Deceiving appearances: signaling by "dead" and "fractured" receptor protein-tyrosine kinases

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Summary

The mechanisms by which most receptor protein-tyrosine kinases (RTKs) transmit signals are now well established. Binding of ligand results in the dimerization of receptor monomers followed by transphosphorylation of tyrosine residues within the cytoplasmic domains of the receptors. This tidy picture has, however, some strange characters lurking around the edges. Cases have now been identified in which RTKs lack kinase activity, but, despite being ''dead'' appear to have roles in signal transduction. Even stranger are the cases in which genes encoding RTKs produce protein products consisting of only a portion of the kinase domain. At least one such ''fractured'' RTK appears to be involved in signal transduction. Here we describe how these strange molecules might function and discuss the questions associated with their evolution. ***BioEssays*** **23:69–76, 2001.**

Introduction

The mechanisms by which most RTKs transduce signals have been dissected extensively at the molecular level. This has led to the development of a model explaining how RTKs work.[1,2] The basic features of the model are as follows: (1) ligand-induced dimerization of receptor monomers, (2) transphoshorylation by the dimerized receptors, and (3) docking of proteins to receptor phosphotyrosines and additional phosphorylation of these docked proteins and other substrate proteins (Fig. 1A). In cases where only a single gene for a particular receptor type is present (e.g. the epidermal growth factor receptor (EGFR) in *Caenorhabditis elegans*), only homodimerization can occur. In other cases, such as the EGFR and fibroblast growth factor receptor (FGFR) families in mammals, multiple family members allow formation of heterodimers and homodimers. Heterodimeric receptors can have different signaling capacities than homodimeric receptors.[3,4]

This paradigm satisfactorily explains the behavior of the majority of RTKs characterized to date. However, it is clear that not all RTKs function this way and data indicate that some RTKs have diverged to become signal transducing molecules that lack kinase activity. We will describe these various cases below and discuss how these seemingly dead or fractured kinases may function in signal transduction pathways. Following this, we will discuss the most puzzling feature of these RTKs, their evolution.

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Abbreviations: EGF, epidermal growth factor; GHR, growth hormone receptor; GST, glutathione S-transferase; PDGF, platelet-derived growth factor; PTB, phosphotyrosine finding; PTK, protein-tyrosine kinase; PLC, phospholipase C; PC, posterior commissure; RTK, receptor protein-tyrosine kinase; SH2 domain, src homology 2 domain; SH3 domain, src homology 3 domain.

"Dead kinases"

ErbB3: a recently deceased RTK

One of the most studied examples of a "dead" kinase is ErbB3, one of the four members of the vertebrate EGFR family. The sequence of the ErbB3 kinase domain reveals that several conserved amino acids, including the proposed catalytic base, are replaced by non-conservative amino acids[5] and subsequent studies demonstrate that ErbB3 has dramatically impaired protein-tyrosine kinase activity.[6,7] Despite this, the *ErbB3* gene is essential; mice lacking the gene die as embryos.[8,9] Furthermore, chimeric EGFR/ErbB3 receptors can transduce mitogenic signals in NIH3T3 fibroblasts.[10–12] Insight into the ErbB3 signaling mechanism began with the finding that ErbB3 can bind members of the NDF/heregulin family of ligands. A putative heterodimer between ErbB3 and ErbB2 binds heregulin (neuregulin) 10–100 fold more efficiently than ErbB3 alone.[13,14] Alimandi et al.[15] showed that co-expression of both *ErbB2* and *ErbB3* could transform NIH3T3 cells under conditions where neither gene alone could do so. Other reports document similar phenomena involving the ability of EGFR family members to elicit different biological activities when expressed in the presence of ErbB3 (reviewed in Ref. 16). Co-expression of EGFR, ErbB2, or ErbB4 with

