Complexity of Receptor Tyrosine Kinase Signal Processing

Natalia Volinsky and Boris N. Kholodenko

Systems Biology Ireland, University College Dublin, Belfield, Dublin 4, Ireland

Correspondence: boris.kholodenko@ucd.ie

Our knowledge of molecular mechanisms of receptor tyrosine kinase (RTK) signaling advances with ever-increasing pace. Yet our understanding of how the spatiotemporal dynamics of RTK signaling control specific cellular outcomes has lagged behind. Systems-centered experimental and computational approaches can help reveal how overlapping networks of signal transducers downstream of RTKs orchestrate specific cell-fate decisions. We discuss how RTK network regulatory structures, which involve the immediate posttranslational and delayed transcriptional controls by multiple feed forward and feedback loops together with pathway cross talk, adapt cells to the combinatorial variety of external cues and conditions. This intricate network circuitry endows cells with emerging capabilities for RTK signal processing and decoding. We illustrate how mathematical modeling facilitates our understanding of RTK network behaviors by unraveling specific systems properties, including bistability, oscillations, excitable responses, and generation of intricate landscapes of signaling activities.

Since the first cloning of the cDNA encoding the epidermal growth factor (EGF) receptor (EGFR), signaling by receptor tyrosine kinases (RTKs) has been in the limelight of scientific interest owing to their central role in the regulation of development, cell motility, proliferation, differentiation, glucose metabolism, and apoptosis (Hunter 2000; Schlessinger 2000; Lemmon and Schlessinger 2010). The RTK family comprises more than 50 cell-surface receptors with intrinsic tyrosine kinase activity. All RTKs consist of three major domains: an extracellular domain for ligand binding, a membrane-spanning segment, and a cytoplasmic domain, which possesses tyrosine kinase activity and contains phosphorylation sites with tyrosine, serine, and threonine residues. Following

ligand binding, RTKs undergo dimerization (e.g., EGFR) or allosteric transitions (e.g., insulin receptor [IR] and insulin-like growth factor-1 receptor [IGF-1R] that are associated into oligomers before the ligand binding), resulting in receptor activation. Auto- and/or *trans*-phosphorylation of RTKs transmit biochemical signals to cytoplasmic adaptor proteins and enzymes, which contain characteristic protein domains, such as Src homology (SH2 and SH3), phosphotyrosine binding (PTB), and pleckstrin homology (PH) domains. These domains act as docking sites for phosphotyrosines or phospholipids thereby triggering the mobilization of these proteins to the cell surface (Pawson and Nash 2003). Subsequently, signals propagate through a tangled network of inter-

Editors: Joseph Schlessinger and Mark A. Lemmon

Additional Perspectives on Receptor Tyrosine Kinases available at www.cshperspectives.org

Copyright © 2013 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a009043

Cite this article as *Cold Spring Harb Perspect Biol* 2013;5:a009043

N. Volinsky and B.N. Kholodenko

connecting proteins and signaling cascades to the nucleus, inducing transcriptional responses of immediate early genes (IEG) and delayed early genes (DEG) (Avraham and Yarden 2011).

The idea of isolated linear pathways that relate signals and receptors to specific genes has given way to the concept of signaling networks, which allow a limited number of RTKs to generate an exponentially larger number of functional outcomes as a result of combinatorial interactions. Although early genetic experiments seem to support a linear pathway view, the current biochemical and imaging data suggest that any given RTK, downstream adaptors, small G-proteins (small GTPases, such as Ras, Rac, Rho, or Cdc42), and activated kinases interact with a large variety of signaling molecules resulting in highly interconnected networks (von Kriegsheim et al. 2009). These signaling networks not only transmit but also process and integrate signals. For instance, signals from different RTKs are integrated through common adaptor proteins and other points of pathway cross talk that include small GTPases and cytoplasmic kinases, such as the Src family kinases, phosphatidylinositol 3-kinase (PI3K), and mitogen activated protein kinases (MAPK) (Kholodenko et al. 2010).

Given that the protein complements of adaptor proteins, small GTPases, and kinases, which mediate RTK-induced signal transduction, overlap for all known RTKs (Lemmon and Schlessinger 2010), questions arise as to how cells can maintain specificity when activated by multiple cues? Also how can coherent cellular decisions, including whether to undergo proliferation, differentiation, or die, be made? The concept is emerging that for any RTK pathway, there is no single protein or gene responsible for signaling specificity. Rather, specificity is determined by the spatiotemporal dynamics of activation of signaling proteins, IEGs, and DEGs, downstream of RTKs (Murphy et al. 2004; Kholodenko 2006; Nakakuki et al. 2010). Yet many signaling events activated by RTKs are partially redundant, and different RTKs can compensate for each other in many cellular functions (Xu and Huang 2010).

A hallmark of complex signaling and early gene networks downstream of RTKs are multiple feed forward and feedback loops, both negative and positive. These regulations operate on different timescales, precisely tuning the signaling outcome and often convert analog input signals into digital outputs (Kholodenko et al. 2010). Immediate feedback or feedforward regulations occur through interactions between two proteins or a protein and a lipid or through posttranslational modifications, such as phosphorylation that alters protein activity. For instance, active extracellular signal regulated kinase (ERK) phosphorylates and inactivates the kinase Raf-1, which is upstream of ERK in the Ras/ERK signaling cascade (Dougherty et al. 2005). These immediate feedforward and feedback loops operate on the timescale of seconds to minutes, which are the characteristic times of the corresponding interactions or catalytic reactions. Another large group of feedback regulators, such as Sprouty, Spred, and Mitogen-inducible gene-6 (Mig-6)/receptor-associated late transducer (RALT) (Gotoh 2009; Murphy et al. 2010; Segatto et al. 2011) requires RTK-induced gene transcription and translation. These delayed feedbacks adapt cells to a more permanent external stimulation, persisting on the timescale of tens of minutes and hours. For instance, transcriptionally induced IEGs, such as dual specificity phosphatases (DUSP), attenuate RTK-induced MAPK signaling. Owing to these multiple feedback and feedforward loops, the understanding of the spatiotemporal dynamics of RTK networks requires more than knowledge of binding partners, their structures, and interactions (Kholodenko 2009).

In this review, we focus on a systems-centered view of the biology of RTK signaling networks. We discuss cross talk between different RTKs and pathways and illustrate how this cross talk may alter the functional outcome of signaling by individual RTKs. We analyze feedforward and feedback loops and show how this regulatory circuitry can bring about the intricate dynamic behavior of RTK networks, including bistability, oscillations, and excitable overshoot transitions. We illustrate how computational modeling and systems analysis can facilitate our

understanding of the complex spatiotemporal behavior of RTK pathways based on the knowledge of molecular mechanisms of signal transduction. Finally, we discuss the challenges ahead that will stimulate further research.

RTK PATHWAY CROSS TALK: FINE BALANCES AT MULTIPLE LEVELS

Cells in situ are exposed to a plethora of signaling cues that activate multiple RTKs. Although individual RTKs have been extensively studied, how signaling networks integrate multiple cues is less understood. How do cells integrate and process external information by exploiting pathway cross talk? Cross talk between RTK-stimulated pathways exists at different signaling levels, which include the level of receptors, adaptor proteins, scaffolds, small GTPases, downstream kinases, and transcriptional responses (Pawson et al. 2001).

Cross talk at the receptor level can be mediated by several mechanisms. An RTK can induce activation of structurally unrelated RTKs, for instance, stimulation of IGF-1R can lead to EGFR (Ahmad et al. 2004) or ErbB2 activity (Balana et al. 2001). At least two mechanisms of cross talk were suggested: direct dimerization between structurally independent RTKs, such as IGF-1R or c-Met with ErbB family receptors (Balana et al. 2001; Ahmad et al. 2004; Tanizaki et al. 2011) and transactivation mediated by a cytoplasmic tyrosine kinase, such as Src. In the latter case, once activated by IGF-1R, Src binds and phosphorylates EGFR, thus promoting EGFR catalytic activity (Jones et al. 2006). Using high-throughput Western blotting and Bayesian interference techniques, different cross talk mechanisms were recently described (Ciaccio et al. 2010). Whereas EGF induced rapid phosphorylation of multiple sites on EGFR, phosphorylation of other RTKs, as well as some EGFR tyrosines, was detected at later time points. In particular, phosphorylation of the c-Met receptor was only detected after about 15 min of the EGFR phosphorylation. These delayed responses suggest the involvement of downstream signaling cascades in mediating RTK cross activation (Ciaccio et al. 2010).

