We identified a 46-kDa ERK, whose kinetics of activation was similar to that of ERK1 and ERK2 in most cell lines and conditions, but showed higher fold activation in response to specific stimuli and evidenced growth factor- and stress-activated signaling pathways. Unlike ERK2, examined here in various cell lines, ERK1b was detected only in some of the tissues examined and seems to be abundant in the rat and human heart. Interestingly, in Ras-transformed Rat1 cells, the ERK1b isoform that responds to exogenous stimulation in Ras-46-kDa ERK isoform, which seems to be the major ERK precipitated by the antibodies against ERK1b together with MEK1 or by lack of coimmunoprecipitation of the two proteins. Thus, ERK1b is a novel ERK isoform that responds to exogenous stimulation in Ras-transformed cells probably due to its differential regulation by MEK.

Central components in the signaling machinery of many extracellular stimuli are the mitogen-activated protein kinase cascades (MAPKs). Each of these signaling cascades consists of three to five tiers of protein kinases that sequentially activate each other by phosphorylation (1–4). Four distinct MAPK cascades, which are currently known to operate in mammals, cooperate to transmit signals from the plasma membrane to intracellular targets and, thereby, regulate proliferation, differentiation, stress responses, and other cellular processes. The first MAPK cascade that was elucidated is the extracellular signal-regulated kinases (ERK; Ref. 5) cascade. Upon mitogenic stimulation, this cascade is initiated by activated Ras, which in turn causes a sequential activation of Raf1, MEK, ERK, RSK, and under some conditions, ERK2, which in turn causes a sequential activation of GSK3 (1) to eventually induce proliferation. Beside its mitogenic properties, ERK1 and ERK2 are 44- and 42-kDa MAPKs, respectively, that reside in a TEY phosphorylation motif (11, 12), and phosphorylate substrates in the nucleus (e.g. Elk1 (18, 19), or transmit the signal to the MAPKAPK level. The main MAPKAPK of the ERK cascade is RSK (20), which, upon activation, can phosphorylate a set of substrates that are different from those phosphorylated by ERK. Other MAPKAPKs of the ERK cascade are MNK and MSK, but these enzymes can also be activated by p38 MAPK (21, 22).

The signaling mechanism of ERK involves a change in its subcellular localization. In resting cells, ERK appears to be localized primarily in the cytosol, where a fraction is attached to cytoskeletal elements (23). However, the authors have full confidence in the findings and conclusions of this paper.
conditions the 105-kDa ERK5, the DP-ERK detected a band with a molecular mass of 46 kDa. This 46-kDa band might be related to a 46-kDa band previously detected with anti-C-terminal domain of ERK-Ab and tentatively termed ERK4 (28). Herein we report the cloning of this 46-kDa ERK, that turned out to be an alternatively spliced form of ERK1 and therefore was termed ERK1b. This ERK isoform is abundant mainly in rat tissues, and lesser amounts were detected in other organisms, including human. Although ERK1b was readily activated by MEK, we found that its activation did not always parallel that of ERK1, possibly due to different subcellular localization of ERK1b, which could modify the mode of its regulation. This was apparent primarily in Ras-transformed Rat1 cells where ERK1b responded differently than ERK1 and ERK2 to exogenous stimulation, suggesting that ERK1b is particularly important for the transmission of signals via the ERK cascade under conditions where ERK1 and ERK2 are under tight phosphatase regulation.

MATERIALS AND METHODS

Buffers—Buffer A contains 50 mM β-glycerophosphate, pH 7.3, 1.5 mM EGTA, 10 mM sodium vanadate, 1 mM sodium metavanadate, Buffer H (homogenization buffer) contains 50 mM β-glycerophosphate, pH 7.3, 1.5 mM EGTA, 1 mM dithiothreitol, 0.1 mM sodium vanadate, 1 mM benzamidine, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 2 μg/mL pepstatin A. Buffer R (reaction mixture at 3-fold final concentration) contains 30 mM MgCl2, 4.5 mM dithiothreitol, 75 mM β-glycerophosphate, pH 7.3, 0.15 mM sodium vanadate, 3.75 mM EGTA, 30 μM calmodulin, and 2.5 mg/mL bovine serum albumin.