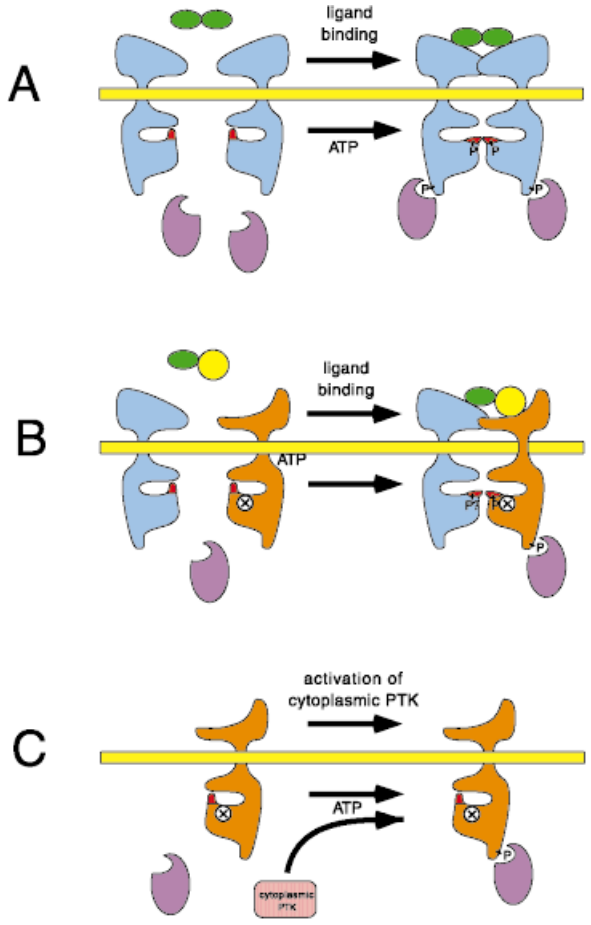

Figure 1. The paradigm for RTK activation and possible mechanisms for signal transduction by a kinase-inactive RTK. **A:** A typical RTK molecule is shown in blue with the activation loop in red. A homodimeric ligand is shown in green and a protein that docks with a phosphorylated tyrosine on the tail of the receptor is shown in purple. Ligand binding results in receptor dimerization and transphosphorylation of tyrosines in the activation loop and on the tail of the receptor. The phosphorylated tyrosine on the tail serves as a docking site for a downstream signaling protein, which binds via an SH2 or PTB domain. In some cases, the docked proteins are subsequently phosphorylated on tyrosine by the activated RTK. **B,C:** An inactive RTK is shown in orange. The circled X indicates the DFG motif, which is replaced by a divergent amino acid sequence in Ryk and CCK-4 family RTKs. A possible heterodimeric ligand is indicated in green and yellow. In B, the inactive RTK dimerizes with an active RTK after binding of ligand. The active RTK then phosphorylates the inactive RTK to create a docking site for a phosphotyrosine-binding signaling molecule. Whether the activation loop would be phosphorylated in the active RTK in this situation is unclear, since the inactive RTK would be unable to phosphorylate the loop. In C, the inactive RTK is phosphorylated by a cytoplasmic PTK which has been activated via another pathway.

ErbB3 resulted in phosphorylation of the ErbB3 cytoplasmic domain.[6,13,14,17] These results add support to a model involving the formation of ligand-induced receptor complexes composed of kinase-inactive ErbB3 and kinase-active EGFR, ErbB2 or ErbB4. In this model, ErbB3 is phosphorylated by its kinase-active partner. Thus, ErbB3 signaling is dependent on the kinase activity of another EGFR family member.[6] The ErbB3 tyrosines which are phosphorylated by the active receptor partner serve as docking sites for downstream signaling molecules. These molecules include phosphoinositol 3-kinase (PI 3-kinase)[10,12] and Shc.[12] Mutation of the Shc-binding site renders ErbB3 incapable of activating mitogen-activated protein (MAP) kinase.[18]