Cross talk between distinct RTKs was shown in non-small cell lung cancer-derived cell lines. Using specific inhibitors of EGFR and c-Met, it was shown that these two RTKs positively influence each other, and that inhibition of one receptor negatively regulates the other (Guo et al. 2008). Another example is a direct interaction between c-Met and insulin receptors in hepatocytes, which facilitate optimal activation of downstream signaling pathways and glucose metabolism (Fafalios et al. 2011).

Different cross talk mechanisms, in which inhibition of one RTK leads to activation of the other RTK have also been observed and suggested to be one of the causes of chemoresistance in cancer patients (Jones et al. 2006; Xu and Huang 2010). In fact, a cocktail of inhibitors targeting several RTKs, such as EGFR, c-Met, and PDGF has been shown to be more effective than a single drug treatment (Stommel et al. 2007). RTK cross talk can also be mediated solely by downstream signaling interactions without affecting the receptors themselves. In this case, costimulation by two ligands, which often occurs in vivo, can lead to synergic or mutually inhibiting responses (Borisov et al. 2009; Martin et al. 2009).

When signals propagate through different branches converging at a common target, both branches can add to the overall response of the target (Kholodenko et al. 1997). Feedforward and feedback loops embracing interacting RTK pathways make the input–output response relationships of one pathway dependent on the activity of the other pathway, thereby creating context-dependent signaling output. A recent study explored how a concordant interplay between RTK pathways stimulated by EGF and insulin can potentiate signaling by the ERK/MAPK at physiological, low EGF levels in HEK293 cells (Borisov et al. 2009). Although the EGFR and IR networks share many downstream components, their responses to cognate stimuli are different. In HEK293 cells, EGF causes strong ERK activation, whereas insulin poorly activates ERK. The main insulin function is metabolic, including the control of glucose metabolism, and stimulation of protein and lipid syntheses. Using a combined experimental and computa-

N. Volinsky and B.N. Kholodenko

tional analysis, it was shown that at low physiological concentration of EGF, cross talk between the EGFR and IR networks converts the insulin-induced increase in the PIP₃ concentration into enhanced ERK activation, whereas at saturating EGF levels the insulin effect becomes insignificant (Borisov et al. 2009).

The data and computational modeling results suggest that major cross talk mechanisms that amplify ERK signaling by insulin are localized upstream of Ras and at the Ras/Raf level (Borisov et al. 2009). Among the variety of cross talk interactions affecting multiple Ras activation and inactivation routes, five key network nodes are shown to be crucial for the EGF-insulin synergy. These nodes involve the adaptor proteins, Grb2-associated binder-1 (GAB1) and insulin receptor substrates (IRS), PI3K/PIP₃ node, soluble tyrosine kinase Src, and the SH2-domain containing protein tyrosine phosphatase-2 (SHP2) that is located upstream of the ERK kinase MEK. The computational model reveals key features of the EGFR and IR network signal processing brought about by: (1) coincidence detection of EGF and insulin stimuli through GAB1 phosphorylation response, (2) coherent feedforward loop from EGFR to Raf via Src (this is a network motif in which an initial signal induces an intermediate input, and both the initial and intermediate inputs are needed to generate the final output [Mangan

et al. 2003], see also below), and (3) multiple positive (PI3K → PIP₃ → GAB1 → PI3K) and negative (ERK ⊣ GAB1, ERK ⊣ Son of Sevenless (SOS), mammalian target of rapamycin (mTOR) ⊣ IRS) feedback loops. The simplified scheme, shown in Figure 1, illustrates how insulin enhances EGF-induced mitogenesis through two partially redundant and compensating signaling branches via IRS and GAB1.

Receptor cross activation can also be mediated via transcriptional induction of an RTK (or its ligands) by another RTK (Jones et al. 2006; Esposito et al. 2008; Gujral et al. 2012; Velpula et al. 2012). For instance, in neuroblastoma-derived cell lines retinoic acid induces activation of the RTK Ret, leading to cell differentiation, as indicated by morphological changes and expression of several differentiation markers. Several studies suggested that TrkB (another RTK and indicator of poor prognosis for neuroblastoma tumors) can link retinoic acid stimulation and the phenotype change. Ret activation induces expression of both TrkB and its primary ligand brain-derived neurotrophic factor (BDNF) (Kaplan et al. 1993; Esposito et al. 2008). Moreover, TrkB knockdown prevents cell differentiation (Esposito et al. 2008). “Reverse” cross talk, in which TrkA and TrkB receptors induce activation of Ret, was also observed (Tsui-Pierchala et al. 2002; Esposito et al. 2008). Although in this case positive regulation of Ret

Figure 1. Mechanisms of insulin-EGF signal integration. Synergistic ERK activation arises from coincidence detection of insulin and EGF stimuli at the level of GAB1 adaptor protein. GAB1 is massively recruited to the membrane by IR signaling (GAB1 → PIP₃-GAB1) and subsequently phosphorylated by EGFR and activated Src (PIP₃-GAB1 → PIP₃-pGAB1). (Modified from Borisov et al. 2009; reprinted, with permission, from the authors.)

does not involve transcriptional regulation (Esposito et al. 2008), $\text{TrkB} \rightarrow \text{Ret} \rightarrow \text{TrkB}$ positive feedback may lead to emerging systems properties, such as bistability (see section below “Intricate RTK Network Dynamics Are Brought about by Feedback Loops”).

In a recent study (Gujral et al. 2012), breast cancer samples were subjected to “reverse-phase” protein microarrays (Sevecka and MacBeath 2006) and phosphorylation or total protein levels of multiple signaling molecules were tested. The resulted data were analyzed by unsupervised hierarchical clustering (Herrero et al. 2001), revealing 12 major clusters of correlating proteins. Interestingly, c-Met RTK phosphorylated on Tyr1349 was clustered together with the total protein level of Axl, which is a structurally independent RTK. This finding was further experimentally validated; siRNA-mediated c-Met targeting resulted in a significant decrease of Axl mRNA levels. These receptors were also shown to interact on a protein level, c-Met stimulation with hepatocyte growth factor-induced activation of Axl. Moreover, both receptors physically interacted with each other, at least when ectopically expressed. Surprisingly,

Axl down-regulation or stimulation did not affect c-Met function on any level, suggesting complex interplay of these two RTKs (Gujral et al. 2012).

Additional levels of complexity, which we do not discuss here, involve cross talk between RTKs and other receptors, such as G-protein-coupled receptors, adhesion molecules, and nuclear receptors.

RTK NETWORKS ARE TIGHTLY CONTROLLED BY FEEDBACK AND FEEDFORWARD LOOPS

Coherent and Incoherent Feedforward Loops

A useful approach toward understanding of signaling and transcriptional networks is the analysis of so-called network motifs (Milo et al. 2002). In addition to the simplest linear signaling design in which the initial signal A regulates the intermediate output B, and B regulates the output C, signaling networks contain more complicated motifs including feedforward loops (FFL) where A regulates B, while A and B jointly regulate C (Fig. 2) (Mangan and Alon

Figure 2. Coherent and incoherent feedforward motifs. (A) Basic structure of coherent feed forward loop (coherent FFL [Mangan and Alon 2003]). (B) Schematic representation of ERK-induced c-Fos expression and activation that includes a cascade of coherent FFLs. Active ERK (ppERK) and RSK (pRSK) activate transcription factors required for c-Fos expression and therefore, *c-fos* mRNA expression. ERK and RSK stabilize and activate the nascent c-Fos protein by phosphorylation making an additional AND gate (based on data from Nakakuki et al. 2010). (C) Basic structure of incoherent feed forward loop of type I (incoherent FFL [Mangan and Alon 2003]). (D) Schematic representation of EGFR regulated *c-fos* mRNA availability in terms of incoherent FFL. On stimulation, EGFR induces expression of *c-fos* (B). ZFP36 is also induced by EGFR and mediates *c-fos* RNA degradation. (Based on data from Avraham and Yarden 2011.)

N. Volinsky and B.N. Kholodenko

2003; Shoal and Alon 2010). In physiological systems, FFL motifs may be combined into larger integrated structures, leading to complex signaling and transcriptional circuits (Shoal and Alon 2010).

