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MET meet adaptors: Functional and structural implications in downstream signalling mediated by the Met receptor

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Abstract

The tyrosin kinase Met receptor regulates multiple cellular events, ranging from cell motility and angiogenesis to morphological differentiation and tissue regeneration. To conduce these activities, the cytoplasmic C-terminal region of this receptor acts as a docking site for multiple protein substrates, including Grb2, Gab1, STAT3, Shc, SHIP-1 and Src. These substrates are characterised by the presence of multiple domains, including the PH, PTB, SH2 and SH3 domains, which directly interact with the multisubstrate C-terminal region of Met. How this receptor recognises and binds a specific substrate in a space-temporal mode is a central question in cell signalling. The recently solved crystal structure of the tyrosine kinase domain of the Met receptor and that of domains of diverse Met substrates provides the molecular framework to understand Met substrate specificity. This structural information also gives new insights on the plasticity of Met signalling and the implications of Met deregulation in tumorigenic processes. In the light of these advances, the present work discusses the molecular basis of Met-substrate recognition and its functional implications in signalling events mediated by this pleiotropic receptor. (Mol Cell Biochem **276:** 149–157, 2005)

Key words: cell signalling, docking proteins, Gab1, Grb2, Met binding domain, Met receptor, Met substrates, STAT3, tyrosine kinase

Introduction

The activation of the Met receptor by its natural ligand, the hepatocyte growth factor (HGF), stimulates a wide variety of cellular responses including angiogenesis [1], cellular motility [2], growth [3], invasion [4], morphological differentiation [5], embryological development [6, 7], tissue regeneration [8], and wound healing [9].

Met activation leads to autophosphorylation of multiple tyrosine residues located in its cytoplasmic domain, specifically Y1151, Y1345 and Y1356. A binding site for multiple substrates is located adjacent to these newly formed phosphotyrosines. Met substrates are multidomain proteins characterised by the presence of specific domains, including the Src homology 2 (SH2), the phosphotyrosine binding (PTB) and the Src homology 3 (SH3) domain. Some of the substrates that directly interact with Met include the growth factor receptor-bound protein (Grb) 2 [10], STAT3 [11], the p85 subunit of phosphatidylinositol 3-kinase (PI3K) [12], Shc [13], phospholipase C-γ (PLCγ) [14], c-Src [10], and Gab1 [14]. Genetic and biochemical studies on Grb2, Shc and Gab1, which are commonly referred as docking proteins [15], have boosted our knowledge of the mechanisms by which growth factor and cytokine receptors transmit signals to downstream effectors in order to regulate cell growth and differentiation. Until recently, the molecular basis of Met-substrate recognition remained obscure. The present work is devoted to the analysis of the structural and functional basis of the direct Met-substrate interactions, the implications of these interactions in Met-mediated signalling events and

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the state of art of biomedical investigations on Met regulation/deregulation.

Multiple proteins directly interact with the Met multisubstrate region

Diverse proteins including Gab1, Grb2, Shc, Src, SHIP-1, SHIP-2, PI3K, PLCγ1 and STAT3, act as substrates when they meet and interact with the Met receptor. They specifically interact with the C-terminal flexible region of Met, which is located downstream the kinase domain. The domain organization of these Met substrates is shown in Fig. 1. The biological role of these proteins, including their participation in Met-mediated signalling events, is described as follows.

Gab1

Gab (Grb2-associated binder) proteins are members of the insulin receptor substrate 1 (IRS-1) family of proteins, which also includes Daughter of sevenless (DOS), Fibroblastic growth factor receptor substrate 2 (FRS2), IRS-1, IRS-2, IRS-3, p62 Downstream of tyrosine kinase (Dok) family, Dok-related (Dok-R) and Linker for activation of T cells (LAT) [16]. Three mammalian gab genes have been identified to date: gab1, gab2 and gab3 [17]. Each member of the Gab protein family presents multiple functional motifs that mediate interactions with several signalling molecules. Only Gab1 directly interacts with the Met receptor [18]. In fact, Gab1 was firstly identified as a Met-receptor interacting protein and as the major tyrosine-phosphorylated protein in cells transformed by Tpr-Met, an oncogenic, constitutively active version of Met [19]. Gab1-deficient mice have a phenotype reminiscent of mice presenting mutations in the HGF and Met genes because they display a reduced liver size and have defects in the migration of muscle precursor cells [20].

