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Regulation of mRNA localization by transmembrane signalling: local interaction of HB-GAM (heparin-binding growth-associated molecule) with the cell surface localizes β-actin mRNA

Carole Fages[1,2], Marko Kaksonen[1,2], Tarja Kinnunen[1,2], Eeva-Liisa Punnonen[3] and Heikki Rauvala[1,2,*]

[1]Laboratory of Molecular Neurobiology, Institute of Biotechnology, the [2]Division of Biochemistry, Department of Biosciences, and [3]Electron Microscopy Unit, Institute of Biotechnology, FIN-00014 University of Helsinki, Finland
Author for correspondence (e-mail: heikki.rauvala@helsinki.fi)

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SUMMARY

Localization of mRNAs is currently thought to be partially responsible for molecular sorting to specific compartments within the cell. In mammalian cells the best-studied example is the β-actin mRNA that is localized to the cell processes, and its localization is necessary in migratory responses of cells. It is reasonable to assume that mRNA localization within cells is coupled to transmembrane signalling due to extracellular factors, but little is known about such putative mechanisms. We show here that HB-GAM, an extracellular matrix-associated factor that enhances migratory responses in cells, is able to localize β-actin mRNA when locally applied to cells via microbeads. The HB-GAM-induced mRNA localization is specifically inhibited by low concentrations of heparin and by heparitinase treatment of cells, showing that cell-surface heparin-type glycans are required for the effect. The finding that soluble N-syndecan is also inhibitory suggests that the transmembrane proteoglycan N-syndecan, previously identified as an HB-GAM receptor, is involved in the mRNA-localizing effect of HB-GAM. Inhibition of the mRNA localization by the src-kinase inhibitor PP1 is compatible with an N-syndecan-mediated effect since the receptor function of N-syndecan has been recently found to depend on the src-kinase signalling pathway. The mRNA-localizing activity of N-syndecan is also suggested by the finding that affinity-purified anti-N-syndecan antibodies coated on microbeads are able to localize β-actin mRNA.

Key words: C6 glioma cell, β-actin mRNA, Regulation of mRNA localization, HB-GAM, N-syndecan

INTRODUCTION

Localization of specific mRNAs is currently regarded as one mechanism for sorting proteins to their target regions. In *Drosophila* and *Xenopus*, many studies have shown that mRNA localization allows the creation of a gradient of the protein and therefore generates the polarity of the oocyte or the egg (for a review, see St Johnson, 1995). In somatic cells, proteins involved in macromolecule assembly are particular examples suitable for this kind of targeting (for a review, see Wilhelm and Vale, 1993). Many cytoskeletal proteins self-assemble after synthesis. Therefore, the local translation in the regions where the filaments are required, is indispensable. One of the most studied localized mRNAs is β-actin. In many cell types, e.g. fibroblasts (Lawrence and Singer, 1986; Hill et al., 1994), endothelial cells (Hoock et al., 1991), and C2 myoblasts (Hill and Gunning, 1993), β-actin protein and mRNA are co-localized in regions of moving cytoplasm, where newly synthesized actin filaments are required.

The mechanisms by which mRNAs are targeted are not fully understood. The general features seem to involve *cis*-and *trans*-acting signals. For example, the *cis*-acting sequences required for the localization of β-actin mRNA, reside in the 3′UTR part of the mRNA. Fine mapping of this region reveals a segment of 54 nt called zipcode, which is necessary and sufficient to localize β-actin mRNA to the periphery of the cell (Kislauskis et al., 1993, 1994). This 54 nt element contains an AC rich region that is conserved among several species (Yaffe et al., 1985), suggesting an important role in the localization. Recently, a zipcode-binding protein was cloned and shown to bind this AC rich region in a specific manner (Ross et al., 1997). Moreover, this zipcode-binding protein has been shown to immunoprecipitate with a complex of proteins that bind to the AC rich region but also to the actin network. In fibroblasts, localization of β-actin mRNA was inhibited by cytochalasin D but not by colcemid, indicating that the targeting of this mRNA is dependent on the microfilament network (Sundell and Singer, 1991). Furthermore, many studies have detected the localized mRNA in granules, suggesting that mRNA is targeted to the cell periphery in a complex with a number of proteins required for the localization and translation of the mRNA: β-actin mRNA in fibroblasts (Sundell and Singer, 1991), MBP mRNA in oligodendrocytes (Ainger et al., 1993), bcd mRNA in *Drosophila* (Ferrandon et al., 1994; Wang and Hazelrigg,

1994), Vg1 and Xcat2 mRNA in *Xenopus* (Forristall et al., 1995; Kloc and Etkin, 1994; Murray et al., 1991).

Despite the fact that several features of the intracellular mechanisms localizing mRNAs are already understood, little is known about the possible transmembrane mechanisms of mRNA localization. One would expect that extracellular factors that regulate cell behavior would inform the cell to localize a particular mRNA. For example, extracellular matrix proteins that enhance process extension in cells during migratory responses, might also direct the localization of a cell motility-associated mRNA, like the β-actin mRNA. In the present study, we have addressed these problems using microbeads onto which different cell matrix proteins were coated. We show that HB-GAM, which enhances neurite outgrowth in several types of neurons (for a review, see Rauvala and Peng, 1997), is very effective in localizing the β-actin mRNA. Furthermore, heparin-type glycans of the cell surface are clearly required for the HB-GAM-induced β-actin mRNA localization. We suggest that these glycans are bound to N-syndecan that acts as an HB-GAM receptor in neurite outgrowth.

MATERIALS AND METHODS

Cell culture

C6 rat glioma cells used in this work were maintained under standard culture conditions. Cells were cultured on Nunc (Naperville, IL) Lab-Tek chamber slides that were either coated with laminin (5 μg/ml in phosphate-buffered saline, overnight at 4°C) or with HB-GAM (20 μg/ml in phosphate-buffered saline, overnight at 4°C) in order to induce rapid process outgrowth. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) or with 10 mg/ml bovine serum albumin (BSA). After the desired culture period, the cells were fixed with 4% paraformaldehyde/0.05% glutaraldehyde in PBS and stored at 4°C.