Although a clearer picture of ErbB3 signaling is evolving, new reports suggest an increasingly intricate and complex mechanism. Following the cloning of a second neuregulin-like growth factor, called neuregulin-2, Carraway et al.[19] showed that neuregulin-1 and neuregulin-2 elicit different biochemical responses in cells despite the fact that both bind to ErbB3 and ErbB4. Other reports suggest that secondary dimerization or oligomerization must occur during ErbB3/ErbB2 signaling since both receptors become phosphorylated following ligand binding.[14,20,21] Additionally, the role of the inactive ErbB3 kinase domain is not clear. Transactivation of the kinase domain of ErbB2 requires a three amino acid segment at the C-terminal end of ErbB3 and may require the inactive ErbB3 kinase domain itself.[22]

The identification of an ErbB3 homologue in the pufferfish[23] puts the origin of ErbB3, at least, at the base of the vertebrates. Only one member of the EGFR family has been identified in *C. elegans*[24] and *Drosophila*,[25] suggesting that the expansion of the EGFR family occurred after the divergence of protostomes. However, an earlier origin of ErbB3, followed by loss from *C. elegans* and *Drosophila* cannot yet be ruled out.

Recent work indicates that another member of the EGFR family, EGFR itself, has a hidden life as a "dead" kinase. Growth hormone stimulates phosphorylation of EGFR via the non-receptor PTK Jak2.[26] The resulting phosphorylated tyrosine serves as a docking site for Grb2 and this association ultimately leads to the activation of MAP kinase. The surprising aspect of this process is that it does not require EGFR to have kinase activity, as a kinase-defective version of EGFR can substitute for active EGFR in this pathway. In this case, EGFR serves simply as a bearer of a phosphotyrosine produced by Jak2. This residue is one that would normally be phosphorylated by the EGFR when it is activated by EGF binding. Activation of various G-protein-coupled receptors can also lead to ligand-independent phosphorylation of the EGFR, in this case by Src PTK, and subsequent downstream signaling events.[27–29] However, it is unclear whether the kinase activity of the EGFR is required for these signaling events.

CCK-4: 500 million years without kinase activity

While ErbB3 lost its activity relatively recently, there is a RTK that has lacked kinase activity from very early in the metazoan radiation. In 1991, Chou and Hayman[30] reported the cloning of *Klg*, a gene from chicken which encodes a novel RTK that lacks kinase activity. A striking feature of the Klg RTK is the replacement of the conserved DFG triplet in the kinase domain with ALS. The aspartate of the DFG triplet binds the magnesium ion that coordinates with the beta and gamma phosphates of ATP and is essential for kinase activity. Subsequently, a human orthologue of Klg was identified;[31,32] it too lacks kinase activity and contains ALG in place of DFG. In 1992, the *Drosophila* Dtrk RTK was described[33] and found to contain YPA instead of DFG. Surprisingly, an immunoprecipitate of Dtrk has kinase activity, although the possibility that this is due to an active kinase complexed with Dtrk has not been investigated.

Recently an RTK (termed *Lemon*) has been identified in *Hydra*[34] which, like CCK-4, Klg, and Dtrk, lacks the conserved DFG motif. The kinase domain of *Lemon* lacks activity when expressed in yeast. Phylogenetic analysis of these four RTKs convincingly demonstrates that they are orthologous.[34] Thus, this kinase class was present in the last common ancestor of *Hydra* and vertebrates that occupied a near-basal position in the metazoan radiation. Interestingly, no obvious orthologue of these kinases is encoded in the *C. elegans* genome. However, several PTKs are known to be present in cnidarians and vertebrates but absent from *C. elegans*,[35,36] indicating that some PTK genes have been lost during the evolution of *C. elegans*.

In addition to the alteration in the DFG motif, the four members of the CCK-4 family share an additional unusual feature. The transmembrane domains of these four proteins show an unprecedented degree of sequence conservation for RTKs.[34] Furthermore, the sequences contain elements that suggest they could mediate protein–protein interactions[37,38] This finding is intriguing as it suggests that the CCK-4 class of RTKs may interact with a kinase-active partner via dimerization of the transmembrane domain.