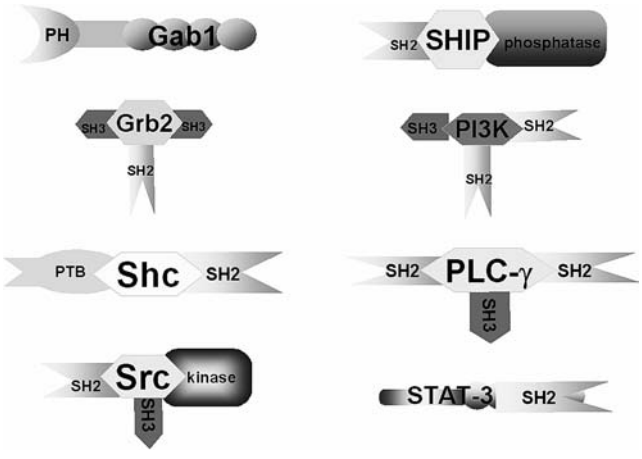

Fig. 1. Structural organisation of Gab1, SHIP, Grb2, PI3K, Shc, PLCγ1, Src and STAT3, all of which bind to the C-terminal multisubstrate region of Met. As shown here, these protein substrates exhibit multiple domains, which mediate specific protein–protein interactions with multiple partners, including this tyrosine kinase receptor.

Gab1 can also couple with PI3K, phospholipase Cγ1, the tyrosine phosphatase SHIP-2 and the adapter protein Crk and recruit them to the Met receptor [21]. The multiple roles of Gab1 in cell signalling can be better appreciated considering that the association of Gab1 with SHIP-2 is essential for the formation of branched tubules, as seen in cultured MDCK epithelial cells [22], while the association of Gab1 with PI3K seems to be important for the prevention of apoptosis [23].

In contrast to the role of Gab1 in EGF and Met signalling, the mechanism(s) of recruitment of Gab1 to other receptor tyrosine kinases including T-cell and B-cell antigen, insulin, TrkA, cytokine and erythropoietin receptors are not well understood. The association of Gab1 with the EGF receptor requires two tyrosine residues in the receptor that act as Grb2-binding sites [24], suggesting that the recruitment of Gab1 via the Grb2 adapter protein may be a common mechanism for the indirect recruitment of Gab1 to receptor tyrosine kinases. Thus, the distinct biological functions of the EGF and Met receptors might be due, at least in part, to the different mechanisms of Gab1 recruitment.

Gab1 is a protein that contains several domains. The PH domain [25] is the most conserved among IRS-1 family members, including Gab1. The PH domain of Gab1, which is located in the amino terminus region, specifically binds to PI3K and mediates Gab1 translocation to the plasma membrane in response to EGF stimulation [26]. Although the PH domain of Gab1 is not involved in Met binding, mutants of Gab1 lacking the entire PH domain, or point mutations at a conserved phospholipid-binding site (i.e. W26A/C, R29A/C) are unable to induce Met-dependent branching tubulogenesis in Madin-Darby canine kidney cells [26], evidencing that this domain is not only responsible for Gab1 membrane localization but also plays a role in Met regulation.