Immunocytochemistry

The fixed cells, permeabilized with methanol for 5 minutes, were blocked first in 50 mM NH_4Cl in PBS for 10 minutes, then with 3% BSA for 15 minutes and immunostained with a mouse anti-β-actin antibody (Sigma, St Louis, MO) or with a rabbit polyclonal anti-N-syndecan antibody (Nolo et al., 1995; Carey et al., 1992). Bound antibodies were then detected either with affinity-purified fluorescein-conjugated goat anti-mouse IgG or with affinity-purified fluorescein-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). All dilutions were prepared in 3% BSA/PBS. The cells were mounted in 30% Mowiol, pH 7, containing 50 mg/ml DABCO and analyzed under a fluorescence microscope (AX-70, Olympus Optical Co Ltd, Japan).

Library screening

Rat β-actin cDNA was cloned from a commercial hippocampus library (lambda ZAP II, Stratagene, La Jolla, CA) using *Hinc*II-*Eco*V fragment of chicken β-actin cDNA (kindly provided by Dr Edward Kislauskis) as a probe. The screening was performed under stringent conditions at 68°C in hybridization buffer (50% formamide/6× standard saline citrate (SSC)/5× Denhardt's reagent/0.5% SDS). The cloned cDNA (1,793 bp) was analyzed by sequencing in both directions using an automated laser fluorescence (ALF, Pharmacia, Uppsala, Sweden) sequencer and by restriction enzymes. The sequence was found to be the same as that of the rat β-actin cDNA reported previously (Nudel et al., 1983), except that part of the 5′UTR was found to be missing in the isolated clone.

In situ hybridization

Linearized Bluescript KS plasmid containing the *Hinc*II fragment (850 bp) of the rat β-actin cDNA or the full coding region of N-syndecan cDNA (Carey et al., 1992) was transcribed in vitro using DIG RNA Labeling kit (Boehringer Mannheim, Mannheim, Germany) as instructed by the manufacturer. In situ hybridization was performed using modification of a protocol described by Wilkinson (1992). The fixed cells on chamber slides were washed free from fixative and treated with ice-cold methanol for 5 minutes. After washes, the cells were treated with proteinase K (Sigma, 5 μg/ml in 0.5 M NaCl/10 mM Tris-HCl, pH 8) for 5 minutes, and postfixed with 4% paraformaldehyde in PBS for 5 minutes. The hybridization buffer was composed of 50% formamide/0.3 M NaCl/2 mM EDTA/20 mM sodium phosphate/2% blocking powder (Boehringer Mannheim)/0.5% CHAPS/0.5% SDS/80 μg/ml denatured salmon sperm DNA. The cells were probed both with the sense and the anti-sense probes overnight at 60°C. After hybridization, the slides were rinsed twice in 2× SSC, twice in 1× SSC for 15 minutes, twice in 0.1× SSC/0.05% CHAPS for 15 minutes at 60°C, and finally twice in 0.1× SSC for 10 minutes. The probes were detected with alkaline phosphatase-conjugated anti-digoxigenin antibody according to the protocol provided by Boehringer Mannheim.

Bead assays

Polystyrene beads (diameter 4.5 or 10 μm, Polysciences, Inc., Warrington, PA) were washed with 95% ethanol and incubated with recombinant HB-GAM (Raulo et al., 1992), affinity-purified antibodies against an N-terminal extracellular peptide of N-syndecan (LRETAMRFIPDC, described by Nolo et al., 1995), laminin, fibronectin, lentil lectin, BSA or non-immune anti-rabbit IgG at a concentration of 100 μg/ml, expected to essentially saturate the bead surface, in PBS for 1 hour at room temperature. The beads were then washed twice with PBS and blocked with BSA (10 mg/ml) in PBS for 30 minutes at 37°C to mask any free bead surface. After coating, the beads were applied to cells (15 beads per cell) that had been grown on laminin (5 μg/ml) for 3 hours or on tissue culture plastic for 4 hours. After an incubation of 15 or 60 minutes at 37°C, as defined in each experiment, the non-attached beads were washed off with the DMEM medium. The cells were then fixed and immunocytochemistry or in situ hybridization was performed as described above. The percentage of beads localizing β-actin mRNA was calculated from 4 to 8 randomly selected fields (60 to 70 cells/field), as indicated in each experiment, using a ×40 objective. Statistical analysis of the data was carried out using the non-parametric one-way analysis of variance (ANOVA). Independent experiments that included preparation of a new batch of cells for the experiment followed by analysis of the mRNA-localizing activity was carried out at least twice for each bead type analyzed.

Inhibition assays

Increasing concentrations of low molecular weight (LMW) heparin (from 125 to 500 ng/ml; Sigma), N-syndecan (from 0.5 to 2 μg/ml; Raulo et al., 1994), recombinant HB-GAM (from 10 to 40 μg/ml; Raulo et al., 1992), Heparitinase III (from 1 to 20 units/ml; Sigma), the src-kinase inhibitor PP1 (from 1 to 10 μM; Calbiochem, La Jolla, CA) or an N-syndecan peptide (LRETAMRFIPDC, 1 to 100 μg/ml; see Nolo et al., 1995) were added to the cells before adding the beads. Heparitinase was incubated with the cells in the assay medium for 2 hours before adding the beads. During incubation with the beads, these molecules were still present in the medium at the same concentrations. For N-syndecan and heparin dialysis against DMEM containing 1% BSA was carried out for 2 hours before the assay. In situ hybridization was carried out as described above to analyze the mRNA-localizing effects of the beads coated with HB-GAM, fibronectin, laminin or the affinity-purified antibodies that bind to the N-terminal extracellular peptide of N-syndecan.