In coherent FFLs, the initial input A activates the intermediate output B, while A and B form the logical gates “AND” or “OR,” thereby providing different regulation of the outcome C. This final outcome can be a downstream effector or process, such as gene promoter or protein activation, that is responsive to two inputs, in which only one or both inputs are required in OR or AND gates, respectively. For an AND gate, increasing time delays related to accumulation of B may be required to activate C, and consequently, an AND motif shows delayed “ON” and immediate “OFF” responses (Mangan and Alon 2003). An OR gate motif is characterized by immediate ON and delayed OFF responses (Shoal and Alon 2010). Interestingly, an AND coherent FFL motif distinguishes between transient and sustained signals. This regulatory motif is found in the networks stimulated by two different RTK ligands, EGF and platelet-derived growth factor (PDGF), which induce transient and sustained ERK activation, respectively (Murphy et al. 2002, 2004). Expression of several IEGs, including *c-fos*, is induced by active ERK and its downstream effector, the p90 ribosomal S6 kinase (RSK). However, the nascent c-Fos protein is unstable, and phosphorylation by ERK and RSK is required for its stabilization and activation. If ERK and RSK signals are transient, there will be no appreciable kinase activity at the time when the c-Fos protein is de novo synthesized. Thus, c-Fos will not be phosphorylated, and will be rapidly degraded. Therefore, only sustained ERK and RSK signaling can allow for a strong c-Fos induction (Fig. 2B) (Murphy et al. 2002, 2004; Nakakuki et al. 2010). This mechanism of discrimination between transient and sustained ERK activity at the IEG level is common for different RTKs and cell types and may lead to distinct cell-fate decisions, such as proliferation or tumorigenicity (Nakakuki et al. 2010).

Incoherent FFLs where A activates C, but also activates B, which is the repressor of C, is

another common motif in network regulation (Fig. 2C). In case of an AND gate, both the presence of an activator A and the lack of a repressor B are required for obtaining the outcome C. For instance, if two independent elements on a promoter bind an activator A and a repressor B, appreciable transcriptional response will only be observed when A is bound and B is absent (Mangan and Alon 2003). Depending on parameters, this motif can endow the system with noise filtering and adaptation (Goentoro et al. 2009; Ma et al. 2009). The repressor B serves as a “memory” element keeping track of the previous levels of the activator A, and the output C detects fold-changes of A rather than the absolute A values (Goentoro et al. 2009; Ma et al. 2009). For instance, a sustained input will result in peak-like transient response, followed by full or partial adaptation, because of accumulating levels of the repressor (Ma et al. 2009).

An incoherent FFL in EGFR-mediated transcriptional circuits was recently described (Amit et al. 2007). EGFR induces expression of the Zink Finger Protein 36 (ZFP36), which binds to AU-rich elements, predominantly found in the 3′-untranslated regions (3′-UTR) of unstable mRNA molecules, and promotes their deadenylation and subsequent degradation (Chen et al. 2001). A systematic analysis of EGF-induced genes revealed that many of these genes contain such 3′-UTR elements, thus suggesting a widespread role of ZFP36 in IEG and DEG regulation (Amit et al. 2007). Although ZFP36 does not affect gene transcription directly, it regulates the availability of multiple mRNA molecules, thereby forming an incoherent FFL (Fig. 2D) (Amit et al. 2007; Avraham and Yarden 2011).

Positive and Negative Feedback Regulation

The concept of feedback control goes back to the ancient Greeks who used float regulators to keep a constant level of water in a tank or oil in a lamp (Sauro and Kholodenko 2004). In biological control systems, negative and positive feedback loops are the most fundamental features. Positive feedback amplifies the signal, whereas negative feedback attenuates it. RTK network topologies involve comprehensive and intricate

regulatory structures of immediate and delayed feedback loops (Fig. 3) (Kiyatkin et al. 2006; Amit et al. 2007; Gotoh 2009; Lemmon and Schlessinger 2010; Sturm et al. 2010; Avraham and Yarden 2011; Segatto et al. 2011).

Positive and Negative Feedbacks at the Receptor Level

In RTK pathways, both the receptor abundance and ligand availability are tightly controlled by

positive and negative feedback loops. For instance, RTK ubiquitination by the E3 ubiquitin ligase Cbl and subsequent degradation of the receptor in lysosomes creates negative feedback at the receptor level. Cbl is recruited to the phosphorylated receptor either directly or via the Grb2 adaptor and is further phosphorylated by RTK or c-Src to become activated (Zwang and Yarden 2009). Positive feedback can be mediated by reactive oxygen species, which are produced in response to RTK activation and inhibit

Figure 3. Simplified scheme presenting multiple feedback loops in the EGFR pathway. Upon EGFR ligation and activation, protein complexes nucleated by scaffolds are formed at the plasma membrane. Activated Ras induces activation of the Raf-MEK-ERK signaling cascade. Active ERK phosphorylates several upstream signaling regulators, including Raf and SOS, forming negative feedback loops. However, ERK also phosphorylates and inhibits RKIP, which is a negative regulator of Raf, thus creating positive feedback. Active ERK translocates to the nucleus where it phosphorylates several transcription factors, inducing transcription of several IEGs that are negative regulators of EGFR signaling. DUSPs are rapidly induced upon ERK activation, and some DUSPs also require phosphorylation by ERK to become fully active in order to dephosphorylate ERK. Another inducible inhibitor of EGFR, Mig-6, inhibits EGFR by blocking its kinase activity and mediating ubiquitin-independent degradation of EGFR. Several transcription factors induced by EGFR, such as MafF, inhibit transcription of genes that contain SRE in the promoter. Additional mechanism of negative regulation is mediated by ZFP36 that binds to the AU-rich 3'-UTR of mRNA molecules, such as *c-fos* mRNA and other IEG, targeting them for degradation. Dashed arrows represent indirect or unknown regulation; blue arrows represent mechanisms involving transports between the cytoplasmic and nuclear compartments.

N. Volinsky and B.N. Kholodenko

protein tyrosine phosphatases (PTPs) that inactivate RTKs. Internalization of active RTKs into endosomes also creates feedback loops, which serve as both positive and negative regulators (Kholodenko 2002; Wiley 2003; Polo and Di Fiore 2006; Sorkin and von Zastrow 2009).

One of downstream targets of RTKs is the ADAM (a disintegrin and metalloproteinase) family of proteases responsible for shedding and release of growth factors such as heparin-binding EGF (Hynes and Schlang 2006; Zhou et al. 2006; Mendelson et al. 2010; Maretzky et al. 2011a,b; Rao et al. 2011). A recent study of head and neck squamous cell carcinoma cell lines has shown that ErbB2 and EGFR mediate ADAM12 protease expression in a PI3K and mTORC1 dependent manner (Rao et al. 2011). In turn, ADAM12 positively regulates ErbB2 expression, thus forming a positive feedback loop. Activated ADAM proteins are also associated with receptor cross talk. Ligands such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) induce ERK1/2 activation and cell migration as a result of EGFR stimulation by heparin-binding EGF, which is released in ADAM-dependent manner (Maretzky et al. 2011a).

Positive and Negative Feedbacks at the Signaling Level

ERK, which is the terminal kinase in the three-layered MAPK/ERK cascade, phosphorylates multiple signaling molecules and transcription factors (Yoon and Seger 2006; von Kriegsheim et al. 2009; Yang et al. 2013). An example of ERK-mediated negative feedback is the phosphorylation of the guanine nucleotide exchange factor SOS. Upon RTK activation, the SOS-Grb2 complex is recruited to the plasma membrane where SOS mediates Ras activation. SOS phosphorylation by ERK or by its downstream kinase RSK-2 leads to dissociation of the SOS-Grb2 complex, thereby terminating Ras activation (Langlois et al. 1995; Dong et al. 1996; Douville and Downward 1997).

Raf kinase inhibitor protein (RKIP) is an endogenous inhibitor of the ERK pathway. By binding both Raf-1 and MEK, RKIP prevents

their physical interaction and MEK phosphorylation (Yeung et al. 1999, 2000). Activated ERK forms a positive feedback loop by phosphorylating RKIP and inhibiting its interaction with Raf-1. This positive feedback loop is imbedded into a long negative feedback loop from ERK to SOS and contributes to oscillations in ERK activity (Shin et al. 2009).