Gab1 does not contain a canonical PTB domain, but its Met binding domain (MBD) may function similarly to a PTB domain in targeting Gab1 to various receptors, such as EGFR and Ret [27]. The MBD site of Gab1 is distinct from the Grb2-binding sites [22]. A 16-amino acid motif within the Gab1 MBD, encompassing the residues (GMQVPPPAHMGFR), is sufficient for the interaction of this protein with the Met receptor. Extracellular signal-regulated kinase (ERK1/2) also binds to the c-Met-binding domain of growth factor receptor-bound protein 2 (Grb2)-associated binder-1 (Gab1). This suggests that Gab1-ERK1/2 binding and nuclear translocation play a crucial role in Egr-1 nuclear accumulation [28]. The fact that Gab1 MBD also binds PI3K, Grb2, Erk1 and Erk2 [21–23, 29, 30] neatly illustrates the complexity of Met regulation.

Another important aspect that requires further investigation is to determine if Gab1 can be directly recruited to other receptor(s) and whether or not this is relevant for Met-mediated signalling.

Grb2

Grb2 is a small adapter protein comprised of an SH2 domain flanked by two SH3 domains and so, is able to bind through its SH3 domains to class II proline-rich ligands containing the sequence PXXPXR [31, 32]. Grb2 binding to guanine-nucleotide-releasing factor hSos1 established a connection between receptor tyrosine kinases and Ras signalling [33]. Interestingly, a single mutation, Y1356VNV to Y1356VHV, of the Grb2 consensus binding site of Met, reduced its interaction with Gab1 [34]. The Met receptor contains only one Grb2 SH2 domain binding site at Y1356 [35], whereas the EGF receptor and the adapter protein, Shc, which is recruited to and phosphorylated by the Met receptor, both contain two Grb2 SH2 domain binding sites [36, 37]. Thus, it is possible that the formation of a (Grb2-SH3)2-Gab1 complex may promote an association of the Grb2 SH2 domains with the twin Grb2-binding sites on the EGFR or Shc. A similar complex might be formed during the interaction of Grb2-Shc with SHIP. In contrast, it has been shown that a TPR-MET mutant that selectively fails to associate with full length Grb2, yet retains the ability to associate with phospholipase Cδ, phosphatidylinositol 3P-kinase, and SHIP-2 [38]. Because the association of Tpr-Met with Grb2 is essential for efficient transformation of fibroblasts by Tpr-Met, the identification of signalling pathways downstream of Grb2 will contribute to the elucidation of the mechanism by which Tpr-Met transforms fibroblasts.

Shc

Shc is a protein participating in mitogenesis that presents several binding domains, including the phosphotyrosine binding (PTB) and SH2 domains. Indeed, the PTB domain was first identified in the Shc docker protein as a sequence that enabled binding to phosphotyrosine (pY) sites on activated receptor kinases (reviewed in [39, 40]). The NMR structure of the Shc PTB domain complexed to a pY-containing peptide from the nerve growth factor receptor TrkA [41] revealed that the fold of the PTB domain is distinct from that of the SH2 domain. Indeed, as shown in Fig. 2, the fold of the PH domain resembles more the tertiary structure of the pleckstrin homology (PH) domain. This means that SH2 and PTB domains show a different disposition of the amino acids surrounding the pY residue. PTB domains recognize pY in the context N-P-X-pY, whereas the SH2 domains generally recognize the +1 to +4 positions immediately C-terminal to the pY. Besides, the PTB domain transduces migratory signals [42] while the SH2 domain is required for mitogenesis. This "decision-making" ability of Shc proteins may involve the juxtaposition of PTB and SH2 domains and the differential use of them under different conditions. If meaningful biological outputs depend on specific PTB-ligand interactions or if a particular PTB domain or ligand site is involved in multiple interactions, is a fundamental aspect that remains to be elucidated.

