Multiple Regions of Internalin B Contribute to Its Ability to Turn on the Ras-Mitogen-activated Protein Kinase Pathway*

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Internalin B (InlB) is a protein present on the surface of Listeria monocytogenes that mediates bacterial entry into mammalian cells. It is thought that InlB acts by binding directly to the hepatocyte growth factor (HGF) receptor, present on the surface of host cells. Binding of InlB to the HGF receptor results in mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase activation, followed by changes in the organization of the actin cytoskeleton. Here we have compared signaling by HGF and InlB. Whereas stimulation with equivalent concentrations of HGF and InlB elicits similar activation of the HGF receptor, we observed striking differences in downstream activation of MAP kinase. InlB leads to a greater activation of the Ras-MAP kinase pathway than does HGF. The leucine-rich repeat region, which was previously shown to be sufficient for binding and activation of the HGF receptor, lacks the ability to super-activate the Ras-MAP kinase pathway. Analysis of a series of deletion mutants suggests that it is the B repeat region between the leucine-rich repeat and GW domains that endows InlB with an increased ability to turn on the Ras-MAP kinase pathway. These unexpected observations suggest that HGF and InlB use alternative mechanisms to turn on cellular signaling pathways.

The hepatocyte growth factor (HGF) receptor is a protein-tyrosine kinase that is expressed predominantly on the surface of epithelial cells. Its natural ligand, HGF or scatter factor, is expressed in fibroblast cells (1). In vitro, HGF and the HGF receptor play key roles in the development of the liver, kidneys, muscle, and neuronal precursors (2). In vitro activation of the HGF receptor stimulates cell division, cell scattering, and the formation of tubular structures in a three-dimensional matrix (3). Whereas normal HGF receptor signaling plays a crucial role in embryonic development, abnormal HGF receptor signaling has been implicated in both tumor development and metastasis (4).

Activation of the HGF receptor leads to autophosphorylation on several tyrosine residues. Tyrosine phosphorylation sites regulate kinase activity and act as binding sites for cellular signaling proteins. The activated HGF receptor interacts either directly or indirectly with several signaling molecules including Grb2, Gab1, Shc, phosphoinositide 3 (PI 3)-kinase, Crk, CrkL, and Cbl (5–11). Gab1 is a docking protein that provides the receptor with binding sites for a variety of other proteins (12). Grb2 is a cytoplasmic adaptor protein that is involved in linking Gab1 to the HGF receptor (13). Grb2 also works together with Sos to link activated receptor protein-tyrosine kinases to Ras activation (14, 15). Shc is a docking protein that is thought to collaborate with Grb2 upstream of Ras (16–19). PI 3-kinase is a lipid kinase that is composed of an 85-kDa regulatory subunit, which contains two SH2 domains and one SH3 domain, and a 110-kDa catalytic subunit (20–22). Activation of PI 3-kinase affects cytoskeletal dynamics and cell survival. Crk and CrkL are related adaptor proteins that are also involved in regulating cytoskeletal changes (23). Cbl is a ubiquitin ligase that regulates receptor internalization and degradation (24).

The Listeria monocytogenes surface-protein internalin B (InlB) binds to and activates the HGF receptor. Initiation of host cell invasion by this intracellular pathogen occurs through the interaction of InlB with the HGF receptor, leading to host cell-protein-tyrosine phosphorylation and cytoskeletal rearrangements. It has been established that the activation of both the Ras-MAP kinase pathway and PI 3-kinase are essential for uptake of L. monocytogenes into host cells (25, 26). InlB is composed of an amino-terminal receptor-binding domain followed by a B repeat region (B) and three GW domains. The receptor-binding domain contains three motifs: an amino-terminal cap, a leucine-rich repeat (LRR) segment, and an immunoglobulin-like region (IR) (27, 28). The amino-terminal cap and the LRR domain have been shown to be sufficient to bind and activate the HGF receptor (29). The GW domains attach InlB non-covalently to the bacterial cell wall and have been shown recently to bind to host cell components upon release of InlB from the bacterium (30, 31).

