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pp125^{FAK}, a structurally distinctive protein-tyrosine kinase associated with focal adhesions

(phosphotyrosine/Rous sarcoma virus/focal adhesion kinase)

MICHAEL D. SCHALLER*, CHERYL A. BORGMAN*, BRADLEY S. COBB*, RICHARD R. VINES*, ALBERT B. REYNOLDS†, AND J. THOMAS PARSONS*‡

*Department of Microbiology and Cancer Center, Box 441, School of Medicine, University of Virginia, Charlottesville, VA 22908; and †Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105

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ABSTRACT **Expression of the Rous sarcoma virus-encoded oncoprotein, pp60$^{v\text{-}src}$, subverts the normal regulation of cell growth, which results in oncogenic transformation. This process requires the intrinsic protein-tyrosine kinase activity of pp60$^{v\text{-}src}$ and is associated with an increase in tyrosine phosphorylation of a number of cellular proteins, candidate substrates for pp60$^{v\text{-}src}$. We report here the isolation of a cDNA encoding a protein, pp125, that is a major phosphotyrosine-containing protein in untransformed chicken embryo cells and exhibits an increase in phosphotyrosine in pp60$^{v\text{-}src}$-transformed chicken embryo cells. This cDNA encodes a cytoplasmic protein-tyrosine kinase which, based upon its predicted amino acid sequence and structure, is the prototype for an additional family of protein-tyrosine kinases. Immunofluorescence localization experiments show that pp125 is localized to focal adhesions; hence, we suggest the name focal adhesion kinase.**

Infection of cells with Rous sarcoma virus results in pronounced alterations in cell structure and loss of normal growth regulation (1–3). Cellular transformation requires the efficient expression of the Rous sarcoma virus-encoded oncoprotein, pp60$^{v\text{-}src}$, a protein-tyrosine kinase (PTK). Alterations in cell morphology and growth regulation require pp60^{src} kinase activity and are accompanied by an increase in the tyrosine phosphorylation of a number of cellular proteins (2). In normal cells, the phosphorylation of proteins on tyrosine residues by receptor and nonreceptor PTKs appears to be critical for the transmission of signals initiated by a variety of growth factors (4, 5). In addition, PTKs appear to play a central role in lymphocyte activation (6) and in mediating signaling pathways in nonmitotic cells, such as adrenal chromaffin cells (7), platelets (8), and neural cells (9).

The importance of PTKs in cellular signaling has led to research focused upon the identification of the substrates of these enzymes. Monoclonal antibodies (mAbs) that recognize seven distinct phosphotyrosine (*P*-Tyr)-containing proteins from pp60$^{v\text{-}src}$-transformed chicken embryo (CE) cells have been generated (10) and used to identify and characterize these putative pp60$^{v\text{-}src}$ targets. One such mAb, 2A7, recognizes a 125-kDa protein (pp125) that is one of the major *P*-Tyr-containing proteins in untransformed avian and rodent fibroblasts (ref. 10; S. B. Kanner, B.S.C., and J.T.P., unpublished observations). pp125 exhibits enhanced phosphorylation on tyrosine in pp60$^{v\text{-}src}$-transformed cells (10). Here, we report the isolation of cDNAs encoding pp125.§ The deduced amino acid sequence of pp125 revealed that it is a cytoplasmic PTK, whose sequence and structural organization are unlike those in data bases. Thus, pp125 appears to be the prototype member of an additional PTK family. Immunofluorescent localization of pp125 suggests that it is present in cellular focal adhesions, hence we suggest the designation pp125^{FAK} (FAK for focal adhesion kinase).

MATERIALS AND METHODS

Protein Characterization and Analysis. Primary CE cells were prepared and cultured as described (11). Lysates were prepared in modified RIPA buffer as described (12). For the preparation of immune complexes, 0.5–1.0 mg of cellular protein was incubated with 10 μg of protein A-purified mAb 2A7 or a 1:500 dilution of rabbit anti-pp125 serum (BC3) for 1 h at 4°C. Immune complexes were recovered by the addition of 100 μl of protein A-Sepharose beads (Pharmacia). For mAb 2A7 immune complexes, protein A-Sepharose beads were preincubated with 20 μg of rabbit anti-mouse IgG (Jackson ImmunoResearch). Immunoprecipitated proteins were subjected to SDS/PAGE (13) and immunoblotted with either affinity-purified rabbit *P*-Tyr antibody (2 μg/ml) or a 1:500 dilution of BC3 antiserum as described (10). For *in vitro* translation studies, the cDNA clone 23b was subcloned into pBluescript (Stratagene), transcribed *in vitro* by using an mCAP mRNA capping kit (Stratagene), and translated *in vitro* by using a rabbit reticulocyte lysate translation system (Promega) and [^{35}S]methionine (DuPont/NEN). The translation products were directly analyzed by SDS/PAGE or immunoprecipitated prior to SDS/PAGE with specific antiserum. The radiolabeled proteins were detected by fluorography using EN^{3}HANCE (DuPont/NEN). *In vitro* kinase assays were carried out by using immune complexes and [γ-^{32}P]ATP (DuPont/NEN) as described (14), and labeled proteins were subjected to SDS/PAGE. Immunofluorescence localization of pp125^{FAK} was carried out as described (11, 15).

