Activation of Extracellular Signal-regulated Kinase 2 by a Novel Abl-binding Protein, ST5

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The human ST5 gene encodes three proteins with predicted molecular masses of 126, 82, and 70 kDa. These widely expressed proteins share a C-terminal region that bears significant sequence homology to a group of GDP/GTP exchange proteins for the Rab3 family of small GTP binding proteins. The N-terminal region of the largest ST5 protein, p126, contains two proline-rich sequences, PR1 and PR2, with consensus motifs similar to Src homology 3 (SH3) binding regions and to mitogen-activated protein kinase (MAPK) phosphorylation sites. Based on these properties, we sought to investigate the activity of ST5 proteins in signal transduction pathways. In vitro, p126 displayed preferential binding to c-Abl SH3, as compared with other SH3 domains. This interaction was mediated by the PR2 sequence. In vivo, expression of p126, but not p82 or p70, activated MAPK/ERK2 in response to EGF in COS-7 cells. Expression of c-Abl with p126 greatly enhanced this activity. Deletion of PR1 blocked the ability of p126 to activate ERK2. Deletion of PR2 did not affect the basal activity, but blocked the stimulatory effect of c-Abl. Whereas p82 expression had no effect on ERK2 activation by p126, p70 completely abrogated this activity. These observations suggest that ST5 can function as a signaling protein and can provide a link between c-Abl and ERK2.

The human ST5 gene, originally designated as HTS1 (1) was identified as a differentially regulated gene in a system of Hela/fibroblast somatic cell hybrids. This widely expressed gene was mapped proximal to the \( \beta \)-globin locus on chromosome 11 and is expressed as three mRNA species, 4.6, 3.1, and 2.8 kb \(^1\) in length (2, 3). These messages encode three predicted protein products, designated by their molecular masses as p126, p82, and p70. The expression of the ST5 gene was of interest because it correlated directly with cellular morphology and suppression of tumorigenicity in the Hela/fibroblast system as well as in several carcinoma cell lines (1). While the two larger mRNA species were expressed at similar levels in all cell lines examined, expression of the 2.8-kb message was specific for non-tumorigenic cells. In the HeLa/fibroblast system, the 2.8-kb message was detected in the non-tumorigenic hybrids but was undetectable or markedly reduced in their tumorigenic derivatives.

The structure of ST5 mRNAs has been previously characterized. The differentially regulated 2.8-kb message consists of the 3’ terminal sequence common to all three ST5 transcripts and derives from a promoter located within an intron of the ST5 gene. We previously identified and characterized an enhancer sequence within this promoter. This sequence contains one AP-1 and 5 YY1 sites that were shown to play a critical role in the differential regulation of the 2.8-kb message in the Hela/fibroblast system (4).

The 4.6- and 3.1-kb messages are alternatively spliced products sharing 5’ and 3’ ends but differing in that the 4.6-kb transcript has an additional 1260-nucleotide sequence located near its 5’ end. This 1260-nucleotide segment encodes a segment of the protein containing two proline-rich regions, PR1 and PR2, unique to p126. The specific organization of proline residues in this sequence raised the possibility of a function for this protein in signal transduction pathways.

Specific proline-rich sequences constitute consensus motifs that serve as binding sites for specific domains in signaling proteins or as substrates for certain protein kinases (5). The recognition site for Src homology 3 (SH3) domains consists of a proline-rich sequence with at least one PXXP core (6–9). SH3 domains are peptides 60–70 amino acids in length involved in protein-protein interactions leading to the activation of a wide variety of signal transduction pathways, including those leading to the activation of the MAP kinase family members ERK1, ERK2, and JNK(10–14). Characterization of the sequence requirements for binding to various SH3 domains has revealed that the amino acids surrounding the PXXP core participate in determining the specificity of these interactions. Ren et al. (8) identified a 10-amino acid proline-rich consensus sequence that mediates association between the c-Abl SH3 domain and a variety of interacting proteins. Since then, a number of SH3 binding proteins have been identified, including mSos, PI3K, p22phox, AAP1, RIN1, and FBPWW (15–19).