RESULTS

β-actin mRNA is localized to the cytoplasmic processes of C6 glioma cells grown on HB-GAM-containing matrix

Previous studies have shown that β-actin mRNA localization to and consequent protein synthesis in the cell processes in fibroblasts may augment cell motility (Kislauskis et al., 1997). C6 glioma cells extend rapidly cytoplasmic processes when grown on HB-GAM and they also express N-syndecan, identified as a receptor of HB-GAM in neurite extension (Raulo et al., 1994). To determine whether β-actin mRNA is also localized under these conditions, in situ hybridization using the *Hinc*II fragment of β-actin cDNA was carried out using C6 cells in which process extensions had been induced for 3 hours by HB-GAM. The detected signal was distributed throughout the cytoplasm but was especially marked in the varicosities and the leading edges of the cells (Fig. 1C and E, arrows). The C6 cells were categorized as having the mRNA either 'localized', when β-actin mRNA was distributed in the extended parts of the cell, or 'non-localized' when the signal was exclusively found in the cell soma. Almost 80% of the cells showed a localized signal.

In contrast to the peripheral localization of β-actin mRNA, N-syndecan mRNA was only detected in the cell soma (Fig. 1D and F, arrows), although the two proteins, β-actin and N-syndecan, showed a localization to the peripheral parts of the cell (Fig. 1A and B). N-syndecan is a transmembrane component having a signal peptide. Thus, N-syndecan is likely to be targeted to the cell processes at the protein level, whereas β-actin is already localized at the mRNA level.

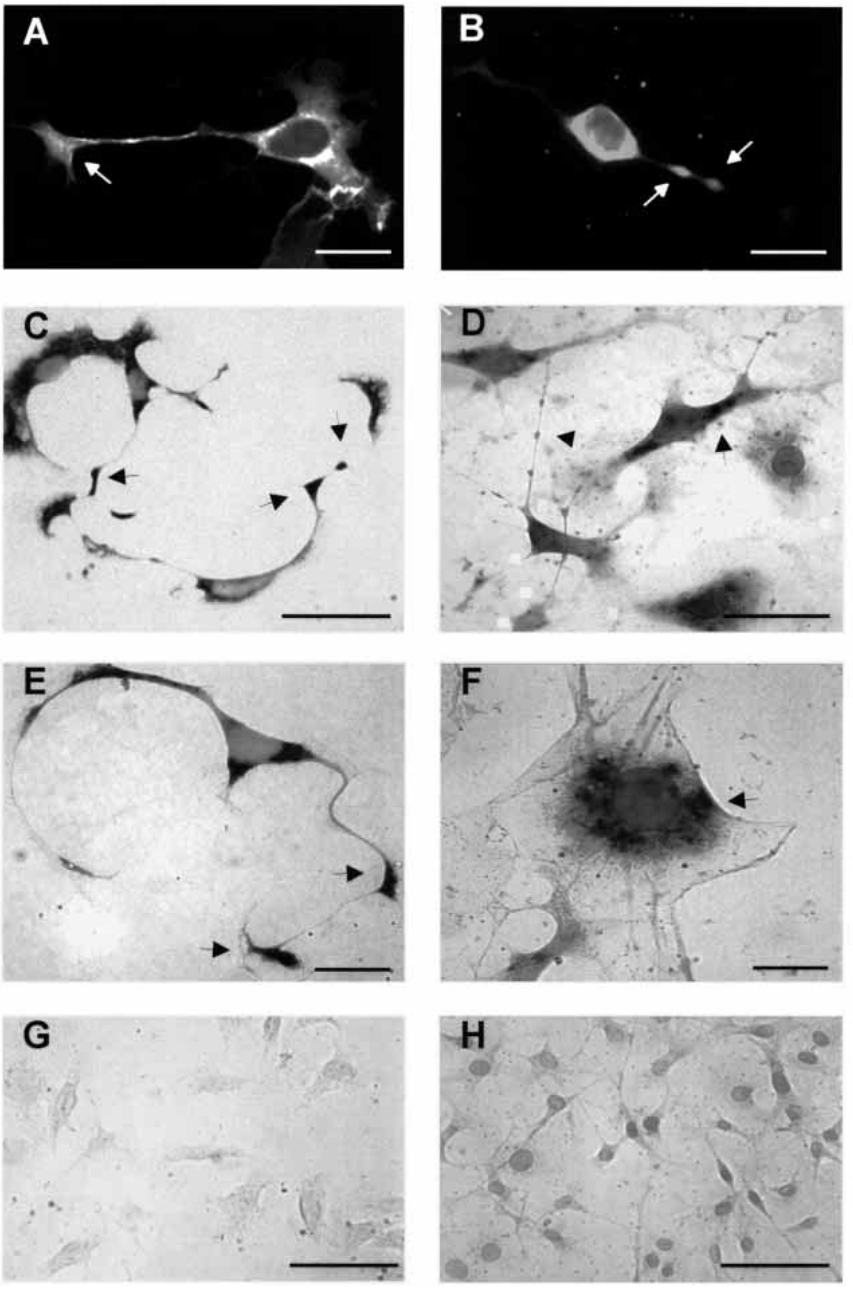

Fig. 1. Localization of β-actin and N-syndecan in C6 cells plated on HB-GAM. Protein localization: (A) β-actin; (B) N-syndecan. Localization of mRNAs: (C and E) β-actin mRNA; (D and E) N-syndecan mRNA; (G) sense control of the β-actin probe; (H) sense control of the N-syndecan probe. Both the β-actin protein (arrow in A) and the mRNA (arrows in C and E) display a peripheral localization. The N-syndecan protein (arrow in B) is also localized to the cell processes; the N-syndecan mRNA localizes to the cell soma (arrows in D and F) but not to the cell processes (arrowhead in D). Bars: 25 μm (A and B); 50 μm (C and D); 15 μm (E and F); 60 μm (G and H).

HB-GAM regulates the localization of β-actin mRNA

Localization of the β-actin mRNA in response to HB-GAM could be an indirect consequence from process growth, or HB-GAM could possess an mRNA-localizing activity due to the binding to its transmembrane receptor. To distinguish between these two alternatives, we coated 4.5 μm polystyrene beads with rat recombinant HB-GAM (Raulo et al., 1992) and applied them to C6 cells grown on laminin for 3 hours. As shown in Fig. 2A,B,D, the local presentation of HB-GAM was very effective in localizing the β-actin mRNA and its protein product. We categorized the localization as 'induced', when a bead was surrounded by the mRNA detection signal, irrespective of the position of the bead on the cell, and 'not induced', when no signal was detected. Almost 50% of the HB-GAM beads induced localization of the β-actin mRNA (Fig. 3), whereas no induced localization was observed for the N-syndecan mRNA (Fig. 2C). The induction mediated by the HB-GAM beads was extremely efficient as we observed that the majority of the β-actin mRNA signal was concentrated around the beads (Fig. 2B and D).