Both HGF and InlB bind and activate the HGF receptor. Interestingly, whereas HGF stimulates mitogenesis, cell migration, and tubulogenesis, InlB induces phagocytosis. This prompted us to compare signaling downstream of these two HGF receptor ligands. Our results show that HGF and InlB are very similar in their ability to activate the HGF receptor, whereas InlB is a stronger activator of the Ras-MAP kinase pathway. We found that InlB induces tyrosine phosphorylation of a 90-kDa PI 3-kinase-associated protein, whereas HGF did not. Analysis of InlB deletion mutants showed that, whereas both the IR and B repeat are necessary for maximal signaling...
downstream of the HGF receptor, it is the B repeat region of InlB that is important for the observed super-activation of the Ras-MAP kinase pathway.

EXPERIMENTAL PROCEDURES

Cells Lines, Antibodies, and Other Reagents—Vero cells were grown in minimal essential medium with Earle’s salts containing 10% fetal bovine serum. Monoclonal antibodies 4G10 against phosphotyrosine, anti-HGF receptor DO-24, anti-HGF receptor DL-21, anti-phospho-Raf-1 (Ser-338) and a polyclonal serum against p85 were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). A polyclonal serum against Raf-1 (E-10) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antibodies against phospho- and phospho-MAP kinase and phospho-MEK 1/2, as well as a monoclonal antibody against phospho-Erk1/2, were obtained from Cell Signaling Technology (Beverly, MA). A monoclonal antibody against Ras was obtained from Transduction Laboratories (Lexington, KY). The MEK1 inhibitor PD98059 was purchased from New England Biolabs (Beverly, MA). HGF was obtained from Sigma.

Cloning, Expression, and Purification of InlB and InlB Derivatives—The LRR domain of InlB was expressed and purified as described previously (32). Intact InlB was expressed and purified as described previously (31). InlBAGGW1 (residues 36–392 and 464–630), InlBABGW1 (residues 36–321 and 464–630), and InlBIR-B-GW1 (residues 36–248 and 464–630) were cloned into pet28b (Novagen) by PCR from the intact InlB expression plasmid using standard protocols. InlBAGGW1, InlBAB-GW1 and InlBIR-B-GW1 were purified using a protocol identical to that used for intact InlB. Protein concentrations were determined using the absorption at 280 nm and the calculated extinction coefficients.

GST Fusion Proteins—The GST-ScH SH2, GST-ScH PTB, GST-Grb2 SH2, and GST-p85 N-SH2 domain fusion proteins have been described previously (33–36). Fusion proteins were isolated as described (37).

Immunoprecipitation—Cells were starved overnight in minimal essential medium with Earle’s salts containing 20 mM Hapes, pH 7.4. Cells were then stimulated for 1 min at 37 °C with HGF, wild-type, or mutant InlB at a final concentration of 1.5 μM unless otherwise noted. Cells were then rinsed 2 times with cold phosphate-buffered saline and lysed in 1 ml of 50 mM Hapes, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EDTA, 100 mM NaF, 10 mM sodium pyrophosphate, 500 μM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 50 μg/ml apronin, and 10 μg/ml leupeptin (PLC-lysis buffer) per 10-cm tissue culture dish. Lysates were cleared by centrifugation at 10,000 rpm in a microcentrifuge at 4 °C, incubated with 5 μl of polyclonal antiserum or 1 μl of monoclonal antibody for 1 h on ice and subsequently for 1 h with 100 μl of 10% protein A-Sepharose or anti-mouse IgG-Sepharose for 1 h at 4 °C on an agitator. Sepharose beads were collected by centrifugation and washed 4 times with PLC-lysis buffer. Immunoprecipitates were boiled 3 min in 62.5 mM Tris/Cl, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, 5 μl dithiothreitol, 2.3% SDS, and 0.025% bromophenol blue (SDS-sample buffer) and resolved by SDS-PAGE.