In this study, we present several lines of evidence supporting a role for the ST5 protein, p126, in the MAP kinase pathway leading to the activation of ERK2. First, analysis of the sequence of the protein now demonstrates a region of homology to guanine nucleotide exchange factors for small GTP binding proteins such as those involved in the activation of ERK2 by EGF. Co-expression of c-Abl with p126 results in a synergistic stimulation of ERK2 activity as compared with p126 or c-Abl alone. We show that while the proline-rich region PR1 is required for the activation of ERK2 by p126, the PR2 region mediates the stimulatory effect of the c-Abl/p126 complex in COS-7 cells.

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‡ The abbreviations used are: kb, kilobase(s); ERK2, extracellular signal regulated kinase 2, SH3, Src homology 3; MAPK, mitogen-activated protein kinase; aa, amino acid(s); PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase; MADD, MAP kinase-activating death domain protein; JNK, c-jun N-terminal kinase; EGFR, epidermal growth factor receptor; HA, influenza hemagglutinin.

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Last, we found that neither p82 nor p70 activates ERK2; however, p70 inhibits the activation of ERK2 by p126. p70 also interferes with ability of the c-Abl-p126 complex to activate ERK2 cooperatively. These observations support a model in which the ST5 proteins are novel components of a growth regulatory signaling pathway, possibly involving the c-Abl protein, linking EGF stimulation to the activation of ERK2.

EXPERIMENTAL PROCEDURES

Cell Lines and Tissue Culture—COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. 

ST5 Antiserum Preparation and Western Blotting—A polyclonal antiserum was raised against an 494–784 of the p126 sequence expressed as a TrpE fusion, following standard protocols (20). Additional characterization of this antiserum will be reported separately. For Western blots, the ST5 proteins were separated on 12% SDS-PAGE, transferred to nitrocellulose and probed with the ST5 antibody. The blot was incubated with protein A-peroxidase, washed three times with phosphate-buffered saline, and developed by chemiluminescence with ECL reagents (Amersham Pharmacia Biotech).

In Vitro Translation of ST5 Proteins—The 4.6-, 3.1-, and 2.8-kb ST5 cDNAs, cloned into the pcDNA3 vector, were in vitro transcribed and translated in the presence of [35S]methionine using the T7 in vitro transcription/translation kit (Promega) according to the specifications of the manufacturer. The translated gene products were analyzed by 12% SDS-PAGE and detected by autoradiography.

Mutagenesis and Plasmid Constructions—For use in expression studies, the 4.6-, 3.1-, and 2.8-kb ST5 cDNAs were cloned in the sense orientation into the pcDNA3 vector (Invitrogen). HA-ERK2, HA-JNK, and c-Abl cDNAs were generously provided by J. Silvio Gutkind (National Institutes of Health). Mutations were introduced directly into the 4.6 kb cDNA pcDNA3 plasmid using the Unique Site Elimination Mutagenesis kit (Amersham Pharmacia Biotech). The mutagenic oligonucleotides were 5'-CCATCACAAGGGCGGCGCTGACCTGCTGACATCAGGACG', which eliminates the XhoI restriction site in the pcDNA vector; 5'-GGGGGACTACAGCCGAGCTGGTATTTGTGACGACG', which deletes amino acids 28–98 (PR1 domain, hyphen at site of deletion); and 5'-GGATCCGGATCCAGAAGTAAGTTGAGGAGGAT-3', which deletes amino acids 401–447 (PR2 domain). The mutations were confirmed by DNA sequencing.

Preparation of GST Fusion Proteins—Using the cDNA of ST5 as a template, DNA fragments corresponding to the various domains were synthesized using the polymerase chain reaction with oligonucleotides that contained appropriate restriction sites and bordered the domains of interest. The amplified DNAs were digested and cloned into pGEX2TK (Amersham Pharmacia Biotech). Large scale cultures were grown, induced with 0.1 mM isopropyl β-D-thiogalactopyranoside for 4 h, and lysed by sonication in NETN buffer (0.5% Nonidet P-40, 20 mM Tris, pH 8.0, 120 mM NaCl). The GST fusion proteins were purified on glutathione-agarose beads (Amersham Pharmacia Biotech) and eluted with 20 mM glutathione in 100 mM Tris, pH 8.0, 120 mM NaCl. The following ST5 fusions were prepared: GST aa 1–27; GST aa 28–447; GST aa 28–100 (PR1 domain); GST aa 401–447 (PR2 domain); GST aa 447–528; and GST aa 531–586. Fusion proteins encoding SH3 domains of Abi, Grb2, and Src were generated as described (15, 21, 22).