Preparation of Cell Extracts and Western Blotting—Cells were grown to subconfluence and were then serum-starved for 18 h in Dulbecco-modified Eagle’s medium containing 0.1% fetal calf serum, and then were exposed to various stimuli for variable amount of time. The extracts were centrifuged, and the supernatants were further kept at 4 °C. The extracts were subjected to Western blotting or used for analysis of ERK1,2 and ERK1b activities we used anion exchange column as described previously (24, 25). For determination of ERK activity, the supernatants were washed once with 0.5 M LiCl, twice with radiolabeled phosphosoribosylphosphate carrier protein (Pierce), and once with buffer A as described previously (29).

Immunoprecipitation—Immunoprecipitates of ERK1 and ERK1b proteins were mixed with either MBP (8.4 μg), Elk1 (1 μg, NEB), or RSK (immunoprecipitated with RSK Abs (Sigma, Rehovot, Israel)) from resting EJ cells) and Buffer R that contained 100 μM [γ-32P]ATP (1–2 cpm/fmol) in a final volume of 30 μL. The phosphorylation reaction was allowed to proceed at 30 °C for 15 min and terminated by sample buffer followed by boiling for 5 min. Phosphorylated proteins were assayed by SDS-PAGE and autoradiography or Western blotting with DP-ERK for phosphorylated ERK. To separate between ERK1,2 and ERK1b activities we used anion exchange column as described previously (7). Briefly, cell extracts (0.75 mL, 0.5 mg) were loaded on DE52 columns (0.4 mL), and the flow-through fraction, containing ERK1b activity, was collected. After wash (1 mL × 3) with Buffer A + 0.02 mM NaCl the ERK1,2 activity were eluted with 1 mL of Buffer A + 0.22 mM NaCl. The ERKs were then immunoprecipitated with ERK1-Ab (C-16) and subject to in vitro phosphorylation as above.

In Vitro Activation of ERKs—For in vitro activation of ERKs Extracts from transformed COS7 cells were subjected to immunoprecipitation with anti-HA-Ab. The HA-ERKs proteins, attached to the beads, were mixed with ΔN-EE-MEK recombinant protein (Sigma, Rehovot, Israel) and Buffer R that contained 100 μM [γ-32P]ATP (1–2 cpm/fmol) at 30 °C. After 15 min, MBP (8.4 μg) was added (final volume of 30 μL), and the phosphorylation was allowed to continue for an additional 15 min. The reaction was terminated and assessed as described above.

Localization Studies—Localization of ERK1b and HA-ERK1b together with MEK1. Immunofluorescence studies were performed as described previously (30). Briefly, 24 h after transfection, the cells were starved (0.1% fetal calf serum, 24 h), and stimulated (15 min, 37 °C) with VOOH, fixed (3% paraformaldehyde), permeabilized (0.2% Triton X-100, 5 min), washed with phosphate-buffered saline, and stained with polyclonal anti-HA-Ab (diluted 1:100; Santa Cruz Biotechnology) and rhodamine-conjugated goat-anti-rabbit Ab (diluted 1:100; Jackson ImmunoResearch). Staining was visualized using a Zeiss fluorescence microscope.
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Results