Many studies have shown that compartmentalization of specific mRNAs in different cell types is dependent on the cytoskeleton (Bassell and Singer, 1997). In fact, disruption of actin filaments using cytochalasin D (0.5 μg/ml) or microtubules using colcemid (5 μg/ml) inhibited significantly the localization of β-actin mRNA around HB-GAM-coated beads (13.31±2.9% and 12.53±3.3%, respectively; calculated as in Fig. 3 that gives control values without cytoskeleton-disrupting agents). The mRNA-localizing activity of the HB-GAM-coated beads therefore depends on both intact microfilaments and microtubules.

Comparison of the HB-GAM-induced mRNA localization to fibronectin-, laminin- and lectin-induced localization

To study whether other types proteins that bind to the cell surface, are able to localize mRNA, we coated the beads with laminin, fibronectin and lentil lectin at the same concentration as HB-GAM (100 μg/ml). Localization of β-actin mRNA was also observed for laminin-and fibronectin-coated beads but with a lower efficiency, 32% and 34.3% respectively (Fig. 3). In contrast, lentil lectin-coated beads had little effect on the localization of β-actin mRNA (10.5%), indicating that all beads binding to the cells are not effective in mRNA localization. BSA-coated beads were also used in the assay but the binding between the beads and the cells was not strong enough to resist the stringent washing conditions of the in situ hybridization. Therefore, only beads displaying cell-binding activity could be studied in the assay.

Soluble heparin, heparitinase and soluble HB-GAM inhibit the localization of β-actin mRNA around HB-GAM-coated beads

In order to study the effect of soluble glycans on the regulation of β-actin mRNA localization through HB-GAM, increasing concentrations of low molecular weight heparin or soluble HB-GAM were added to the culture medium during the bead assay. Increasing concentrations of heparitinase were also tested. Heparin and heparitinase (Fig. 4A and B) significantly inhibited the localization of β-actin mRNA induced by the HB-GAM beads. Soluble heparin had no significant effect on the localization of β-actin mRNA induced by laminin (Fig. 4A).

If the HB-GAM-coated beads operate through a local mode of signalling, one would expect that application of the native protein in the culture medium prevents mRNA localization due to the HB-GAM-coated beads. Soluble HB-GAM indeed significantly decreased the localization of β-actin mRNA induced by the HB-GAM-coated beads (Fig. 4C).

Role of N-syndecan in β-actin mRNA localization

The effects of heparin and heparitinase (see above) indicate an involvement of a heparan sulfate proteoglycan in the mRNA-localizing effect of HB-GAM. Previous studies have shown that N-syndecan binds HB-GAM with high affinity (Raulo et

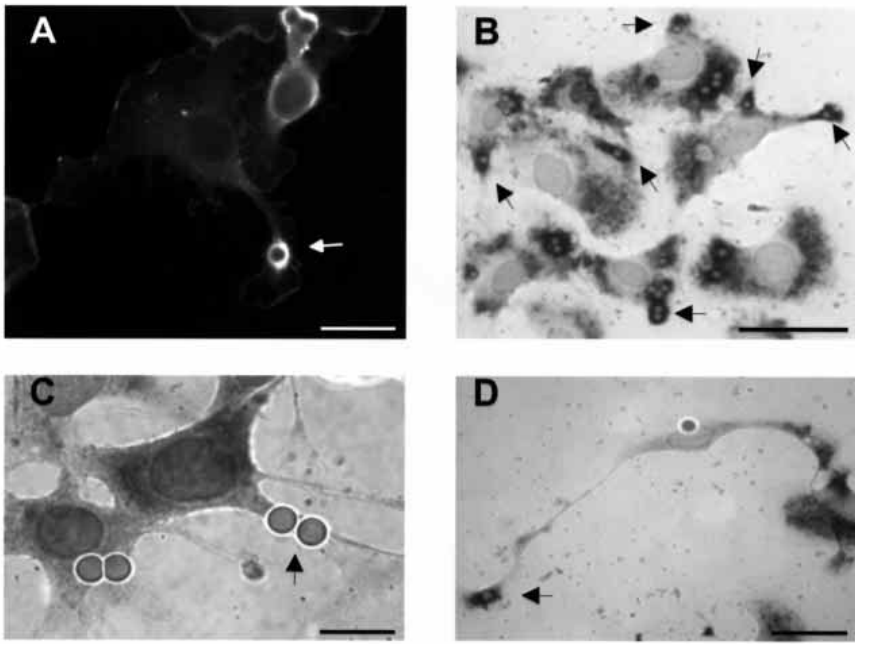

Fig. 2. β-actin protein (arrow in A) and mRNA (arrows in B and D) localize around HB-GAM-coated beads. In contrast, HB-GAM-coated beads have no effect on the localization of N-syndecan mRNA (arrow in C). The cells were incubated with the beads for 15 minutes. Bars: 30 μm (A,B,D); 10 μm (C).

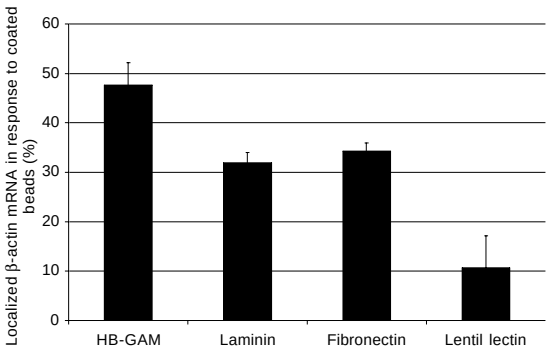

Fig. 3. β-actin mRNA localization in response to different protein-coated beads. Almost 50% of the HB-GAM-coated beads localize β-actin mRNA, whereas lentil lectin has little effect (10.5%). Laminin and fibronectin also localize β-actin mRNA but to a lower degree (32% and 34.3%, respectively) as compared to HB-GAM. The cells were incubated with the beads for 15 minutes. Each value represents the mean ± s.d. calculated from 4 randomly selected microscopic fields (n=4).

al., 1994; Kinnunen et al., 1996). In order to further study the mechanism by which HB-GAM acts, increasing concentrations of soluble N-syndecan were added to the culture medium. Soluble N-syndecan inhibited significantly the localization of β-actin mRNA induced by HB-GAM (Fig. 5A), whereas at the same concentration range it had no effect on the localization of β-actin mRNA induced by laminin (Fig. 5A).