In Vitro Binding Assay—GST fusion proteins to be used as probes were labeled with heart muscle kinase in the presence of [γ-32P]ATP. Equal amounts of each full-length ST5 fusion protein were fractionated by 12% SDS-PAGE, transferred to nitrocellulose, blocked with 5% non-fat dry milk, and probed with 3 μg of [γ-32P]ATP labeled GST Abi SH3, GST Grb2 SH3, or GST Src SH3 domain in TBST (100 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween) plus 5% milk powder. After incubation for 2 h at 4°C, the membrane was washed five times with TBST, and the labeled proteins were detected by autoradiography.

MAP Kinase Assay—Subconfluent COS-7 cells were transfected with 2 μg of HA-epitope-tagged ERK2 and 2 μg of additional expression plasmids by the DEAE-dextran method (23). The total amount of DNA was adjusted with pcDNA3 vector to 6 μg per plate. Two days post-transfection, cells were transferred to serum-free medium and cultured for an additional 24 h. EGF was added to a concentration of 150 ng/ml 15 min prior to harvesting. Cells were washed once with ice-cold phosphate-buffered saline and lysed at 4°C in 1 ml of buffer containing 20 mM HEPES, pH 7.5, 1 mM EDTA, 40 mM β-glycerophosphate, 1% Nonidet P-40, 2.5 mM MgCl2, 2 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethyl-sulfonyl fluoride, 20 μM aprotinin, and 20 μM leupeptin. After high speed centrifugation for 30 min, the epitope-tagged ERK2 was immunoprecipitated from aliquots of supernatants containing equal amounts of protein with 2 μg of the mouse monoclonal antibody 12CA5 (Boehringer Mannheim) at 4°C for 1 h. The immune complexes were recovered with protein G-Sepharose beads (Amersham Pharmacia Biotech), washed three times with 100 mM Tris, pH 7.5, 1% Nonidet P-40, 2 mM sodium orthovanadate, one time in 100 mM Tris, pH 7.5, 0.5 mM lithium chloride, and once in kinase buffer (12.5 mM MOPS, pH 7.5, 12.5 mM β-glycerophosphate, 7.5 mM MgCl2, 0.5 mM EGTA, 0.5 mM sodium fluoride, and 0.5 mM sodium orthovanadate). The MAPK activity was measured by suspending the anti-HA precipitates in 25 μl of kinase buffer containing 1 μCi of [γ-32P]ATP and 40 μg of myelin basic protein (MBP) (Sigma) per reaction. After a 20-min incubation at 30°C, the reactions were stopped by adding 4× Laemmli sample buffer, heat denatured at 95°C for 5 min, separated on a 12% SDS-polyacrylamide gel, and exposed to a screen for detection and quantitation with a Molecular Dynamics Storm imaging system.

Western Blot Analysis—Equal amounts of COS lysates transfected with ERK2 HA and the indicated expression plasmids were separated on 10% SDS-PAGE, transferred to nitrocellulose, blocked with 5% non-fat dry milk, and probed with ST5 antibody. After an incubation with the secondary antibody, anti-rabbit IgG conjugated with horseradish peroxidase, the blot was developed by chemiluminescence with ECL reagents.

JNK Assay—COS-7 cells were cotransfected with 2 μg of HA-epitope-tagged JNK and additional DNAs by the DEAE-dextran method. The total amount of DNA was adjusted with pcDNA3 vector to 6 μg per plate. After overnight serum starvation, transfected cells were stimulated with 150 ng/ml EGF for 15 min. The epitope-tagged JNK protein was immunoprecipitated with the HA antibody (12CA5), and JNK activity was determined in kinase buffer using GST-ATF as substrate. Products were detected as in the MAPK assay.

RESULTS

Identification of ST5 Proteins—The sequence of the three ST5 mRNA species predicts proteins with molecular masses of

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126, 82, and 70 kDa. Proteins consistent with those predicted sizes were readily produced from the cloned, full-length cDNAs either by transient transfection in COS-7 cells or by in vitro transcription/translation (Fig. 1A and B). The migration of p126 was somewhat slower than predicted, presumably due to the presence of proline-rich regions unique to this gene product. Fig. 2 shows the complete amino acid sequence of p126. The structure of the three ST5 proteins is shown schematically in Fig. 3A. The p126 sequence contains 23 S/T-P motifs, of which 16 occur within the segment unique to p126. Five of these, including three present only in p126, conform to the expanded motif P-X-X-P motifs are shown in italics. The predicted initiation codon for p70 at amino acid 529 is shown large and bold.

