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Grb2, a Double-Edged Sword of Receptor Tyrosine Kinase Signaling

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Abstract

Receptor tyrosine kinases (RTKs) exhibit basal tyrosine phosphorylation and activity in the absence of ligand stimulation, which has been attributed to the “leaky” nature of tyrosine kinase autoinhibition and stochastic collisions of receptors in the membrane bilayer. This basal phosphorylation does not produce a signal of sufficient amplitude and intensity to manifest in a biological response and hence is considered to be a passive, futile process that does not have any biological function. This paradigm has now been challenged by a study showing that the basal phosphorylation of RTKs is a physiologically relevant process that is actively inhibited by the intracellular adaptor protein growth factor receptor-bound 2 (Grb2) and serves to “prime” receptors for a rapid response to ligand stimulation. Grb2 is conventionally known for playing positive roles in RTK signaling. The discovery of a negative regulatory role for Grb2 reveals that this adaptor acts as a double-edged sword in the regulation of RTK signaling.

Receptor tyrosine kinases (RTKs) play pleiotropic roles in the biology of metazoans by providing basic communication systems between the extracellular milieu and intracellular signaling pathways. Upon ligand binding, the ectodomains of RTKs dimerize, enabling cytoplasmic kinase domains to transphosphorylate each other on tyrosine residues (1–3). Phosphorylation of tyrosines in the activation loop (A-loop) increases the intrinsic kinase activity, which then mediates secondary phosphorylation events on tyrosines in the juxtamembrane region, kinase insert, and C-terminal tail (4, 5). These phosphorylated tyrosines and their surrounding sequences (i.e., pTyr-ψ-X-ψ and N-P-X-pTyr) provide docking sites for substrates containing Src homology 2 (SH2) or phosphotyrosine binding domains, respectively. This recruitment facilitates substrate phosphorylation, triggering distinct signaling pathways (6, 7).

How ligand-induced dimerization of ectodomains leads to the activation of cytoplasmic tyrosine kinase domains is one of the unresolved mysteries in RTK biology. The two most discussed models include a diffusion-based model and a preformed dimer model. According to the diffusion-based model (Fig. 1A), receptors float freely as monomers in the membrane lipid bilayer and are dimerized upon ligand binding, which places the cytoplasmic kinase domains in sufficient proximity for transphosphorylation to occur (8). The preformed dimer model posits that the receptors are already predimerized in the absence of ligand; however, the orientation of the cytoplasmic kinase domains is not permissive for transphosphorylation (Fig. 1B) (9). Ligand binding induces a conformational change in the receptor ectodomains, which in turn reorients the kinase domains to enable transphosphorylation.

In contrast to the diffusion-based model, the lateral diffusion in the membrane is not a rate-limiting factor for preformed dimers, and thus allows for an immediate response to ligand

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stimulation and also reduces the ligand concentration necessary for receptor activation. The preformed dimer model carries a greater risk of accidental ligand-independent transphosphorylation of the kinase domains, necessitating exquisite autoinhibitory mechanisms for RTK regulation. Indeed, the intrinsic kinase activity is kept in check through multiple defense mechanisms, including the occlusion of the sites for adenosine triphosphate or substrate binding (10, 11) and an autoinhibitory network of hydrogen bonds at the kinase hinge region referred to as the molecular break (12). The autoinhibition is not 100% bullet-proof, however, and random collisions of the unliganded receptors in either model result in a basal “noise” receptor tyrosine phosphorylation and activity (13). Under physiological conditions, this basal phosphorylation is too weak to manifest in a sustained and robust signal that is necessary to give rise to a cellular response. Hence, basal phosphorylation is considered merely a feeble process that reflects the “leakiness” of kinase autoinhibition and random collisions. Harmonious with this model, overcrowding of RTKs by gene amplification or mutation-induced loss of kinase autoinhibition, or both, increases the basal phosphorylation of receptors, leading to uncontrolled RTK signaling in many human diseases (12, 14). Different RTKs exhibit different amounts of background phosphorylation in the absence of ligand, which most likely reflects the differences in the effectiveness of modes of kinase autoinhibition employed by different RTKs.