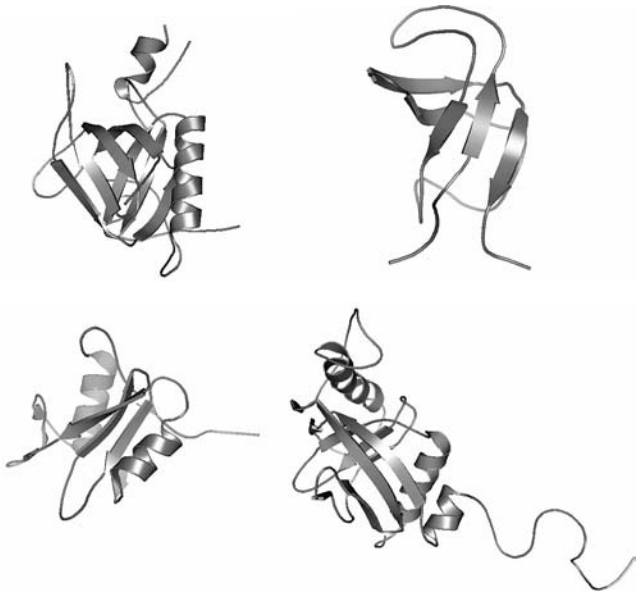

Fig. 2. (A) PH domain of Bruton's kinase (PDB, 1B55); (B) SH3 domain of the p85 subunit of PI3K (PDB, 1PNJ); (C) Three-dimensional structure of the SH2 domain of Grb2 (PDB, 1QG1); (D) PTB domain of Shc (PDB, 1SHC).

Src

Src is a member of a family of non-receptor tyrosine kinases defined by a common modular structure. The amino-terminus of all Src family members are myristoylated, a modification determined by the first seven amino acids of the molecule that causes association with the plasma membrane [43]. Downstream of the myristoylation sequence lies a short sequence (50–80 amino acid residues) that varies highly among the various family members and therefore, is referred as a unique domain. Src family members also contain SH3 and SH2 domains [44]. These domains contribute to the tight regulation of the Src kinase via intramolecular interactions [45]. Thus, disruption of these interactions may be a major mechanism for Src activation. Moreover, the interactions between the SH2 and SH3 domains with other signalling molecules might provide additional (perhaps redundant) levels of control of Src activity [46, 47].

Mutations of certain residues within the Src kinase domain result in inactive kinase forms, while mutation of residues located within the activation loop only affect the Src enzymatic activity [48]. Near the end of the kinase domain it is located a conserved tyrosine residue, Y527, which is critical for Src regulation. Interestingly, in some signalling networks such as that of integrin, Src appears to resemble an adaptor molecule that may function by recruiting or activating other tyrosine kinases [49]. Consequently, Src may play different roles in the context of different signalling systems.

SHIP-1 and SHIP-2

SHIP-1 (SH2-containing inositol 5-phosphatase) and SHIP-2 are SH2- and zinc finger-containing proteins [50]. SHIP-1 was originally identified as a signalling molecule in cytokine-stimulated hematopoietic cells such as macrophage colony-stimulating factor-stimulated cells [51, 52]. SHIP-1 and -2 are binding partners of Met [53]. SHIP-1 binds to one of the tyrosine residues at the Met multiple substrate binding site, pY1356VNV, which is also the binding site for Grb2. Interestingly, the YVNV motif is identical to the common binding site of Shc to SHIP and Grb2, while the point mutation of N1358 into histidine abolishes the branching potential of Met [54]. SHIP-1 is associated with PI3K before HGF stimulation and dissociates from PI3K immediately after HGF stimulation. This indicates that SHIP-1 selectively regulates PI3K and PLCγ1 and suggests that this interaction determines the astrocyte-specific response to HGF. The rapid dissociation of SHIP-1 from PI3K after HGF stimulation contrasts with previous observations showing that their association is promoted after stimulation with various cytokines or growth factors [55]. SHIP-1 does not bind other tyrosine kinase receptors such as c-Kit, TrkA, or the insulin receptor. In contrast, PI3K binds to c-Fms, c-Kit, and the insulin receptor, the sequences of which contain the typical consensus motif, pYxxM, for the PI3K binding site.

