Intersectin Activates Ras but Stimulates Transcription through an Independent Pathway Involving JNK*

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Intersectin (ITSN) is a recently described endocytic adaptor protein consisting of multiple modular domains, including two amino-terminal Eps15 homology (EH)† domains, a central coiled-coil domain, and five carboxyl-terminal Src homology 3 (SH3) domains (1–5). In addition, a splice variant of ITSN, termed ITSN-1, has been identified and encodes a carboxy-terminal extension encoding a guanine nucleotide exchange factor domain specific for Cdc42 (6, 7). Although initial studies implicated ITSN in regulation of clathrin dependent endocytosis (1, 3–5, 8), we have shown that ITSN also regulates mitogenic signaling pathways (9). For example, ITSN cooperated with progesterone to accelerate maturation of Xenopus oocytes in vivo, induced morphological transformation of rodent fibroblasts, and stimulated Elk-1-dependent transcriptional events (9). However, the biochemical mechanism for this activity was unclear.

Preliminary characterization of the pathway(s) involved in Elk-1 activation by ITSN revealed that this activity was independent of MEK1/2 and Erk MAPK (9). This result was surprising given the proposed involvement of ITSN in activation of Ras, a potent activator of the Raf-MEK-Erk pathway (10, 11). However, a recent study demonstrated that Ras activates distinct signaling pathways at different endomembrane compartments (12). Thus, we were interested in determining the contribution of Ras to ITSN signaling. The results presented herein demonstrate for the first time that ITSN stimulates RasGTP levels and forms a complex with Ras in vivo on a previously uncharacterized vesicular compartment. However, ITSN stimulation of Elk-1 does not require Ras function. In addition, ITSN signaling does not require the kinase activity of the epidermal growth factor receptor although EGFR and ITSN cooperate to synergistically activate Elk-1 and are co-localized on vesicles. Rather, ITSN stimulates Elk-1 through a JNK-dependent pathway. Inhibition of JNK attenuates Elk-1 activation by ITSN. Conversely, ITSN overexpression activates JNK. These data further define the biochemical pathways through which ITSN functions and support the model that ITSN is a pivotal component in integrating numerous cellular processes, including activation of GTPase cascades, endocytosis, and mitogenesis.

**MATERIALS AND METHODS**

Cells and Reagents—293T and NIH/3T3 cells were maintained as previously described (13). Growth factors were purchased from the following: recombinant human platelet-derived growth factor BB, recombinant human hepatocyte growth factor, and recombinant human basic fibroblast growth factor were from R & D Systems, Inc.; human recombinant epidermal growth factor was from Upstate Biotechnology; and bovine insulin was from Invitrogen. SB203580 and SP600125 (also known as Jnk inhibitor II) were purchased from Calbiochem. For each 10 μM of JNK inhibitor added to the media, 0.1% Me2SO was also added (e.g. 20 μM SP600125/0.2% Me2SO). All the inhibitors were resuspended in Me2SO as carrier. PY20 antibody was purchased from BD Transduction Laboratories. FLAG monoclonal antibody was purchased from Sigma. Phosphospecific JNK antibody was purchased from New England Biolabs. Monoclonal anti-hemagglutinin antibody was purchased from Covance. Polyclonal GST antibody conjugated to horseradish peroxidase was purchased from Santa Cruz Biotechnology. Polyclonal antibodies to Sos-1 were purchased from Santa Cruz Biotechnology and Upstate Biotechnology.

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§ The abbreviations used are: EH, Eps15 homology; SH3, Src homology 3; ITSN, intersectin; FRET, fluorescence resonant energy transfer; JNK, c-Jun amino-terminal kinase; RBD, Ras binding domain; RTK, receptor tyrosine kinase; HA, hemagglutinin; Erk, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; GEF, GEF receptor; GST, glutathione S-transferase; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein; CMV, cytomegalovirus; PBS, phosphate-buffered saline; RLU, relative light unit; Icfp, CFP fluorescence intensity; TBS, Tris-buffered saline.
Intersectin Activates Ras and JNK Independently

