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Processing Temporal Growth Factor Patterns by an Epidermal Growth Factor Receptor Network Dynamically Established in Space

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Abstract

The proto-oncogenic epidermal growth factor (EGF) receptor (EGFR) is a tyrosine kinase whose sensitivity and response to growth factor signals that vary over time and space determine cellular behavior within a developing tissue. The molecular reorganization of the receptors on the plasma membrane and the enzyme-kinetic mechanisms of phosphorylation are key determinants that couple growth factor binding to EGFR signaling. To enable signal initiation and termination while simultaneously accounting for suppression of aberrant signaling, a coordinated coupling of EGFR kinase and protein tyrosine phosphatase activity is established through space by vesicular dynamics. The dynamical operation mode of this network enables not only time-varying growth factor sensing but also adaptation of the response depending on cellular context. By connecting spatially coupled enzymatic kinase/phosphatase processes and the corresponding dynamical systems description of the EGFR network, we elaborate on the general principles necessary for processing complex growth factor signals.

Contents

1. INTRODUCTION

Communication via growth factors coordinates the collective behavior of cells that generate their identity within the developing tissues of an organism. Cell-surface receptors recognize the ligands as inputs from the environment and transmit the signals to the inside of the cell. An example is the epidermal growth factor (EGF) receptor (EGFR), whose signaling affects collective processes in the embryonic development of skin, lung, heart, and neuronal tissues (Lemmon et al. 2014; Miettinen et al. 1995, 1999; Sibilia & Wagner 1995; Threadgill et al. 1995) but also wound healing in fully developed organisms (Sibilia et al. 2007, Yu et al. 2010). As one of four members of the ErbB family of tyrosine kinase receptors, EGFR can be activated upon binding of the different cognate ligands: EGF, transforming growth factor alpha, amphiregulin, heparin-binding EGF (HB-EGF), betacellulin (BTC), epigen (EGN), and epiregulin (EPR) (Burgess 2008, Lemmon et al. 2014). The binding of these known ligands leads to EGFR dimerization (Burgess et al. 2003, Lax et al. 1989) and subsequent activation of its intrinsic tyrosine kinase activity. EGFR can also form heterodimers with the other family members (Lemmon et al. 2014, Yarden & Sliwkowski 2001), particularly the orphan receptor ErbB2 and the kinase-dead receptor ErbB3 when these are coexpressed in cells. Although the bispecific ligands HB-EGF, EPR, and BTC regulate both EGFR and ErbB4 (Wilson et al. 2009), the latter is mainly activated by the specific class of neuregulins (Falls 2003).

The activation of the intrinsic tyrosine kinase activity by dimerization causes autophosphorylation of the receptor in *trans* and thereby intracellular signal transduction that determines cell fate (Jura et al. 2009, Zhang et al. 2006). Some of the low-affinity ligands such as EPR and EGN function as partial agonists of EGFR dimerization but full agonists of EGFR phosphorylation (Freed et al. 2017), indicating that EGFR phosphorylation (and thereby signaling) depends not only on the generation of stable dimers but also on the ligand-induced kinetics of the reversible dimerization reaction (Macdonald-Oberman & Pike 2014). In physiological settings, the ligands are produced locally depending on intracellular signaling, which further affects the receptor in a recursive autocrine or paracrine manner (Conte & Sigismund 2016, Singh & Harris 2005). Due to the central role of EGFR in tissue generation and homeostasis, its overexpression and/or hyperactivation through genetic alterations can also lead to malignant transformations and tumor development (Rowinsky 2004). In such pathological instances, EGFR mutations can not only affect the behavior of individual cells, leading to disorganized growth, but also change the population response in a tissue by altering how cells sense and communicate via growth factors.

Through structural and biochemical studies, it became apparent how information on growth factor binding to EGFR is transmitted through the membrane by self-association that leads to phosphorylation and signaling in the cytoplasm (Burgess 2008, Lemmon et al. 2014). Such studies have also revealed that different cognate ligands induce distinct structural changes in EGFR, leading to different signaling patterns (Freed et al. 2017, Sweeney & Carraway 2000, Wilson et al. 2009). However, multiple and sometimes even opposed phenotypic responses can arise from the same growth factor depending on context, and time-varying EGF signals can also alter the phenotypic response to resemble the responses induced by other growth factors (Ryu et al. 2015, Yarden & Sliwkowski 2001). These information-processing capabilities therefore indicate that EGFR is embedded in a sensing system that can interpret complex growth factor patterns. To describe how EGFR processes information as a function of stimulus history and current context, we discuss how the EGFR phosphorylation response emerges through coordinated action of EGFR reaction kinetics, vesicular dynamics of the receptor, and spatially established interactions with protein tyrosine phosphatases (PTPs). We hereby argue that this interdependence between the different scales of receptor organization establishes a unified growth factor–sensing system that manifests within each scale and enables a balance between signaling commitment and continuous responsiveness in a changing environment.

2. FROM THE STRUCTURAL BASIS OF EGFR ACTIVATION TO ITS REACTION DYNAMICS

2.1. Conformational Mechanisms of EGFR Kinase Activation

EGFR activation through EGF-induced dimer formation relies on allosteric release of the intramolecular interaction between two of the four extracellular subdomains (II and IV). This release results in a transition from a closed to an open configuration with freed dimerization arms of subdomain II that allow the association between two EGFR receptor molecules (Burgess et al. 2003, Ferguson et al. 2003, Garrett et al. 2002, Lax et al. 1989, Ogiso et al. 2002). A symmetric extracellular domain module configuration with a 2:2 EGF:EGFR stoichiometry is thereby generated at saturating EGF concentrations (**Figure 1*a***) (Garrett et al. 2002, Ogiso et al. 2002), whereas an asymmetrical extracellular domain module with a 1:2 stoichiometry and altered subdomain II binding interface (Alvarado et al. 2010, Liu et al. 2012) is likely adopted at low physiological EGF concentrations. EGF binding thus removes the steric constraints on the self-association imposed by the tethered extracellular module, favoring dimerization of the transmembrane helices as well