Recent studies on the signal transduction pathway of N-syndecan have shown an involvement of src tyrosine kinases (Kinnunen et al., 1998a). For this reason, we added to the mRNA localization assays PP1 that has been reported to specifically inhibit src-family tyrosine kinases (Hanke et al., 1996). PP1 had a significant inhibitory effect on the localization of β-actin mRNA induced by HB-GAM (Fig. 5B).

If N-syndecan is involved in the mRNA-localizing effect of HB-GAM (see above), it is to be expected that an artificial N-syndecan-binding ligand would mimick the effect of HB-GAM. Anti-N-syndecan antibodies coated on microbeads were indeed able to induce localization of the β-actin mRNA (Fig. 6). The effect of the antibodies was somewhat less pronounced as compared to that of HB-GAM analyzed in parallel (Fig. 6), which might be due to a difference in the binding affinity to N-syndecan or to a lower density of binding sites on the beads coated with the antibodies having a much higher molecular size as compared to HB-GAM that as a small molecular size protein is expected to give a high surface density of N-syndecan binding sites. The effect of the anti-N-syndecan antibodies appeared specific since under the same conditions non-immune rabbit IgG had little effect (10.5±1.95% of beads localizing β-actin mRNA, compare to values in Fig. 6 that were calculated in the same manner). The synthetic peptide against which the anti-N-syndecan antibodies had been produced inhibited the mRNA-localizing effect close to the value (about 10%) that was observed by the non-immune IgG (Fig. 6). Binding of the antibody-coated beads to the cell surface and the ensuing localization of the β-actin mRNA therefore depend on N-syndecan expressed at the cell surface.

DISCUSSION

Mechanisms, by which cells change shape and/or direction of migration, involve rapid actin polymerization at the leading edges. Local peripheral synthesis of actin monomers can provide a pool for actin elongation. In this paper, we have evaluated a mechanism by which β-actin mRNA can be

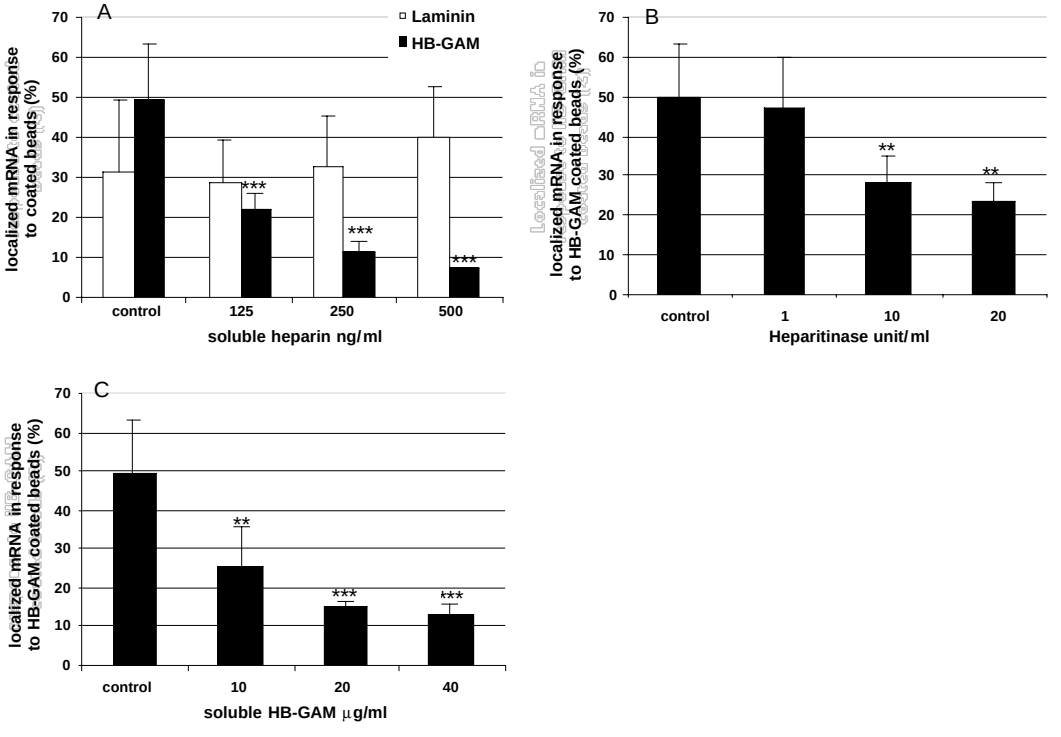

Fig. 4. Effect of increasing concentrations of soluble heparin (A), heparitinase (B) and of soluble HB-GAM (C) on the β-actin mRNA localization induced by HB-GAM-coated beads. Soluble heparin, heparitinase and soluble HB-GAM inhibit significantly the localization of β-actin mRNA around HB-GAM-coated beads. In contrast, heparin has no effect on the localization of β-actin mRNA induced by laminin-coated beads (A). The cells were incubated with the beads for 15 minutes. All values represent the mean ± s.d. (n=6). *P<0.05; **P<0.01; ***P<0.001.