Disruption of RTK-mediated signaling complexes assembled on scaffolds is a common mechanism of negative feedback. For instance, following IR or IGF-1R-mediated phosphorylation of numerous tyrosine residues on the IRS scaffold proteins, these scaffolds become capable of recruiting multiple signaling complexes, including PI3K (Schmitz-Peiffer and Whitehead 2003; Boura-Halfon and Zick 2009). This facilitates PI3K activation, production of PIP₃ and activation of the PI3K/AKT/mTOR cascade. Subsequent phosphorylation of IRS on serine residues by downstream kinases, such as mTOR, its effector S6K1, and MAPKs, disrupts the signaling complexes assembled on IRS and down-regulates the PI3K pathway (Gual et al. 2005; Boura-Halfon and Zick 2009).

Feedback Loops via Transcription

RTK signaling induces transcriptional activation of multiple IEGs and DEGs, which, in turn, creates multiple positive and negative feedback loops (Avraham and Yarden 2011). Several induced proteins, including Mig-6/RALT and the leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1), generate feedback by acting on the receptor. The transmembrane protein LRIG1 is expressed several hours after the onset of EGFR stimulation. While LRIG1 interacts with EGFR via the extracellular domain, the intracellular domain of LRIG1 recruits Cbl, thereby providing additional mechanism of Cbl-mediated EGFR degradation (Segatto et al. 2011). Whereas Cbl activation depends on EGFR activity, LRIG1 reduces the number of surface EGFR molecules both in basal conditions and upon stimulation, suggesting additional mechanisms of EGFR down-regulation (Segatto et al. 2011). Mig-6/RALT is expressed within an hour after EGFR activation

and binds the kinase domain of all ErbB receptors in a ligand-dependent manner, suppressing their catalytic activities (Gotoh 2009; Segatto et al. 2011). Mig-6 targets EGFR to lysosomes and mediates EGFR degradation independently of receptor phosphorylation or ubiquitination, thus showing the second mechanism of negative regulation (Segatto et al. 2011). The Mig-6 protein also contains a Cdc42/Rac interaction and binding (CRIB) domain that selectively binds an active form of the small G protein Cdc42, which is an important regulator of cell migration. Indeed, by sequestering Cdc42, Mig-6 inhibits hepatocyte growth factor (HGF)-induced cell migration (Gotoh 2009).

DUSP proteins dephosphorylate and inactivate MAPKs. Different DUSPs have different preferred MAPK substrates and distinct intracellular localization. Also, often MAPK binding selectively facilitates DUSP activation. For instance, binding of ERK but not JNK or p38 MAPKs is required for catalytic activation of the cytoplasmic DUSP6 (Camps et al. 1998). The class I DUSPs (1/2/4/5) are inducible nuclear phosphatases, whose expression can be detected within an hour after activation of the ErbB receptors (Owens and Keyse 2007; Nakakuki et al. 2010). A recent study found transient ERK activity in the nucleus, whereas ERK activity in the cytoplasm was sustained. These different ERK kinetics in the cytoplasm and the nucleus were explained by inducible expression of several nuclear DUSPs since simultaneous knockdown of these phosphatases substantially prolonged ERK activation in the nucleus (Nakakuki et al. 2010).

Sprouty, whose expression is also induced by RTKs, is another regulator of upstream signaling. De novo synthesized Sprouty translocates to the plasma membrane where it is phosphorylated by the Src family kinases (Mason et al. 2006; Edwin et al. 2009). This phosphorylation (at Tyr55 for the Sprouty2 sequence) induces a conformational change that allows Sprouty to bind Grb2, disrupting the Grb2–SOS complex and leading to inhibition of Ras activation. The same phosphorylation at Tyr55 triggers Sprouty–Cbl interaction that sequesters Cbl from RTK, thereby preventing RTK degra-

dation. Because of this dual role in RTK regulation, Sprouty acts as an RTK signaling modulator rather than inhibitor (Edwin et al. 2009).

Additional example of positive feedback regulation was observed in breast tumor initiating cells (Aceto et al. 2012). Src-homology 2 domain-containing phosphatase 2 (SHP2) can act as tumor promoter by facilitating RTK-induced mitogenic signaling (Ostman et al. 2006). In a recent study SHP2 was shown to activate transcription factors ZEB1 and c-Myc. Whereas ZEB1 drives epithelial-to-mesenchymal transition (EMT) (Schmalhofer et al. 2009), c-myc induces expression of lin-28 homolog B, a repressor of microRNA biogenesis (Chang et al. 2009). This leads to let-7 microRNA repression and, therefore, results in overexpression of let-7 targets, including Ras and c-Myc itself (Aceto et al. 2012). Thus, SHP2-mediated positive feedback loop is required for maintenance and invasiveness of breast tumors (Aceto et al. 2012). Importantly, this is only one example of many in which microRNA molecules take part in signaling and transcriptional regulation downstream to RTKs (Avraham and Yarden 2012).

Feedback Loops Operating within Transcriptional Circuits

Additional mechanisms of positive or negative regulation involve de novo expression of proteins that affect mainly transcriptional events rather than upstream signaling. The first wave of the EGFR-induced transcriptional response (IEGs; immediate early genes) contains predominantly transcriptional activators; the second, delayed response wave (DEGs), involves multiple inhibitors of gene transcription that act at different levels (Amit et al. 2007). For instance, the proteins MafF and Kruppel-like factor 2 (KLF2) induced by RTKs (Amit et al. 2007; Dijkmans et al. 2009; Dutta et al. 2011) are negative regulators of several promoter elements, including the serum response element (SRE) (Amit et al. 2007). SRE is found within promoters of multiple IEG, such as transcription factors *c-fos* and *egr-1*, and a negative signaling regulator DUSP6 (Christy and Nathans 1989; Rivera et al.

N. Volinsky and B.N. Kholodenko

1990; Treisman 1995; Bluthgen et al. 2009). Although the molecular mechanism of MafF- and KLF2-mediated transcriptional inhibition is unknown, the diversity of SRE containing genes clearly indicates that this regulation might have both positive and negative impact on signaling and transcriptional events.

Feedback mediated by posttranslational modifications develops on the scale of minutes, whereas feedback generated by de novo expressed proteins is much slower and operates on a longer timescale ranging from 30 min to several hours and more. The delayed negative feedback mechanisms may prevent permanent activation by continuous or recurrent signals, if de novo expressed proteins are present in the system for a prolonged time. In this case, a refractory period when cells are not responsive to stimuli is often observed. The refractory mechanism might be necessary for preventing continuous proliferation of cells exposed to multiple pulses of growth factors.

INTRICATE RTK NETWORK DYNAMICS ARE BROUGHT ABOUT BY FEEDBACK LOOPS: BISTABILITY, OSCILLATIONS, AND EXCITABILITY

Immediate and delayed feedback loops not only change steady-state input–output responses, but they also bring about dynamic instabilities. When a steady state becomes unstable, a system can jump to another stable state, start to oscillate, or show chaotic behavior. For instance, signals downstream of RTKs are often amplified by positive feedback, but if the feedback is sufficiently strong and a part of the pathway within the feedback shows ultrasensitive behavior with respect to the input signal, this system can become *bistable* (Ferrell 1999). A bistable system can switch between two distinct stable steady states, but cannot rest in intermediate states. Because of the coexistence of two alternative states, a bistable system displays *hysteresis*, meaning that the stimulus needs to exceed a threshold value to flip the system to another steady state at which it may remain when the stimulus returns to its initial value. In fact, hysteresis and bistability in MAPK cascades brought about by positive

feedback loops have been suggested to contribute to cell differentiation and long-term synaptic potentiation (Santos et al. 2007; Smolen et al. 2008). In addition, positive feedback either alone or in combination with negative feedback can trigger oscillations. Such positive–negative feedback oscillations generally do not have sinusoidal shapes and operate in a pulsatory manner: A part of a dynamic system is bistable, and there is a slow process induced by negative feedback that periodically forces the system to jump between “Off” and “On” states, generating oscillations (Pomerening et al. 2003; Sha et al. 2003).