Phosphorylation of p46 ERK in Several Cell Lines — Mitogenic signaling in Rat1 cells was examined using the DP-ERK, which specifically recognizes the dually phosphorylated TEY motif in the activation loop of ERKs. In Western blot analysis of cell extracts derived from serum-starved Rat1 cells, the Ab detected a faint band of 42 kDa in addition to the expected bands of 42-kDa ERK2 and 44-kDa ERK1 (Fig. 1A). Stimulation of the cells with either EGF or TPA resulted in an enhanced staining of the 46-kDa band, with kinetics similar to those of ERK1 and ERK2. The reactivity of all three bands with DP-ERK was reduced when the Rat1 cells were pretreated with the specific MEK inhibitor PD98059 prior to stimulation. When the antigenic peptide, used to generate the Ab, was added to the immunoblots, all three bands disappeared (data not shown), which confirmed that the staining of the 46-kDa band by DP-ERK was specific. The 46-kDa band was also recognized by Abs directed against the C and N terminus of ERK1, against subdomain XI of ERK1 (weaker staining), and against the C terminus of ERK2 (weaker staining; data not shown). In contrast, Ab directed against JNK1, which is a 46-kDa MAPK isoform, did not recognize this 46-kDa band (data not shown). Thus, the 46-kDa band appears to represent a genuine, TEY-containing, ERK isoform, which is phosphorylated by MEKs. This band may be similar to the poorly characterized ERK4, which was previously detected in rat (28, 31)- and human (32)-derived tissue culture cells.

A 46-kDa ERK appeared to be a major phosphorylated isoform of ERK in Rat1 cells, but its expression and activation in other cell lines had to be examined. Thus, in the rat-derived PC12 cell line, typical transient and sustained kinetics (33) of the 46-kDa ERK as well as of ERK1 and ERK2, were observed when the cells were stimulated with nerve growth factor and EGF (Fig. 1B). A sustained activation was observed for ERK1, ERK2, and the 46-kDa ERK also in FcERI-induced rat mucosal-type mast cells (RBL-2H3 line) and in heat-shocked rat glia cells (data not shown). In addition, a lesser amount of the 46-kDa ERK, which had kinetics similar to those of ERK1 and ERK2, was detected also in several other cell lines derived from mouse, calf, and guinea pig (data not shown), as well as in human breast cancer-derived MCF-7 cells where all three apparent ERKs (ERK1, ERK2, and p46 ERK) were significantly phosphorylated upon stimulation with okadaic acid (an inhibitor of protein serine/threonine phosphatases 1 and 2a; Fig. 2, A, C, and E). Thus, 46 kDa ERK is present mainly in malignant cells in slightly lower amounts.

In several cell cultures, the phosphorylation of the 46-kDa ERK varied significantly from its counterpart kinases. However, in some instances, the phosphorylation of the 46-kDa ERK was similar to that of ERK1 and ERK2. For example, in transformed Rat1 cells (EJ), the phosphorylation of ERK1 and ERK2 was reduced at 5–10 min and then increased at up to 60 min after the application of an osmotic shock to the cells (Fig. 2, A and B). On the other hand, the kinetics of the 46-kDa ERK phosphorylation increased shortly after the osmotic shock, peaked at 30 min, and declined thereafter. Similarly, the kinetics of the 46-kDa ERK's phosphorylation was different from that of ERK1 and ERK2 also upon EGF stimulation of the same EJ cells (Fig. 2, C and D), as well as in MAFa (34)-stimulated mast cells (data not shown). To confirm that the 46-kDa ERK is regulated differentially from ERK1 and ERK2, we separated activated forms of ERK1 and ERK2 from the activated form of the 46-kDa ERK on a small bed of DE52 resin (7). While the 46-kDa ERK eluted in the flow-through, active ERK1 and ERK2 were retained on the resin and could be eluted with 0.2 M NaCl. As expected from the result with the total extracts above, staining of the flow-through fractions with anti-active ERK antibodies revealed a 5–6-fold increase in the 46-kDa ERK staining after NaCl and EGF stimulation, without a change in the total amount of the 46-kDa protein (Fig. 2E, left). On the other hand, staining of the 0.2 M NaCl eluted fractions from the same columns showed that the staining of ERK1 and ERK2 was reduced after NaCl treatment and not significantly changed upon EGF stimulation, without a change in the total amount of proteins in these fractions (Fig. 2E, right). We then studied the catalytic activities of the ERKs by immunoprecipitating the ERK in the various fraction followed by an in vitro kinase assay using MBP as a substrate. The results obtained with this procedure demonstrated a direct correlation to the results obtained with the antibodies above (Fig. 2F), confirming the differential activation of the 46-kDa proteins in EJ cells. Therefore, these data indicate that although the mode of ERK1, ERK2, and the p46...
ERK regulation is usually similar, a differential mode of p46 ERK regulation may also exist. This deferential regulation may be due to differences either in the phosphorylation or dephosphorylation processes, which may both culminate in the differential kinetics of phosphorylation observed under some conditions.