Isolation of trpE Fusion Proteins and Preparation of Rabbit Antibodies. The 10a and 34b FAK cDNAs were subcloned into the pATH procaryotic expression vectors (16). The resultant constructs encoded fusion proteins containing the 37-kDa amino-terminal fragment of trpE fused to codons 651–1028 and 286–1028, respectively. The trpE–10a construct contains five unrelated residues between the trpE and FAK sequences. The expression of the fusion proteins was induced as described (17). The trpE–10a fusion protein was purified by SDS/PAGE and used to prepare polyvalent antisera (18).

Isolation of cDNA Clones and DNA Sequence Analysis. A λgt11 chicken embryo cDNA library (Clontech) was screened with mAb 2A7 as described (15). Additional cDNA clones were identified by using radiolabeled cDNA 10a to screen a

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Abbreviations: PTK, protein-tyrosine kinase; FAK, focal adhesion kinase; CE, chicken embryo; mAb, monoclonal antibody; *P*-Tyr, phosphotyrosine.

‡To whom reprint requests should be addressed.

§The nucleotide sequence data reported in this paper have been deposited in the GenBank data base (accession number M86656).

λgt10 cDNA library prepared from CE cell RNA (J. Huff and J.T.P., unpublished work). Phage from positive plaques were purified by three rounds of plaque purification. The cDNA inserts were isolated and subcloned into pBluescript. DNA sequencing was carried out by using the dideoxy sequencing procedure as described (19). DNA and amino acid sequences were analyzed by using the sequence analysis software EUGENE and SAM (MBIR software package of the Baylor College of Medicine), GCG package of the University of Wisconsin Genetics Computer Group (20), and FASTA (21).

RESULTS

Identification of cDNA Clones Encoding pp125. A λgt11 library expressing cDNAs prepared from the mRNA from 10-day-old chicken embryos was screened using mAb 2A7 (15), and a single clone containing a 1.6-kilobase-pair cDNA insert was isolated. This cDNA was used as a probe to isolate a number of overlapping cDNAs (22), seven of which are illustrated in Fig. 1. Selected cDNAs were subcloned into pBluescript, and the nucleotide sequence was determined by using the strategy diagrammed in Fig. 1. The sequence assembled from these cDNAs extends 4285 nucleotides and contains a polyadenylylation signal (23) near the 3′ end, followed closely by 13 consecutive terminal adenosine residues (Fig. 2). The 5′ most cDNA clone, 23b, contains two methionine codons conforming to the canonical sequence for initiation of translation (nucleotides 117–123 and 138–144) (24, 25). The first initiation codon (nucleotides 120–123) is preceded by three in-frame termination codons and is followed by a 1028-codon open reading frame encoding a protein of predicted molecular mass of 116,455 Da. Northern blot analysis indicated that the mRNA corresponding to this cDNA is ≈4.8 kilobases in length (M.D.S. and J.T.P., unpublished observations) and thus may contain an additional 500 nucleotides of 5′ untranslated sequence.

pp125 is a Distinctive PTK. Comparison of the nucleotide sequence and the deduced amino acid sequence of the encoded protein with the National Biomedical Research Foundation and GenBank data bases revealed that this cDNA encoded a distinctive PTK (referred to hereafter as FAK for focal adhesion kinase; see below). The predicted amino acid sequence of pp125^{FAK} contains the structural motifs common to all protein kinases (Figs. 2 and 3), including an ATP-binding site (Gly^{404}-Xaa-Gly^{406}-Xaa-Xaa-Gly^{409}) (26), three residues that are predicted to interact with the γ-phosphate group of the bound ATP molecule (Lys^{429}, Glu^{446}, and Asp^{539}) (27), and an amino acid postulated to be the catalytic site of these enzymes (Asp^{521}) (27). In addition, pp125^{FAK} contains two peptide sequences that are highly conserved among PTKs and are not found in serine/threonine protein kinases (Asp^{521}-Ile^{522}-Ala^{523}-Ala^{524}-Arg^{525}-Asn^{526} and Pro^{560}-Ile^{561}-Lys^{562}-Trp^{563}-Met^{564}). The isoleucine at residue 522 is unusual since leucine is found at the analogous position in all other PTKs, with the exception of the *ltk* PTK, which also contains an isoleucine (28, 29).