RESULTS

ITSN Activation of Elk-1 Does Not Require EGFR Kinase Activity—We previously demonstrated cooperativity between ITSN and EGF in the synergistic activation of Elk-1 and in the transformation of rodent fibroblasts (9). Given this finding coupled with the fact that ITSN overexpression is sufficient to inhibit endocytosis (4, 17), it is possible that the ability of ITSN to activate Elk-1 may stem from inhibition of EGFR down-regulation thereby leading to enhanced or sustained signaling from the activated receptor at the cell surface. Alternatively, ITSN may stimulate an autocrine loop involving EGFR ligands thereby leading to receptor activation and Elk-1 stimulation. To test whether ITSN signaling was dependent on the EGFR kinase activity, we treated cells with a specific pharmacological inhibitor of EGFR, PD153035 (18) (Fig. 1). Inhibition of EGFR did not attenuate ITSN activation of Elk-1, although there was a complete loss of Elk-1 activation with EGF stimulation alone thereby leading to receptor activation and Elk-1 stimulation. Alternatively, EGFR phosphorylation and detection of endogenous ITSN was accomplished by incubating cells with 5 mg/ml Texas Red-conjugated transferrin (Molecular Probes) for 30 min at 37 °C followed by removal of the unconjugated probe. Fluorescent labeling of activated EGFRs was accomplished by incubating cells with 40 ng/ml Texas Red-conjugated EGF (Molecular Probes) for 20 min at 37 °C. Dual-color digital laser scanning confocal microscope was used to continuously monitor ligand internalization by individual cells.

JNK Assays—HEK 293T cells were transiently co-transfected with the indicated expression constructs and a FLAG-epitope-tagged JNK expression construct. Following an overnight incubation in serum-free media, cell lysates were harvested and Western blots were performed to assess the expression of FLAG-JNK. Lysates were normalized so that equal amounts of FLAG-JNK were immunoprecipitated with a FLAG monoclonal antibody. Precipitates were washed three times with ice-cold PLC-LB (50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 150 mM MgCl2, 1 mM NaF) supplemented with 1 mM Na3VO4, 10 mM Na3P04, 1 mM pepstatin, 1 mM aprotinin, and 10 mg/ml aprotinin then resuspended in 25 ml of 4% NuPAGE sample buffer supplemented with 5% β-mercaptoethanol. After heating to 70 °C for 10 min, equivalent amounts of samples were fractionated on duplicate 4–12% NuPAGE gels (Invitrogen), transferred to Immobilon-P membranes, and probed with antibodies to the FLAG epitope to determine total levels of JNK or antibodies to activated, dually phosphorylated JNK (New England Biosciences). GST Pull-down Assays—Bacterially expressed GST fusion proteins were prepared essentially as described (16) except that bacterial pellets were lysed in PLC-LB. Ten micrograms of the individual GST-SH3 domains bound to Sepharose beads was combined with 250 μg of lysate from cells stimulated with EGF for varying lengths of time and then incubated at 4 °C for 2 h with gentle mixing. The beads were then pelleted at 20,800 × g for 2 min at 4 °C, washed five times with 500 μl of PLC-LB with inhibitors, resuspended in 25 μl of 4% NuPAGE loading buffer (Invitrogen) supplemented with 5% β-mercaptoethanol, boiled at 100 °C for 5 min, fractionated on NuPAGE gels, and then transferred to Immobilon-P filters. The filters were then blocked in 5% nonfat dry milk, 1X Tris-buffered saline, 0.05% Tween 20 (Sigma), pH 7.4 (TBS-T), overnight at 4 °C. The top half of the filters was then probed with antibodies to Soy1 (2 μg/ml) in PBS containing 3% nonfat dry milk overnight at 4 °C. The lower half of the blots were probed with α-GST-horseradish peroxidase (BioRad) containing 3% nonfat dry milk for 1 h at room temperature. Filters were washed five times for a total of 50 min. Signals were developed with SuperSignal chemiluminescence reagent (Pierce).

RESULTS

ITSN Activation of Elk-1 Does Not Require EGFR Kinase Activity—We previously demonstrated cooperativity between ITSN and EGF in the synergistic activation of Elk-1 and in the transformation of rodent fibroblasts (9). Given this finding coupled with the fact that ITSN overexpression is sufficient to inhibit endocytosis (4, 17), it is possible that the ability of ITSN to activate Elk-1 may stem from inhibition of EGFR down-regulation thereby leading to enhanced or sustained signaling from the activated receptor at the cell surface. Alternatively, ITSN may stimulate an autocrine loop involving EGFR ligands thereby leading to receptor activation and Elk-1 stimulation. To test whether ITSN signaling was dependent on the EGFR kinase activity, we treated cells with a specific pharmacological inhibitor of EGFR, PD153035 (18) (Fig. 1). Inhibition of EGFR did not attenuate ITSN activation of Elk-1, although there was a complete loss of Elk-1 activation with EGF stimulation alone in the presence of this inhibitor. In addition, inhibition of EGFR abolished the cooperative activation of Elk-1 by ITSN and EGF.