Lin *et al.* propose an interesting model whereby the background RTK phosphorylation is a regulated process and plays a physiological role in RTK signaling by “priming” the kinase domains for rapid activation upon ligand binding to the receptor (15). Specifically, they demonstrate that homodimeric Grb2, an adaptor protein consisting of an SH2 domain flanked by N- and C-terminal SH3 domains, binds through its C-SH3 to a proline-rich sequence at the extreme C-terminal tail of two fibroblast growth factor receptor 2 (FGFR2) molecules. This intracellular Grb2-mediated receptor dimerization results in basal transphosphorylation of the kinase domains of FGFR2 on the A-loop tyrosines (Fig. 1C). The authors propose that the geometry of the Grb2-induced FGFR2 dimer is not permissive for secondary tyrosine phosphorylation events that link receptor activation to downstream signaling pathways and hence that the A-loop phosphorylated (activated) kinase domain FGFR2 is incapable of signaling. The authors further suggest that Grb2-mediated dimerization of the cytoplasmic domain of FGFR2 cooperates with the ectodomain in stabilizing preformed inactive receptor dimers.

By using an *in vitro* kinase assay, they show that Grb2 enhances autophosphorylation activity of FGFR2. Furthermore, fluorescence resonance energy transfer (FRET) and cell-based experiments demonstrate that the Grb2 concentration-dependent receptor dimerization and the accompanying basal phosphorylation obey a Gaussian curve peaking at a 1:1 Grb2:FGFR2 ratio. Excess Grb2 attenuates dimerization and basal phosphorylation of receptor because each Grb2 dimer would bind to a single FGFR2 molecule. Isothermal titration calorimetry (ITC) data suggest a stepwise mode of binding: Dimeric Grb2 first binds a single receptor with higher affinity (0.1 μ M) and then a second receptor with lower affinity (25 μ M).

The authors suggest that the inhibitory effect of Grb2 ceases upon dual phosphorylation of two tyrosines, one in the FGFR2 C-tail and one in the C-terminal SH3 domain of Grb2, both of which map to the presumptive Grb2-FGFR2 interface. The dual phosphorylation of these residues is proposed to cause an electrostatic repulsion, forcing Grb2 to dislodge from the receptor. The liberated receptors are then free to transphosphorylate each other on tyrosines in the C-tail and juxtamembrane regions to recruit downstream signaling substrates, including phospholipase C- γ , the adaptor CrkL, and the docking/scaffolding adaptor FRS2 α , and activate multiple signaling pathways (Fig. 1D).

Consistent with the proposed negative role for Grb2, an FGFR2 isoform lacking its C-tail displays transforming potential, although this also could be due to the fact that loss of the C-tail inhibits receptor internalization (16). The distal C-terminal polypro-line sequence on FGFR2 that mediates Grb2 binding is not conserved in FGFR1, FGFR3, or FGFR4, although these members of the FGFR family have proline-rich sequences further upstream in their C-tails that could serve as binding sites for SH3 domain of Grb2 (17). It remains to be seen whether Grb2-mediated control of background phosphorylation of FGFR2 also applies to other members of the FGFR subfamily and other RTKs. Equally intriguing would be to discover whether differences in basal phosphorylation of different RTKs correlate with the differences in the affinity of RTKs for Grb2.

Grb2, initially discovered as the missing link between the epidermal growth factor receptor (EGFR) and the Ras–mitogenactivated protein kinase (MAPK) pathway, is required for signaling by nearly all RTKs (18–20). Grb2 uses its N-terminal SH3 domain to associate constitutively with the guanine nucleotide exchange factor (GEF) Son of Sevenless (Sos) and becomes recruited to phosphorylated RTKs through its SH2 domain (21, 22). This event translocates Sos to the vicinity of its substrate, Ras, allowing Sos to catalyze the exchange of guanine diphosphate (GDP) to guanine triphosphate (GTP) (22, 23). GTP hydrolysis by Ras then initiates activation of kinase Raf and the downstream kinase cascade (24). FGFRs lack the canonical Grb2 SH2 domain-binding motif and thus are incapable of recruiting the Grb2-Sos complex directly to the activated receptor. Instead, FGFRs phosphorylate FRS2 α and β , which are myristoylated membrane-anchored docking proteins, and these phosphorylated proteins serve as the platform for the recruitment of the Grb2-Sos complex to the receptor complex (25, 26). In addition to translocating Sos to the membrane, Grb2 also positively regulates FGFR signaling by binding to the phosphatase Shp2, which then dephosphorylates Sprouty proteins, key intracellular inhibitors of FGF signaling (27). Lastly, Grb2 associates with Gab1, another adaptor protein, which participates in the phosphoinositide 3-kinase (PI3K)–Akt pathway (28) (Fig. 1D).