The amino acid sequence alterations responsible for the lack of activity of the CCK-4 class of RTKs have not been investigated systematically. However, mutation of the FLS to DFG in Lemon does not restore kinase activity.[34] Thus Lemon, and likely the other CCK-4 class RTKs, have additional impairments.

Ryk: resurrecting a dead kinase

Another group of RTKs that lack kinase activity are those of the Ryk family. These RTKs have been identified in *C. elegans*,[39] *Drosophila*,[40–44] and vertebrates.[45] Ryk family RTKs have alterations in a number of conserved amino acids in the kinase catalytic domain, including the DFG motif, and lack kinase activity.[46] Yet, despite the absence of kinase activity, Ryk can activate downstream signaling pathways.[46] It has been convincingly demonstrated that *Derailed*, a Ryk family member in *Drosophila*, plays a critical role in axon guidance.[47] Probing fixed embryos with an epitope-tagged version of the Derailed extracellular domain demonstrated binding to neurons in the posterior commissure (PC),[47] in keeping with the hypothesis that a repellant ligand prevents Derailed axons that express from crossing in the PC. Thus it appears that Derailed is a ligand-"activated" RTK with no kinase activity. Unpublished studies (J. Thomas, Salk Institute, pers. comm.) have shown that substitution of the catalytic lysine residue with alanine in the Derailed protein has no effect on its ability to rescue loss-of-function mutations in the Derailed locus. Taken together, these findings make it seem unlikely that Derailed functions as a kinase.

Doughnut, another *Drosophila* Ryk family member, is phosphorylated on tyrosine when expressed in S2 cells.[43] This phosphorylation occurs even when the catalytic lysine is replaced by arginine, a mutation that should prevent kinase activity. Thus, it appears that *Doughnut* (and by extension other Ryk family members) may be a substrate for another PTK.

Interestingly, restoration of the DFG motif in Ryk by site-directed mutagenesis restores kinase activity.[46] This suggests that the critical event in conversion of Ryk to a dead kinase is mutation of the DFG motif to DNA. The kinase function of Ryk has been inactive since the divergence of mammals, nematodes and arthropods, and has accumulated a number of non-conservative changes in critical residues in the catalytic domain. Yet its activity can be returned by restoring only the DFG motif. This result indicates that we still have a great deal to learn about structure–function relationships within the catalytic domains of RTKs.

Nothing is known about the ligands or possible active RTK partners of the Ryk family of RTKs. Interestingly, the extracellular portion of Ryk RTKs has recently been shown to contain a domain that is related to the Wnt-binding domain of Wnt inhibitory factors,[48] suggesting that Ryks might interact with Wnts. One mechanism by which Ryk might act as a signaling molecule is as a heterodimer with an active RTK partner, in a way similar to that of ErbB3 (Fig. 1B). If this is the case, it must involve a highly divergent partner, since the *C. elegans* genome contains only one obvious Ryk family member, Ryk itself.[49] It is possible that Ryk acts through a similar mechanism to EGFR and GHR, in which a non-receptor PTK phosphorylates the RTK (Fig. 1C). If Ryk functions solely in this ligand-independent manner, however, the function of the conserved extracellular domain is unclear.

Although this review focuses on dead RTKs, dead protein-serine/threonine kinases are also known. One such kinase, the integrin-linked kinase (ILK), lacks kinase activity and functions in integrin-based signaling by acting as an adaptor molecule in the protein kinase B/AKT signaling pathway.[50]