For in vitro binding studies, cell lysates were incubated for 1 h at 4 °C on a rocker with 10 μl of glutathione-agarose beads containing 1–2 μg of fusion protein. Beads were washed 4 times with PLC-lysis buffer, and bound proteins were analyzed by SDS-PAGE and immunoblotting.

Immunoblotting—Proteins were transferred to polyvinylidene difluoride membranes using a Bio-Rad semi-dry blotting apparatus at 50 mA per gel for 60 min at room temperature. For each individual antibody, immunoblotting was performed per the manufacturer’s protocol. Reactive proteins were visualized by ECL (Amersham Biosciences). Immunoblotting was performed per the manufacturer's protocol.

Ras Activation Assay—GST-RBD was expressed in Escherichia coli and purified on glutathione-agarose beads. The beads were washed several times in Tris-buffered saline containing 1% Triton X-100 (TBST) and stored at 4 °C in TBST containing 0.02% azide. For affinity precipitation, lysates were incubated with GST-RBD pre-bound to glutathione-agarose (~10 μl of beads containing ~10–20 μg of protein) for 1 h at 4 °C on an agitator. Bound proteins were resolved by SDS-PAGE on a 12% acrylamide gel and subjected to Western blotting with an anti-Ras monoclonal antibody.

RESULTS

HGF and InlB Differ in Their Ability to Induce Tyrosine Phosphorylation of Cellular Proteins—It has been reported that InlB binds to and activates the HGF receptor (29). This suggests that InlB and HGF activate the same signal transduction pathways. To confirm this, we compared protein tyrosine phosphorylation following stimulation with either InlB or HGF. Vero cells, which naturally express the HGF receptor, were grown to confluence, starved overnight in serum-free medium, and stimulated for 1 min at 37 °C with 1.5 nM HGF or 1.5 nM InlB. Whole cell lysates were analyzed by anti-Tyr(P) immunoblotting (A). Anti-HGF receptor immunoprecipitates (IP) of control (lane 1) and HGF- (lane 2) or InlB (lane 3)-stimulated Vero cells were analyzed by anti-Tyr(P) (B) and anti-HGF receptor (C) immunoblotting. The results show that there are several proteins that become tyrosine-phosphorylated upon stimulation with either HGF or InlB (Fig. 1A). Most proteins, including the HGF receptor, were phosphorylated to similar levels in response to either HGF or InlB (Fig. 1A). Interestingly, one protein, with an apparent molecular mass of ~40 kDa (Fig. 1A, p40), was more highly tyrosine-phosphorylated in response to InlB than in response to HGF. These results suggest that HGF and InlB differ in their ability to turn on cellular signaling cascades.

HGF and InlB Have Different Abilities to Activate the Ras-MAP Kinase Pathway—To identify p40, we focused on members of the MAP kinase family because several of its members, including p38MAPK, Erk-1 (p44MAPK), and Erk-2 (p42MAPK), have been implicated in signal transduction downstream of the HGF receptor (38).

To investigate whether p40 is either Erk-1 or Erk-2, cells were stimulated with HGF or InlB, and whole cell lysates were...
analyzed by immunoblotting with an antibody that recognizes the phosphorylated, active forms of Erk-1 and Erk-2 (Fig. 2A). An anti-Erk-2 blot was included as a control (Fig. 2B). Both Erk-1 and Erk2 are activated in response to either HGF or InlB. InlB appears to be a stronger activator of Erk-1 and Erk-2 than HGF (Fig. 2, A and C). Stimulation in the presence of PD98059, a potent inhibitor of MEK, blocks activation of Erk-1 and Erk-2 (Fig. 2A). Anti-Tyr(P) immunoblotting of whole cell lysates from cells stimulated with InlB or HGF shows that p40 is no longer phosphorylated when cells are stimulated in the presence of PD98059 (Fig. 2C). These results indicate that p40 represents Erk-2.