Based upon its sequence and structural organization, pp125^{FAK} represents an additional family of PTKs. The amino acid sequence of the catalytic domain of pp125^{FAK} is only 31–41% identical to the catalytic domains of a number of PTKs (Fig. 3). This is substantially lower than the identity observed between members of a PTK family. Analysis of the hydrophobicity of the protein revealed that pp125^{FAK} does not contain a transmembrane region (30), and there are not recognizable sites for acylation at the amino or C termini. This result is consistent with the observation that pp125^{FAK} is found in the cytosolic fraction following hypotonic lysis and differential centrifugation to separate cytoplasm and membranes (S. B. Kanner and J.T.P., unpublished observations). Thus, pp125^{FAK} is one of the few PTKs that is neither a transmembrane receptor nor membrane associated. FAK contains neither SH2 nor SH3 domains, which are structural elements involved in protein–protein interactions (31), and, in fact, does not exhibit significant homology with any known PTK outside of the catalytic domain. Interestingly, the C-terminal region of pp125^{FAK} contains two proline-rich stretches, residues 681–738 and 829–887, where the proline content exceeds 19% (Fig. 2). Finally, the overall architecture of pp125^{FAK} is unique in that the catalytic domain is flanked by large N-terminal and C-terminal domains.

Verification That the FAK cDNA Encodes pp125. Since the initial FAK cDNA was isolated using a single mAb to pp125, further evidence confirming the identity of pp125 and the FAK gene product was required. A rabbit antiserum (designated BC3) was generated against a bacterially expressed trpE fusion protein containing the 377 amino acids C-terminal to the kinase domain (residues 651–1028). BC3 and mAb 2A7

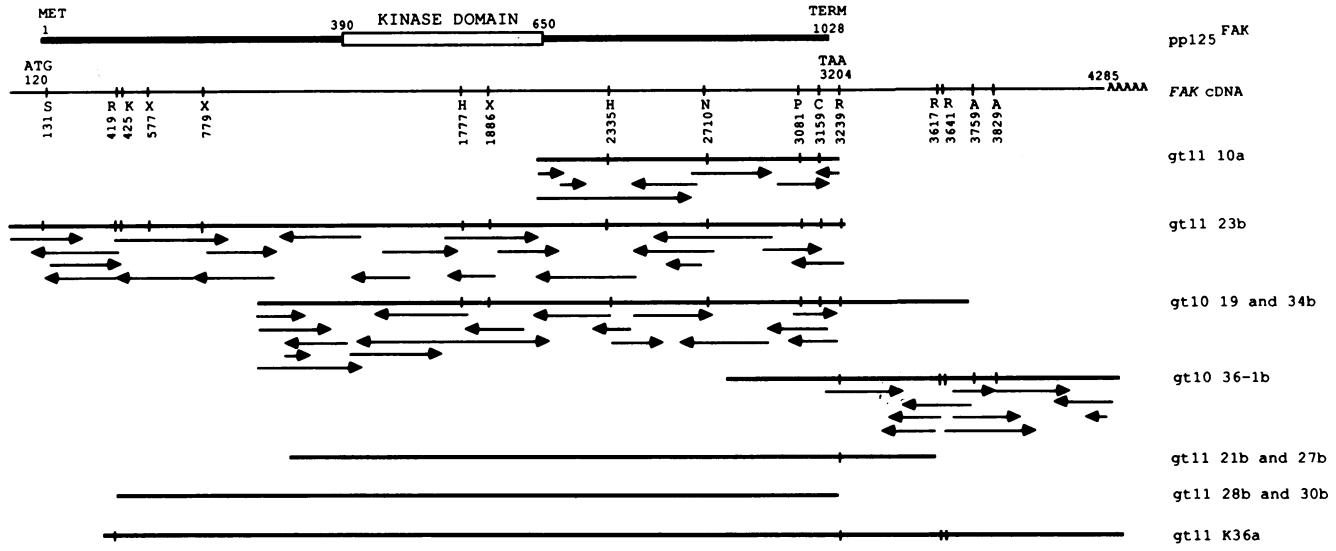
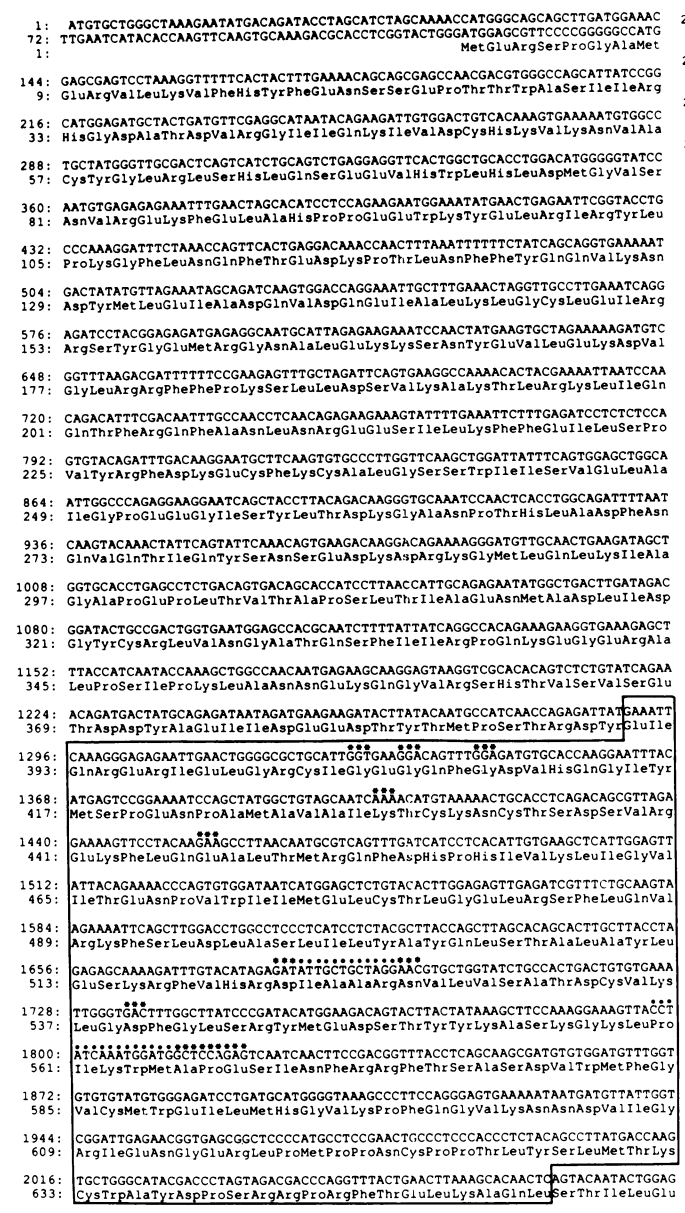