Fractured kinases

tr-kit: half a kinase is better than none

The so-called dead RTKs, although lacking kinase activity and containing alterations at various amino acid residues, are still intact viz-a-viz their domain organization and basic structural features. We now know, however, of two genes that encode RTKs that produce drastically altered protein products, termed "fractured kinases." The first example of a "fractured kinase" comes from the *Kit* locus in mammals. The *Kit* gene encodes a RTK, which is a member of the PDGF receptor subfamily of RTKs.[1] The kinase domain of Kit contains an amino acid sequence (the kinase insert) located between the ATP-binding lobe and the substrate-binding lobe.[51,52] *Kit* maps to the *white spotting locus* (*W*) of the mouse,[53–55] and mutations of *Kit* have pleiotropic developmental effects, causing an impairment in hematopoiesis, melanogenesis and gametogenesis.[56,57] The *Kit* ligand is a growth factor encoded at the *Steel* locus.[58]

The *Kit* gene is widely expressed as a single 5.5 kb transcript[52] that encodes the entire receptor. Full-length *Kit* RNA is present in primordial germ cells in the embryonic gonad and in type A spermatogonia of the postnatal testis. *Kit* expression ceases at the meiotic phase of spermatogenesis.[59,60] Expression resumes in postmeiotic cells of the testis, but full-length RNA is not detected in these cells. Rather, two novel transcripts of 3.2 and 2.3 kb are found.[59] The 3.2 kb transcript encodes a truncated version (tr-kit) of the Kit receptor, which is missing the extracellular domain, the transmembrane domain, and the N-terminal half of the kinase domain, i.e. the ATP-binding lobe.[61] Also missing is the kinase insert sequence, which contains the tyrosine residue which, when phosphorylated, forms the binding site for the 85 kDa subunit of PI 3-kinase.[62] Tr-kit does, however, retain the tyrosine which, upon phosphorylation, is the binding site for phospholipase Cγ.[63]

In the mouse, tr-kit is expressed following transcriptional activation from a promoter located within the intron between exons 16 and 17.[61,64] As a result, the N terminus of tr-kit consists of 12 novel hydrophobic residues which are absent from Kit. At least two *cis*-acting elements in the tr-kit promoter bind spermatid-specific factors.[65] As a consequence, tr-kit accumulates late in spermatogenesis.[65] Tr-kit is located in the sperm cytoplasm, especially in the midpiece of the flagellum.[66] This expression pattern suggests that tr-kit may function during the process of sperm differentiation or, alternatively, that it has a role in the function of mature sperm. Micro-injection of recombinant tr-kit protein or in vitro-synthesized tr-kit RNA into metaphase II-arrested mouse eggs causes complete parthenogenetic activation of the oocytes, including cleavage.[66]

Treatment of these injected eggs with an inhibitor of phospholipase C (PLC) blocks the activating ability of tr-kit.[66] Co-expression of PLC and tr-kit in mammalian cells stimulates the production of diacylglycerol (DAG) and inositol phosphates and the level of phosphotyrosine on PLC is elevated in the co-expressing cells.[67] An antibody against the SH3 and SH2 domains of PLC blocked the ability of tr-kit to activate mouse eggs.[67] The fact that tr-kit retains the tyrosine residue that, when phosphorylated, binds an SH2 domain of PLC, suggests that the effects of tr-kit are mediated by phosphorylation of this tyrosine followed by binding to an SH2 domain of PLC. Several pieces of data, however, conflict with this interpretation. First, injection of a GST fusion protein containing the PLC SH3 domain inhibits egg activation by tr-kit, while a fusion protein containing the SH2 domains of PLC does not.[67] Second, tr-kit is not phosphorylated on tyrosine when expressed in mammalian cells, even though it stimulates PLC activity in these cells.[67] Finally, tr-kit does not bind to a GST fusion protein containing the PLC SH2 and SH3 domains, suggesting the possibility that it does not associate stably with PLC in vivo.[67]

So, what can one conclude about the role of tr-kit? One interpretation of the available data would be that the sperm releases tr-kit into the cytoplasm of the egg upon fertilization which interacts with the SH3 domain of PLC and, thereby, participates in the activation of PLC (Fig. 2). It is interesting to note that the N-terminal tail of tr-kit contains a PXXP motif, which is a potential binding site for an SH3 domain. What is unexplained in this scenario is the source of the tyrosine kinase activity that phosphorylates PLC in tr-kit-expressing cells.