A search of the nonredundant GenBank™ data bank with the p126 sequence using the BLASTP program (26) revealed highly significant homologies (Expect values ranging from 3 \times 10^{-11} to 4 \times 10^{-7}) between several known proteins and amino acids 833–1000 in the C-terminal region common to all three ST5 proteins. Among the homologous proteins are two anonymous Caenorhabditis elegans cDNAs and a group of highly similar proteins, including the C. elegans axc-3 gene product, a Ras3 GDP/GTP exchange factor from Rattus norvegicus, and three nearly identical human proteins identified as MADD (MAP kinase activating death domain protein), DENN, and KIAA0358 (27–29). The alignment of these proteins is presented in Fig. 3C. The region of homology includes the leucine zipper motif of MADD, suggesting that this region of ST5 may function as a site for dimerization or interaction with other proteins.

The 420 amino acid segment unique to p126, extending from amino acids 28–447, contains two proline-rich regions, designated PR1 and PR2 (Fig. 3, A and B). Within these two stretches are several occurrences of the 10-amino acid consensus sequence XPXXPPPXP (X = any amino acid; \(\Psi\) = a hydrophobic amino acid) reported as a consensus binding site for the Abi SH3 domain (8). Motifs with prolines at positions 2, 7, and 10, which were found to be important for c-Abl binding, are repeated four and three times in PR1 and PR2, respectively (Fig. 3B). However, the occurrences of this motif in PR1 lack the hydrophobic amino acid at position 9, which was found to be important for Abi binding (Fig. 3B). Therefore, this sequence suggested the possibility of a specific interaction between the p126 and the c-Abl SH3 domain and PR2.

The PR2 Sequence in ST5 Is Necessary for the Association with the c-Abl SH3 Domain—Since PR1 and PR2 share a consensus characteristic of c-Abl SH3 ligands (8), we tested the ability of segments of the ST5 proteins to interact directly with c-Abl in vitro. GST fusion proteins containing various regions of the ST5 coding sequence were fractionated by SDS-PAGE, immobilized on nitrocellulose, and probed with \(^{32}\)P-labeled c-Abl SH3 domain. The SH3 binding domain of the 3BP-1 protein (8) expressed as a GST fusion served as the positive control for c-Abl SH3 binding.

As shown in Fig. 4B, the SH3 domain of c-Abl bound strongly to fusion proteins containing aa 401–447 (PR2) or 28–447, the sequence unique to p126, containing both PR1 and PR2. In contrast, c-Abl SH3 did not recognize aa 28–100 (PR1), GST alone, or GST fused to any region of the ST5 protein C-terminal to PR2. To further examine the specificity of c-Abl-p126 interaction, we studied the binding of the Grb2 protein and the Src SH3 domain to the ST5 fusion proteins. Like the c-Abl fusion, the Grb2 probe recognized the PR2 fusion, GST-aa 401–447, and GST-aa 27–447 (Fig. 4C). In addition, this probe bound to aa 102–401, the sequence between PR1 and PR2. In contrast, the Src SH3 domain failed to bind to either PR1 or the PR2 fusion proteins but interacted very weakly with fusions containing the region between these two proline-rich domains (aa 28–447 and 101–400, Fig. 4D). The results show that p126 can...
interact with certain SH3 domains in vitro and that the PR2 region interacts preferentially with the c-Abl SH3 domain, as predicted from the sequence.

*P126 Stimulates ERK2 Activation in Response to EGF*—The observations that p126 shares some structural features with signaling proteins and can bind the intracellular kinase c-Abl in vitro prompted us to explore whether ST5 can participate in signaling pathways involving c-Abl leading to the activation of either ERK2 or JNK (14, 30). Initially, we tested the effect of ST5 proteins alone on these pathways. COS-7 cells were transfected with the epitope-tagged ERK2 or JNK cDNAs together with expression vectors carrying the cDNA for the ST5 proteins, p126, p82, or p70. Transfection with the active form of Ras (Ras 12V) served as a positive control. After 72 h, cells were treated with EGF (150 ng/ml) for 15 min. The HA-tagged kinases were immunoprecipitated from cell lysates with the 12CA5 antibody to the HA epitope, and the precipitates were assayed for kinase activity on an appropriate substrate, either myelin basic protein (MBP) for ERK2 or GST-ATF for JNK.