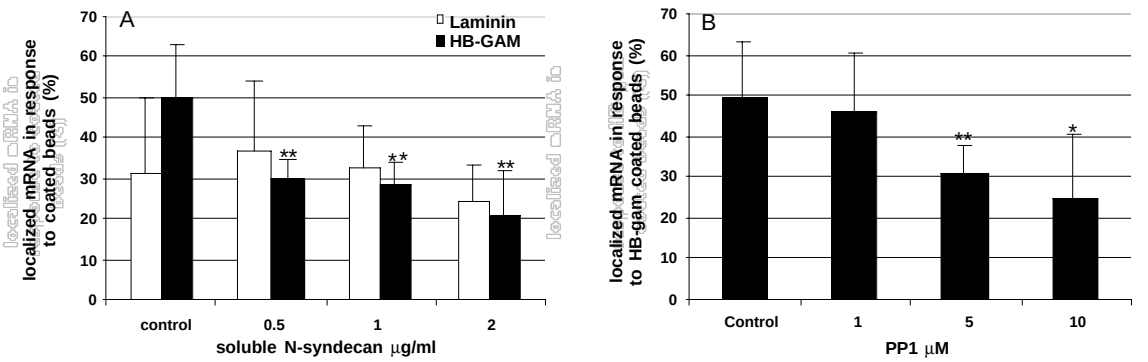

Fig. 5. Effect of increasing concentrations of soluble N-syndecan (A) and PP1 (B) on the mRNA-localizing effect of HB-GAM. N-syndecan decreases significantly the localization of β-actin mRNA around HB-GAM beads, whereas it has no significant effect on the localization around laminin-coated beads (A). PP1 also inhibits significantly the localization of β-actin mRNA induced by HB-GAM. The beads were incubated with the cells for 15 minutes, and each value represents the mean ± s.d. (n=6). *P<0.05; **P<0.01.

regulated at the leading edges in C6 glioma cells. The results show that extracellular factors, that promote migratory responses, regulate the distribution of β-actin mRNA within the cell.

Localization of β-actin mRNA is directly coupled to transmembrane mechanisms involved in process extension

Previous studies have shown that the β-actin mRNA becomes delocalized in serum-starved cells, indicating a requirement for serum factors on continued localization (Latham et al., 1994; Hill et al., 1994). Furthermore, addition of growth factors like PDGF in serum-starved cells can restore the peripheral localization of the β-actin messenger (Latham et al., 1994). However, this delocalization and relocalization might be indirectly due to the process extension or the growth stage of the cell. To test if extracellular factors can induce the distribution of β-actin mRNA irrespectively of the growing processes, HB-GAM was applied locally via microbeads on the cells. Accumulation of the β-actin messenger around the beads both in the cell soma and the processes indicates that HB-GAM is able to regulate the mRNA localization independently on the process growth. This result shows that the β-actin mRNA localization is tightly connected to the mechanisms involved in process extension.

Sundell and Singer have demonstrated that the β-actin mRNA localization is a microfilament-dependent process (Sundell and Singer, 1991). Recently, it has been shown that the localized or delocalized β-actin mRNA is bound to the cytoskeleton (Latham et al., 1994). Based on the inhibitory effects of cytochalasin D and colchicine, the mRNA-localizing effect of HB-GAM depends on both microfilaments and microtubules.

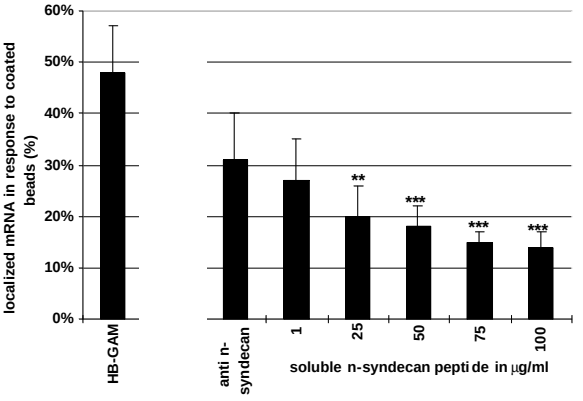

Fig. 6. N-syndecan-dependent localization of β-actin mRNA. The cells were grown on tissue culture plastic for 4 hours, and beads coated either with HB-GAM or with affininity-purified antibodies against the N-terminal peptide of N-syndecan were incubated with the cells for 1 hour. Increasing concentrations of the N-syndecan peptide antigen in solution inhibit the mRNA-localizing effect of the antibodies. All values represent the mean ± s.d. (n=8). **P<0.01; ***P<0.001.

Localization of β-actin mRNA contributes to migratory mechanisms

The β-actin mRNA localization and the ensuing local synthesis of the protein determine cell polarity and increase cell migration in chicken embryonic fibroblasts (Kislauskis et al., 1994, 1997). Cell migration plays a fundamental role in many biological phenomena. For example, in metastasis, tumor cells migrate from the initial tumor mass through the basement membranes to the circulation system and to the target tissues. The mechanism of migration involves a combination of different phenomena. The first steps, morphological polarization and membrane extension with formation and stabilization of attachments, are often coupled to local actin polymerization (for a review, see Lauffenberg and Horowitz, 1996). Therefore, newly translated monomers could provide a pool of actin to locally polymerize new actin filaments. HB-GAM enhances migratory responses in neurons (for a review, see Rauvala and Peng, 1997) and also in C6 glioma cells. The fact that HB-GAM can control the distribution of the β-actin mRNA within the cell indicates that redistribution of cell motility-associated mRNA(s) is one mechanism in the suggested function of HB-GAM as a molecule controlling process extension and guidance (Rauvala, 1989; Rauvala et al., 1994).

Mechanisms of synaptic plasticity, like LTP (long-term potentiation), are thought to involve reorganization of neuronal connections that depends on extension of cytoskeletal structures (for a review, see Edwards, 1995). We have recently implicated HB-GAM in the mechanism of LTP (Lauri et al., 1996, 1998). Whether the role of HB-GAM in LTP is related

to its mRNA-localizing activity is an interesting notion that warrants further studies. It is reasonable that local synthesis of cytoskeletal structures in synaptic areas might be an efficient way to reorganize neural connections during stabilization of LTP.

β-actin mRNA can be localized through different transmembrane components

Our results show that HB-GAM, laminin and fibronectin are able to regulate the localization of β-actin mRNA. Nevertheless, HB-GAM has the strongest effect on this regulation, as seen by the quantitative analysis of mRNA signal around beads. The ability to cluster transmembrane components under the beads might be more efficient for HB-GAM than for laminin or fibronectin in short assays used in the study.