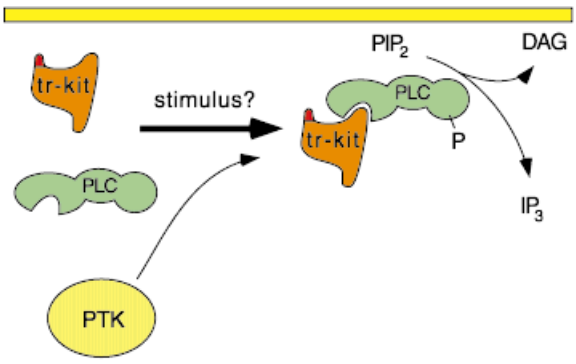

Figure 2. A possible mechanism for involvement of tr-kit in signal transduction. Tr-kit is colored orange with the activation loop red. PLC is shown in green. Tr-kit causes activation of PLC via an unknown mechanism. Direct contact between tr-kit and PLC is shown, although the evidence for this is contradictory. The SH3 domain of PLC blocks the activation of PLC by tr-kit, but a stable PLC–tr-kit complex has not been detected.[67] PLC is also phosphorylated on tyrosine by an unidentified PTK (shown in yellow) in tr-kit-expressing cells.[67]

There is some evidence that Kit and its ligand may play a role in the maintenance of meiotic arrest in mouse oocytes.[68] If this is the case, then it is possible that Kit forms part of the tr-kit–PLC pathway and its kinase activity could play a role in activation of PLC. A clearer picture of the role of tr-kit will, hopefully, be provided by studies of mice in which the tr-kit promoter in the intron of *Kit* has been inactivated.

Two truncated *Kit* transcripts have been identified in a variety of human cancer cell lines.[69,70] Human *tr-kit* transcripts also arise by transcription initiation in an intron of the *Kit* gene, but in this case in the intron immediately upstream of the one used for mouse *tr-kit*. The two human *tr-kit* transcripts differ because of alternative splicing that leads to either retention or removal of a short sequence near the 5′ end of the transcript.[70] The human tr-kit proteins contain novel N termini, a portion of the kinase insert and the substrate-binding lobe of the kinase domain. The activity of human tr-kit is unknown. Its expression in normal cells has not been examined, and functional studies have not been carried out. Neither of the predicted human tr-kit proteins contain a potential SH3-binding domain.

Hinterteil: a complex fractured kinase in a simple animal

Recently a gene encoding a truncated PTK has been identified in *Hydra*.[71] This gene, termed *Hinterteil* (*Hint*), encodes only the substrate-binding domain of a PTK such as that seen in tr-kit. *Hint* is a member of a family of PTKs, some of which have been shown to encode full-length RTKs.[72,73] *Hint*, itself, does not produce a transcript encoding a complete RTK. Rather, it appears that the *Hint* gene is dedicated solely to the production of truncated protein. The RTK family to which *Hint* belongs is not closely related to *Kit*. Thus, there is no obvious evolutionary relationship between *Hint* and tr-kit. The *Hint* gene undergoes a complex set of alternative *trans* and *cis* splices, some of which change the sequence of the N-terminal portion of the protein.[71] Finally, a novel *Hint* transcript is detected in sexual male *Hydra*, suggesting a possible role for the protein encoded by this transcript in spermatogenesis or sperm function.[71]

The picture that emerges from the available data on fractured RTKs is that they are an interesting group about which we still know very little. The fact that, in both *Hydra* and mammals, the truncation yields a transcript encoding the same part of the kinase domain, the substrate-binding lobe, is intriguing. Comparisons of the *Hydra* and mammals suggest that truncated RTKs have evolved more than once during metazoan evolution. Whether transcripts encoding truncated RTKs are present in *C. elegans* or *Drosophila* is unknown. Although a role in egg activation is suggested by the data on mouse tr-kit, genetic data (i.e. knockouts which specifically target the tr-kit promoter) are needed to further illuminate this aspect of the story.