It is possible that differences between HGF and InlB in their ability to activate Erk-1 and Erk-2 are restricted to a specific ligand concentration. To investigate this, Vero cells were stimulated with a range of HGF or InlB concentrations (Fig. 3). Anti-HGF receptor immunoprecipitates were analyzed by anti-Tyr(P) and anti-HGF receptor immunoblotting, whereas whole cell lysates were analyzed for the presence of activated Erk-1 and Erk-2. The results show that receptor phosphorylation levels off between 0.75 and 1.5 nM HGF or InlB (Fig. 3A) and confirm that InlB is a better activator of Erk-1 and Erk-2 than HGF at all concentrations tested.

Erk-1 and Erk-2 are activated following a cascade of events known as the Ras-MAP kinase pathway (39). Finding differences in the extent of MAP kinase activation following stimulation with HGF or InlB prompted us to investigate other elements of this pathway. MEK activation was evaluated by blotting whole cell lysates of control and stimulated cells with an antibody that recognizes the active form of MEK. The results show that MEK activation paralleled MAP kinase activation (Fig. 4A). To measure levels of active Raf, Raf immunoprecipitates were probed with an antibody that recognizes active Raf (Fig. 4B). As a control the immunoprecipitates were probed with an anti-Raf antiserum (Fig. 4C). The results show that InlB activation paralleled MAP kinase activation (Fig. 4A). To measure the levels of active Ras, we used a GST fusion protein containing the Ras-binding domain (RBD) of Raf. This fusion protein binds only to active, GTP-bound Ras (40, 41). Lysates from control, HGF-, or InlB-stimulated Vero cells were incu-
Fig. 4. InlB is a better activator of the Ras-MAP kinase pathway than HGF. Whole cell lysates of control (lane 1), HGF- (lane 2), and InlB-stimulated (lane 3) Vero cells were analyzed by immunoblotting with an antibody against active MEK (A). Anti-Raf immunoprecipitates from these same cells were probed with an antibody against active Raf (B) and an antiserum against Raf (C). Lysates of control, HGF-, and InlB-stimulated Vero cells were incubated with the Ras-binding domain of Raf immobilized on agarose beads. Bound proteins were analyzed by anti-Ras immunoblotting (D). Whole cell lysates from these same cells were probed by anti-Ras immunoblotting (E).

bated with the GST-RBD fusion protein immobilized on glutathione-agarose beads. Bound proteins were analyzed by anti-Ras immunoblotting (Fig. 4D). Anti-Ras immunoblots of whole cell lysates were included as a control (Fig. 4E). The data show slightly higher levels of Ras activation in response to InlB than in response to HGF (Fig. 4D). Together, these observations show that InlB is a better activator of the Ras-MAP kinase pathway than HGF.

Tyrosine Phosphorylation of an Unidentified 90-kDa Protein in Response to InlB but Not in Response to HGF—To find out whether there are differences in signal transduction events between the HGF receptor and active Ras, we focused on proteins known to be involved in Ras activation, including Shc, Grb2, and PI 3-kinase. Analysis of Shc and Grb2 immunoprecipitates by anti-Tyr(P) blotting showed that all three isoforms of Shc (p46, p52, and p66) are tyrosine-phosphorylated in response to either HGF or InlB (results not shown). GST fusion proteins containing either the Shc PTB domain, the Shc SH2 domain, or the Grb2 SH2 domain associated with several unidentified proteins that became phosphorylated in response to either HGF or InlB. No qualitative differences in either Shc or Grb2 associated proteins were observed (results not shown).