Negative feedback in the MAPK/ERK cascade endows robustness to variations of parameters within the feedback loop and stabilizes the active ERK concentration when demand for active ERK fluctuates (Sauro and Kholodenko 2004). Using a combination of modeling and validating experiments, it was shown that the integration of a three-tier MAPK cascade structure with negative feedback generates emergent systems properties that resemble the features of a negative feedback amplifier (NFA), known from engineering (Sturm et al. 2010). These properties have decisive effects on the MAPK cascade drug sensitivity and adaptation to perturbations. In particular, the active ERK concentration was shown to be stabilized in response to drug-induced perturbations of the upstream ERK kinase, MEK (Sturm et al. 2010). Yet, above a certain threshold strength, negative feedback can induce damped or sustained oscillations in the system. These oscillations are caused by the time delay within the negative feedback loop and also require some degree of ultrasensitivity within the cascade (Kholodenko 2000). In fact, oscillations in the MAPK/ERK pathway were recently discovered experimentally, confirming previous theoretical predictions (Nakayama et al. 2008; Shankaran et al. 2009; Shin et al. 2009; Hu et al. 2013). An intriguing systems property of protein modification cascades and even simpler signaling systems is excitable and overshoot signaling responses to transient stimuli (Kaimachnikov and Kholodenko 2009). In this case, the entire RTK pathway behaves as an excitable device with a built-in excitability

threshold. Depending on the magnitude and duration of a transient stimulus, activation responses of key kinases and/or small GTPases fit into one of two distinct classes of either low or high amplitude responses, whereas there are no intermediate responses, merely proportional to the stimulus. For instance, the excitable behavior of small GTPases such as Rac or Rho, which does not respond to under-threshold stimuli, but yields high-activity pulses in response to over-threshold stimuli, can precisely control cell movement (Ridley 2001; Tsyganov et al. 2012).

A fundamental question is how cell-to-cell variability affects the RTK network function and dynamics. In any cell, transcription and translation are inherently stochastic and give rise to large cell-to-cell variability in protein levels observed even in genetically identical cells, such as clonal cell populations (McAdams and Arkin 1997; Spencer et al. 2009). Theoretical findings suggest that nonlinear signal processing by signaling networks *always* broadens the distribution of cellular responses to any signal, perturbation, or drug. Although bimodal distributions of cellular responses to input signal are generally thought to manifest bistable or ultrasensitive behavior in single cells, it has been shown recently that such bimodal responses can occur as a result of the protein abundance variability (Birtwistle et al. 2012; Kim and Sauro 2012). In particular, experiments and computer simulations show that a simple MAPK/ERK-cascade model with negative feedback that displays graded, analog ERK responses at a single cell level may show bimodal responses to EGF at the cell population level (Birtwistle et al. 2012). These results show that bimodal signaling response distributions do not necessarily imply digital (ultrasensitive or bistable) single cell signaling. The interplay between protein expression noise and network topologies can bring about digital population responses from analog single-cell dose responses. Thus, cells can retain the benefits of robustness arising from negative feedback, while simultaneously generating population-level on/off responses that are thought to be critical for regulating cell-fate decisions.

The extremely rich repertoire of different dynamic behaviors allow RTK signaling pathways to serve as analog-to-digital converters, transforming a transient or sustained growth factor activation into distinct responses, such as all-or-none, oscillatory, and pulsatory responses. Subsequent deconvolution of these different responses by the transcriptional machinery can be translated into different cell-fate decisions (Murphy et al. 2004; Kholodenko et al. 2010; Nakakuki et al. 2010).

SPATIAL SIGNAL PROPAGATION

Imaging data and computational models suggest that the propagation of RTK signaling from the plasma membrane to targets in the nucleus is tightly controlled by a variety of regulatory mechanisms (Kholodenko et al. 2010; Vartak and Bastiaens 2010; Grecco et al. 2011; Alam-Nazki and Krishnan 2012). RTK signaling pathways are highly spatially organized within cells. Activator enzymes, such as kinases or guanine nucleotide exchange factors (GEFs), and inactivating enzymes (e.g., phosphatases and GTPase-activating proteins [GAPs]) often localize to different cellular locales (Kholodenko 2009). For a protein phosphorylated by a membrane-bound kinase and dephosphorylated by a cytosolic phosphatase, it was predicted that there can be a gradient of the phosphorylated form, which is high close to the membrane and low within the cell (Brown and Kholodenko 1999). Instructively, the shape of the gradient depends mainly on the phosphatase activity. If the phosphatase is not saturated, the concentration profile of the active, phosphorylated form of the target protein decays almost exponentially with the distance from the membrane. Such exponential decrease in the activity of G proteins can also be observed if GEF activity is associated with a cellular structure, such as chromatin, and when GAP activity is excluded from this structure (Kholodenko 2006). Spatial gradients of protein activities organize signaling around cellular structures, such as membranes, chromosomes and scaffolds, and provide positional cues for key processes, including cell division. Such intracellular gradients of protein

N. Volinsky and B.N. Kholodenko

activities have been detected in live cells using imaging technologies based on fluorescence resonance energy (Maeder et al. 2007).

Raf-1, the initial kinase in the MAPK/ERK cascade is activated near the plasma membrane where activated downstream of RTKs, the small GTPase Ras resides. How can the phosphorylation signal that was initiated at the plasma membrane propagate through the cytoplasm where it is terminated by phosphatases? Central mechanisms of spatial signal propagation have been suggested. First, a kinase cascade can be assembled on a scaffold protein that protects transmission of phosphorylation against phosphatase activity. Second, even for a soluble cascade of (de)phosphorylation cycles, the phosphorylation signal reaches further into the cell interior, when the cascade has more levels, and this might be one of the reasons that cascades exist (Munoz-Garcia et al. 2009). Third, kinesin motor-mediated movement of the endosomes and kinase complexes along microtubules can transfer phosphorylation signals, guarding against dephosphorylation (Kholodenko 2002; Perlson et al. 2005, 2006). Finally, and most intriguingly, it was suggested that RTK signals can propagate as nonlinear traveling waves that create global spatial switches or pulses of kinase and GTPase (in)activation (Munoz-Garcia and Kholodenko 2010). These mechanisms facilitate signal propagation from activated RTKs across single cells.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Versatility and diversity of RTKs and their malfunctioning in major pathological conditions have made RTK signaling a focus point of extensive genetic and biochemical studies. Recent changes in our perception of RTK signaling pathways from linear pipelines to combinatorial complex interaction networks have highlighted the need for novel conceptual and technological approaches to move forward our comprehension of cell signaling by RTKs. An ever-growing contribution of mathematical models helps us understand RTK signaling in a cell as an integrated system rather than a list of proteins and genes.

Signaling from different RTKs leads to distinct cellular outcomes, yet these signals are transmitted through the overlapping cascades of common signal transducers. As described in this review, we have begun to realize that signal specificity is generated by a multiplicity of feedforward and feedback loops, combinatorial protein assemblies and their spatiotemporal dynamics rather than by a large number of genes with specific functions. However, many questions remain open. For instance, how is specificity maintained, if activation of one RTK results in cross talks with many other RTKs? We can speculate that whereas activation of a “primary” RTK occurs almost immediately following the ligand binding, the activation of “secondary” receptors is delayed. This time delay, combined with multiple negative feedbacks that are initiated by “primary” receptor, is expected to have a negative impact on the amplitude and duration of signals induced by the “secondary” receptor. Therefore, in a physiologic system that contains intact feedbacks, secondary activation is expected to serve only for fine-tuning of the responses.

Additional complexity arises from multiple protein isoforms that can contribute to both signaling specificity and redundancy. For instance, the growing body of evidence suggests that ERK1 and ERK2 might also have isoform specific, nonredundant functions in proliferation, differentiation, and EMT (Li and Johnson 2006; Vantaggiato et al. 2006; Shin et al. 2010). Future research will elaborate how different protein isoforms, such as the phosphatase SHP1 versus SHP2 or distinct isoforms of the scaffolds IRS and GAB, contribute to specific signaling responses and, thereby, to specific cellular phenotypes.

A feature of RTK networks highlighted here is the coexistence of multiple mechanisms that often play similar regulatory roles. For instance, negative feedback loops from ERK to SOS and from ERK to Raf-1 and EGFR degradation are redundant because each of these processes is sufficient for signal termination (Orton et al. 2008). In fact, mathematical models show that the system control is always distributed over multiple processes (von Kriegsheim et al. 2009).

This high degree of redundancy is expected to make the system robust toward a multitude of perturbations. Although system robustness is important for cell survival, it also makes cancer cells resistant to multiple drug treatments. This is the place where systems approach can be helpful in predicting the fragility points of pathological RTK networks to be exploited by combinatorial drug therapies.

ACKNOWLEDGMENTS

We thank David Croucher and Walter Kolch for discussions and critical reading of the manuscript. This work is supported by Science Foundation Ireland under grant No. 06/CE/B1129. We apologize that we could not cite many pertinent contributions to the field because of space limitations.