Evolutionary considerations

The routes by which inactive and truncated RTKs evolved is an intriguing question. A gene encoding an inactive RTK can be generated by changing a nucleotide. However, mutation of one copy of a gene encoding an RTK will potentially be problematic for the cell. Because RTKs dimerize as part of the signal transduction process, an inactivating mutation in the kinase domain for one copy of an RTK gene could lead to dominant-negative inhibition of the wild-type RTK. A mutant EGFR in which the catalytic lysine is replaced by alanine acts in a dominant-negative manner to suppress signaling from the wild-type EGFR.[74] A point mutation in Kit that results in loss of kinase activity is responsible for the W^{42} dominant, white spotting phenotype in the mouse,[75] which leads to defects in gametogenesis, hematopoiesis and pigmentation. Other examples of kinase-inactivating RTK mutations that are dominant include a human insulin receptor mutation that is associated with dominantly inherited insulin resistance[76] and a mutation of human Ret RTK associated with Hirschsprung disease.[77]

Given that a point mutation that leads to loss of kinase activity is likely to lead to a dominant-negative effect, how might RTKs such as Ryk and CCK-4 arise? A simple solution would be for gene duplication events to precede the appearance of the inactivating mutation (Fig. 3). In this case, the dominant negative effect of the mutant RTK would be diluted to the point where it would not be expected to cause a problem. The system would then be free to evolve a signaling pathway involving the inactive RTK. If signaling by the inactive RTK involves dimerization with an active RTK partner, it would be logical for that partner to be a member of the same family as the inactive RTK. Thus, the gene duplication scenario both mitigates potential dominant-negative effects and provides potential partners for the inactive RTK. This scenario is tenable for ErbB3, which is a member of a family of four related RTKs which has arisen from gene duplications.[3]

However, in the scenario depicted in Fig. 3, the kinase-inactive RTK will still presumably act in a dominant-negative manner with the active RTKs with which it dimerizes. So although the dominant-negative effect will be reduced to an acceptable level by duplication of normal genes, how such an inactive kinase would be positively selected for still needs to be explained. One possibility is that kinase-dependent and kinase-independent functions co-existed in the original RTK. The mutant kinase-inactive version could potentially still possess the kinase-independent functions and could, then, be selected for its ability to carry out these functions. In fact, the participation of the EGFR in the growth-hormone-signaling pathway is independent of its kinase activity,[26] providing an example in which both kinase-dependent and kinase-independent functions co-exist in a single RTK. In the case of ErbB3, the situation has evolved to the point where activation of ErbB2 in the ErbB2–ErbB3 heterodimer is dependent on a