FIG. 2. Kinetics of ERK phosphorylation and activation upon osmotic shock or EGF treatment of EJ cells. EJ cells (7 × 10⁵ cells/6-cm plate) were serum-starved for 16 h and then stimulated with either 0.7 M NaCl (A) or with 50 ng/ml EGF (B) for the indicated times. Extracts (50 μg) were analyzed by immunoblotting with DP-ERK (DP) and anti-C terminus of ERK-Ab (C16). B and D, quantitative determination of the results in A and C. Phosphorylation of ERK2 (●), ERK1 (▲), and p46 ERK (■) was calculated as the intensity of staining with DP-ERK divided by the intensity of staining with C16. This is a representative experiment that was reproduced four times. E and F, EJ cells were serum-starved and then stimulated (+) for 10 min with either 0.7 M NaCl (NaCl), 50 ng/ml EGF (EGF), or left untreated (−). ERK1 and ERK2 were separated from p46 ERK on an anion exchange column as described above. ERK activity in the different fractions was analyzed by immunoblotting with DP-ERK (DP in E) as compared with the staining by anti-C terminus of ERK-Ab (C16 in E) or by immunoprecipitation with ERK-Ab (C16) followed by kinase assay with MBP as a substrate (F). The results in E and F were reproduced four times.

ERK regulation is usually similar, a differential mode of p46 ERK regulation may also exist. This deferential regulation may be due to differences either in the phosphorylation or dephosphorylation processes, which may both culminate in the differential kinetics of phosphorylation observed under some conditions.

**ERK1b:** Cloning, Purification, and Preparation of Antibody—The presence of p46 ERK in a variety of cell types and its
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Differential regulation in transformed cells, prompted the in-depth study of this protein by molecular biology. Since p46 ERK was recognized by several Abs, directed to various regions of ERK1, sense and antisense oligonucleotide primers derived from the sequence of ERK1 were used for RT-PCR with an RNA template obtained from EJ cells. With one of these primer pairs, two amplified products were obtained (Fig. 3A): the expected ~300-bp band (ERK1) and another of ~400 bp. Both products were cloned and sequenced. The ~300-bp product corresponded to the sequence of ERK1; sequencing of the upper product revealed a unique 78-bp sequence flanked by sequences identical to those of the rat ERK1 (Fig. 3B). Using RT-PCR with oligonucleotides derived from the unique 78-bp insert, oligonucleotides derived from the 5′- and 3′-ends of the rat ERK1, and RNA from EJ cells as a template, we cloned the full-length cDNA of this ERK. This full-length cDNA contained the 78-bp inserted between bases 1020 and 1021 of ERK1, and its sequence, except for the insertion, was identical to that of ERK1 (Fig. 3C), which indicates that this is an alternative spliced form of ERK1, and was therefore termed ERK1b. The alternative splicing probably occurs at the end of exon 7 of the ERK1 gene as this exon ends with a sequence corresponding to the site of insertion (35). The 78-bp insertion encodes 26 amino acids (Fig. 3C), which are localized between Glu-340 and Pro-341 of the rat ERK1 in a large loop (L16) between two α helices (αi and αL16) just C-terminal to the conserved core of the kinase domain (Fig. 3B). The sequence of insertion did not exhibit significant homology with any known protein or cDNA in the database, and no particular protein motif could be attributed to this sequence. Thus, in rat cells, besides ERK1 and ERK1psi (5), an additional mRNA from EJ cells that encodes for a putative protein kinase with a calculated molecular mass of 45.6 kDa.