Interestingly, a tyrosine-phosphorylated 90-kDa protein that is present in the PI 3-kinase immunoprecipitates from InlB-stimulated cells is absent from immunoprecipitates from cells that were stimulated with HGF (Fig. 5A). To investigate this further, lysates of HGF- or InlB-stimulated cells were analyzed for the presence of proteins that bind to the PI 3-kinase amino-terminal SH2 domain in vitro. The results confirmed the presence of a 90-kDa protein present in lysates of InlB-stimulated cells that can bind to the PI 3-kinase amino-terminal SH2 domain (Fig. 5B). This protein is absent in lysates of HGF-stimulated cells. Our data show that stimulation of Vero cells with InlB results in tyrosine phosphorylation of a 90-kDa protein that is not tyrosine-phosphorylated in response to HGF.

Sequences Outside the HGF Receptor-binding Domain Contribute to the Ability of InlB to Turn on MAP Kinase—InlB is composed of an amino-terminal LRR domain, an immunoglobulin-like region (IR), a B repeat region, and three carboxy-terminal glycine and tryptophan-containing (GW) domains (Fig. 6A). It is known that the LRR domain by itself can bind to the HGF receptor. LRR can be isolated as a non-physiological disulfide-linked dimer (LRR<sub>d</sub>) or as a monomer (LRR<sub>m</sub>).<sup>2</sup> Dimeric LRR activates the HGF receptor, whereas the monomer is unable to do so.<sup>2</sup> To find out whether the LRR domain is sufficient for InlB-mediated signaling, we compared the signaling capabilities of full-length InlB with those of LRR<sup>d</sup>. Vero cells were stimulated with InlB, LRR<sup>d</sup>, and HGF and analyzed for HGF receptor phosphorylation and MAP kinase activation (Fig. 6). Our data show that stimulation with 1.5 nM HGF, InlB, or LRR<sup>d</sup> results in very similar levels of HGF receptor activation, as measured by receptor tyrosine phosphorylation (Fig. 6, B and C). Interestingly, we found that there is a dramatic difference between InlB and either LRR<sup>d</sup> or HGF in their abilities to activate Erk-2 (Fig. 6, D and E). LRR<sup>d</sup> resembled HGF in its ability to activate Erk-2, whereas InlB was found to be a more potent activator. This suggests that portions of InlB besides the LRR domain are required for ability of InlB to super-activate the Ras-MAP kinase pathway.

The IR and B Repeats Play an Important Role in Signaling by InlB—To find out which regions of InlB are involved in MAP kinase super-activation, we generated a series of carboxy-terminal deletion mutants. Progressive deletion from the carboxyl terminus affected receptor activation.<sup>2</sup> This made it difficult to separate receptor activation from MAP kinase activation. To test the importance of the IR, B, and GW domains to the ability of the IR to activate cell signaling, mutant proteins with internal deletions were generated (Fig. 7A). Vero cells were stimulated with the various mutants and analyzed for HGF receptor and MAP kinase activation (Fig. 7, B and D). Control blots showing receptor levels and MAP kinase levels were included (Fig. 7, C and E). Our data show that the InlB mutant lacking the first GW repeat (InlB<sub>B-GW1</sub>) is unaffected in its ability to turn on either the HGF receptor or MAP kinase (Fig. 7). A second mutant that lacks both the B repeat and the first GW repeat (InlB<sub>B-GW1</sub>) is unaffected in its ability to turn on the receptor but has lost the ability to super-activate MAP kinase (Fig. 7). Finally, a mutant that lacks the IR, the B repeat, and the first GW repeat (InlB<sub>ΔIR-B-GW1</sub>) loses the ability to turn on the HGF receptor (Fig. 7A). These data clearly show that the IR and B repeat are important for signaling by InlB.

DISCUSSION

InlB is a protein that is expressed on the surface of L. monocytogenes and that initiates host cell entry by binding to the HGF receptor (29, 42). Binding of InlB to the HGF receptor stimulates phagocytosis (3, 43). In contrast, stimulation with HGF results in mitogenesis, cell scattering, and tubulogenesis (44, 45). This prompted us to compare signaling in response InlB and HGF.