ITSN Complexes with Sos to Activate Ras—Given that the signaling activity of ITSN was independent of the EGFR ki-
Fig. 1. ITSN activation of Elk-1 does not require the kinase activity of the epidermal growth factor receptor. HEK cells were co-transfected with the reporter plasmids and either vector control (pCGN-Hyg) or an ITSN expression plasmid as described previously (9). Following serum starvation overnight, either in the presence of the EGFR kinase inhibitor (PD153035) or vehicle control (Me2SO), cells were stimulated with EGF (100 ng/ml) for 5 h, lysed, and then analyzed for luciferase activity. All results were normalized to vector-transfected HEK cells were co-transfected with the reporter plasmids and either vector control (pCGN-Hyg) or an ITSN expression plasmid as described previously (9). Following serum starvation overnight, either in the presence of the EGFR kinase inhibitor (PD153035) or vehicle control (Me2SO), cells were stimulated with EGF (100 ng/ml) for 5 h, lysed, and then analyzed for luciferase activity. All results were normalized to vector-transfected cells in the absence of stimulation. The data are expressed as the mean ± S.E. from four independent experiments performed in duplicate.

In vitro

nase, we sought to determine the signaling pathway(s) activated by ITSN. Previous results revealed that ITSN complexed with the Ras guanine nucleotide exchange factor Sos1, and this interaction was mediated by the SH3 domains of ITSN (10). To determine the specificity of this interaction, we used GST-SH3 fusion proteins of the individual ITSN SH3 domains to purify Sos1 from cell lysates. As illustrated in Fig. 2A, SH3A, SH3C, and SH3E each bound Sos1 with apparent equal affinity. GST, SH3B, and SH3D did not complex with Sos1. Given the cooperativity between EGF and ITSN and the ability of growth factor stimulation to regulate complex formation between Sos and Grb2 through phosphorylation (19, 20), we tested whether EGF stimulation of cells altered the ability of Sos1 to bind the SH3 domains of ITSN. As illustrated in Fig. 2A, EGF stimulation did not affect in vitro binding of the SH3 domains to Sos1.

Overexpression of the SH3 region of ITSN inhibited EGF activation of both MAPK and Ras suggesting that ITSN might regulate Ras activation through interaction with Sos (9, 11). Based on the localization of ITSN to the plasma membrane (21) and the interaction with Sos, we hypothesized that ITSN expression would enhance Ras activation through recruitment of Sos to the plasma membrane (22, 23). Indeed, overexpression of either Xenopus or mouse ITSN was sufficient to increase Ras GTP levels (Fig. 2B). The difference in activity between Xenopus ITSN and mouse ITSN is due to higher expression of mouse versus Xenopus ITSN (data not shown).