Overexpression of SHIP-1 drastically enhances the tubulogenesis potency of Met without altering the Met-mediated cell scattering and proliferation. However, cells overexpressing a mutant SHIP-1 lacking catalytic activity, fail to form branching tubules in the presence of HGF, suggesting that the effects of SHIP-1 overexpression to accelerate tubulogenesis are due to the enhanced phosphoinositide phosphatase activity.

SHIP-1 and -2 may also participate in the STAT pathway since it interacts with the protein inhibitor of activated STAT1, PIAS1 [56]. As discussed below, another member of the STAT family, STAT3, is an important substrate for Met-mediated branching tubulogenesis [11].

The elucidation of the role of SHIP-2 in Met signalling began few years ago. To date, it has been established that SHIP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the Met receptor [22, 26] and that this phosphatase plays a crucial role in the HGF/SF-induced cell scattering through the regulation of two distinct small G proteins, Ras and Rho [57]. Because the epidermal growth factor (EGF) receptor, which is closely related to Met, forms a ternary complex with SHIP-2 and Grb2 [58], it is tempting to speculate that novel aspects of the role of SHIP-2 in Met signalling will be unveiled in the coming years.

PI3K and PLCγ1

The SH2 domain of the phosphatidylinositol 3-kinase (PI3K) regulatory subunit binds Gab1 in a phosphorylation-independent manner and mediates the association of Gab1 and receptor protein-tyrosine kinases including the Met, insulin, EGF, and NGF receptors. Thus, the PI3K regulatory subunit can act as an adaptor protein via a phosphotyrosyl-independent SH2 interaction, allowing Gab1 to serve as a substrate for several tyrosine kinases.

Mutants of the Met receptor responsible for hereditary papillary renal carcinoma enhanced PI3K/PKB activation [59]. The enhanced PI3K signalling has been associated with protection of transformed cells from apoptosis. Moreover, this enhancement indicates that PI3K plays an essential role in cell survival and transformation induced by oncogenic receptor variants or receptor amplification.

In astrocytes, Met tyrosine phosphorylation selectively activates PI3K but not PLCγ1. The latter seems to be essential for the HGF-induced cell migration. In contrast with this activation effect of PI3K in astrocytes, activation of both PI3K and PLCγ1 occurs in most of the cell types [10, 60]. Thus, the selective activation of PI3K seems to determine the cellular response of astrocytes to HGF, suggesting that, depending on the cell type, multiple mechanisms may participate in the selective activation of PLCγ1 and PI3K.

STAT3

Although there are functional differences among members of the STAT protein family, the structure determination of the cores of STAT1 and STAT3 and the NH2 terminus of STAT4, in addition to sequence comparisons and deletion mutagenesis, revealed that STAT proteins share common structural features [61]. Among the various domains of STAT proteins, some of particular relevance are: 1) the central DNA-binding domain, which determines DNA sequence specificity of individual STATs [61]; 2) the so-called TAD domain, which is also located at the C-terminal end of the molecule, is involved in communication with transcription complexes [62];

3) the critical tyrosine residue that is required for SH2-phosphotyrosine interaction and therefore, STAT activation, is located near the SH2 domain; 4) the coiled-coil domain, which provides potential contacts for transcription factors and other regulatory proteins [63]. In addition to these domains, oligomerisation also plays a role in STAT regulation [64]. For example, it was early established that the tetramerisation of STAT proteins contributes to stabilise its DNA-binding activity on weak promoters [65].

The signal transducers and activators of transcription (STAT) factors function as downstream effectors of cytokine and growth factor receptor signalling [66]. In contrast with normal cells and tissues, constitutive activation of STATs has been detected in a wide variety of human cancer cell lines and primary tumours. Concerning its interaction with the Met receptor, it is worth to mention the characteristic association of STAT3 with Met kinase mutations. The Met ($p + 1$ loop) $\rightarrow$ Thr point mutation of Met (also commonly referred as the Met 2B mutation), leads to the formation of tumours with high metastatic potential, including oral squamous cell carcinoma (OSCC) [67]. This dramatic event is apparently the result of the constitutive phosphorylation of STAT3 by Met [68].