Several members of the MAP kinase family, including Erk-1, Erk-2, and stress-activated p38 MAPK, are activated in response

<sup>2</sup>M. Banerjee, J. Copp, M. Marino, T. Chapman, P. van der Geer, and P. Ghosh, manuscript in preparation.
to HGF (46). We found that InlB is a better activator of Erk-1 and Erk-2 than HGF. This is of interest for two reasons. First, because InlB and HGF are both thought to signal exclusively through the HGF receptor, we expected MAP kinase activation to correlate with receptor activation. Our data show that this is not the case (Figs. 3, 6, and 7). Second, MAP kinase activation has been shown to be essential for InlB-mediated host cell invasion (25). Our data raise the possibility that InlB uses a distinct signaling mechanism, one that differs from that used by HGF, to turn on MAP kinase.

To explain the differences in activation of the Ras-MAP kinase pathway, we examined proteins that are thought to act proximal in the HGF receptor signal transduction process. Phosphorylation of Shc- and Grb2-associated proteins in response to HGF has been reported previously (7, 47). We did not observe qualitative differences between InlB and HGF in their abilities to stimulate the phosphorylation of Shc- and Grb2-associated proteins (results not shown). It is worth noting that the identity of some of these associated proteins remains to be determined.

It has been shown previously (26, 48) that activation of the HGF receptor leads to tyrosine phosphorylation of p85, the regulatory subunit of PI 3-kinase. There are several tyrosine-phosphorylated proteins that co-immunoprecipitated with the 85-kDa subunit of PI 3-kinase in response to either InlB or HGF. These include a 120-kDa protein that most likely represents Gab1, a 110-kDa protein, p85, and a 55-kDa protein. In addition, there is a 90-kDa protein that is tyrosine-phosphorylated in response to InlB but not in response to HGF. In vitro binding experiments showed that this protein binds to the amino-terminal SH2 domain of p85. It is tempting to speculate that the difference in the ability to activate the Ras-MAP kinase pathway between InlB and HGF is a result of the difference in ability to stimulate phosphorylation of this 90-kDa protein. A variety of downstream targets for PI 3-kinase have been identified (49). However, we are unaware of the identification of a 90-kDa protein known to bind to the amino-terminal SH2 domain of p85. Identification and further characterization of this protein will be necessary to find out whether this protein performs an essential function during InlB-dependent signal transduction. We are currently using an affinity purification approach to identify this protein.

InlB is composed of an amino-terminal domain that contains an amino-terminal cap, several LRR, and an immunoglobulin-like region (IR). These three motifs comprise a single structural domain that is followed by a B repeat region (B) and a carboxyl-terminal domain that contains three tandem GW repeats (31, 50) (Fig. 6A). The LRR domain was found previously (29) to mediate binding and activation of the HGF receptor. The GW domain has been shown to mediate the association of InlB with the bacterial surface (50, 51). When we compared InlB and LRRΔ, we found that both proteins are similar in their ability to turn on the HGF receptor but different in their ability to turn on MAP kinase. InlB is a stronger activator of MAP kinase.

To find out why InlB is a better activator of MAP kinase than either LRRΔ or HGF, we analyzed a series of carboxyl-terminal InlB deletion mutants for their ability to stimulate cell signaling to the Ras-MAP kinase pathway. Because progressive deletion of the carboxyl terminus affected the abilities of these mutant proteins to activate the HGF receptor, it was impossible to look at downstream signaling. Therefore, mutant proteins with internal deletions were generated and tested for their abilities to activate cell signaling. Deletion of the first GW repeat (GW1) resulted in a protein that is unaffected in its ability to activate the HGF receptor or the Ras-MAP kinase pathway. This demonstrates that internal deletions can be made in InlB without completely destroying its biological ac-

Fig. 5. An unidentified tyrosine-phosphorylated protein associates with PI 3-kinase in response to InlB but not in response to HGF. Immunoprecipitates (IP) of the p85 subunit of PI 3-kinase from control (lane 1), HGF- (lane 2), and InlB-stimulated (lane 3) Vero cells were analyzed by anti-Tyr(P) immunoblotting (A). Lysates from control, HGF-, and InlB-stimulated Vero cells were incubated with a GST fusion protein containing the amino-terminal SH2 domain of p85 immobilized on agarose beads. Bound proteins were analyzed by anti-Tyr(P) immunoblotting (B).