ITSN and Ras Interact on Cytoplasmic Vesicles—Previous studies reported localization of endogenous ITSN on clathrin-coated pits in COS-7 cells and cytoplasmic vesicles in primary hippocampal neurons (21). YFP-tagged mouse ITSN expressed in COS-1 cells was observed in the cytosol and on distinct cytoplasmic vesicles that were clustered in the paranuclear region adjacent to Golgi but were also evident in the cell periphery (Fig. 3). This pattern was very similar to that observed for endogenous ITSN in hippocampal neurons (21). Although the subcellular distribution of these vesicles and the reported co-localization with clathrin are consistent with endosomes, they did not accumulate Texas Red-conjugated transferrin suggesting that they are distinct compartments (Fig. 4). However, these ITSN-positive vesicles do contain EGFR consistent with the notion that these vesicles are sites of active signaling complexes (Fig. 4). To determine if H-Ras was associated with ITSN-bearing vesicles, we co-transfected COS-1 cells with CFP-H-Ras and YFP-ITSN (Fig. 3A). H-Ras was associated with a subset of ITSN-bearing vesicles. To determine if H-Ras and ITSN interacted with each other on this compartment, we measured FRET between the two molecules. A FRET signal could be detected as sensitized emission on vesicles bearing both H-Ras and ITSN (Fig. 3A). No FRET signal was observed when YFP-ITSN was co-expressed with CFP alone (data not shown). FRET was further validated by release of donor quenching upon acceptor photobleaching (24). The FRET efficiency was calculated as 10 ± 1% (mean ± S.E., n = 25 vesicles), within the range reported for intracellular protein-
ITSN and H-Ras interact in living cells. COS-1 cells were co-transfected with (A) YFP-tagged mouse ITSN (YFP-ITSN) and CFP-tagged 
human H-Ras (CFP-H-Ras) or (B) CFP-tagged Ras binding domain of c-Raf (CFP-RBD), YFP-ITSN and untagged H-Ras, grown in 
surface, and imaged alive with a Zeiss 510 laser scanning confocal microscope. In each series, CFP-H-Ras (A) or CFP-RBD (B) is displayed in 
the red channel and YFP-ITSN is shown in the green channel. The bottom panel shows FRET images as sensitized emission (excitation at 
458 nm, emission >560 nm). A vesicle positive for FRET is indicated with arrows. Arrowheads indicate Golgi. Scale bars represent 10 μm. C, 
ITSN and Ras co-precipitate from cell lysates. 293T cells were co-transfected with the indicated plasmids. ITSN immunoprecipitates 
were then fractionated on gels and probed with antibodies to Ras (top panel). Lysates were probed with antibodies to Ras or to HA-tagged 
ITSN (middle and bottom panels) to confirm expression levels.

Protein interactions (15). Furthermore, immunoprecipitation of 
ITSN from cell lysates resulted in the co-precipitation of wild 
type H-Ras (Fig. 3C). Thus, H-Ras and ITSN interact on a 
subset of ITSN-bearing cytoplasmic vesicles.

To determine if the ITSN-H-Ras association led to Ras activation, we co-expressed the Ras binding domain of Raf-1 (RBD) 
tagged with CFP and YFP-ITSN along with untagged H-Ras (Fig. 3B). CFP-RBD was associated with a subset of ITSN-bearing vesicles indicating that at least a portion of the H-Ras on this compartment was in the GTP-bound state. Consistent with this result, we were able to co-precipitate constitutively activated H-Ras with ITSN (Fig. 4C). In contrast to CFP-H-Ras, we detected no FRET signal between CFP-RBD and YFP-ITSN.

ITSN Activation of Elk-1 Is Independent of Ras—Given that 
ITSN complexed with Ras and stimulated RasGTP levels, we 
tested whether ITSN activation of Elk-1 was dependent on Ras. 
Co-expression of Ras dominant-negatives along with ITSN did 
not block Elk-1 activation by ITSN (Fig. 5). However, EGF 
activation of Elk-1 was inhibited by the Ras dominant-negatives as was the cooperative activation of Elk-1 by EGF and ITSN. These results indicated that ITSN signals to Elk-1 activation through a Ras-independent mechanism.

ITSN Activation of Elk-1 Is Differentially Regulated by the 
JNK and p38 Pathways—The observation that ITSN activated 
Elk-1 independently of Ras and the Erk MAPK pathway coupled with the fact that p38 and JNK activate Elk-1 in various cell 
types (25) suggested that alternative MAPK pathways were involved in ITSN signaling. To test this hypotheses, we treated 
ITSN-expressing cells with a pharmacological inhibitor of JNK (26). As illustrated in Fig. 6A, ITSN activation of Elk-1 was 
significantly impaired with JNK inhibition. Our previous studies with truncation mutants of ITSN revealed that the signaling 
activity of ITSN was localized to the amino-terminal EH domains (9). Treatment of EH-expressing cells with the JNK inhibitor also blocked JNK activation. Given a recent report demonstrating that the SP600125 inhibits kinases other than 
JNK (27), we tested whether a JNK dominant-negative (JNK- 
APF) (28) would also block ITSN signaling. However, expression of JNK-APF did not alter ITSN expression (data not shown). Together, these results demonstrate that ITSN activation of Elk-1 was dependent on JNK activity.