Grb2 preferentially uses its N-terminal SH3 domain to bind the polyproline sequence in Sos (29–31). Analysis of the crystal structure of the Grb2 dimer shows an unobstructed N-terminal SH3 domain amenable for binding (32). These observations indicate that, in the Grb2:FGFR2 heterotetramer, the N-terminal SH3 should be free to bind Sos and Gab1, risking the possibility of translocation of Sos or Gab1 to the membrane and activating the Ras-MAPK and PI3-Akt pathways in a ligand-independent fashion (33). Hence, the proposed model (Fig. 1C) begs the question of how the negative and positive roles of Grb2 are balanced such that the integrity of the signaling pathways is not compromised. It remains to be seen whether the positive and negative roles of Grb2 are mediated by distinct intracellular pools.

The proposed model adds another interesting dimension to our understanding of the role of adaptor proteins in RTK signaling. Grb2-mediated dimerization of the cytoplasmic domains of RTK could influence the organization of the receptor ectodomain, potentially affecting ligand binding. Indeed, a study by McKeehan and co-workers (34) suggested that the intracellular orientation of the cytoplasmic domains in FGFR2 affects binding of FGF7 ligands to receptor ectodomain. This inside-out “like” regulatory mechanism resonates with previously described inside-out signaling systems, most notably between talin and integrin (35) and between Ephrin and Eph (36). Grb2-mediated regulation of FGFR basal phosphorylation opens a new window of opportunity for design of a novel class of modulators of FGF signaling. Specific inhibitors of dimeric Grb2 binding to FGFR could be used to promote FGF signaling in tissue repair and wound healing, whereas agents that augment Grb2-FGFR binding could find use for inhibition of FGFR signaling in FGFR-driven cancers.

The model proposed by the author is in tune with the current notion that dimerization per se is not sufficient to activate RTKs, but rather precise orientation of the receptors within the dimer is also critically important. Although it was previously thought that RTK conformation is exclusively regulated by homotypic interactions between receptors and by ligand binding, the identification of Grb2 as a previously unknown regulator of cytosol-mediated receptor dimerization adds an additional layer of complexity in the regulation of RTK signal transduction. Future research should focus on answering the central question of how ligand-induced conformational changes in the receptor ectodomain are parsed into precise reorientation of the cytoplasmic kinase domain, enabling productive receptor transphosphorylation and activation.

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Fig. 1.

Models of basal receptor phosphorylation and ligand-mediated activation. **(A)** Diffusion-based model. Receptors float laterally within the plasma membrane, making occasional collisions that result in basal phosphorylation and activation of the receptor. This stochastic process is limited by the diffusion rate constant of the receptor. **(B)** Preformed dimer model. Receptor ectodomains interact homotypically, resulting in preformed dimers. However, the orientation of these dimers is not conducive to transphosphorylation of kinase domains. Diffusion is not a rate-limiting factor. **(C)** Intracellular Grb2-mediated receptor dimerization model. Receptors are held in close proximity by a Grb2 dimer. The C-terminal SH3 domains of Grb2 bind the C-terminal tail P-X-X-P motif of the receptor. The close proximity of the kinase domains allows for increased basal phosphorylation on the A-loop, but downstream signaling is not permitted because of steric hindrances imposed by Grb2 on phosphorylation of recruitment-site tyrosines. **(D)** Ligand-induced receptor activation. Ligand binding enables the receptor to adopt the proper orientation required for effective receptor transphosphorylation of tyrosine residues. This transphosphorylation links the receptor to activation of specific downstream pathways, eliciting a robust signaling response and unique cellular outcomes. Distinct pools of Grb2 relay the signal from the activated receptors to different proteins and secondary messengers.