Fig. 6. The LRR domain dimer resembles HGF in its ability to turn on MAP kinase. A schematic diagram of InlB and the LRR domain dimer (LRRΔ) is shown (A). Anti-HGF receptor immunoprecipitates from control (lane 1), HGF- (lane 2), InlB- (lane 3), and LRRΔ-stimulated (lane 4) Vero cells were analyzed by anti-Tyr(P) (B) and anti-HGF receptor (C) immunoblotting. Whole cell lysates were analyzed by immunoblotting with antibodies that recognize active Erk-1 and Erk-2 (D) and an antibody against Erk-2 (E).
Neither GW1 nor the B repeat region of InlB seems necessary for maximal activation of the HGF receptor. Comparing the abilities of these proteins to mediate signaling downstream of the HGF receptor, however, shows that the mutant that is missing both GW1 and the B repeats region is severely compromised in its ability to activate the Ras-MAP kinase pathway. The IR appears to be essential for HGF receptor activation because the protein that is missing this region, as well as GW1 and the B repeats, has lost the ability to turn on the HGF receptor. An alternative explanation is that the IR is essential for normal folding of InlB. However, we do know that folding of LRR is independent of IR, as LRRd itself is active (Fig. 6). Our results show that it is possible to separate activation of the HGF receptor from the super-activation of MAP kinase. Furthermore, it appears that it is the B repeat that is essential for the increased ability of the InlB to turn on MAP kinase.

One possible explanation for the observed differences in the ability to activate MAP kinase is that the kinetics for the ligand-receptor interaction differs between HGF and InlB. This has been observed for the T cell receptor, where downstream signaling is affected by the kinetics of the interaction between receptor and ligand (52). However, it should be noted that the activation of the T cell receptor requires the interaction of two different cell types and is the result of several interactions among various proteins present on the surface of the interacting cells (53, 54).

An alternative explanation is that the B repeat region of InlB could act by recruiting an unrelated receptor into the signaling complex. InlB thus may signal in a manner analogous to the nerve growth factor (NGF). Two unrelated receptors for NGF have been identified (55). One is a receptor protein-tyrosine kinase and is called the NGF receptor or Trk (56). The second one is called p75 and is a member of the tumor necrosis factor receptor family (57). The NGF receptor can act as a receptor for NGF by itself. Co-expression of p75 increases NGF binding, and it influences the ability of NGF to stimulate NGF receptor autophosphorylation (58–60). This second receptor could activate distinct signaling pathways that are different from those activated by the HGF receptor but act in synergy with the signaling downstream of the HGF receptor to lead to the super-activation of MAP kinase that is seen in response to InlB.

What is the identity of the second receptor? It has been reported that both gC1qR and glycosaminoglycans are membrane-bound host-cell components that interact with InlB and are important for InlB-mediated invasion of L. monocytogenes (30, 61). However, it has been established that InlB interacts with both of these molecules via its GW domains and not the B repeat region (30, 31). The identification of a second receptor that can interact with the B repeat of InlB...
would be of importance to understanding how InlB mediates host-cell signaling.

In summary, we have found that there are differences in the abilities of InlB and HGF to turn on the Ras-MAP kinase pathway. Analysis of deletion mutants shows that the LRR, the B repeat region and the C-terminal region that is responsible for the signaling differences between InlB and HGF in their abilities to activate MAP kinase. We believe that the LRR is involved in binding to the HGF receptor, whereas one or more of the other domains could interact with a second receptor.

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