The kinase domain of Met presents unique features

The crystal structure of the kinase domain of Met demonstrates that this protein presents the typical bilobal architecture observed in many other kinases [69–71] (see Fig. 3A). Met kinase also exhibits a high sequence identity with the kinase domains of insulin receptor (IR) kinase and fibroblast growth factor (FGF) receptor kinase (i.e., 44% and 41%, respectively). However, the crystal structure of Met kinase shows the following particular characteristics: 1) a functionally relevant salt bridge formed between residues Lys-1110 and Glu-1127 seen in IR kinase is not observed in the two crystal structures of the Met kinase reported to date; 2) adjacent to the core Met kinase domain, residues 1060–1069 form a 3_{10} helix. This 3_{10} helix is absent in IR kinase and FGF receptor kinase; 3) the orientation of the Met C-terminal α-helix, as in this protein, such α-helix seems to be part of the structural elements involved in kinase activation. A similar orientation of the C-terminal α-helix of FGF receptor kinase and IR kinase-3P, for example, would lead to steric clashes with the N terminal region of the activation loop; 4) the activation loop of Met, downstream the Phe-1223, adopts a β-turn conformation (i.e., residues 1223–1226), while residues 1222–1230 assume a conformation that does not allow proper catalytic positioning of Glu-1127. Indeed, in the apo-Met structure, residues 1231–1244 of the activation loop are not defined in the electron density map, indicating that they are disordered. In contrast, the crystal structure of Met kinase complexed with the inhibitor K-252a shows that the entire activation loop adopts an ordered structure.

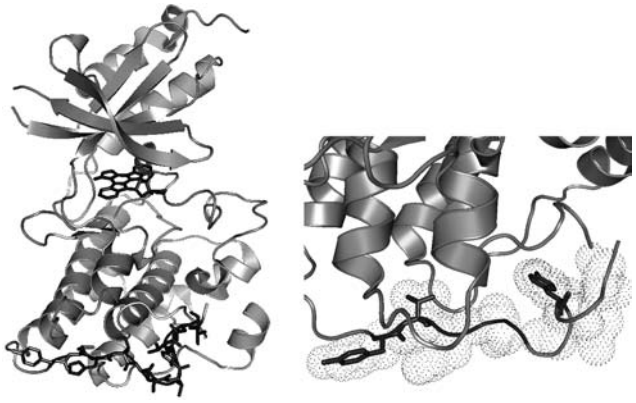

Fig. 3. (A) Three-dimensional structure of the kinase domain of the human Met receptor. The kinase inhibitor K-252a and the residues of the multisubstrate binding region are shown in stick representation. (B) Zoom-in of the multisubstrate binding region showing the Y1256 and 1249 residues, which are essential for substrate binding, in stick representation.

Interestingly, another Met kinase inhibitor, SU11274, differentially affects the kinase activity and subsequent signalling of various mutant forms of Met [72]. Two Met variants, M1268T and H1112Y, are potently inhibited by SU11274 at low concentration (i.e. 2 μM). Inhibition of the Met kinase activity altered cell proliferation, morphology and motility. Cells containing resistant mutants appeared unaffected by the compound. The basis for the sensitivity or resistance to SU11274 in terms of the position of these mutations has been recently discussed [72]. Of course, additional mechanisms participate in the regulation of the activity of the Met kinase, such as alternative splicing of the Met juxtamembrane domain, which in turns affects the pattern of tyrosine phosphorylation [73, 74].