Whether the p46 ERK is indeed encoded by the ERK1b mRNA was determined by transfecting COS7 cells. EJ tumors, induced in nude mice, were excised and lysed in the presence of proteinase and phosphatase inhibitors. The cytosolic fraction obtained was subjected to anion exchange chromatography on a Resource Q column, in which the p46 ERK did not bind and was eluted in the flow-through, whereas the ERK1 and ERK2 bound and eluted with 0.1 M NaCl (Fig. 4A). Two bands that contained the p46 ERK were applied to an affinity column that consisted of the anti-C terminus of ERK Ab bound to agarose. After the column was extensively washed, the bound proteins were eluted at high pH (Fig. 4B). This protocol resulted in ~4000-fold purification of a 46-kDa protein that reacted with anti-ERK-Ab (Fig. 4C) and was about 70% pure. The purified 46-kDa protein was excised from the SDS gel, digested with trypsin, and the resulting peptides analyzed by mass spectrometry and when necessary also Edman degradation. Eight peptides were recovered from the main protein in the band, seven of them were identical to those expected from ERK1, including a peptide containing the exact sequence around and including the TEY motif of ERK1. The other peptide that was obtained corresponded to the sequence GISVPVSR (part of the predicted protein sequence of the 78-bp insert of ERK1b), which indicated that the isolated 46-kDa band is, at least in part, the cloned ERK1b protein.

The identity of the 46-kDa band was further confirmed using a specific polyclonal Ab that was generated against the ERK1b insert. This Ab, termed 4086, recognized two bands in a cytosolic extract of EJ cells (Fig. 5), one of which migrated to the same location as the 46-kDa protein recognized by the anti-C-terminal of ERK-Ab (Fig. 5). The staining of the 46-kDa band, but not of the 30-kDa band, was competitively inhibited by the antigenic peptide (data not shown), which verified the specificity of the Ab. This Ab was used to assess the overexpression of the translation product of ERK1b in COS7 cells. A HA tag was fused to the N-terminal portions of ERK1 and ERK1b, and these constructs were transfected into COS7 cells, which were examined for ERK1b protein content using Ab 4086. In mock-transfected COS7 cells, no 46-kDa protein could be detected by Ab 4086. However, this Ab clearly recognized the expected 48-kDa band (ERK1b with HA tag) in the transfected cells, which was also recognized by an anti-HA-Ab and was retarded on SDS-PAGE upon VOOH stimulation of the COS7 cells. Thus, Ab 4086 recognizes both endogenous and recombinant ERK1b and therefore provides further evidence that ERK1b is indeed the p46 ERK.

Functional Characterization of ERK1b—The functional characteristics of ERK1b were studied by transfecting COS7 cells with HA-ERK1b, HA-ERK1, and an empty vector. Protein extracts obtained from serum-starved, transfected cells exposed to EGF, VOOH, or buffer were subjected to immunoprecipitation with anti-HA-Ab. After extensive washing to remove...
impurities, the precipitated proteins were subjected to Western blotting with DP-ERK and anti-HA-Ab or to a kinase assay with MBP as a substrate. Two days after transfection, the amount of HA-ERK1b produced in COS7 cells was usually less than that of ERK1 (Fig. 6A). However, the basal state of the specific activation (phosphorylation of the TEY motif per amount of HA-ERK and specific activity (phosphorylated MBP per amount of HA-ERK) of HA-ERK1b in the transfected cell was somewhat higher than those of HA-ERK1 (ERK1b and ERK1), which were immunoprecipitated from EGF- or VOOH-stimulated transfected COS7 cells. Two days after transfection, the phosphorylation of the ERK1b as compared with ERK1 and ERK2 (Fig. 8, B and D) was significantly lower than the stimulation of ERK1 phosphorylation in Ras-transformed Rat1 cells (13.5-fold). The stimulation of ERK1b phosphorylation in Ras-transformed EJ cells was significantly higher (6–7-fold) than in Rat1 cells (C16, Fig. 8A). Similar elevation of expression of ERK1b was noticed also with other oncogenes and additional cell lines (data not shown). With DP-ERK, we observed that the basal TEY phosphorylation of ERK1 and ERK2 as well as ERK1b was slightly higher in Rat1 than in EJ cells (Fig. 8A). However, similarly to the stimulation by EGF and NaCl (Fig. 2), TPA stimulation led to similar differences in the extent of phosphorylation of ERK1, ERK2, and ERK1b compared with ERK1 and ERK2 (Fig. 8, C and D). The stimulation of ERK1-TEY phosphorylation reached (up to 6-fold, Fig. 8B) and the detection of ERK1b phosphorylation was higher than that of ERK1 and ERK2 in both transfected mammalian cell lines (12.5-fold). To verify the results obtained with these methods (Fig. 8, A and B), the anti DP-ERK antibodies (Fig. 3C) were used. The results clearly indicate a differential regulation of ERK1b in EJ and Rat1 cells. Thus, our results clearly indicate a differential regulation of ERK1b in EJ cells and may suggest that in transformed cells, ERK1b is the major ERK isoform to respond to exogenous stimulation and thereby allow the physiological responses of transformed cells.