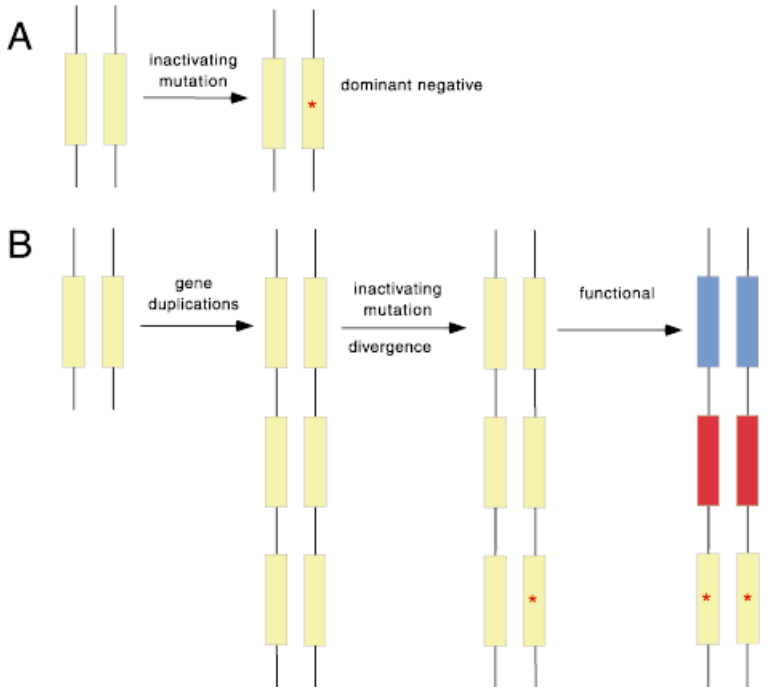

Figure 3. Possible route for evolution of a gene encoding an inactive RTK. Genes encoding RTKs are shown as rectangles. The initial situation shows the two copies of the gene present in a diploid cell. The red asterisk indicates a mutation in the kinase domain that leads to loss of activity. **A:** Mutation occurs without gene duplication, leading to potential dominant-negative inhibition of the wild-type kinase. **B:** Two rounds of gene duplication precede the mutational event. For the sake of simplicity, the genes resulting from duplication are shown in tandem linkage. One or more of the duplicated genes could also become unlinked from the parent gene.

sequence in ErbB3.[22] Thus ErbB3 has changed from a dominant-negative RTK to a co-activator of ErbB2.

Dimerization appears to occur primarily between members of the same RTK family. Thus, the gene duplication scenario in Figure 3 is complicated by the apparent absence of a gene family for at least one of the inactive RTKs, Ryk. *C. elegans* has a Ryk homologue with a DFG to DSA alteration. However, no other Ryk-related RTKs are contained within the *C. elegans* genome,[78] and, thus, there is no evidence that gene duplication occurred prior to the loss of kinase activity. Until we know more about the function of Ryk and its potential dimerization partners in the nematode and other organisms, the evolutionary history will remain unclear.

We do not yet know whether the CCK-4 family contains any kinase-active members that could be binding partners for the inactive RTKs of this family. It will be interesting to see whether the Human Genome Project identifies additional members of this family. However, given the possibility that the CCK-4 family of RTKs might interact via their transmembrane domains (see above), the range of possible classes of partners is potentially large.

Because we know so little about the function of fractured RTKs, it is difficult to hypothesize about their evolution. It is interesting to consider, however, that fractured RTKs would provide useful raw material for the evolution of new signaling molecules. By retaining phosphorylation sites and, thereby, potential as substrates for activated RTKs, they could serve as the starting points for evolution of signaling molecules of the type typified by insulin receptor substrate proteins.[79]

Conclusions

The presence of dead and fractured RTKs in animal cells indicates that we have more to learn about RTK signaling pathways than the textbook paradigm would suggest. Of particular interest is the evolutionary history of such kinases. Their small number, relative to the number of intact RTKs, suggests that they do not evolve readily. Yet, at least one of the families of dead RTKs has been present for most of metazoan existence, suggesting that they are important. As more metazoan gene sets are cataloged, we hope to gain a better understanding of how these RTKs arose and whether they are truly as rare as they currently seem. Structural studies of the kinase domain of an inactive RTK might also be informative. To date, the data available on the interaction between ErbB2 and its inactive partner, ErbB3, suggest that interesting features are likely to be revealed by such structural information.

Finally, a cautionary note regarding deductions about activity from the amino acid sequences of kinases is in order. A recently published paper describes WNK1, a novel mammalian protein-serine/threonine kinase in which the conserved lysine residue that is critical for ATP binding in kinases is replaced by a cysteine; yet this protein has kinase activity.[80] Structural modeling and site-directed mutagenesis

indicate that WNK1 has a lysine at a position usually occupied by a conserved glycine residue in the catalytic lobe and that this lysine apparently substitutes for the catalytic lysine which is missing from its usual location. Appearances are indeed deceiving.

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