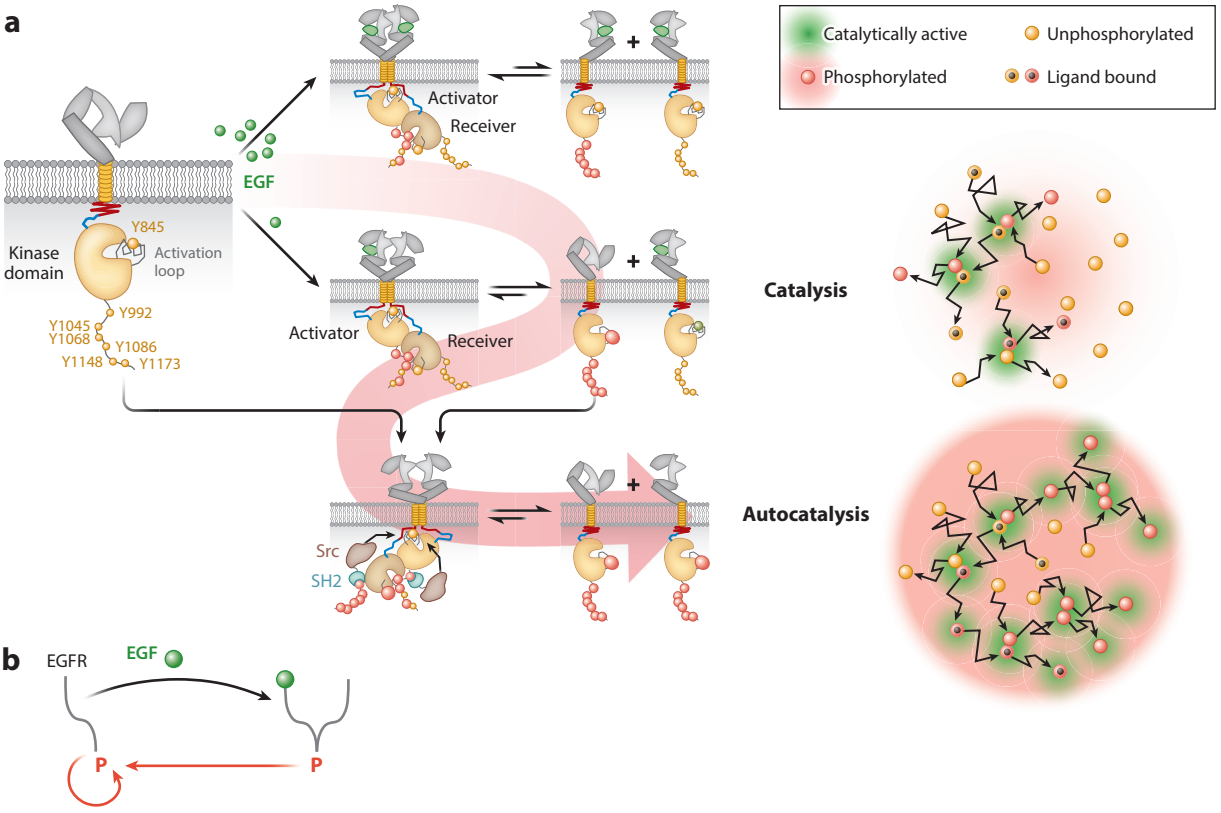

Figure 1

Catalytic and autocatalytic EGFR activation. (*a*, *left*) In the absence of growth factors, EGFR is predominantly monomeric with unphosphorylated tyrosine residues (e.g., regulatory Y845 in the activation loop of the kinase domain or signaling tyrosines on the C-terminal tail). (*Middle*) Upon EGF stimulation (*green spheres*), distinct short-lived, catalytically active dimers form that are in equilibrium with monomers. A high EGF dose generates an extracellular domain symmetrical/intracellular domain asymmetrical dimer (2:2 EGF:EGFR stoichiometry, *top row*) that is longer lived than the extracellular domain asymmetrical/intracellular domain asymmetrical dimer (1:2 EGF:EGFR stoichiometry, *middle row*) formed at a low EGF dose. Tyrosine residues are directly (receiver kinase) or indirectly (e.g., via EGFR-phosphorylation-dependent recruitment of Src) phosphorylated within the dimers. Disassociated EGFR monomers that are phosphorylated on Y845 are catalytically active and can form transient dimers with ligandless EGFR, resulting in its phosphorylation (*downward arrows* pointing to *bottom row*). (*Right*) On the population level, the transient dimers with a 1:2 EGF:EGFR stoichiometry turn over EGFR monomers, resulting in a catalytic amplification of monomer phosphorylation (*top*). This catalytic amplification is spatially restricted by the diffusion of the monomeric receptors (*faded pink haze*, *top*). Phosphorylation on Y845 results in a cascade of autocatalytic EGFR phosphorylation–mediated activation events that enable the spread of the phosphorylation signal (*solid pink haze*, *bottom*). The large pink arrow in the background is a visual guide through the reaction steps that lead to autocatalysis. (*b*) Causal representation of the bidirectional relationship between ligand-bound dimers and autocatalytically activated monomers. Monomers are converted to dimers through EGF binding (*black arrow*), whereas dimers promote autocatalytic phosphorylation of EGFR monomers (*red arrows*). Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; P, phosphorylated; Src, tyrosine-protein kinase Src; SH2, Src homology 2 domain; Y, tyrosine.

as the juxtamembrane segments and the formation of asymmetrical dimers of the intracellular kinase domains (Zhang et al. 2006). During this self-association, the intracellular C-lobe of the kinase domain of one protein acts as an allosteric activator while the N-lobe of the binding partner acts as an allosteric receiver in such a way that its activation loop position is altered so that an open, active conformation of the kinase domain is favored.

The activation of the intracellular kinase domain of the receiver results in the transphosphorylation of tyrosine residues (pY) on the C-terminal tail of the activator receptor within the EGFR dimer. Subsequent recruitment of adaptor proteins that contain Src homology 2 domains (SH2s to pY992, 1068, 1086, 1114) or phosphotyrosine-binding domains (PTBs to pY992, 1068, 1173, 1148) then couple activated receptor dimers to diverse signaling pathways in the cytoplasm, such as the Ras-mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K), protein kinase B (Akt), and phospholipase C-gamma (PLC-γ) pathways (Yarden & Sliwkowski 2001).

Concurrently, recruitment of the E3 ligase Cbl directly to pY1045 and/or indirectly via the adaptor protein Grb2 to pY1068 and pY1086 ubiquitinates the receptor on lysines (de Melker et al. 2004, Jiang et al. 2003, Schmidt & Dikic 2005, Stang et al. 2000, Thien & Langdon 2001). Monoubiquitination creates docking sites for proteins such as AP1 or CIN85 that couple to the endocytic machinery via specialized ubiquitin-docking domains (Haglund et al. 2002, Huang et al. 2006) and thereby marks the dimeric receptor for degradation via the endocytic lysosomal route, leading to signal termination.

Ligand binding to EGFR thus triggers a unidirectional sequence of molecular self-association and subsequent conformational rearrangement events that lead to activation of the intrinsic kinase and phosphorylation within the dimer. This profiles the phosphorylated dimeric receptor as a stable end product that initiates signal transduction through cell-specific signaling pathways, which determine specific phenotypic responses.

2.2. Catalysis and Autocatalysis

Quantitative single-cell, EGF-dose/EGFR-phosphorylation-response experiments have demonstrated EGFR phosphorylation levels that largely exceed the measured level of EGF-induced self-association, indicating that the information about extracellular ligand is communicated to ligandless receptors through their phosphorylation (Reynolds et al. 2003, Stanoev et al. 2018). Such amplification of EGFR phosphorylation can occur via turnover of EGFR monomers through an EGFR dimer that serves as a catalytic intermediate. This requires that EGFR dimers are transient complexes through which the exchange of monomers can occur (Ichinose et al. 2004). Optical tracking of single EGFR molecules in cells has indeed demonstrated that both ligand-bound and ligandless dimers are formed transiently with relatively short lifetimes ($k_{off} \sim 0.1$–$0.3\ s^{-1}$) (Chung et al. 2010, Coban et al. 2015, Valley et al. 2015), which is consistent with the low micromolar affinity of extracellular as well as intracellular EGFR segment dimerization (Burgess et al. 2003, Odaka et al. 1997, Ogiso et al. 2002). Furthermore, for physiological, subsaturating EGF concentrations (below or in the range of the nanomolar EGF dissociation constant), most EGFR dimers are occupied by a single EGF but can still exhibit transphosphorylating activity (Liu et al. 2012). Negative cooperativity for the second EGF binding (Macdonald & Pike 2008, Pike 2012, Wofsy et al. 1992), which originates from an altered asymmetrical dimer interface in the extracellular domains (Alvarado et al. 2010), suggests that EGF-EGFR dimers with 1:2 stoichiometry are even shorter-lived intermediates (Salazar-Cavazos et al. 2020). That transient dimers can give rise to catalytic amplification of phosphorylation is further supported by the fact that low-affinity ligands such as epiregulin and epigen act as partial agonists of dimerization (due to a weakened dimerization interface) but are full agonists of receptor phosphorylation (Freed et al. 2017). EGFR phosphorylation amplification through phosphorylation on ligandless monomers by transient association with ligand-bound EGFR is depicted in **Figure 1*a***. On the population level, the catalytic amplification of the phosphorylation is spatially restricted by the diffusion of the ligand-bound receptors.

However, this catalytic phosphorylation can also trigger autocatalytic EGFR activation by phosphorylation on regulatory tyrosines that affect kinase domain conformation. Molecular dynamics simulations have shown that Y845 phosphorylation in the EGFR activation loop suppresses the intrinsic disorder in the αC-helix region, thereby stabilizing an active kinase conformation as well as increasing EGFR dimerization (Shan et al. 2012). Using a conformational EGFR sensor based on genetic code expansion, Baumdick et al. (2018) provided further evidence that Y845 phosphorylation does indeed stabilize an active activation loop conformation in EGFR monomers. This conserved regulatory Y845 has been reported to be phosphorylated by Src, which in turn is activated by phosphorylated EGFR (Osherov & Levitzki 1994, Sato et al. 1995), indicating that an autocatalytic loop can also be realized indirectly. In this respect, it has been demonstrated that the two kinases in an asymmetric dimer cannot access each other's activation loops and therefore require the recruitment of a kinase such as Src on phosphorylated Y1086 (Kovacs et al. 2015). This is consistent with the findings that phosphorylation on signaling tyrosines precedes phosphorylation on Y845 (Kim et al. 2012), and that phosphorylation of the distal segment of the tail (Y999–Y1186) enhances phosphorylation of Y845 (Kovacs et al. 2015). Alternatively, the phosphorylation of Y845 might require the formation of higher-order oligomers of asymmetric EGFR dimers (Huang et al. 2016). In this case, the transient oligomers formed between ligand-bound dimers and ligandless receptors could facilitate the activation of ligandless receptors (Ichinose et al. 2004).

Figure 1*a* depicts a schematic representation of the autocatalytic amplification through phosphorylation in transient ligandless dimers. This bimolecular reaction leads to a cascade of phosphorylation and activation events that can enable the spread of the phosphorylation signal (Tischer & Bastiaens 2003). As shown in **Figure 1*b***, these reactions can be abstracted through a causal bidirectional relationship between ligand-bound dimers and autocatalytically activated monomers.

2.3. The Role of Nanoscale EGFR Organization in the Plasma Membrane for Controlled Signaling

The catalytic and autocatalytic reaction–diffusion mechanisms of EGFR activation run the risk of generating an uncontrolled amplification of signals and thereby aberrant signaling. One layer of constraint over these reactions can be exerted by diffusional barriers that limit free diffusion of EGFR to nanoscopic membrane domains (50–300 nm wide) that are delimited by actin-based membrane skeleton fences (Kusumi & Sako 1996, Kusumi et al. 2005). These domains are considered to be well-mixed protein reaction vessels within which EGFR self-association and the resulting phosphorylation is facilitated. The crossing of signaling proteins through these fences thus becomes the rate-limiting factor in lateral information transfer by reaction–diffusion mechanisms such as autocatalysis. However, equipartitioning among the domains results in few receptors per domain at physiological EGFR expression levels of $\sim 5 \times 10^4$ receptors per cell, thereby limiting EGFR reactivity (Clayton et al. 2005, Grecco et al. 2011). However, preformed 100–200-nm-sized nanoclusters containing 10–100 EGFR molecules have been observed, indicating the presence of diffusional traps based on weak interactions with cytoskeletal or other proteins (den Hartigh et al. 1992, Holowka & Baird 2017, Masip et al. 2016). Alternatively, these nanoclusters might be additionally maintained by homotypic interactions between the extracellular domains of EGFR molecules (Needham et al. 2016, Zanetti-Domingues et al. 2018). What is clear, however, is that EGF stimulation leads to a ubiquitination-dependent enhanced trapping of EGFR in the formation of clathrin-coated pits (CCPs) (Ibach et al. 2015, Rappoport & Simon 2009). The rapid exchange between diffusive and immobile states of EGFR within CCPs enables a spatially confined reaction–diffusion mechanism that amplifies EGFR phosphorylation

(Ibach et al. 2015). An additional positive feedback loop between CCP formation and phosphorylated EGFR trapping thereby leads to the acceleration of the formation of invaginated membrane structures that accumulate EGFR clusters, which effectively nucleate intracellular signaling complexes (Liang et al. 2018) and commit these complexes to signaling endosomes (Miaczynska et al. 2004, Sorkin et al. 1996, Villasenor et al. 2016, Wouters & Bastiaens 1999).

In these submicrometer-scale clathrin-coated membrane structures there is thus a balance between the generation of relatively stable large EGFR signaling complexes that commit the system to intracellular endosomal signaling and reversibility by transient EGFR self-association that matters not only for growth factor sensing but also for distinct signaling responses in time. Rapid and continuous phosphorylation/dephosphorylation reaction cycles (Bohmer et al. 1995, Offterdinger et al. 2004, Ruff et al. 1997) are essential for both the sensing of and the response to extracellular changes in growth factors. Indeed, it has been demonstrated that these phosphorylation/dephosphorylation cycles operate on a timescale of seconds, which is approximately two to three orders of magnitude faster than the total duration of receptor phosphorylation (Kleiman et al. 2011). This reveals protein tyrosine phosphatases to be key regulators of EGFR phosphorylation dynamics.

2.4. The Role of Protein Tyrosine Phosphatases in Regulating EGFR Phosphorylation

PTPs that have distinct localization in the cell (Andersen et al. 2001, Tonks 2006) provide the major EGFR-dephosphorylating activities (Alonso et al. 2004, Fischer et al. 1991, Tonks & Neel 1996). Different PTPs that have preferential activity and thereby specificity toward given EGFR tyrosine residues have been identified (Liu & Chernoff 1997, Tiganis et al. 1998, Yuan et al. 2010). Additionally, large-scale studies based on enzymatic assays of purified PTPs (Barr et al. 2009), membrane two-hybrid assays (Yao et al. 2017), or biochemical assays on cell extracts after small interfering RNA (siRNA) knockdown (Tarcic et al. 2009) have been performed to identify which PTPs dephosphorylate EGFR. Recently, microscopic imaging of EGFR phosphorylation upon opposed genetic PTP expression perturbations have identified not only the receptor-like R-PTP-γ and R-PTP-η and the endoplasmic reticulum–associated TC-PTP as the strongest dephosphorylating activities toward EGFR, but also when and where they dephosphorylate EGFR (Stanoev et al. 2018). The catalytic activity of fully active PTPs is, however, two to three orders of magnitude higher than that of tyrosine kinases (Fischer et al. 1991). This would effectively suppress spurious EGFR phosphorylation (Baumdick et al. 2015) but also inhibit EGFR phosphorylation upon growth factor binding and thereby impair signal initiation. This conundrum implies that PTP activity is regulated through feedback coupling to EGFR kinase activity. Such regulation of EGFR phosphorylation by EGFR-PTP feedback enables suppression of spurious EGFR activation to be unified with robust growth factor dose sensing and subsequent signaling duration before resetting to a basal state. As discussed below, the EGFR-PTP feedback can be locally realized either by biochemical reactions coupled to EGFR phosphorylation that modulate PTP activity or in space via phosphorylation-dependent vesicular trafficking of the receptor through spatially distributed PTP activities.

2.4.1. Regulation of PTP activity by EGFR-induced oxidation. A major mechanism of biochemical coupling between EGFR and PTPs occurs by EGFR-induced reactive oxygen species (ROS)-mediated oxidation of the catalytic cysteine of PTPs (Bae et al. 1997, Rhee et al. 2000). This coupling is realized via the multisubunit NADPH oxidases (NOXs), where EGFR-induced PI3K activation induces phosphatidylinositol (3,4,5) trisphosphate (PIP3) production in the plasma

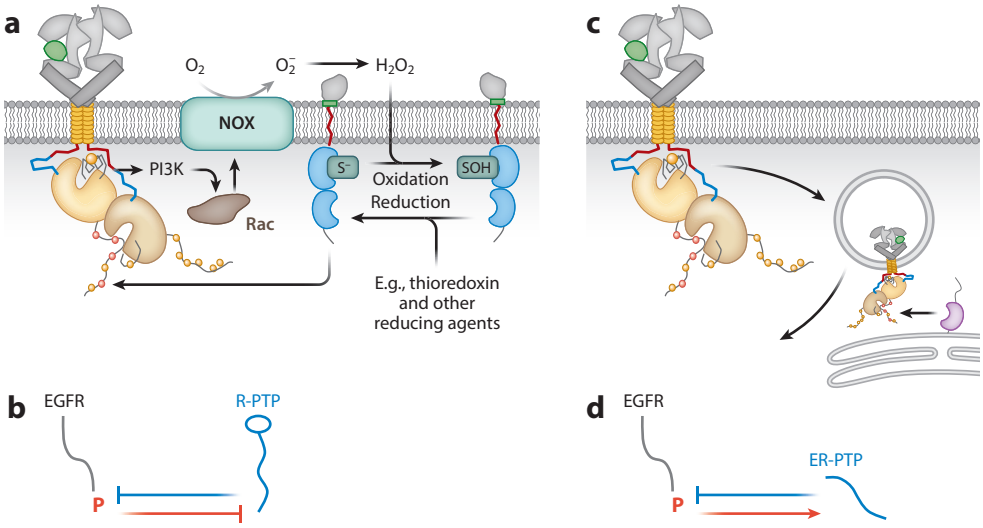

Figure 2

Feedback regulation of EGFR phosphorylation by PTPs. (*a*) R-PTPs dephosphorylate phosphorylated tyrosine (*red* to *yellow dots*) on EGFRs at the plasma membrane. Phosphorylated EGFR induces PI3K activation, which in turn activates the GTPase Rac and NOX assembly. NOX generates extracellular superoxide from oxygen that is rapidly dismutated to H_2O_2 and reenters the cell, where it reversibly inactivates R-PTP by oxidation of a catalytic cysteine to sulfenic acid. Phosphorylated EGFR thereby inhibits R-PTP activity. (*b*) Causal representation of the generated double-negative (toggle-switch) interaction of EGFR and R-PTP. The monomeric phosphorylated EGFR species is represented. (*c*) EGF-induced phosphorylation of EGFR promotes its internalization via endocytosis. The PTPs associated with the cytoplasmic side of the ER membrane dephosphorylate EGFRs on endosomes, implicitly coupling EGFR-phosphorylating to PTP-dephosphorylating activity through endocytosis. (*d*) Causal representation of this spatially established negative feedback between EGFR and ER-PTPs. The monomeric phosphorylated EGFR species is represented. Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ER-PTP, endoplasmic reticulum–associated protein tyrosine phosphatase; H_2O_2, hydrogen peroxide; NOX, NADPH oxidase; O_2^-, superoxide; P, phosphorylated; PI3K, phosphoinositide-3-kinase; PTP, protein tyrosine phosphatase; R-PTP, receptor-like protein tyrosine phosphatase; S^-, reduced cysteine thiol; SOH, sulfenic acid.

membrane, resulting in the activation of the GTPase Rac and thereby the NOX assembly (Abo et al. 1991; Bae et al. 1997, 2000; Paulsen et al. 2012). Activated NOX generates extracellular superoxide from oxygen that is rapidly dismutated to H_2O_2 and reenters the cell by diffusion or via aquaporins (Bienert et al. 2006, Suh et al. 1999). These become the source of a steep membrane-proximal intracellular H_2O_2 gradient (Bae et al. 1997), which can locally oxidize the catalytic cysteine of PTPs to the catalytically impaired cysteine sulfenic acid (SOH, **Figure 2*a***) (Salmeen et al. 2003). This primary oxidation product is reversible, enabling PTP oxidation/reduction cycles to generate a tightly confined area containing inactive PTPs near the ROS-generating membrane due to the vast cytoplasmic pool of antioxidants (Schieber & Chandel 2014, Grecco et al. 2011). This local ROS-mediated inhibition of PTP activity due to phosphorylated EGFR amounts to a negative regulatory causality (**Figure 2*b***) that, together with the negative regulation of EGFR phosphorylation by PTP, generates a double-negative feedback loop (Reynolds et al. 2003). This type of feedback regulation has been demonstrated for the candidate tumor suppressor R-PTP-γ, which enables both suppression of spurious EGFR activation and robust phosphorylation upon growth factor stimulation (Stanoev et al. 2018).

2.4.2. Direct EGFR-PTP negative feedback. Negative feedback regulation of EGFR phosphorylation can be accomplished by phosphorylation-induced EGFR-PTP interaction that activates the PTP. The protein tyrosine phosphatase SHP2, which is encoded by the proto-oncogene *PTPN11* (Bennett et al. 1996, Lechleider et al. 1993, Sugimoto et al. 1993), participates in such feedback and exists in the cytoplasm in a low-activity state in which the active site is occluded by an intramolecular interaction with its N-terminal SH2 domain. Interaction of the SH2 domain with pY on EGFR releases the autoinhibitory interaction, creating an active form of the phosphatase that dephosphorylates the receptor. This negative feedback regulation has been shown to maintain a constant mean amount of phosphorylated EGFR on endosomes irrespective of their size and EGFR loading (Villasenor et al. 2015). However, this negative feedback regulation of EGFR phosphorylation can also result in a positive effect on EGFR signaling from the plasma membrane as demonstrated for dephosphorylation of Y992 that recruits Ras GTPase–activating protein (RasGAP) (Agazie & Hayman 2003). Furthermore, H_2O_2-mediated oxidation of SHP2 on redoxosomes (Spencer & Engelhardt 2014), as demonstrated for PDGFR (Tsutsumi et al. 2017), could add another layer of regulation in which a negative feedback switches to a ROS-mediated toggle switch to sustain signaling of endocytosed receptor tyrosine kinases in the peripheral cytoplasm.

2.4.3. Spatial establishment of negative EGFR-PTP feedback. Negative feedback regulation of EGFR phosphorylation can also be realized in space by receptor-mediated endocytic trafficking of activated, phosphorylated EGFR from the plasma membrane to an area with high PTP activity. Toward that end, the two PTPs associated with the cytoplasmic side of the endoplasmic reticulum membrane (PTPN1/PTP1B and PTPN2/TC-PTP) (Cool et al. 1989, Tonks et al. 1988) have been shown to dephosphorylate EGFR on endosomes (Haj et al. 2002, Yudushkin et al. 2007), implicitly coupling EGFR phosphorylation activity to PTP dephosphorylating activity through endocytosis (**Figure 2*c***). This amounts to a spatially established negative feedback that determines the duration of EGFR phosphorylation (**Figure 2*d***). PTP1B has also been shown to suppress the spontaneous activation of EGFR by dephosphorylating Y845 on constitutively recycling receptors (Baumdick et al. 2015). The activities of these PTPs are additionally redox regulated (Haque et al. 2011, Lee et al. 1998, Meng et al. 2004) through space to enable EGFR signal propagation in the cytoplasm (Yudushkin et al. 2007).

3. REGULATION OF GROWTH FACTOR SIGNALING BY THE DYNAMICS OF SPATIAL EGFR CYCLES

The concept that signaling molecules downstream of cell-surface receptors can influence vesicular trafficking (Dykes et al. 2017, Er et al. 2013, Hanafusa et al. 2011, Laketa et al. 2014, Salazar & Gonzalez 2002, Wang et al. 2015) generates a reciprocal relationship between receptor activation and vesicular dynamics that can shape the cellular response to stimuli (Mettlen et al. 2018). Ligand-bound EGFR complexes are predominantly internalized via clathrin-mediated endocytosis (CME) at low EGF doses and clathrin-independent endocytosis (CIE) at high EGF concentrations (Bakker et al. 2017, Collinet et al. 2010, Goh et al. 2010). Receptor-ligand complexes packaged into vesicles from both pathways enter early endosomes (EEs) by fusion (Bucci et al. 1992, Goh & Sorkin 2013, Vieira et al. 1996); EEs further mature in the perinuclear area into late endosomes (LEs) and eventually fuse into lysosomes where receptors (and ligand) are degraded (Ceresa 2006, Levkowitz et al. 1999, Rink et al. 2005, Vanlandingham & Ceresa 2009). Positioned both temporally and physically between the plasma membrane and the lysosomal compartment, the highly dynamic, interconverting vesicular system propagates and processes receptor tyrosine

kinase signals in the cytoplasm (Baass et al. 1995; Bakker et al. 2017; Grecco et al. 2011; Grimes et al. 1996; Rashid et al. 2009; Schenck et al. 2008; Sorkin et al. 2000; Villasenor et al. 2015, 2016; Wouters & Bastiaens 1999).

3.1. Regulation of Vesicular EGFR Dynamics by EGFR Signaling

Akt activity that is coupled to EGFR activity via PI3K drives EGFR vesicular trafficking through the endosomal system (Er et al. 2013) by activating the early endosomal effector PIKfyve (FYVE-containing phosphatidylinositol-3-phosphate 5-kinase). The kinase activity of PIKfyve converts phosphatidylinositol-3-phosphate to phosphatidylinositol-3,5-bisphosphate on endocytic vesicles, which enhances the transition of EGFRs from EEs to LEs as well as to Rab11-positive recycling endosomes (REs), thereby recycling EGFR to the plasma membrane. Cbl-mediated ubiquitination of the receptor couples the active, phosphorylated ligand-bound receptor to the endocytic machinery at the plasma membrane (Haglund et al. 2002, Huang et al. 2006). However, ubiquitination also functions as a sorting signal in the vesicular trafficking of the EGFRs (Bakker et al. 2017, Henne et al. 2011, Marmor & Yarden 2004, Waterman et al. 2002). Whereas ligand-bound, ubiquitinated receptor complexes are unidirectionally trafficked from the plasma membrane through the EEs and LEs to lysosomes, the internalized nonubiquitinated, predominantly monomeric receptors are redirected from the EEs to the REs and back to the plasma membrane (Baumdick et al. 2015) (**Figure 3*a***).

3.2. The Effects of EGF Concentration on EGFR Trafficking Dynamics

EGFR trafficking dynamics depends on the extracellular concentration of EGF, because distinct EGF concentrations establish a different balance between ligandless monomers and ligand-bound EGFR complexes. At saturating EGF concentrations, a high steady-state amount of ligand-bound, ubiquitinated dimers is generated, which are unidirectionally trafficked from the plasma membrane to the lysosome for degradation. However, at the low subsaturating EGF concentrations typically found in human tissue secretions (0.4 to 20 ng/mL) (Konturek et al. 1989), only a fraction of receptors are ligand bound, whereas the majority of receptors remain monomeric, are not ubiquitinated, and undergo recycling (**Figure 3*b***). Once these recycled receptors are at the plasma membrane, they are available to bind growth factors, which generates the dimeric species. Such an EGFR trafficking system, whose differential dynamics depends on EGF concentration, enables sensing of growth factor signals that vary over time. The ligand-bound entities alone cannot sense further growth factor changes due to the high-affinity binding of EGF with dissociation rates on a timescale of minutes to tens of minutes ($k_{off} \sim 0.5$–$0.05\ min^{-1}$) (Bellot et al. 1990, Defize et al. 1989) and their rapid endocytosis through aggregation in CCPs. However, before being endocytosed, the ligand-bound dimeric EGFR transfers information about growth factor concentration by promoting the autocatalytic phosphorylation of the recycled ligandless receptors (**Figure 1**). These ligandless receptors therefore function as sensing entities of upcoming growth factor signals. For the system to sense changes in growth factor signals, the ligandless receptors have to be dephosphorylated before they return to the plasma membrane.

4. FROM SPATIAL CYCLES TO CAUSALITY AND DYNAMICAL MODE OF EGF SENSING

Sensing of growth factor signals that vary over time arises through the coordinated action of vesicular dynamics, EGFR reaction kinetics, and the PTP-EGFR interactions that regulate the

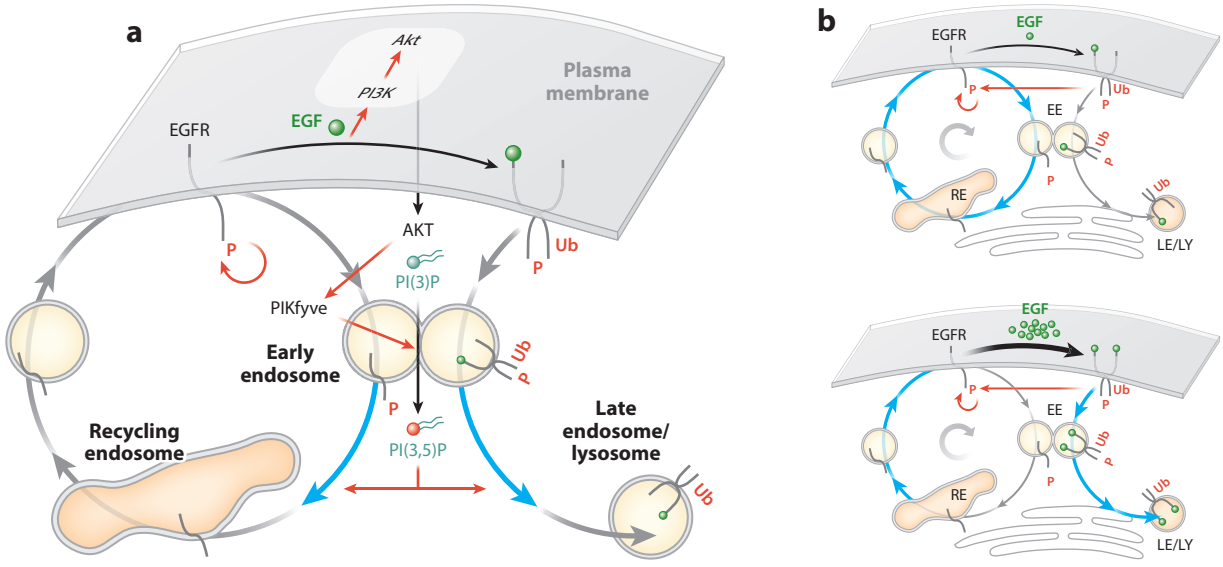

Figure 3

Dynamics of spatial EGFR cycles. (*a*) EGFR vesicular trafficking through the endosomal system is promoted through the EGFR-PI3K-Akt-dependent activation of the early endosomal effector PIKfyve, which phosphorylates PI(3)P to PI(3,5)P. This enhances the transition of EGFR from EEs to other endocytic compartments, namely LEs or REs. Ub functions as a sorting signal in the vesicular trafficking of EGFR through the endosomal system: Ligand-bound, ubiquitinated receptor complexes are unidirectionally trafficked from the plasma membrane through the EEs and LEs to be degraded in LYs, whereas the internalized, nonubiquitinated monomeric receptors are redirected from the EEs to the REs back to the plasma membrane. (*b*) EGF (*green spheres*) doses potentiate a distinct vesicular route. (*Top*) At physiologically low EGF doses, only a minority of receptors are ligand bound, whereas the majority of receptors remain monomeric, are not ubiquitinated, and undergo recycling. (*Bottom*) At saturating EGF dose, there is a high steady-state amount of dimeric ligand-bound receptors that are ubiquitinated and unidirectionally traffic from the plasma membrane to the LY for degradation. Abbreviations: Akt, protein kinase B; EE, early endosome; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; LE, late endosome; LY, lysosome; P, phosphorylation; PI3K, phosphoinositide-3-kinase; PI(3)P, phosphatidylinositol-3-phosphate; PI(3,5)P, phosphatidylinositol-3,5-bisphosphate; PIKfyve, FYVE-containing phosphatidylinositol-3-phosphate 5-kinase; RE, recycling endosome; Ub, ubiquitin.

receptors' phosphorylation dynamics. A systemic description of the response dynamics of such a complex system first requires translating these interdependencies into a causal description. The obtained network allows the analysis of which qualitatively different EGFR response dynamics can arise. The predicted qualitative differences in response dynamics can then be verified by experimentally altering the system's parameters to identify how EGFR senses changes in growth factor signals.

4.1. Reconstruction of the EGFR-PTP Network

Causal links between EGFR-PTP pairs can be identified by using an EGFR phosphorylation response to EGF dose as a dynamical signature of the underlying feedback motif together with opposed genetic perturbations (Rahi et al. 2017, Reynolds et al. 2003, Stanoev et al. 2018). Ectopic expression of the pairwise interaction partners potentiates the dynamic signature and thereby the underlying motif over those with other endogenous PTPs, whereas PTP knockdown validates the identified motif. This approach revealed double-negative EGFR-R-PTP-γ feedback (toggle switch) and negative regulation of EGFR phosphorylation by R-PTP-η on the plasma membrane, as well as negative feedback regulation by TC-PTP spatially established by the vesicular recycling

of the receptor (**Figure 4*a***). The recycling thereby unifies these pairwise EGFR-PTP interactions into a core receptor network that is distributed in space (**Figure 4*b***).

4.2. Identification of Dynamical Modes of Operation

Given the identified network topology, the EGFR phosphorylation response depends on two experimentally controllable parameters. The first is the fraction of ligand-bound EGFR (EGF-EGFR %), an extrinsic parameter that depends on the extracellular EGF concentration, whereas

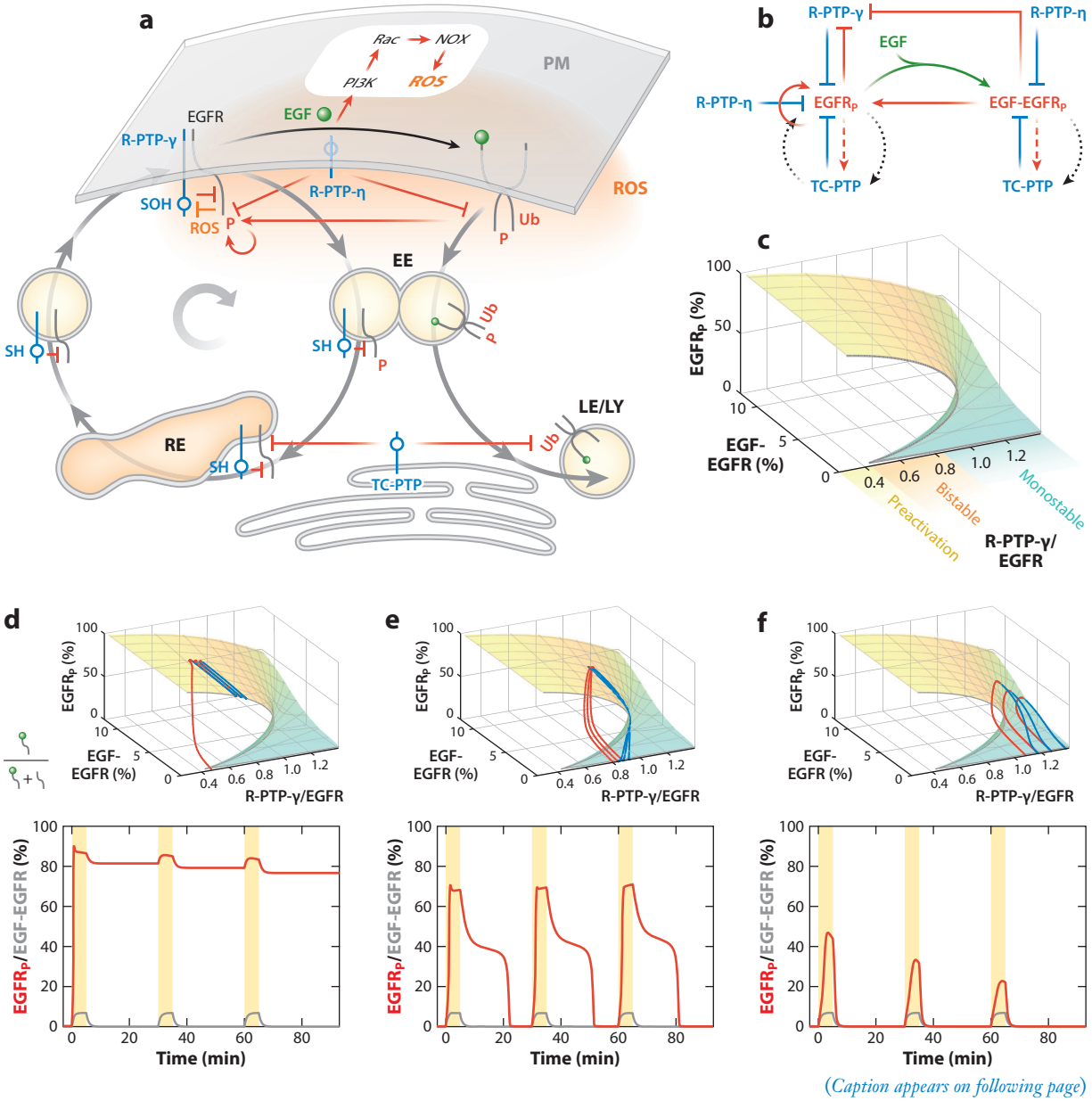

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Figure 4 (*Figure appears on preceding page*)

EGFR phosphorylation responses are determined by the dynamical modes of the spatially distributed EGFR-PTP network. (*a*) EGFR phosphorylation dynamics is regulated through interaction with PTPs in space. At the plasma membrane, the steep H_2O_2 gradient (ROS, *red haze*) mediates a double-negative feedback between monomeric EGFR and R-PTP-γ, whereas R-PTP-η negatively regulates EGFR phosphorylation. A negative feedback between EGFR and TC-PTP is established by vesicular recycling. (*b*) Causal representation of the spatially distributed EGFR-PTP network shown in panel *a*. Red and blue lines indicate causal interactions, green arrows represent ligand binding, and dashed black arrows denote that the enclosed causal links are established through vesicular trafficking. (*c*) Three-dimensional bifurcation diagram of the network in panel *b* showing the dependence of EGFR phosphorylation response (percent of $EGFR_P$) on the relative R-PTP-γ to EGFR concentration on the plasma membrane (R-PTP-γ/EGFR) and the fraction of ligand-bound receptors (percent of EGF-EGFR). (*d–f*, *bottom*) Red lines show temporal EGFR phosphorylation responses upon time-varying growth factor stimulation obtained by numerical simulations for organization in the (*d*) bistable, (*e*) critical, and (*f*) monostable regions. Gray lines indicate the fraction of ligand-bound receptors, and yellow bars show growth factor pulses. (*d–f*, *top*) Overlay of the corresponding EGFR phosphorylation trajectories on the three-dimensional bifurcation diagram in panel *c*. Red and blue lines show trajectory segments during pulse presence and absence, respectively. Abbreviations: EE, early endosome; EGF, epidermal growth factor; $EGFR_p$, phosphorylated epidermal growth factor receptor; H_2O_2, hydrogen peroxide; LE, late endosome; LY, lysosome; NOX, NADPH oxidase; P, phosphorylated; PI3K, phosphoinositide-3-kinase; PM, plasma membrane; PTP, protein tyrosine phosphatase; RE, recycling endosome; ROS, reactive oxygen species; R-PTP-η, receptor-like protein tyrosine phosphatase eta; R-PTP-γ, receptor-like protein tyrosine phosphatase gamma; SH, reduced cysteine thiol; SOH, sulfenic acid; TC-PTP, T cell protein tyrosine phosphatase; Ub, ubiquitin. Panels *a–c* are adapted from Stanoev et al. (2018) under a Creative Commons CC-BY license. Results presented in panels *d–f* were obtained with the two-compartmental model from Stanoev et al. (2020).

the second is the concentration of R-PTP-γ relative to that of EGFR on the plasma membrane (R-PTP-γ/EGFR), an intrinsic parameter. Due to the nonlinearities in the system, qualitatively distinct EGFR phosphorylation responses can be expected for organization in different parameter ranges. The possible dynamical modes of operation for the EGFR-PTP network have been analyzed using bifurcation analysis, which is a theoretical tool that for a given network allows the detection of the parameter ranges in which the possible dynamical regimes occur (Strogatz 2018). Three possible dynamical modes of operation of the EGFR-PTP network have thereby been identified and experimentally verified: a bistable regime bordering two monostable regimes characterized by basal and high EGFR phosphorylation (**Figure 4*c***) (Reynolds et al. 2003, Stanoev et al. 2018). These dynamical states are an intrinsic property of the network topology that is centered around an EGFR–R-PTP-γ toggle-switch motif with autocatalytic EGFR activation. Stimulation of EGFR with different ligands affects only the bifurcation parameter ranges and thereby the regimes in which the network operates.

For a given range of R-PTP-γ/EGFR on the plasma membrane, the system operates in the bistable regime where EGFR can be in either a basal or a high phosphorylation state in the absence of any stimulus. When starting from the basal EGFR phosphorylation state, increasing the ligand and thereby EGF-EGFR fraction, the system switches to the monostable high EGFR phosphorylation state via the high bistable state. However, for higher EGFR concentrations relative to those of R-PTP-γ, the system operates in the monostable regime of high EGFR phosphorylation. In this case the autocatalytic EGFR phosphorylation amplification always leads to R-PTP-γ inhibition via H_2O_2 production, regardless of growth factor stimulus. Conversely, at lower membrane EGFR concentrations, a monostable regime of basal EGFR phosphorylation is attained. In this regime, EGFR phosphorylation gradually increases with growth factor receptor occupancy (**Figure 4*c***).

In the presented dynamical framework, the temporal evolution of the average EGFR phosphorylation state has been considered, but equivalent results have been also obtained using single-molecule, reaction–diffusion simulations (Stanoev et al. 2020). In both instances, homogenous distribution of the receptor and the phosphatase on the plasma membrane of the cell has been considered as a well-mixed reaction vessel. As discussed in the section titled The Role of Nanoscale EGFR Organization in the Plasma Membrane for Controlled Signaling, there is likely

a nanoscale organization of EGFR consisting of discrete clusters with 10–100 receptors (Masip et al. 2016) that poses constraints on the free diffusion of the reactants. However, the diffusion of the EGFR molecules within as well as in and out of the nanoclusters (Fujiwara et al. 2016, Ibach et al. 2015) enables all of the dynamical regimes to be manifested as an ensemble behavior of all nanoclusters, which is the experimental observable for microscopic imaging (Stanoev et al. 2018).

4.3. Dynamical Modes Determine Phosphorylation Responses

When the system is poised in the bistable mode of operation, the response of EGFR phosphorylation to low-threshold EGF doses is rapid and robust. However, this bistable organization limits the system's responsiveness to temporal growth factor changes by switching it to a high EGFR phosphorylation state after the initial growth factor stimulus, which irreversibly persists after growth factor removal (**Figure 4*d***). EGF pulse experiments with R-PTP-γ knockdown have provided experimental confirmation of such growth factor–triggered sustained activity (Stanoev et al. 2018). Organization in this regime also enables lateral propagation of EGFR phosphorylation at the plasma membrane, because receptors can sustain their own activity in membrane areas not exposed to ligand (Reynolds et al. 2003, Tischer & Bastiaens 2003). However, for organization in the monostable regime, the system quickly loses its ability to sense and respond to growth factor signals that vary over time (**Figure 4*f***). This was experimentally confirmed by inhibition of receptor recycling that lowered the steady-state EGFR concentration on the membrane, leading to a weaker and dampened phosphorylation response to pulsed EGF stimulation (Stanoev et al. 2018). The recursion between theory and experiments thereby demonstrates that sensing growth factor signal changes cannot be realized when the system organization is in either the bistable or the monostable operation regime. Namely, sensing time-varying EGF signals requires rapid amplification of EGFR phosphorylation at low growth factor levels together with the ability to reset to basal EGFR phosphorylation when growth factor levels decline.

4.4. The Role of Metastable Dynamics in the Sensing of Changing Environments

EGFR phosphorylation was rapidly amplified as well as transiently maintained before resetting to basal levels in response to low-dose EGF pulses (Stanoev et al. 2018). Theoretical analysis revealed that the combination of these features arises from critical organization of the system between the mono- and bistable regimes of operation (**Figure 4*e***). For this organization, an emergent metastable state is what gives rise to prolonged EGFR phosphorylation after growth factor removal, generating a transient memory of previous growth factor signals (Stanoev et al. 2020). This metastable state enables the duration of EGFR phosphorylation to depend on previous stimulus history, effectively displaying signal-integration capabilities. This could provide a possible explanation of how cell fates can be biased depending on growth factor frequencies rather than growth factor identity alone. This has been observed for PC12 cells, in which a range of frequencies in EGF stimuli led to differentiation instead of proliferation (Ryu et al. 2015).

However, the EGFR-PTP sensing system exhibits limits in responsiveness to time-varying EGF signals due to the ubiquitination-dependent removal of ligand-bound receptors from the plasma membrane. This lowers the receptor's steady-state concentration upon each transient stimulus, effectively shifting the system toward the monostable regime. Consequently, the system loses its ability to robustly sense and respond to upcoming growth factor signals depending on dose and duration of EGF stimuli. However, low-affinity ligands such as epiregulin that exhibit a rapid dissociation from EGFR and generate a weakened dimerization interface lead to an increased number of monomeric receptors that recycle and thereby maintain the EGFR

concentration on the membrane. Therefore, the system is expected to tend to maintain its organization in the critical dynamical regime upon prolonged epiregulin stimulation, which could explain the more sustained EGFR phosphorylation at the plasma membrane when compared to saturating EGF stimulus, as observed experimentally (Freed et al. 2017).

4.5. Implications of Dynamical Modes of Operation for Oncogenic Signaling

In many pathological settings, EGFR signaling is altered due to EGFR mutations that are specific either to the extracellular or kinase domains (deletions/insertions or point mutations) as well as gene amplifications/overexpression (Pines et al. 2010). These mutations commonly enhance EGFR kinase activity, either by loss of suppressive regulatory extracellular domain functions or by altering the allosteric regulation of the kinase activity (Choi et al. 2007, Ekstrand et al. 1992, Ozer et al. 2010). Among the most common kinase domain mutations that account for aberrant EGFR activity in lung, pancreatic, and other cancers is the exon 21 missense mutation where arginine is substituted for leucine at position 834 in the activation loop (L834R) (Sharma et al. 2007). This mutation distally suppresses the local disorder of the N-lobe dimerization interface, thereby facilitating kinase domain dimerization (Shan et al. 2012). However, full-length EGFR L834R dimers are transient ($k_{off} \sim 0.15\ s^{-1}$) (Valley et al. 2015) and enzymatically more active than wild-type (WT) receptors (Yun et al. 2007, Zhang et al. 2006), giving rise to spontaneous EGFR phosphorylation. This suggests that the enhanced intracellular kinase domain dimerization can surpass the steric constraints imposed by the tethered extracellular domain. EGFR L834R phosphorylation can still be enhanced upon EGF stimulation, suggesting that its catalytic activity can still be regulated upon ligand binding (Chen et al. 2006).

Oncogenic mutations typically occur in one EGFR allele (Nomura et al. 2007), suggesting that EGFR phosphorylation in pathological settings is determined by interactions between mutant and WT receptors (Red Brewer et al. 2013). Further, the L834R mutant has been shown to preferentially adopt an allosteric acceptor position in the heterotypic interaction with WT receptors, leading to their hyperphosphorylation. The structural basis for increased aberrant L834R activity through suppression of the local intrinsic disorder of the αC-helix region has also been proposed for Y845 phosphorylation on WT receptors (Shan et al. 2012). The L834R mutant thus resembles the autocatalytically activated EGFR state, thereby being prone to induce autocatalytic phosphorylation amplification of WT receptors. Analogous to this situation, oncogenic EGFR overexpression also leads to its hyperphosphorylation (Arteaga & Engelman 2014).

From a dynamical point of view, in both cases the EGFR hyperphosphorylation results from a mutation-induced shift between the dynamical operational modes of the system. Given that EGFR phosphorylation dynamics is regulated by the spatially established EGFR-PTP network (**Figure 4*a***), receptor overexpression leads to an increase in EGFR density at the plasma membrane, effectively shifting the operational mode of the system to either the bistable or preactivated monostable regime. EGFR expression is, however, proportional to the maximal kinase activity in the system, suggesting that enhanced receptor kinase activity, as in the L834R/WT system, effectively results in an equivalent shift. A similar argument also applies to coexpression of oncogenic extracellular domain mutants with WT receptors. An oncogenic mutation-induced shift to the bistable mode of operation likely enables a highly aberrant EGFR phosphorylation state to be maintained once a growth factor stimulus is encountered. As outlined in the section titled The Effects of EGF Concentration on EGFR Trafficking Dynamics, EGFR trafficking—and thereby its phosphorylation response—depends on the concentration of EGF. Because the majority of receptors are internalized and degraded at consistently high EGF doses, kinase-mutant-driven oncogenic EGFR signaling is likely to be shut off under these conditions. However, at low

physiological EGF concentrations or, similarly, with transient binding of weak-affinity ligands, vesicular recycling keeps most EGFRs at the plasma membrane, thereby maintaining the system in the irreversible bistable regime. The resulting collectively enhanced kinase activity thereby underlies persistent signaling from the plasma membrane.

A persistent signaling from the plasma membrane can also result from an interaction of EGFR with another protein, which traps the dynamically maintained receptors on the plasma membrane, thereby effectively shifting the dynamical mode of operation of the EGFR-PTP network toward the bistable or preactivation regime. In this respect, it was previously shown that EGFR association with the oncoprotein erbB2 is sufficient to prolong and enhance the net phosphorylation of EGFR at the plasma membrane, independent of the kinase activity of erbB2 (Haslekås et al. 2005, Offterdinger & Bastiaens 2008).

5. CELLULAR CONTEXT-DEPENDENT PLASTICITY IN EGFR SIGNALING RESPONSES

The ability of cells to adapt to highly dynamic environments must extend beyond the processing capabilities discussed in the section titled Dynamical Modes Determine Phosphorylation Responses in ways that allow distinct cellular responses to be generated depending on the current environmental context. Toward this end is the observation that EGFR phosphorylation is blocked if breast, liver, or tongue squamous cell carcinoma cells are stimulated with hepatocyte growth factor (HGF) before stimulation with cognate EGFR ligands, thereby affecting cell migration (Gusenbauer et al. 2013).

A complex changing environment is generally present during wound healing, in which a switch from a migratory to a stationary mode of behavior coupled to cell proliferation is necessary to ensure efficient wound closure. In this setting, the receptors from the Eph family act as sensors of cell density, becoming activated at cell–cell contacts through interaction with membrane-bound ephrin ligands present on the surface of neighboring cells (Pasquale 2010). Subsaturating, physiological EGF stimulation promotes an increase in Akt-dependent EGFR recycling (Laketa et al. 2014, Stallaert et al. 2018), which maintains sensitivity to EGF and thereby a migratory mode of operation by sustaining receptors at the plasma membrane (**Figure 5*a***). However, cell contact–induced EphA2-receptor activity suppresses Akt activity and thereby traps both recycling and ligand-bound EGFR in EEs by not activating PIKfyve. This change in the steady-state spatial distribution of EGFR switches the cellular response from EGF-induced signaling from the plasma membrane, which promotes migration, to extracellular signal-regulated kinase (Erk) MAP kinase–dependent signaling from EEs, which promotes proliferation (Stallaert et al. 2018) (**Figure 5*b***). The cellular environment can thus generate context-dependent responses to EGF stimulation by modulating EGFR vesicular recycling.

6. SUMMARY AND OUTLOOK

To decipher how cells interpret EGF patterns, the description of intracellular signaling can be decoupled from the multicellular context by investigating information-processing capabilities within single cells in an experimentally controlled mimic of a changing environment. This can be accomplished using microspectroscopic imaging approaches to spatially resolve signaling reactions in live cells (Dehmelt & Bastiaens 2010) together with microfluidic devices that enable spatial–temporal control of growth factor patterns (Blum et al. 2019, Ryu et al. 2015). Such approaches have revealed how multiscale collective computations are established within single cells. On the nanometer scale, catalytic and autocatalytic EGFR phosphorylation reactions are

confined by clathrin-mediated diffusional traps on the plasma membrane, enabling both controlled signal propagation in the cytoplasm and responsiveness to changing extracellular signals through dynamic exchange of receptor monomers (Ibach et al. 2015, Rappoport & Simon 2009). The responsiveness is generated through a feedback coupling with spatially distributed PTPs by

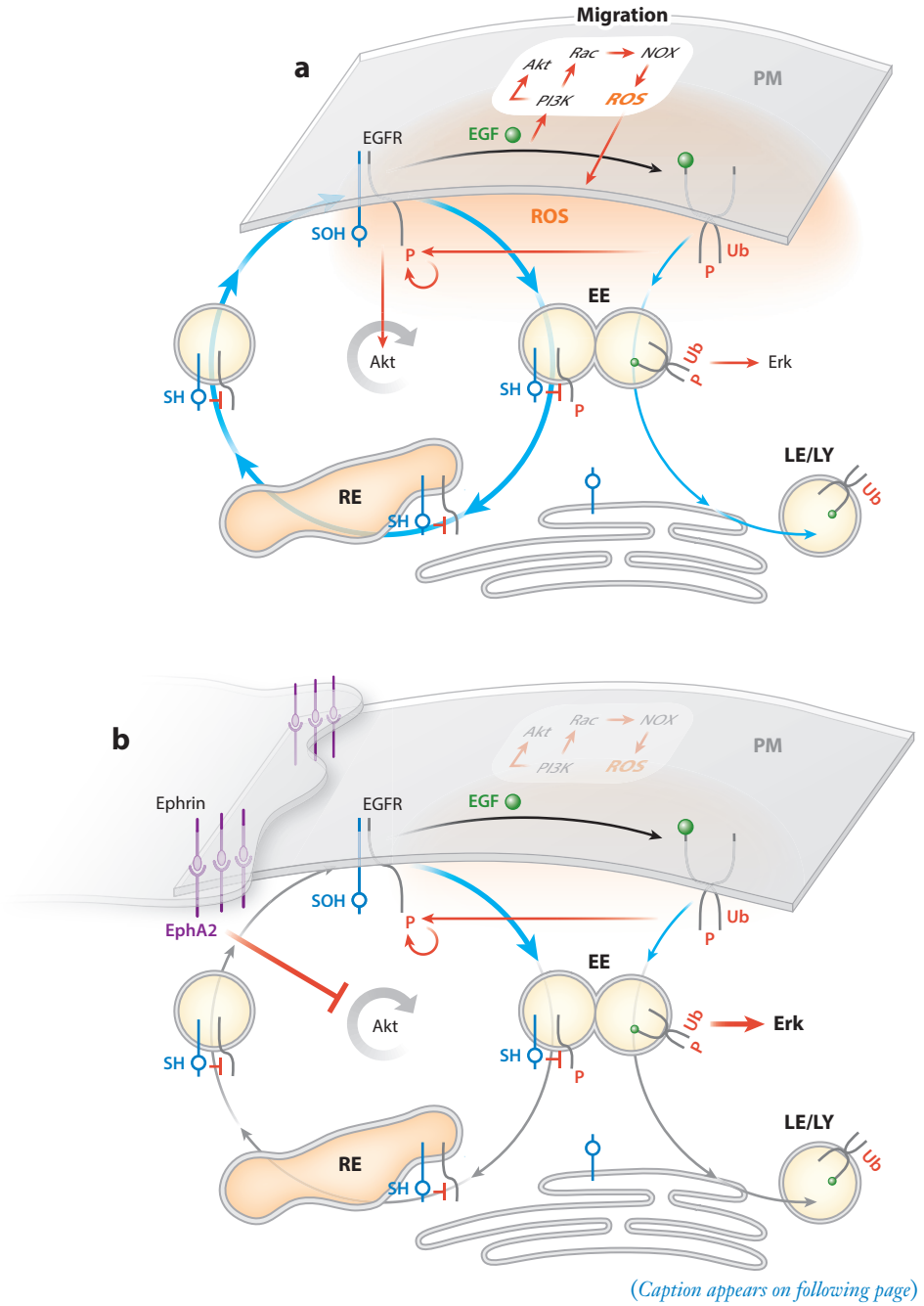

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Figure 5 (*Figure appears on preceding page*)

Context-dependent switching in EGFR signaling responses. (*a*) Low-level sustained EGF (*green spheres*) stimulation promotes EGFR-Akt-dependent EGFR monomer recycling (*blue arrows*), and autocatalytic phosphorylation amplification of monomers is sustained by a minority of EGFR dimers as long as EGF in the extracellular medium generates EGFR dimers from monomers (*black curved arrow*). The resulting high steady-state level of activated, phosphorylated EGFR at the plasma membrane activates the PI3K-mediated signaling networks (Akt, Rac) that produce ROS by NOX and promote the migratory state of cells (*white box* within *gray plasma membrane*). (*b*) Cell contact–induced ephrin-EphA2-receptor activity suppresses Akt kinase activity and thereby traps both nonubiquitinated recycling and ligand-bound, ubiquitinated EGFR in EEs, from where Erk signaling promotes proliferation. Abbreviations: Akt, protein kinase B; EE, early endosome; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; LE, late endosome; LY, lysosome; NOX, NAPDH oxidase; P, phosphorylated; PI3K, phosphoinositide-3-kinase; PM, plasma membrane; RE, recycling endosome; ROS, reactive oxygen species; SH, reduced cysteine thiol; SOH, sulfenic acid; Ub, ubiquitin.

vesicular recycling, thereby generating a higher-scale organization. This spatial cycling depends on growth factor–induced signaling from the receptors, enabling responsiveness to time-varying growth factor signals and context-dependent phenotypic outcomes to EGF (Stanoev et al. 2018). Due to interdependence across scales, the lower levels of organization manifest in the higher-level response dynamics. By measuring these response dynamics, genetic perturbations enable the identification of how signaling activities affect each other. Theoretical analysis of the dynamics of these networks as a function of experimentally accessible parameters can elucidate how EGFR growth factor sensing operates as well as predict how oncogenic EGFR mutations affect the responsiveness of the system.

Cells in developing or repairing tissues, however, both receive and emit information about the continuously changing environment through paracrine growth factor signaling. If the reception and the emission of information from growth factors are coupled, a unified system is generated in which the collective computation coordinates the phenotypic responses of the cells in the population. The higher-scale organization of the tissue thereby affects the intracellular signaling and vice versa. For example, EGF paracrine communication is demonstrably a determinant for the coordinated behavior of migrating cells during wound closure, where spatially distinct collective EGFR signaling responses arise within the tissue (Aoki et al. 2017, Hiratsuka et al. 2015).

The next step is therefore to understand how paracrine EGF communication enables heterogeneous identities to be generated and maintained in tissues (Koseska & Bastiaens 2017). For this, the same theoretical formalism described in the section titled From Spatial Cycles to Causality and Dynamical Mode of EGF Sensing can help identify under which conditions EGFR phosphorylation responses that differ from those of individual cells are possible. This can guide experimental efforts to understand how cell–cell communication via the EGFR-sensing network affects collective responses in developing tissues.

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