Moreover, soluble heparin or heparitinase have no significant effects on the mRNA-localizing activity of laminin, although they strongly inhibit the effect of HB-GAM. These extracellular components therefore act through different transmembrane mechanisms. Laminin and fibronectin might act through integrin receptors and HB-GAM through N-syndecan.

The findings that anti-N-syndecan antibodies can specifically localize β-actin mRNA and that the HB-GAM-induced mRNA localization is inhibited by heparitinase, heparin and soluble N-syndecan support the inference that N-syndecan is able to act as a transmembrane receptor in mRNA localization. Furthermore, N-syndecan is specifically isolated as an HB-GAM-binding protein from C6 cells (A. Kinnunen and H. Rauvala, unpublished results), in a manner that was previously shown in the HB-GAM receptor isolation from brain (Raulo et al., 1994).

It has been suggested that syndecans, including N-syndecan, have a role in organizing the cytoskeleton upon binding to extracellular matrix (Bernfield et al., 1992; Carey, 1997). Localization of N-syndecan to the surface of the cell soma and the cell processes, including the filopodia and the varicosities, during contact with an HB-GAM-containing matrix (Raulo et al., 1994; Kinnunen et al., 1998a) is compatible with this view. Recent biochemical and cell-biological data suggests that the intracellular moiety of N-syndecan binds a complex of cytosolic proteins, including c-src, fyn, cortactin and a still unidentified 30 kDa protein (Kinnunen et al., 1998a). Furthermore, binding of HB-GAM to N-syndecan increases the phosphorylation of c-src and cortactin, suggesting that N-syndecan acts as an HB-GAM receptor by modulating the cytoskeleton via the src kinase/cortactin pathway.

Like the neurite outgrowth-promoting effect of HB-GAM (Kinnunen et al., 1998a), the mRNA-localizing activity is also significantly inhibited by PP1, a specific inhibitor of the src-family tyrosine kinases (Hanke et al., 1996). Induction of src-kinase activity by HB-GAM via N-syndecan might lead to phosphorylation of proteins required for the localization of the β-actin mRNA. Ross et al. (1997) have recently isolated a complex of proteins responsible for the localization of β-actin mRNA. Some of them possess a phosphorylation site.

It is also noteworthy that, in addition to the src-kinase, cortactin and the 30 kDa protein, tubulin has been found as an intrinsic component in the protein complex bound to the cytosolic tail of N-syndecan (Kinnunen et al., 1998a). In addition, β3 tubulin and N-syndecan co-localize in early axon pathways in brain (Kinnunen et al., 1998b). Therefore, microtubules associated to the cytosolic tail of N-syndecan may act as an mRNA-transporting filament structure. The effect of colchicine indeed suggests that an intact microtubule network is required in N-syndecan-mediated mRNA localization. It is also known that in cells a fraction of tubulin associates with the plasma membrane and can act as a major substrate of the src-kinase (Matten et al., 1990; Abu-Amer et al., 1997).

In conclusion, this study shows that a local cue of a cell matrix-associated protein is able to control the β-actin mRNA localization. Therefore, local protein translation and actin elongation in response to a local extracellular cue may be an important mechanism to enhance and guide migratory responses of cells.

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REFERENCES

Abu-Amer, Y, Ross, F. R., Schlesinger, P., Tondravi, M. M. and Teitelbaum, S. L. (1997). Substrate recognition by osteoclast precursors induces c-src/microtubule association. *J. Cell Biol.* **137**, 247-258.

Ainger, K., Avossa, D., Morgan, F., Hill, S. J., Barbossa, E. and Carson, J. H. (1993). Transport and localization of exogenous myelin basic protein mRNA microinjected into oligodendrocytes. *J. Cell Biol.* **123**, 431-441.

Bassell, G. and Singer, R. H. (1997). MRNA and cytoskeletal filaments. *Curr. Opin. Cell Biol.* **9**, 109-115.

Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L. and Lose, E. J. (1992). Biology of the syndecans: A family of transmembrane heparan sulfate proteoglycans. *Annu. Rev. Cell Biol.* **8**, 365-393.

Carey, D. J., Evans, D. M., Stahl, R. C., Asundi, V. K., Conner, K. J., Garbes, P. and Cizmeci-Smith, G. (1992). Molecular cloning and characterization N-syndecan, a novel transmembrane heparan sulphate proteoglycan. *J. Cell Biol.* **117**, 191-201.

Carey, D. J. (1997). Syndecans: multifunctional cell surface co-receptors. *Biochem J.* **327**, 1-16.

Edwards, F. A. (1995). LTP- A structure model to explain the inconsistencies. *Trends Neurosci.* **18**, 250-255.

Ferrandon, D., Elphick, L., Nusslein-Volhard, C. and St. Johnston, D. (1994). Staufen protein associates with the 3′UTR of bicoid mRNA to form particles that move in a microtubule-dependent manner. *Cell* **79**, 1221-1232.

Forristall, C., Pondel, M. and King, M. (1995). Patterns of localization and cytosketal association of two vegetally localized RNAs. *Development* **121**, 201-208.

Hanke, J. H, Gardner, J. P., Dow, R. L., Changelian, P. S., Brissette, W. H., Weringer, E. J., Pollok, B. A. and Connelly, P. A. (1996). Discovery of a novel, potent, and src family-selective tyrosine kinase inhibitor. *J. Biol. Chem.* **271**, 695-701.

Hill, M. A. and Gunning, P. (1993). Beta and gamma actin mRNAs are differentially located within myoblasts. *J. Cell Biol.* **122**, 825-832.

Hill, M. A., Schedlich, L. and Gunning, P. (1994). Serum-induced transduction determines the peripheral location of β-actin mRNA within the cell. *J. Cell Biol.* **5**, 1221-1230.

Hoock, T., Newcomb, P. and Herman, I. M. (1991). Beta actin and its mRNAs are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury. *J. Cell Biol.* **112**, 653-664.

Kinnunen, T., Raulo, E., Nolo, R., Maccarana, M., Lindahl, U. and Rauvala, H. (1996). Neurite outgrowth in brain neurons induced by heparin-binding growth-associated molecule (HB-GAM) depends on the

specific interaction with heparan sulfate at the cell surface. *J. Biol. Chem.* **271**, 2243-2248.