The structural basis of Met substrate specificity

It has been early recognised that Y1234 and Y1235 of native Met kinase are phosphorylated after receptor activation. The crystal structure of Met kinase suggests that phosphorylation of Y1194 might also contribute to the establishment of the active conformation by contacting the pivot point of the C-terminal α-helix. The crystal structure of Met kinase also indicates the establishment of an extended conformation in the region 1349–1352, followed by two β-turns that encompass the residues 1353–1356 and 1356–1359, respectively (Fig. 3B). The extended conformation of the region 1349–1352 is similar to that of phosphopeptides bound to canonical SH2 domains [75, 76]. Indeed, the extended phosphopeptide

bound to the SH2 domain of the kinase Lck superimposes to residues 1349–1352 of Met in such a way that binding of the SH2 domain of Lck overlaps with parts of the C-terminal lobe of Met kinase, suggesting that during Met activation, the SH2-interacting residues reorient toward the surface of the kinase domain. Residues 1353NATY resemble the consensus sequence for Shc-PTB domain-binding peptides (NPXY). The NMR structure of the PTB domain of a Shc-peptide complex showed the NPXpY motif at the C-terminal region of a β-strand forms a type I β-turn structure [41]. Interestingly, the superposition of this β-turn onto the C^{α} positions of Met 1353–1356 shows a remarkable conformational similarity between both β-turns. Also in this case, docking of the PTB domain with the C-terminal lobe of Met kinase would lead to steric clashes.

In contrast, Y1356 and the three residues that follow it form a type II β-turn. This is very different to what is observed in phosphopeptides that bind to Grb2: a type I β-turn conformation, which is related to type II β-turns by a 180° flip of the central peptide unit. The comparison of the conformation of residues 1356–1359 from the crystal structure of Met kinase with that of a corresponding phosphopeptide bound to Grb2 SH2, shows that the conformational differences involve the residues Y1356, V1357 and N1358 [76]. However, a rotamer conformational change would move the N1358 side chain into the position adopted in the Grb2-complex. This manner, the SH2 domain of Grb2 can be docked to Met by superimposition of residues 1356–1359. Because this rotation does not result in significant steric clashes with the C-lobe of Met kinase, it seems that the conformations of these three motifs are close to those recognized by their binding partners. If this is the case, then the conformation of the C-terminal region observed in the crystal structures should be similar to that of active, full-length Met.

The crystal structure of the kinase domain of Met illustrates the conformational flexibility of tyrosine kinases. It also reveals that the conformation of the N-terminal residues of the activation loop contribute to the binding of the inhibitor K-252a (Fig. 3A). Very importantly, this region might be a suitable target for specific inhibitors. Additionally, the structural information of the kinase domain of Met allows the description of the position and conformation of the C-terminal region of Met in its unphosphorylated form, which in turn makes possible to suggest a mechanism of the interaction of this region with the SH2 domain of Grb2.

Of the major relevance for Met substrate specificity, is the fact that the multifunctional docking site of Met binds to only one signalling molecule [77]. When several effectors meet Met simultaneously, the following hierarchical binding Grb2 > Gab1 > SHIP-1 > PI3K has been observed *in vitro*.