**MEK-dependent Subcellular Localization of ERK1b**—A possible cause for the differential regulation of ERK1b could be a different subcellular localization. Therefore, the subcellular localization of ERK1b was examined in CHO cells that had been transfected with either HA-ERK1 or HA-ERK1b. Anti-HA-Ab revealed that before and after stimulation of the cells, most of the transfected proteins were localized in the nucleus without significant differences between them (data not shown). This observation is in agreement with the localization of ERK in the nucleus when overexpressed in various cells (24, 25). Since overexpression of MEK results in cytoplasmic retention of ERK (24, 25), both HA-ERK1 and HA-ERK1b were coexpressed in CHO cells, together with wild type MEK1. As reported previously for ERK2 (25), in resting cells, HA-ERK1 was localized primarily in the cytoplasm (~90%), but upon stimulation with VOOH, a large amount of it was translocated into the nucleus (Fig. 9). In contrast, in resting cells HA-ERK1b was already localized primarily in the nucleus (70%) and upon stimulation became even more nuclear (80%, Fig. 9A). Therefore, the 26-amino acid unique sequence of ERK1b might be involved in
disruption of the MEK-dependent cytoplasmic localization of ERK1b in resting cells. A plausible explanation for this is that the 26-amino acid insertion changes the conformation of the neighboring MEK-dependent cytoplasmic localization sequence (residues 312–320 of ERK2) that was identified by our laboratory (25). To further verify the differential MEK-dependent localization of ERK1b, we examined the physical association of ERK1 and ERK1b with wild type MEK. As reported before for ERK2 and Xenopus ERK (8, 25), ERK1 was associated with MEK1 in resting cells and the interaction was prevented upon stimulation (Fig. 9B). However, ERK1b did not significantly interact with MEK1 even in resting cells (Fig. 9B), supporting the idea that the 26-amino acid insertion interferes with the MEK-induced cytosolic anchoring of ERK1, causing the constitutive nuclear localization of ERK1b. This differential localization may cause a differential regulation of ERK1b by phosphatases and brings about its higher response to stimuli in transformed cells.

**DISCUSSION**

The ERK cascade is involved in diverse physiological processes that result in different outcomes. Thus, elucidation of the mechanism by which different signals can be transmitted by similar signaling cascades, but still evoke different downstream outcomes, is important. Several mechanisms for the specificity of the ERK cascade have been proposed. For example in PC12 cells the duration and strength of ERK activation seem to be important for specificity (33). In these cells, transient activation of ERK results in proliferation, whereas a sustained activation causes differentiation. Other proposed mechanisms for determining specificity are (i) compartmentalization, which can be achieved by association with either cytoskeletal elements (23) or scaffold proteins (36), and may direct signals to the correct destination; (ii) cross-talk with different signaling pathways (37), which modifies the signals in the ERK cascade; (iii) differential intensities of signals (38).
**ERK1β, an ERK Isoform with Unique Regulation**