Expression of p126 induced a 4-fold increase in ERK2 activity (Fig. 5A). In contrast, expression of p82 or p70 had little effect. Deletion of the PR1 region (p126 lacking aa 28–100, ΔPR1) resulted in the complete loss of the ability to stimulate ERK2 activation, whereas deletion of PR2 (p126 lacking aa 401–447, ΔPR2) had little effect (Fig. 5B). Fig. 5C shows approximately equal expression of the wild-type and mutant ST5 proteins in COS lysates.

Each of the three ST5 proteins failed to stimulate JNK activity significantly in response to EGF (Fig. 5D). Without EGF treatment, the stimulatory effect of p126 on ERK2 was less pronounced (data not shown). Thus, wild type p126 can function in the signal transduction pathway leading to ERK2 activation in response to EGF, and this activity requires the PR1 region of the protein.

*C-Abl Stimulates ERK2 Activation Mediated by p126*—In vitro, p126 bound strongly to the SH3 domain of c-Abl. The ERK2 stimulation assay provided a tool to study the effects of the interaction between p126 and c-Abl leading to ERK2 activation in response to EGF, and this activity requires the PR1 region of the protein.

*p70 Blocks p126-mediated Activation of MAPK*—The pattern of expression of p70 in Hela/fibroblast hybrids suggests that this ST5 isoform functions in maintaining the nontumorigenic phenotype (4). We hypothesized that p70 acts to inhibit signal transduction through p126. To test this possibility, the effect of p70 on p126-mediated activation of ERK2 was examined. Expression of p70 strongly inhibited the induction of ERK2 by p126 (Fig. 6A). Transfection of p82 slightly reduced the stimulatory effect of p126 on ERK2, but to a lesser extent than p70. Co-transfection of p70 with p126 and c-Abl reduced ERK2 activity to basal levels (Fig. 6A). These data support the conclusion that p70 negatively regulates signaling through p126.
We have presented several lines of evidence supporting the involvement of ST5 proteins in growth regulatory signaling pathways. First, homology searches revealed significant sequence similarities to rab3-specific GDP/GTP exchange factors (GEFs) from rat and C. elegans and to a human protein named MADD, for “MAP kinase activating death domain protein” (27–29). MADD was identified as a protein that interacts with the tumor necrosis factor type I receptor (28). The homology to receptor binding proteins with GEF activity raises the possibility that the ST5 proteins may represent additional members of a novel class of such proteins. This possibility is consistent with the other findings reported in this study regarding both the protein binding properties observed in vitro and the ability of p126 to activate ERK2 in vivo.

Short sequence motifs, particularly in the 420 aa segment unique to p126, also suggested involvement of the ST5 proteins in signal transduction pathways. These motifs include the proline-rich domains PR1 and PR2, and many copies of a consensus that conforms to phosphorylation target sites by MAP kinases (24, 25). The organization of prolines in PR1 and PR2 suggested the possibility that these regions might interact with SH3 domains. PR2 was noted to contain several copies of a motif that has been shown to bind preferentially to the c-Abl SH3 domain (8). In vitro filter binding assays confirmed the ability of PR2 to bind to the c-Abl SH3 domain and, furthermore, supported the inference from the sequence analysis that PR2 would bind preferentially to c-Abl over other SH3 containing proteins. PR1 was not required for the association with Abl SH3. The binding specificity was most pronounced when compared with results obtained with the c-Src SH3 domain, which did not bind PR1 or PR2. The Grb2 protein also bound to PR2, as well as to the sequence between PR1 and PR2, presumably through short proline-rich sequences in this segment of the protein. The ability of the protein to interact with Grb2, together with homology to the GEFs, raises the possibility that p126 could function as a link between an adaptor protein, such Grb2, and a small GTP binding protein, such as Ras, in a manner analogous to the well studied Sos protein (11, 12).

Based on inferences from the amino acid sequence and the ability of p126 to bind SH3 domains, experiments were designed to investigate the possible function of this protein in signal transduction pathways in vivo. In COS-7 cells, transfection of p126 resulted in the stimulation of the enzymatic activity of the MAP kinase ERK2. In contrast, no stimulation of JNK activity was detected under the same conditions. Through the use of deletion mutants, the PR1 region was shown to be critical for this activity, whereas PR2 was not required. These results show that p126 can function as an activator of the EGFR-mediated ERK2 pathway.