REFERENCES

- Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, Confalonieri S, Quarto M, Hu G, Balwierz PJ, et al. 2012. Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. *Nat Med* **18**: 529–537.
- Ahmad T, Farnie G, Bundred NJ, Anderson NG. 2004. The mitogenic action of insulin-like growth factor I in normal human mammary epithelial cells requires the epidermal growth factor receptor tyrosine kinase. *J Biol Chem* **279**: 1713–1719.
- Alam-Nazki A, Krishnan J. 2012. An investigation of spatial signal transduction in cellular networks. *BMC Syst Biol* **6**: 83.
- Amit I, Citri A, Shay T, Lu Y, Katz M, Zhang F, Tarcic G, Siwak D, Lahad J, Jacob-Hirsch J, et al. 2007. A module of negative feedback regulators defines growth factor signaling. *Nat Genet* **39**: 503–512.
- Avraham R, Yarden Y. 2011. Feedback regulation of EGFR signalling: Decision making by early and delayed loops. *Nat Rev Mol Cell Biol* **12**: 104–117.
- Avraham R, Yarden Y. 2012. Regulation of signalling by microRNAs. *Biochem Soc Trans* **40**: 26–30.
- Balana ME, Labriola L, Salatino M, Movsichoff F, Peters G, Charreau EH, Elizalde PV. 2001. Activation of ErbB-2 via a hierarchical interaction between ErbB-2 and type I insulin-like growth factor receptor in mammary tumor cells. *Oncogene* **20**: 34–47.
- Birtwistle MR, Rauch J, Kiyatkin A, Aksamitiene E, Dobrzynski M, Hoek JB, Kolch W, Ogunnaike BA, Kholodenko BN. 2012. Emergence of bimodal cell population responses from the interplay between analog single-cell signaling and protein expression noise. *BMC Syst Biol* **6**: 109.
- Bluthgen N, Legewie S, Kielbasa SM, Schramme A, Tchernitsa O, Keil J, Solf A, Vingron M, Schafer R, Herzog H, et al. 2009. A systems biological approach suggests that transcriptional feedback regulation by dual-specificity phosphatase 6 shapes extracellular signal-related kinase activity in RAS-transformed fibroblasts. *FEBS J* **276**: 1024–1035.
- Borisov N, Aksamitiene E, Kiyatkin A, Legewie S, Berkhout J, Maiwald T, Kaimachnikov NP, Timmer J, Hoek JB, Kholodenko BN. 2009. Systems-level interactions between insulin-EGF networks amplify mitogenic signaling. *Mol Syst Biol* **5**: 256.
- Boura-Halfon S, Zick Y. 2009. Phosphorylation of IRS proteins, insulin action, and insulin resistance. *Am J Physiol Endocrinol Metab* **296**: E581–E591.
- Brown GC, Kholodenko BN. 1999. Spatial gradients of cellular phospho-proteins. *FEBS Lett* **457**: 452–454.
- Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, Chabert C, Boschert U, Arkinstall S. 1998. Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* **280**: 1262–1265.
- Chang TC, Zeitels LR, Hwang HW, Chivukula RR, Wentzel EA, Dews M, Jung J, Gao P, Dang CV, Beer MA, et al. 2009. Lin-28B transactivation is necessary for Myc-mediated let-7 repression and proliferation. *Proc Natl Acad Sci* **106**: 3384–3389.
- Chen CY, Gherzi R, Ong SE, Chan EL, Rajmakers R, Puijck GJ, Stoecklin G, Moroni C, Mann M, Karin M. 2001. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* **107**: 451–464.
- Christy B, Nathans D. 1989. Functional serum response elements upstream of the growth factor-inducible gene zif268. *Mol Cell Biol* **9**: 4889–4895.
- Ciaccio ME, Wagner JP, Chu C, Lauffenburger DA, Jones RB. 2010. Systems analysis of EGF receptor signaling dynamics with microwestern arrays. *Nat Methods* **7**: 148–155.
- Dijkmans TE, van Hooijdonk LW, Schouten TG, Kamphorst JT, Fitzsimons CP, Vreugdenhil E. 2009. Identification of new nerve growth factor-responsive immediate-early genes. *Brain Res* **1249**: 19–33.
- Dong C, Waters SB, Holt KH, Pessin JE. 1996. SOS phosphorylation and disassociation of the Grb2–SOS complex by the ERK and JNK signaling pathways. *J Biol Chem* **271**: 6328–6332.
- Dougherty MK, Muller J, Ritt DA, Zhou M, Zhou XZ, Copeland TD, Conrads TP, Veenstra TD, Lu KP, Morrison DK. 2005. Regulation of Raf-1 by direct feedback phosphorylation. *Mol Cell* **17**: 215–224.
- Douville E, Downward J. 1997. EGF induced SOS phosphorylation in PC12 cells involves P90 RSK-2. *Oncogene* **15**: 373–383.
- Dutta P, Koch A, Breyer B, Schneider H, Dittich-Breiholz O, Kracht M, Tamura T. 2011. Identification of novel target genes of nerve growth factor (NGF) in human mastocytoma cell line (HMC-1 [V560G c-Kit]) by transcriptome analysis. *BMC Genomics* **12**: 196.
- Edwin F, Anderson K, Ying C, Patel TB. 2009. Intermolecular interactions of Sprouty proteins and their implica-