Fig. 8. ERK1β is differentially regulated by TPA in EJ cells. A, EJ and Rat1 cells (~5 x 10⁶ cells/6-cm plate) were serum-starved for 16 h and then stimulated (15 min) with TPA (250 nm) or left untreated (Basal). The cells were then harvested, and 50 μg cytosolic and nuclear extract of each treatment were subjected to SDS-PAGE and immunoblotting with either DP-ERK (DP) or anti-C terminus of ERK-Ab (C16). The position of ERK1β, ERK1, and ERK2 is indicated. B, quantitative determination of fold stimulation of ERK1 (denoted I) and ERK1β (denoted Ib). The intensity of the bands in A was determined by densitometer (model 690, Bio-Rad). Specific phosphorylation of ERK1 and ERK1β was calculated as the intensity of staining with DP-ERK of each of the band divided by the intensity of their counterpart band detected by the C16 antibody. Fold stimulation was calculated as the phosphorylation of TPA per basal phosphorylation and is the average of four independent experiments. Lightly shaded columns, Rat1 cells; dark shaded columns, ERK1.

Fig. 9. MEK-dependent localization of ERK1 and ERK1β. A, CHO cells were transfected with either HA-ERK1 or HA-ERK1β together with wild type or ERK1b (denoted Ib) or left untreated (Basal). The ERKs were visualized with monoclonal anti-HA-Ab and a fluorescent-labeling technique as described under “Material and Methods.” The lower panel is a similar in the cells examined (data presented three times). B, EJ cells were serum-starved for 16 h and then treated with VOOH (100 μM) or left untreated (Basal). The amount of phosphorylated MEK was found to be similar in the cells examined (data not shown). Moreover, the sequence of two peptides from the digested, purified p44 ERK differed slightly from the predicted sequence of ERK1β. Therefore, an ERK isoform of 46 kDa, distinct from ERK1b, may also exist in transformed rat cells. Ras transformation amplified the amount of ERK1β in a MEK-dependent manner (Fig. 2A). Oncogenic transformation was previously shown to influence alternative splicing of several signaling proteins (e.g. Ref. 45). This effect of oncogenic transformation might be due to enhanced phosphorylation in the transformed cells, since phosphorylation is known to regulate splicing (46) by affecting several steps of the splicing proc-
ERK1b, an ERK Isoform with Unique Regulation

ERK1 and ERK2. When overexpressed in mammalian cells (Fig. 8).

ERK1b appears to escape from the tight down-regulation in the latter suggests that these two isoforms (ERK1 and ERK2) as well as other transformed cells (49), constitutive activation of phosphorylation by a component of the ERK cascade is involved.

found that the amount of ERK1b is reduced by overexpression of the upstream components of the ERK cascade was not accompanied by a comparable activation of ERK1 and ERK2. The latter suggests that these two isoforms (ERK1 and ERK2) might be under tight down-regulation, and thus in EJ cells their fold stimulation by extracellular stimuli is low. However, ERK1b appears to escape from the tight down-regulation in the EJ cells and appears to be the major responsive ERK isoform (Fig. 8).

The subcellular distribution of ERK1b differs from that of ERK1 and ERK2. When overexpressed in mammalian cells Xenopus ERK, ERK2, and ERK1 are localized to the nucleus of resting cells (Refs. 24 and 25 and data not shown). This subcellular localization changes when MEK1 is overexpressed together with the ERKs, which results in mostly cytosolic distribution of the ERKs (Refs. 24 and 25 and Fig. 9). In contrast, ERK1b was not retained in the cytosol, even upon high-overexpression of MEK1 (Fig. 9). ERK1b also did not interact with the overexpressed MEK1. Recently, we identified a sequence (residues 312–320) of ERK2 that is responsible for its induced cytosolic retention (25). Changing these alanines resulted in a nuclear distribution of ERK1b in the presence of overexpressed MEK1. The sequence of ERK1b residues 312–320 of ERK2 is almost identical to residues 340 of ERK1, in the end of which a 26-amino acid insertion is localized. Therefore, this insertion probably affects the association of ERK1b with MKPs. Our findings are consistent with ERK1b being the major ERK isoform that responds to exogenous stimulation in Ras-transformed Rat1 cells.

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WITHDRAWN
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