Consistent with other studies, transfection of c-Abl by itself had little effect on ERK2 activation. However, co-transfection of c-Abl with p126 resulted in a pronounced stimulation of the activity obtained with p126 alone. This result provides direct evidence that c-Abl can interact with p126 in vivo. Further support for this conclusion came from transfection of the ΔPR2 mutant, which lacks the sequences necessary for interaction with the c-Abl SH3 domain in vitro. This mutant was able to stimulate ERK2 activity to approximately the same extent as wild-type p126. However, the c-Abl protein was no longer able to stimulate the basal activity obtained by transfecting ΔPR2 alone. The results indicate that the stimulatory effect of c-Abl on p126 results from a physical interaction of the two proteins in vivo and that this interaction is dependent on the PR2 region of p126.

Stimulation of ERK2 activity by ST5 proteins was only observed with the largest gene product, p126. Neither p82 nor p70 has any effect on ERK2 activation. However, co-transfection of c-Abl with p126 resulted in a pronounced stimulation of the activity obtained with p126 alone. This result provides direct evidence that c-Abl can interact with p126 in vivo. Further support for this conclusion came from transfection of the ΔPR2 mutant, which lacks the sequences necessary for interaction with the c-Abl SH3 domain in vitro. This mutant was able to stimulate ERK2 activity to approximately the same extent as wild-type p126. However, the c-Abl protein was no longer able to stimulate the basal activity obtained by transfecting ΔPR2 alone. The results indicate that the stimulatory effect of c-Abl on p126 results from a physical interaction of the two proteins in vivo and that this interaction is dependent on the PR2 region of p126.

Although the specific mechanism by which p70 inhibits p126 signaling has not been established in the present study, several...
plausible possibilities exist. For example, p126 may function as a homodimer, perhaps utilizing the region homologous to the rab3 GEFs as a dimerization domain. In this scenario, p70 might compete with homodimerization by forming inactive p126/p70 heterodimers, thereby blocking activity of the protein. An alternative model, based on the similarities noted between p126 and GEF proteins such as Sos, envisions p126 as a link between two proteins, A and B, in the signal transduction pathway, with the N-terminal portion of the protein interacting with protein A and the C-terminal segment with protein B. Transmission of the signal from A to B would require both segments of p126; interaction of the C-terminal segment alone (i.e. p70) with protein B would fail to activate the downstream components of the pathway. The role of p70 in this model would be to compete with p126 for binding to protein B, again blocking signaling through p126.

The results of this study identify the ST5 gene product p126 as a component of a signal transduction pathway leading to activation of the MAP kinase ERK2. To gain a greater understanding of how the ST5 proteins participate in regulating the non-tumorigenic phenotype, it will be important to identify the specific signals to which these proteins respond, the specific function of p126 in the pathway, and the mechanism of inhibition of the pathway mediated by the differentially expressed ST5 gene product, p70.

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FIG. 5. Effect of p126 on ERK2 activity. A, COS-7 cells were cotransfected with 2 μg of HA-epitope-tagged ERK2 together with the pcDNA3 vector (first data point shown, control) or expression plasmids (2 μg) carrying cDNAs for p126, p82, or p70. Ras12V was used as a positive control. Extracts were prepared and assayed for MAP kinase activity as described under “Experimental Procedures.” B, effect of PR1 and PR2 deletions on ERK2 activation. The deletion mutants APR1 and APR2 were transfected and assayed as in panel A. C, lysates from COS-7 cells transfected with ERK2 HA and expression plasmids for wild type p126, p126 mutants, APR1 and APR2, p82, or p70 were subjected to SDS-PAGE (10% gel) and Western blot analysis with ST5 antibody. D, the ST5 cDNAs were cotransfected into COS-7 cells with HA-JNK instead of HA-ERK2, and lysates were assayed for JNK activity with GST-ATF as substrate. Each transfection was done in triplicate. The autoradiograms shown represent the results obtained with one of three parallel transfections. The data shown in the charts represent the mean ± S.E. of the results obtained from three independent transfections.

FIG. 6. Effect of c-Abl and p70 on p126-mediated activation of ERK2. COS-7 cells were cotransfected with the indicated plasmids and processed for ERK2 activation assays as in Fig. 5.
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ST5 and ERK2 Signaling