Although ITSN signaling activity was JNK-dependent, contrast results were obtained with an inhibitor to p38. Pharma 
ocological inhibition of the p38 pathway with SB203580 (29) 
led to a significant increase in ITSN activation of Elk-1 as well as an increase in the cooperativity between EGF and ITSN in 
activating Elk-1 (Fig. 1C). Western blot analysis of cell lysates indicated that ITSN was expressed at equivalent levels in cells 
treated with each inhibitor as compared with vehicle-treated 
cells (data not shown). These results indicated the ITSN sig 
naling was differentially regulated by p38 and JNK.

ITSN Stimulates JNK Phosphorylation—Because ITSN ac 
tivation of Elk-1 was JNK-dependent, we next tested whether 
ITSN activated JNK. Co-expression of either Xenopus or mouse 
ITSN with FLAG-tagged JNK led to JNK activation (Fig. 6D). 
Mouse ITSN was more potent at stimulating JNK due to its 
higher expression. Additionally, expression of the EH domains 
was sufficient to stimulate JNK activation consistent with the 
Elk-1 reporter assays (Fig. 6A). Expression of a constitutively 
activated allele of Ras, H-RasQ61L, led to slight JNK activa 
ion. However, co-expression of ITSN with activated Ras led to 
a dramatic induction in JNK activation. In contrast, there was little cooperativity in the activation of JNK by co-expression 
of ITSN with wild type Ras. These results indicated that, al 
though ITSN did not require Ras for activation of Elk-1, ITSN 
cooperated with Ras-activated pathways to potentiate JNK 
activation.

DISCUSSION

Previous work from our laboratory demonstrated that the 
edecytoadaptor protein ITSN regulates mitogenic signaling 
pathways leading to cell growth and differentiation (9). Al 
though ITSN synergized with EGF as well as other growth 
factors (e.g. hepatocyte growth factor and basic fibroblast 
growth factor, data not shown) to stimulate Elk-1, we found 
that Elk-1 activation by ITSN was independent of EGFR ki 
nase activity. These data indicate that ITSN signaling does not result merely from inhibition of EGFR endocytosis thereby 
prolonging signaling from the receptor at the cell surface. Fur 
thermore, the inability of PD153035 to affect ITSN signaling 
suggests that ITSN does not stimulate an autocrine loop lead 
ing to Elk-1 activation, at least not one involving EGFR ligands

![Fig. 3. ITSN and H-Ras interact in living cells. COS-1 cells were co-transfected with (A) YFP-tagged mouse ITSN (YFP-ITSN) and CFP-tagged human H-Ras (CFP-H-Ras) or (B) CFP-tagged Ras binding domain of c-Raf (CFP-RBD), YFP-ITSN and untagged H-Ras, grown in surface, and imaged alive with a Zeiss 510 laser scanning confocal microscope. In each series, CFP-H-Ras (A) or CFP-RBD (B) is displayed in the red channel and YFP-ITSN is shown in the green channel. The bottom panel shows FRET images as sensitized emission (excitation at 458 nm, emission >560 nm). A vesicle positive for FRET is indicated with arrows. Arrowheads indicate Golgi. Scale bars represent 10 μm. C, ITSN and Ras co-precipitate from cell lysates. 293T cells were co-transfected with the indicated plasmids. ITSN immunoprecipitates were then fractionated on gels and probed with antibodies to Ras (top panel). Lysates were probed with antibodies to Ras or to HA-tagged ITSN (middle and bottom panels) to confirm expression levels.](image-url)
Rather, our findings suggest that ITSN directly stimulates biochemical pathways that activate Elk-1 independent of EGFR activity (see below). Recent studies have demonstrated an interaction between ITSN and the Ras guanine nucleotide exchange factor Sos1 suggesting an involvement in Ras activation (10, 11). Indeed, overexpression of the SH3 domains of ITSN blocked EGF activation of both Ras and MAPK (9, 11). Our current results demonstrate that ITSN stimulates RasGTP levels and physically complexes with Ras in vivo on intracellular vesicles. This interaction is likely mediated through Sos binding to the SH3 domains of ITSN, particularly SH3A, -C, and -E, although we cannot rule out the potential involvement of additional Ras exchange factors in this process. In contrast, however, our initial studies revealed that ITSN activated Elk-1 in an MEK- and MAPK-independent manner suggesting a lack of Ras involvement in ITSN signaling (9). Indeed several lines of evidence suggest that Ras is not involved in ITSN signaling to Elk-1. First, the EH domains are potent activators of Elk-1 yet do not associate with Sos1. Second, pharmacological inhibitors of MEK1/2, a prominent Ras target, do not block Elk-1 activation by ITSN (9). Finally, ITSN activation of Elk-1 is unaffected by expression of Ras dominant-negative proteins, although these proteins potently inhibited EGF activation of Elk-1 as well as abolished the synergy between EGF and ITSN. Together these data indicate that ITSN signals to Elk-1 through a Ras-independent pathway.