Kinnunen, T., Kaksonen, M., Saarinen, J., Kalkkinen, N., Peng, H. B. and Rauvala, H. (1998a). Cortactin-src kinase signalling pathway is involved in N-syndecan-dependent neurite outgrowth. *J. Biol. Chem.* **273**, 10702-10708.

Kinnunen, A., Kinnunen, T., Kaksonen, M., Nolo, R., Panula, P. and Rauvala, H. (1998b). N-syndecan and HB-GAM (heparin-binding growth-associated molecule) associate with early axonal tracts in the rat brain. *Eur. J. Neurosci.* **10**, 635-648.

Kislauskis, E. H., Li, Z., Singer, R. H. and Taneja, K. L. (1993). Isoform-specific 3′-untranslated sequences sort alpha-cardiac and beta-cytoplasmic actin messenger RNAs to different cytoplasmic compartments. *J. Cell Biol.* **123**, 165-172.

Kislauskis, E. H., Zhu, X. and Singer, R. H. (1994). Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J. Cell Biol.* **127**, 441-451.

Kislauskis, E. H., Zhu, X. and Singer, R. H. (1997). β-actin messenger RNA localization and protein synthesis augment cell motility. *J. Cell. Biol.* **136**, 1263-1270.

Kloc, M. and Etkin, E. D. (1994). Delocalization of Vg1 mRNA from the vegetal cortex in Xenopus after destruction of Xlsirt RNA. *Science* **65**, 1101-1103.

Lathman, V. M., Kislauskis, E. H., Singer, R. H. and Ross, F. A. (1994). β-actin mRNA localization is regulated by signal transduction mechanisms. *J. Cell Biol*. **126**, 1211-1219.

Lauffenberg, D. A. and Horwitz, A. F. (1996). Cell migration: a physically integrated molecular process. *Cell* **84**, 359-369.

Lauri, S. E., Taira, T., Kaila, K. and Rauvala, H. (1996). Activity-induced enhancement of HB-GAM expression in rat hippocampal slices. *NeuroReport* **7**, 1670-1674.

Lauri, S. E., Rauvala, H., Kaila, K. and Taira, T. (1998). Effect of heparin-binding growth-associated molecule (HB-GAM) on synaptic transmission and early LTP in rat hippocampal slices. *Eur. J. Neurosci*. **10**, 188-194.

Lawrence, J. B. and Singer, R. H. (1986). Intracellular Localization of Messenger RNAs for cytoskeletal proteins. *Cell* **45**, 407-415.

Matten, W. T., Aubry, M., West, J. and Maness, P. (1990). Tubulin is phosphorylated at tyrosine by $pp60^{c\text{-}crc}$ in nerve growth cone membranes. *J. Cell Biol.* **111**, 1959-1970.

Murray, M. T., Krohne, G. and Franke, W. W. (1991). Different forms of soluble cytoplasmic mRNA binding proteins and particles in Xenopus laevis oocytes and embryos. *J. Cell Biol*. **112**, 1-11.

Nudel, U., Zadut, R., Neuman, S., Levy, Z. and Yaffe, D. (1983). The nucleotide sequence of the rat cytoplasmic β-actin gene. *Nucl. Acids Res*. **11**, 1759-177.

Nolo, R., Kaksonen, M., Raulo, E. and Rauvala H. (1995). Co-expression of heparin-binding growth associated molecule (HB-GAM) and N-syndecan (syndecan-3) in developing rat brain. *Neurosci. Lett*. **191**, 39-42.

Raulo, E., Julkunen, I., Merenmies, J., Pihlaskari, R. and Rauvala, H. (1992). Secretion and biological activities of heparin-binding growth-associated molecule. *J. Biol. Chem*. **267**, 11408-11416.

Raulo, E., Chernousov, M. A., Carey, D. J., Nolo, R. and Rauvala H. (1994). Isolation of neuronal cell surface receptor of Heparin Binding Growth-associated Molecule (HB-GAM). Identification as N-syndecan (syndecan-3). *J. Biol. Chem*. **269**, 12999-13004.

Rauvala, H. (1989). An 18-kD heparin-binding protein of developing brain that is distinct from fibroblastic growth factors. *EMBO J.* **8**, 2933-2941.

Rauvala, H., Vanhala, A., Castren, E., Nolo, R., Raulo, E., Merenmies, J. and Panula, P. (1994). Expression of HB-GAM (Heparin-binding growth-associated molecule) in the pathways of developing axonal processes in vivo and neurite outgrowth in vitro induced by HB-GAM. *Dev. Brain Res.* **79**, 157-176.

Rauvala, H. and Peng, H. B. (1997). HB-GAM and heparin-type glycans in the development and plasticity of neuron-target contacts. *Prog. Neurobiol.* **52**, 127-144.

Ross, A. F., Oleynikov, Y., Kislauskis, E. H., Taneja, K. L. and Singer, R. H. (1997). Characterization of a β-actin mRNA zipcode-binding protein. *Mol. Cell. Biol*. **17**, 2158-2165.

St Johnston, D. (1995). The intracellular localization of messenger RNAs. *Cell* **81**, 161-170.

Sundell, C. L. and Singer, R. H. (1991). Requirement of microfilaments in sorting of actin messenger RNA. *Science* **253**, 1275-127.

Wang, S. and Hazelrigg, T. (1994). Implications for bcd mRNA localization from spatial distribution of exu protein in *Drosophila* oogenesis. *Nature* **369**, 400-403.

Wilhelm, J. E. and Vale, R. D. (1993). RNA on the move: the mRNA localization pathway. *J. Cell Biol*. **123**, 269-274.

Wilkinson, D. G. (1992). Whole mount in situ hybridization of vertebrate embryos. In *In Situ Hybridisation: a Practical Approach* (ed. D. G. Wilkinson), pp. 75-83. IRL Press, Oxford.

Yaffe, D., Nudel, U., Mayer, Y. and Neuman, S. (1985). Highly conserved sequences in the 3´ untranslated region of mRNAs coding for homologous proteins in distantly related species. *Nucl. Acids Res*. **13**, 3723-3737.