many duplicates after local and whole genome duplications and created many new ligand/receptor interactions. But obviously, the coevolution paradigm has restricted this to occur only within families. At least to our knowledge, there is no case that a ligand of a certain gene family has switched its binding affinity to another family of RTKs outside its original affiliation.

Coevolution would also predict that following a WGD, each set of duplicated RTKs should have kept a corresponding set of duplicated ligands, but this is obviously not the case as can be seen, for instance, for the lack of any 3R duplicates in fish for the EGFR ligands, despite two paralogues of the receptor have been retained in evolution.

With the same argument, it is predicted that the intracellular signal transduction network downstream of RTKs should have expanded together with the RTK families. To trace the evolutionary history and origins of this extremely complex system where indeed many components are paralogues is a challenging task for evolutionary systems biology [41]. An intriguing hypothesis has been put forward in this field. It is proposed that WGDs would have initially duplicated RTKs with a single cognate ligand and a linear private signal transduction machinery. Over time, these uncoupled cascades helped by the structural similarity of their paralogous members started to horizontally interact, leading finally to the cross-talking pathways [6].

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