N. Volinsky and B.N. Kholodenko

- tions in development and disease. *Mol Pharmacol* **76**: 679–691.
- Esposito CL, D'Alessio A, de Franciscis V, Cerchia L. 2008. A cross talk between TrkB and Ret tyrosine kinases receptors mediates neuroblastoma cells differentiation. *PLoS ONE* **3**: e1643.
- Fafalios A, Ma J, Tan X, Stoops J, Luo J, Defrances MC, Zarnegar R. 2011. A hepatocyte growth factor receptor (Met)-insulin receptor hybrid governs hepatic glucose metabolism. *Nat Med* **17**: 1577–1584.
- Ferrell JE Jr. 1999. Building a cellular switch: More lessons from a good egg. *Bioessays* **21**: 866–870.
- Goentoro L, Shoval O, Kirschner MW, Alon U. 2009. The incoherent feedforward loop can provide fold-change detection in gene regulation. *Mol Cell* **36**: 894–899.
- Gotoh N. 2009. Feedback inhibitors of the epidermal growth factor receptor signaling pathways. *Int J Biochem Cell Biol* **41**: 511–515.
- Grecco HE, Schmick M, Bastiaens PI. 2011. Signaling from the living plasma membrane. *Cell* **144**: 897–909.
- Gual P, Le Marchand-Brustel Y, Tanti JF. 2005. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* **87**: 99–109.
- Gujral TS, Karp RL, Finski A, Chan M, Schwartz PE, Macbeath G, Sorger P. 2012. Profiling phospho-signaling networks in breast cancer using reverse-phase protein arrays. *Oncogene* doi: 10.1038/onc.2012.378.
- Guo A, Villen J, Kornhauser J, Lee KA, Stokes MP, Rikova K, Possemato A, Nardone J, Innocenti G, Wetzel R, et al. 2008. Signaling networks assembled by oncogenic EGFR and c-Met. *Proc Natl Acad Sci* **105**: 692–697.
- Herrero J, Valencia A, Dopazo J. 2001. A hierarchical unsupervised growing neural network for clustering gene expression patterns. *Bioinformatics* **17**: 126–136.
- Hu H, Goltsov A, Bown JL, Sims AH, Langdon SP, Harrison DJ, Faratian D. 2013. Feedforward and feedback regulation of the MAPK and PI3K oscillatory circuit in breast cancer. *Cell Signal* **25**: 26–32.
- Hunter T. 2000. Signaling—2000 and beyond. *Cell* **100**: 113–127.
- Hynes NE, Schlange T. 2006. Targeting ADAMS and ERBBs in lung cancer. *Cancer Cell* **10**: 7–11.
- Jones HE, Gee JM, Hutcheson IR, Knowlden JM, Barrow D, Nicholson RL. 2006. Growth factor receptor interplay and resistance in cancer. *Endocr Relat Cancer* **13**: S45–S51.
- Kaimachnikov NP, Kholodenko BN. 2009. Toggle switches, pulses and oscillations are intrinsic properties of the Src activation/deactivation cycle. *FEBS J* **276**: 4102–4118.
- Kaplan DR, Matsumoto K, Lucarelli E, Thiele CJ. 1993. Induction of TrkB by retinoic acid mediates biologic responsiveness to BDNF and differentiation of human neuroblastoma cells. Eukaryotic Signal Transduction Group. *Neuron* **11**: 321–331.
- Kholodenko BN. 2000. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur J Biochem* **267**: 1583–1588.
- Kholodenko BN. 2002. MAP kinase cascade signaling and endocytic trafficking: A marriage of convenience? *Trends Cell Biol* **12**: 173–177.
- Kholodenko BN. 2006. Cell-signalling dynamics in time and space. *Nat Rev Mol Cell Biol* **7**: 165–176.
- Kholodenko BN. 2009. Spatially distributed cell signalling. *FEBS Lett* **583**: 4006–4012.
- Kholodenko BN, Hoek JB, Westerhoff HV, Brown GC. 1997. Quantification of information transfer via cellular signal transduction pathways. *FEBS Lett* **414**: 430–434; erratum, **419**: 150.
- Kholodenko BN, Hancock JF, Kolch W. 2010. Signalling ballet in space and time. *Nat Rev Mol Cell Biol* **11**: 414–426.
- Kim KH, Sauro HM. 2012. In search of noise-induced bimodality. *BMC Biol* **10**: 89.
- Kiyatkin A, Aksamitiene E, Markevich NI, Borisov NM, Hoek JB, Kholodenko BN. 2006. Scaffolding protein Grb2-associated binder 1 sustains epidermal growth factor-induced mitogenic and survival signaling by multiple positive feedback loops. *J Biol Chem* **281**: 19925–19938.
- Langlois WJ, Sasaoka T, Saltiel AR, Olefsky JM. 1995. Negative feedback regulation and desensitization of insulin- and epidermal growth factor-stimulated p21ras activation. *J Biol Chem* **270**: 25320–25323.
- Lemmon MA, Schlessinger J. 2010. Cell signaling by receptor tyrosine kinases. *Cell* **141**: 1117–1134.
- Li J, Johnson SE. 2006. ERK2 is required for efficient terminal differentiation of skeletal myoblasts. *Biochem Biophys Res Commun* **345**: 1425–1433.
- Ma W, Trusina A, El-Samad H, Lim WA, Tang C. 2009. Defining network topologies that can achieve biochemical adaptation. *Cell* **138**: 760–773.
- Maeder CI, Hink MA, Kinkhabwala A, Mayr R, Bastiaens PI, Knop M. 2007. Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signalling. *Nat Cell Biol* **9**: 1319–1326.
- Mangan S, Alon U. 2003. Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci* **100**: 11980–11985.
- Mangan S, Zaslaver A, Alon U. 2003. The coherent feedforward loop serves as a sign-sensitive delay element in transcription networks. *J Mol Biol* **334**: 197–204.
- Maretzky T, Evers A, Zhou W, Swendeman SL, Wong PM, Rafii S, Reiss K, Blobel CP. 2011a. Migration of growth factor-stimulated epithelial and endothelial cells depends on EGFR transactivation by ADAM17. *Nat Commun* **2**: 229.
- Maretzky T, Zhou W, Huang XY, Blobel CP. 2011b. A transforming Src mutant increases the bioavailability of EGFR ligands via stimulation of the cell-surface metalloproteinase ADAM17. *Oncogene* **30**: 611–618.
- Martin B, Brenneman R, Golden E, Walent T, Becker KG, Prabhu VV, Wood W 3rd, Ladenheim B, Cadet JL, Maudsley S. 2009. Growth factor signals in neural cells: Coherent patterns of interaction control multiple levels of molecular and phenotypic responses. *J Biol Chem* **284**: 2493–2511.
- Mason JM, Morrison DJ, Basson MA, Licht JD. 2006. Sprouty proteins: Multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. *Trends Cell Biol* **16**: 45–54.
- McAdams HH, Arkin A. 1997. Stochastic mechanisms in gene expression. *Proc Natl Acad Sci* **94**: 814–819.

- Mendelson K, Swendeman S, Saftig P, Blobel CP. 2010. Stimulation of platelet-derived growth factor receptor β (PDGFR β) activates ADAM17 and promotes metallo-proteinase-dependent cross-talk between the PDGFR β and epidermal growth factor receptor (EGFR) signaling pathways. *J Biol Chem* **285**: 25024–25032.
- Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. 2002. Network motifs: simple building blocks of complex networks. *Science* **298**: 824–827.
- Munoz-Garcia J, Kholodenko BN. 2010. Signalling over a distance: Gradient patterns and phosphorylation waves within single cells. *Biochem Soc Trans* **38**: 1235–1241.
- Munoz-Garcia J, Neufeld Z, Kholodenko BN. 2009. Positional information generated by spatially distributed signaling cascades. *PLoS Comput Biol* **5**: e1000330.
- Murphy LO, Smith S, Chen RH, Fingar DC, Blenis J. 2002. Molecular interpretation of ERK signal duration by immediate early gene products. *Nat Cell Biol* **4**: 556–564.
- Murphy LO, MacKeigan JP, Blenis J. 2004. A network of immediate early gene products propagates subtle differences in mitogen-activated protein kinase signal amplitude and duration. *Mol Cell Biol* **24**: 144–153.
- Murphy T, Hori S, Sewell J, Gnanapragasam VJ. 2010. Expression and functional role of negative signalling regulators in tumour development and progression. *Int J Cancer* **127**: 2491–2499.
- Nakakuki T, Birtwistle MR, Saeki Y, Yumoto N, Ide K, Nagashima T, Brusch L, Ogunnaike BA, Okada-Hatakeyama M, Kholodenko BN. 2010. Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics. *Cell* **141**: 884–896.
- Nakayama K, Satoh T, Igari A, Kageyama R, Nishida E. 2008. FGF induces oscillations of Hes1 expression and Ras/ERK activation. *Curr Biol* **18**: R332–334.
- Orton RJ, Sturm OE, Gormand A, Wolch W, Gilbert DR. 2008. Computational modelling reveals feedback redundancy within the epidermal growth factor receptor/extracellular-signal regulated kinase signalling pathway. *IET Syst Biol* **2**: 173–183.
- Ostman A, Hellberg C, Bohmer FD. 2006. Protein-tyrosine phosphatases and cancer. *Nat Rev Cancer* **6**: 307–320.
- Owens DM, Keyse SM. 2007. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**: 3203–3213.
- Pawson T, Nash P. 2003. Assembly of cell regulatory systems through protein interaction domains. *Science* **300**: 445–452.
- Pawson T, Gish GD, Nash P. 2001. SH2 domains, interaction modules and cellular wiring. *Trends Cell Biol* **11**: 504–511.
- Perlson E, Hanz S, Ben-Yaakov K, Segal-Ruder Y, Seger R, Fainzilber M. 2005. Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. *Neuron* **45**: 715–726.
- Perlson E, Michalevski I, Kowalsman N, Ben-Yaakov K, Shaked M, Seger R, Eisenstein M, Fainzilber M. 2006. Vimentin binding to phosphorylated Erk sterically hinders enzymatic dephosphorylation of the kinase. *J Mol Biol* **364**: 938–944.
- Polo S, Di Fiore PP. 2006. Endocytosis conducts the cell signaling orchestra. *Cell* **124**: 897–900.
- Pomerening JR, Sontag ED, Ferrell JE. 2003. Building a cell cycle oscillator: Hysteresis and bistability in the activation of Cdc2. *Nat Cell Biol* **5**: 346–351.
- Rao VH, Kandel A, Lynch D, Pena Z, Marwaha N, Deng C, Watson P, Hansen LA. 2011. A positive feedback loop between HER2 and ADAM12 in human head and neck cancer cells increases migration and invasion. *Oncogene* **31**: 2888–2898.
- Ridley AJ. 2001. Rho GTPases and cell migration. *J Cell Sci* **114**: 2713–2722.
- Rivera VM, Sheng M, Greenberg ME. 1990. The inner core of the serum response element mediates both the rapid induction and subsequent repression of c-fos transcription following serum stimulation. *Genes Dev* **4**: 255–268.
- Santos SD, Verveer PJ, Bastiaens PI. 2007. Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. *Nat Cell Biol* **9**: 324–330.
- Sauro HM, Kholodenko BN. 2004. Quantitative analysis of signaling networks. *Prog Biophys Mol Biol* **86**: 5–43.
- Schlessinger J. 2000. Cell signaling by receptor tyrosine kinases. *Cell* **103**: 211–225.
- Schmalhofer O, Brabletz S, Brabletz T. 2009. E-cadherin, β -catenin, and ZEB1 in malignant progression of cancer. *Cancer Metastasis Rev* **28**: 151–166.
- Schmitz-Peiffer C, Whitehead JP. 2003. IRS-1 regulation in health and disease. *IUBMB Life* **55**: 367–374.
- Segatto O, Anastasi S, Alema S. 2011. Regulation of epidermal growth factor receptor signalling by inducible feedback inhibitors. *J Cell Sci* **124**: 1785–1793.
- Sevecka M, MacBeath G. 2006. State-based discovery: A multidimensional screen for small-molecule modulators of EGF signaling. *Nat Methods* **3**: 825–831.
- Sha W, Moore J, Chen K, Lassaletta AD, Yi CS, Tyson JJ, Sible JC. 2003. Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc Natl Acad Sci* **100**: 975–980.
- Shankaran H, Ippolito DL, Chrisler WB, Resat H, Bollinger N, Opresko LK, Wiley HS. 2009. Rapid and sustained nuclear-cytoplasmic ERK oscillations induced by epidermal growth factor. *Mol Syst Biol* **5**: 332.
- Shin SY, Rath O, Choo SM, Fee F, McFerran B, Kolch W, Cho KH. 2009. Positive- and negative-feedback regulations coordinate the dynamic behavior of the Ras-Raf-MEK-ERK signal transduction pathway. *J Cell Sci* **122**: 425–435.
- Shin S, Dimitri CA, Yoon SO, Dowdle W, Blenis J. 2010. ERK2 but not ERK1 induces epithelial-to-mesenchymal transformation via DEF motif-dependent signaling events. *Mol Cell* **38**: 114–127.
- Shoval O, Alon U. 2010. SnapShot: Network motifs. *Cell* **143**: 326–e321.
- Smolen P, Baxter DA, Byrne JH. 2008. Bistable MAP kinase activity: A plausible mechanism contributing to maintenance of late long-term potentiation. *Am J Physiol Cell Physiol* **294**: C503–C515.
- Sorkin A, von Zastrow M. 2009. Endocytosis and signalling: Intertwining molecular networks. *Nat Rev Mol Cell Biol* **10**: 609–622.
- Spencer SL, Gaudet S, Albeck JG, Burke JM, Sorger PK. 2009. Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **459**: 428–432.