Our studies indicate that ITSN signals through JNK to stimulate Elk-1. Pharmacological inhibition of JNK or expression of a JNK dominant-negative greatly attenuated activation of Elk-1 by ITSN. Conversely, expression of ITSN stimulated JNK activation. Although ITSN did not depend on Ras for activation of Elk-1, ITSN cooperated with constitutively activated Ras, but not wild type Ras, to potentiate JNK activation. We interpret this result to mean that, although ITSN activates Ras on vesicles, this pool of Ras is not capable of coupling to JNK (Fig. 7). Rather, we speculate that ITSN cooperates with a different pool of Ras. Consistent with this notion, Ras activation of JNK is spatially restricted to certain endomembrane compartments (12). Given the ability of ITSN and EGFR to cooperate in activating Elk-1, our results suggest that ITSN cooperates with a signal from Ras at the plasma membrane, or possibly another endomembrane compartment. Furthermore, the lack of Erk activation with ITSN overexpression suggests that this pool of ITSN-activated Ras is not capable of coupling to the MEK-Erk pathway. These observations suggest that ITSN is either inhibiting Ras signaling or altering the ability of Ras to select effectors that lead to activation of Erk or JNK. Given that ITSN promotes Ras activation, we favor the later possibility. However, formal proof that ITSN-activated Ras...
couples to distinct biochemical pathways awaits experimental support.

Our findings also reveal a dynamic regulation of ITSN by MAPK pathways. Whereas JNK is an important mediator of ITSN function, p38 negatively regulates ITSN signaling and Erk appears to play a minimal role in ITSN signaling. Other groups have reported opposing actions of p38 and JNK in Ras signaling and transformation (31, 32) consistent with our observations for ITSN regulation. The ability of ITSN to stimulate JNK was not due to nonspecific induction of a stress response due to protein overexpression. Whereas ITSN overexpression led to pronounced JNK activation, Ras overexpression had only a modest effect, although both were expressed quite well in 293T cells. This point is clearly illustrated by the difference in synergy between ITSN and activated Ras versus wild type Ras (Fig. 6D). Furthermore, both JNK and p38 are stress-activated kinases yet have opposing roles in ITSN signaling. We do not currently know the mechanism behind p38 inhibition of ITSN signaling. However, one possibility is that p38 phosphorylates ITSN thereby disrupting ITSN signaling complexes. Further work will be necessary to determine the mechanism by which p38 regulates ITSN.

Given that ITSN activated Ras and that Ras potently activates MEK and Erk, how can we reconcile the fact that MEK and Erk are not involved in ITSN signaling? We have recently demonstrated that, in addition to the plasma membrane, Ras proteins are localized to distinct endomembrane compartments where they stimulate different signaling pathways at each site (12, 33). Coupled with our findings, it is intriguing to speculate that ITSN may promote Ras activation at distinct subcellular compartments, particularly intracellular vesicles, and that activation of Ras on these vesicles leads to activation of novel signaling pathways due to spatial restrictions of these pathways. Alternatively, given its scaffolding properties, ITSN may promote the interaction of distinct effectors with Ras thereby leading to the selective activation of non-ERK pathways. Such a model is reminiscent of the regulation of yeast MAPK pathways by scaffolding proteins (34, 35). Although not mutually exclusive, these two possibilities provide possible mechanisms by which ITSN may promote the selective activation of signal-
ITSN activates at least two distinct biochemical signaling pathways. ITSN co-localizes with and activates Ras on cytoplasmic vesicles (Ras(ves)); however, this pool of Ras does not appear to activate Erk or JNK MAPKs. In contrast, ITSN stimulates the JNK pathway through its EH domains. In addition, ITSN synergizes with RTKs (e.g. EGFR and bFGFR) and constitutively activated Ras to stimulate Elk-1 and JNK activation, respectively. These results suggest that ITSN cooperates with an RTK-Ras signal emanating from the plasma membrane. Ras(PM), fraction of Ras localized to the plasma membrane.

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