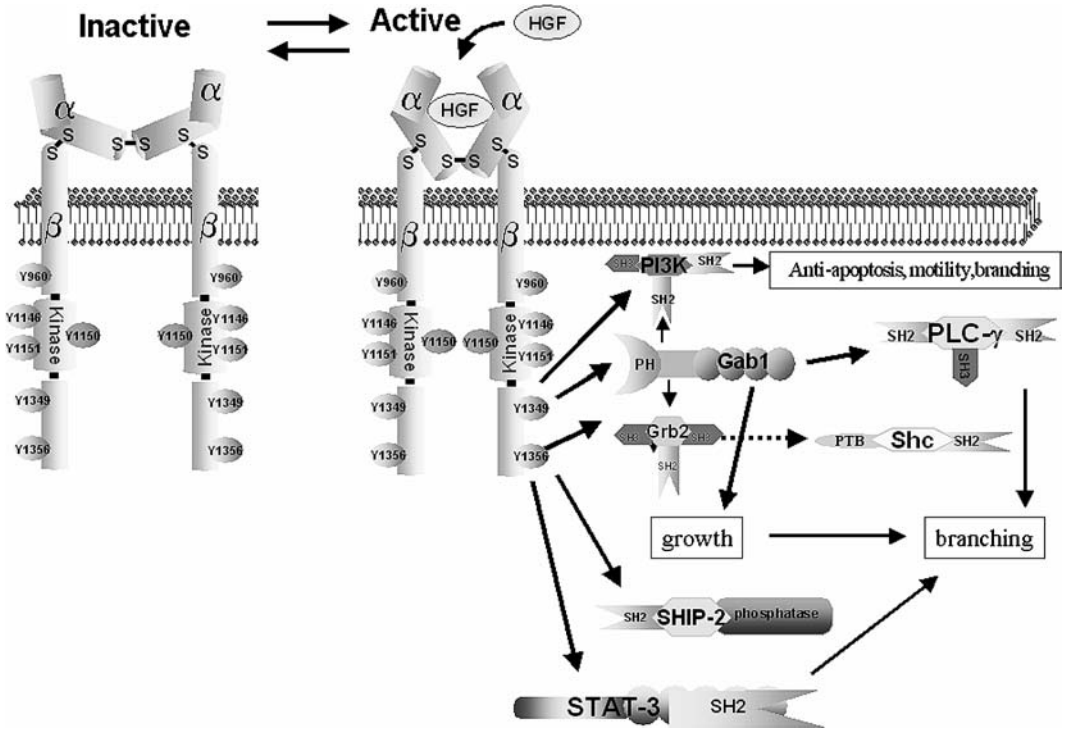

Fig. 4. Biological implications of the direct interaction between the Met receptor and diverse multidomain protein substrates. As shown here, these substrates meet Met and bind to its multidocking C-terminal region, which is located downstream the catalytic kinase domain.

Although Grb2 clearly binds to Met preferentially over PI3K and Gab1, SHIP-1 does not compete with the Met-Gab1 interaction. However, Grb2-independent recruitment of Gab1 requires the C-terminal lobe and structural integrity of the Met receptor kinase domain [78].

Therefore, the binding order of these effectors might be crucial for the multiple, Met-mediated signal transduction events sketched in Fig. 4. Moreover, the *in vivo* binding of full-length molecules may also be dependent upon their allosteric interactions or the formation of multiple higher-order complexes to activate their receptors. Furthermore, novel putative intracellular TPR/Met-substrates, including SNAPIN, DCOHM, VAV-1, Sorting nexin 2, Death associated protein kinase 3, SMC-1, Centromeric protein C, and hTID-1, have been recently identified using mouse embryo cDNA library screenings [79]. The specificity of the interaction of these proteins with TPR-Met has been validated *in vitro* and *in vivo* [79]. These novel TPR-Met substrates provide new insights on Met signalling, including the participation of this receptor in apoptosis-regulating mechanisms (through its interaction with DAPK-3) and in the control of the cell cycle (via its binding to CENPC and SMC-1). They also evidence the complexity and diversity of Met signalling.

Conclusion

As described in this work, the multidocking site of the Met receptor interacts with a number of different ligands. The multifunctional docking site of Met provides binding sites for several protein substrates including PI3K, Grb2, Gab1, Src, Shc and SHIP-1. The fact that PI3K and Gab1 bind to phosphotyrosines pY1349 and pY1356, whereas SHIP-1 and Grb2 bind only at phosphotyrosine pY1356 illustrates the delicacy of the structural basis of Met substrate specificity.

It is likely that future work will uncover additional ligands and/or docking proteins that may participate in multi-input combinatorial switching systems mediated by tyrosine kinases. I anticipate that the structural characterization of those novel Met substrates will be needed to further understand the mechanisms mediating Met recognition. Moreover, whether or not the conformation of the Met C-terminal region in its nonphosphorylated form, as seen in the crystal structures available to date, accurately reflects the mechanisms involved in substrate recognition, will also require the structural characterization of complexes of Met with at least some of the docking proteins described here.

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