N. Volinsky and B.N. Kholodenko

- Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C, et al. 2007. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* **318**: 287–290.
- Sturm OE, Orton R, Grindlay J, Birtwistle M, Vyshemirsky V, Gilbert D, Calder M, Pitt A, Kholodenko B, Kolch W. 2010. The mammalian MAPK/ERK pathway exhibits properties of a negative feedback amplifier. *Sci Signal* **3**: ra90.
- Tanizaki J, Okamoto I, Sakai K, Nakagawa K. 2011. Differential roles of trans-phosphorylated EGFR, HER2, HER3, and RET as heterodimerisation partners of MET in lung cancer with MET amplification. *Br J Cancer* **105**: 807–813.
- Treisman R. 1995. Journey to the surface of the cell: Fos regulation and the SRE. *EMBO J* **14**: 4905–4913.
- Tsui-Pierchala BA, Milbrandt J, Johnson EM Jr. 2002. NGF utilizes c-Ret via a novel GFL-independent, inter-RTK signaling mechanism to maintain the trophic status of mature sympathetic neurons. *Neuron* **33**: 261–273.
- Tsyganov MA, Kolch W, Kholodenko BN. 2012. The topology design principles that determine the spatiotemporal dynamics of G-protein cascades. *Mol Biosyst* **8**: 730–743.
- Vantaggiato C, Formentini I, Bondanza A, Bonini C, Naldini L, Brambilla R. 2006. ERK1 and ERK2 mitogen-activated protein kinases affect Ras-dependent cell signaling differentially. *J Biol* **5**: 14.
- Vartak N, Bastiaens P. 2010. Spatial cycles in G-protein crowd control. *EMBO J* **29**: 2689–2699.
- Velpula KK, Dasari VR, Asuthkar S, Gorantla B, Tsung AJ. 2012. EGFR and c-Met cross talk in glioblastoma and its regulation by human cord blood stem cells. *Transl Oncol* **5**: 379–392.
- von Kriegsheim A, Baiocchi D, Birtwistle M, Sumpton D, Bienvenut W, Morrice N, Yamada K, Lamond A, Kalna G, Orton R, et al. 2009. Cell fate decisions are specified by the dynamic ERK interactome. *Nat Cell Biol* **11**: 1458–1464.
- Wiley HS. 2003. Trafficking of the ErbB receptors and its influence on signaling. *Exp Cell Res* **284**: 78–88.
- Xu AM, Huang PH. 2010. Receptor tyrosine kinase coactivation networks in cancer. *Cancer Res* **70**: 3857–3860.
- Yang SH, Sharrocks AD, Whitmarsh AJ. 2013. MAP kinase signalling cascades and transcriptional regulation. *Gene* **513**: 1–13.
- Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, et al. 1999. Suppression of Raf-1 kinase activity and MAP kinase signaling by RKIP. *Nature* **401**: 173–177.
- Yeung K, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, Kolch W. 2000. Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. *Mol Cell Biol* **20**: 3079–3085.
- Yoon S, Seger R. 2006. The extracellular signal-regulated kinase: Multiple substrates regulate diverse cellular functions. *Growth Factors* **24**: 21–44.
- Zhou BB, Peyton M, He B, Liu C, Girard L, Caudler E, Lo Y, Baribaud F, Mikami I, Reguart N, et al. 2006. Targeting ADAM-mediated ligand cleavage to inhibit HER3 and EGFR pathways in non-small cell lung cancer. *Cancer Cell* **10**: 39–50.
- Zwang Y, Yarden Y. 2009. Systems biology of growth factor-induced receptor endocytosis. *Traffic* **10**: 349–363.

Cold Spring Harbor Perspectives in Biology

Complexity of Receptor Tyrosine Kinase Signal Processing

Natalia Volinsky and Boris N. Kholodenko

Cold Spring Harb Perspect Biol 2013; doi: 10.1101/cshperspect.a009043

Subject Collection [Signaling by Receptor Tyrosine Kinases](#)

CSF-1 Receptor Signaling in Myeloid Cells

E. Richard Stanley and Violeta Chitu

The EGFR Family: Not So Prototypical Receptor Tyrosine Kinases

Mark A. Lemmon, Joseph Schlessinger and Kathryn M. Ferguson

Tie2 and Eph Receptor Tyrosine Kinase Activation and Signaling

William A. Barton, Annamarie C. Dalton, Tom C.M. Seegar, et al.

The Spatiotemporal Organization of ErbB Receptors: Insights from Microscopy

Christopher C. Valley, Keith A. Lidke and Diane S. Lidke

Insulin Receptor Signaling in Normal and Insulin-Resistant States

Jérémie Boucher, André Kleinriders and C. Ronald Kahn

Central Role of RET in Thyroid Cancer

Massimo Santoro and Francesca Carlomagno

Receptor Tyrosine Kinase-Mediated Angiogenesis

Michael Jeltsch, Veli-Matti Leppänen, Pipsa Saharinen, et al.

Biology of the TAM Receptors

Greg Lemke

The Genesis of Tyrosine Phosphorylation

Tony Hunter

Structure-Function Relationships of ErbB RTKs in the Plasma Membrane of Living Cells

Donna J. Arndt-Jovin, Michelle G. Botelho and Thomas M. Jovin

Receptor Tyrosine Kinases: Legacy of the First Two Decades

Joseph Schlessinger

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

Jennifer Green, Roel Nusse and Renée van Amerongen

Regulation of Receptor Tyrosine Kinase Ligand Processing

Colin Adrain and Matthew Freeman

Molecular Mechanisms of SH2- and PTB-Domain-Containing Proteins in Receptor Tyrosine Kinase Signaling

Melany J. Wagner, Melissa M. Stacey, Bernard A. Liu, et al.

Eph Receptor Signaling and Ephrins

Erika M. Lisabeth, Giulia Falivelli and Elena B. Pasquale

Effects of Membrane Trafficking on Signaling by Receptor Tyrosine Kinases

Marta Miaczynska

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**

Oligo Modifications?
Your wish is our command.

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense