Chapter 18
Receptor Dynamics in Signaling

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Abstract Reliable inter- and intracellular communication is central to both the development and the integrity of multicellular organisms. Key mediators of these processes are cell surface receptors that perceive and convert extracellular cues to trigger intracellular signaling networks and ultimately a phenotypic response. Deregulation of signal transduction leads to a variety of diseases, and aberrations in receptor proteins are very common in various cancer types. Therefore, cell surface receptors have been established as major targets in drug discovery. However, in order to efficiently apply therapeutics, it is crucial to gain knowledge about design principles of receptor signaling. In this chapter, we will discuss signal transduction at the receptor level for examples from different receptor classes.

1 Introduction

Tightly regulated cellular communication is key not only to the development of multicellular organisms but also to the functional integrity of tissues, organs,

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I.I. Goryanin and A.B. Goryachev (eds.), *Advances in Systems Biology*,
Advances in Experimental Medicine and Biology 736,
DOI 10.1007/978-1-4419-7210-1_18,

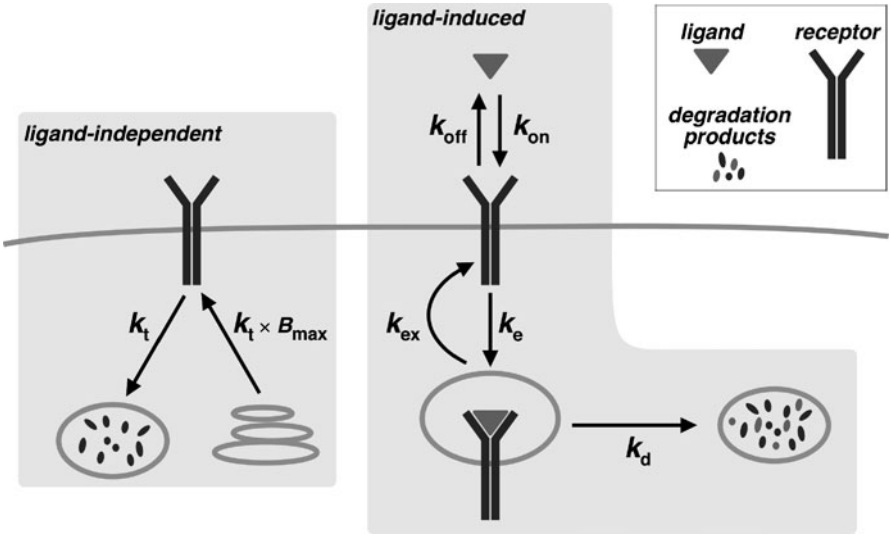

Fig. 18.1 Generalized scheme of ligand and receptor interaction and trafficking processes

and the whole body. There are a plethora of mediators involved in cell-to-cell communications such as small molecules, peptides, cytokines, growth factors, lipid hormones, and physical signals. These molecules bind to specific cell surface receptors, which initiate signal transmission by linking extracellular cues to intracellular cascades of signaling molecules. Integration of different signal transduction networks via crosstalk of intersecting pathways processes the information and finally leads to appropriate phenotypic responses of the cell such as proliferation, differentiation, migration, survival, or apoptosis.

Aberrations in signaling cascades are linked to various disease types including cancer, infections, as well as immunological and metabolic disorders. In the advent of targeted therapeutics, cell surface receptors have become prime objectives in drug discovery [1], and various antibodies impeding ligand binding or small molecule inhibitors interfering with the enzymatic activity of receptor proteins undergo development or are already used in cancer therapy.

However, to efficiently apply targeted therapeutics, it is crucial to understand the complex regulation of the underlying biochemical networks [2–4]. Therefore, the identification of design principles for cell surface receptor signaling holds great promise in furthering rational drug discovery and personalized therapy strategies. Mathematical models have been established to aid the understanding of how ligand–receptor interaction and trafficking shape receptor activation kinetics [5–8]. In a generalized scheme (Fig. 18.1), ligand undergoes binding to receptor proteins with distinct association (k_{on}) and dissociation (k_{off}) rates. Trafficking of receptors can be both ligand-independent and ligand-induced. Receptor transport to the plasma membrane ($k_t \times B_{max}$) can be described by ligand-independent

endocytosis (k_t) and the receptor abundance in the absence of ligand (B_{max}), i.e. at steady state. Endocytosis of ligand–receptor complexes (k_e) can either be followed by recycling (k_{ex}) or by degradation processes (k_d). This generalized model varies with the receptor system under study, and additional processes might be taken into account such as ligand-induced mobilization of newly synthesized receptor from intracellular pools to the plasma membrane.

In this review, we will discuss information processing at the receptor level, exemplified by the erythropoietin receptor (EpoR), the interleukin 3 receptor (IL3R), the epidermal growth factor receptor (EGFR), and the receptor for transforming growth factor beta (TGFβ).

2 Cytokine Receptors

Cytokine receptors are involved in diverse physiological processes such as the development of the hematopoietic system or in pro- as well as anti-inflammatory cellular responses [9, 10]. Members of the cytokine receptor family are single membrane-spanning proteins that lack intrinsic enzymatic activity and, therefore, associate with cytoplasmic Janus kinases (JAK). Mutations that constitutively activate cytokine receptors have been described for a variety of hematological disorders, and they are found either in receptor proteins such as the EpoR [11, 12], the granulocyte colony-stimulating factor (GCSF) receptor [13], and the thrombopoietin receptor [14], or in receptor-associated kinases such as JAK2 [15] and JAK1 [16].

2.1 Erythropoietin Receptor

Erythropoietin (Epo) signaling [17] is crucial for the survival, proliferation, and differentiation of erythroid progenitors at the colony-forming unit-erythroid (CFU-E) stage [18]. Crystallographic studies revealed that the EpoR is expressed as a preformed homodimer [19]. The majority of receptor protein resides in intracellular compartments of the endoplasmic reticulum and the Golgi apparatus as shown for both endogenous EpoR in CFU-E cells as well as exogenous EpoR expression in various cell lines [20–24].

Endocytosis and subsequent degradation of ligand–receptor complexes have been proposed to downregulate EpoR activity [25]. Using a kinetic model, ligand-induced endocytosis could be identified as a mechanism to clear Epo from the extracellular space, and differences in clearance rates between Epo derivatives were assigned to distinct ligand binding rates [26].

By combining time-resolved quantitative data for ligand-independent and ligand-induced endocytosis with ordinary differential equation-based modeling, design principles of EpoR signaling could be further refined [8]. Whereas ligand-induced endocytosis plays a major role in shaping early-response kinetics of EpoR phosphorylation, ligand-independent EpoR turnover at the plasma membrane is crucial

for a linear conversion of extracellular Epo levels into receptor activation. Both computational and experimental evidence showed that intracellular EpoR pools constitute a reservoir for a continuous replenishment of cell surface receptor, a process that is key to linear information processing. While peak levels of EpoR and JAK2 phosphorylation are saturated at higher ligand concentrations, the duration and thereby the integral of signaling activity of these proteins is increased under such conditions.

This principle of dose-to-duration signaling has been analyzed as a means to decode ligand levels beyond saturation and subsequently shown for pheromone signaling in yeast at the level of mitogen-activated protein kinases [27]. In light of this, it will be interesting to examine if the linear relation between extracellular ligand concentration and activation of signaling molecules might be abrogated downstream of the EpoR. Such an observation could indicate at which level EpoR-mediated signaling interacts with other signaling networks through pathway crosstalk, thereby allowing for integration and interpretation of the cellular signaling status.

2.2 Interleukin 3 Receptor

In contrast to the EpoR, the IL3R consists of a cytokine-specific alpha chain and the common beta chain, which is shared with cytokine receptors for IL5 and the granulocyte–macrophage colony-stimulating factor (GM-CSF) [28].

Studying the characteristics of IL3R activation showed that, comparable to the EpoR system, IL3 is rapidly depleted from the medium within the early phase of stimulation [8]. A second key feature shared by the EpoR and the IL3R is the restimulation capacity of both the receptor and the receptor-associated JAK2, demonstrating that cells remain ligand-responsive (Fig. 18.2). However, treatment of cells with IL3 resulted in a massive degradation of the common beta chain and JAK2 (Fig. 18.2b). This observation indicates that in contrast to the EpoR, the majority of IL3R resides at the plasma membrane where it is accessible for ligand binding. Another key difference between these receptor systems is the IL3-induced increase of beta chain expression, which may compensate for dramatic receptor degradation after ligand engagement and prevent a refractory state of the cell.

In summary, the EpoR and the IL3R reveal comparable characteristics of signaling at the receptor level, i.e. (1) rapid clearance of ligand from the medium and (2) receptor recovery at the plasma membrane. However, both receptor systems evolved distinct strategies to accomplish this systems behavior, either employing a constant rapid ligand-independent turnover of the EpoR or a massive ligand-stimulated synthesis of the IL3R (Fig. 18.2). Rapid uptake of ligand from the medium by ligand-induced endocytosis has been discussed to facilitate temporal fidelity of receptor signaling [29, 30]. Thus, the combination of rapid ligand depletion with fast cell surface recovery of the EpoR or the IL3R enables the cell to stay in a ligand-responsive state and at the same time promotes a high temporal resolution of extracellular signaling cues.

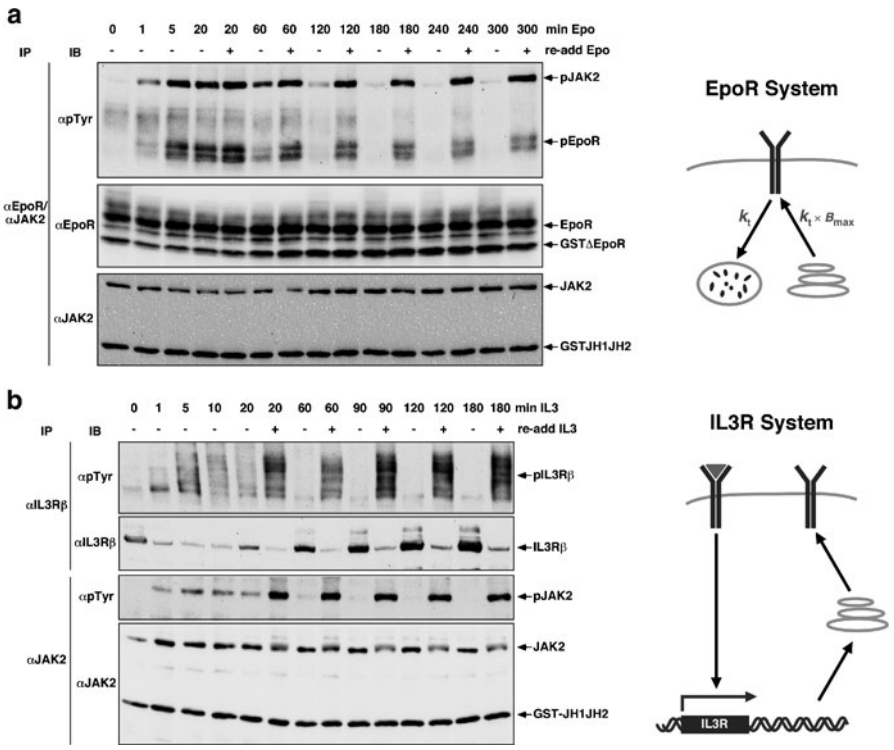

Fig. 18.2 Comparison of overall systems behavior and strategies employed in (**a**) the EpoR and (**b**) the IL3R system. Immunoblot analysis shows that both receptor systems stay in a ligand-responsive state as judged by receptor and JAK2 phosphorylation after re-addition of ligand. (**b**) Left panel adapted from [8]

3 Epidermal Growth Factor Receptor

Members of the receptor tyrosine kinase (RTK) family are single-pass transmembrane proteins that regulate multiple cellular processes such as proliferation, differentiation, migration, angiogenesis, and metabolism [31]. Conversely, deregulation of RTK signaling pathways has been assigned to various human cancers as well as non-malignant diseases [32, 33]. After completion of the Humane Genome Project, 58 RTKs have been identified [34] including ErbB receptors, vascular endothelial growth factor receptor, and c-Met. The EGFR (ErbB1, Her1) is a member of the ErbB receptor family and as the prototypical RTK probably the best-studied receptor, also from a systems point of view [6]. EGFR signaling regulates proliferation and survival in a variety of epithelial cell types, and deregulated signaling through the EGFR is associated with numerous solid tumors [35].

Biochemical studies showed that the EGFR is rapidly internalized from the plasma membrane upon epidermal growth factor (EGF) stimulation and subsequently degraded in the lysosomal compartment. This downregulation is proposed to contribute to signal attenuation [36, 37]. However, this observation is context-dependent since stimulation of the EGFR with transforming growth factor α (TGFα) results in receptor recycling rather than in downregulation [38] due to a higher pH sensitivity of ligand–receptor binding [39]. Differential binding and trafficking of EGF and TGFα have been shown to result in distinct mitogenic potency of EGFR signaling [40]. This knowledge has also been employed to engineer a more effective variant of EGF [41], and a similar study has been carried out for the cytokine GCSF [42]. Distinct receptor trafficking or binding properties also account for the altered biology of IL2 [43] and Epo [26] derivatives, respectively.

Comparing the regulatory role of endocytosis in EGFR and EpoR signaling shows that the contribution of endocytic downregulation D, i.e. the ratio of ligand-induced (k_e) to ligand-independent (k_t) receptor endocytosis, is approximately threefold higher for EGF-stimulated EGFR ($D = 7.5$) [30] than for the EpoR system ($D = 2.3$) [8]. This is due to both a lower rate of ligand-independent endocytosis and a higher rate for ligand-induced endocytosis of the EGFR compared to the EpoR. Whereas EGF mediated a substantial decrease in half-life and total expression of its receptor [44], neither higher levels of Epo nor prolonged exposure to ligand resulted in a change of total EpoR expression [8]. Thus, ligand-mediated loss of receptor protein at the plasma membrane is much more likely to play a role in attenuation of EGF-stimulated EGFR signaling [36, 37] compared to EpoR signaling. In addition, the ratio of ligand-induced endocytosis k_e to ligand–receptor dissociation k_{off} is considerably higher for Epo–EpoR compared to EGF–EGFR complexes [30]. This, in combination with a rapid constitutive receptor turnover, allows the EpoR system to reach a high temporal resolution of sampling extracellular cues, while, at the same time, staying in a ligand-responsive state.

4 Transforming Growth Factor β Receptor

In contrast to cytokine receptors and the EGFR, the TGFβ receptor belongs to the serine/threonine kinase receptor family. Binding of TGFβ ligand induces cooperative complex formation of two receptor subunits, the TGFβ type I and type II receptors. The type II receptor is a constitutively active serine/threonine kinase that, upon ligand binding, activates the dormant TGFβ type I receptor. The type I receptor in turn phosphorylates serine residues of receptor-associated SMAD2 and SMAD3 transcription factors [45]. TGFβ is mainly involved in the development as well as homeostasis of tissues. Although TGFβ signaling is typically thought of mediating anti-proliferative cues and, therefore, being a tumor suppressor, it can fuel tumor progression at later stages by stimulation of tumor angiogenesis and metastasis [46].

Signaling through SMAD transcription factors is promoted by clathrin-mediated endocytosis, whereas endocytosis via caveolae mediates receptor turnover [47, 48].

A recent study suggested that caveolae are also involved to differentially trigger the mitogen-activated protein kinase cascade [49]. Thus, receptor trafficking possesses the capacity to induce distinct biological responses, thereby establishing an additional layer of regulation to TGFβ signal transduction. Mathematical analysis of the TGFβ pathway showed that the connection of receptor activation and trafficking processes allows for sensing absolute and temporal changes in ligand concentrations, regulating signal duration, and controlling cellular responses upon stimulation with multiple ligands [7]. Another study suggested that the ratio of clathrin- and caveolae-mediated endocytosis controls transient versus sustained responses [50]. Similar to the TGFβ receptor, signaling from endosomes has also been proposed for signaling downstream of RTKs as well as G-protein coupled receptors (GPCR) as a mechanism to facilitate temporal and spatial regulation [51].

5 Concluding Remarks

The examples discussed in this review show that various strategies have evolved to shape signal initiation at the receptor level by ligand–receptor interaction and trafficking kinetics. The physiological impact of distinct trafficking routes and signaling endosomes is still not fully explored as illustrated by controversial results for caveolae-mediated EGFR internalization [52, 53]. Deciphering these processes might give rise to an even more complicated picture of how receptor dynamics set the stage for selective regulation of downstream signaling. However, despite these distinct strategies, a unifying regulator of signal transduction at the receptor level appears to be the ratio of ligand-independent and ligand-induced endocytosis and subsequent receptor degradation [7, 8, 30].

Different from homodimeric EpoR, many cytokine receptors are composed of heterotypic subunits. Besides the IL3R that shares its common beta chain with receptors for IL5 and GM-CSF, another subset of cytokine receptors including receptors for IL2, IL4, IL7, IL9, IL13, IL15, and IL21 have a common gamma chain, whereas receptors for e.g. IL6, IL11, or LIF engage the gp130 subunit [28]. This gives rise to potential competition between different receptors for their common chain and additionally, these receptors often signal through the same JAK–STAT cascade. Moreover, induced feedback regulators, for instance members of the suppressor of cytokine signaling (SOCS) family, can affect multiple cytokine receptors either directly or indirectly at the level of JAKs or downstream pathway components. Thus, there are numerous layers of cross-regulation in cytokine signaling as exemplified by studies of IL7 signaling [54]. These phenomena create the necessity to generate complex data and mathematical models, studying the effects of multiple cytokine stimuli or of a specific stimulus on the activity of various cytokine receptors.

Crosstalk also plays a crucial role for EGFR signaling in cancer. The EGFR does not only form hetero-oligomeric structures with other members of the ErbB receptor family, but it is also suggested to directly interact with c-Met [55, 56] and to exhibit

transactivation with c-Met [57] and GPCRs [58] at multiple levels. Such interactions are relevant for both drug resistance and cancer progression.

Although studies of cell lines exposed to single stimuli give rise to important insights, it will be crucial to expand the analysis of cell signaling towards more physiological conditions of multi-factor stimulation for understanding in vivo signaling through cell surface receptors. This also holds true for the repertoire of stimulation schemes: bolus stimulation is a rather non-physiological, yet practical means to examine signal transduction in cell lines. However, the investigation of autocrine or paracrine signaling in the cellular microenvironment or the administration of a constant stimulus at physiological concentrations promises to advance the field of signaling research. Here, technical developments such as microfluidics [59, 60] in combination with mathematical modeling may greatly impact the success of such endeavors and finally refine strategies for drug discovery [3].

Acknowledgments This work was supported by the Helmholtz Alliance on Systems Biology (SBCancer) (VB, JT, UK), the German Federal Ministry of Education and Research (BMBF)-funded MedSys-Network LungSys (JT, UK), and the Excellence Initiative of the German Federal and State Governments (EXC 294) (JT).

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