FIG. 1. cDNAs and nucleotide sequencing strategy. The FAK cDNA is illustrated as a line with the positions of the predicted initiation and termination codons indicated. Also shown are the positions of a number of restriction sites used for subcloning, including the sites recognized by *Eco*RI (R), *Sma* I (S), *Kpn* I (K), *Xho* II (X), *Hin*dIII (H), *Nru* I (N), *Pst* I (P), *Cla* I (C), and *Aha* II (A). The heavy line above the cDNA denotes the predicted translation product, pp125^{FAK}, with the catalytic domain of the protein kinase denoted by an open box. The structures of seven partial cDNAs that were isolated are also shown. The arrows beneath each cDNA indicate regions that have been sequenced.



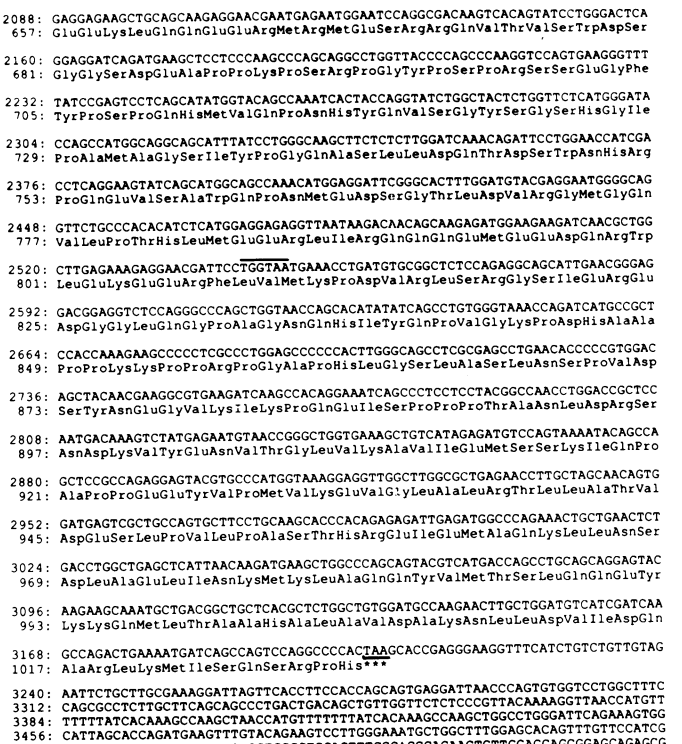


FIG. 2. Nucleotide sequence of FAK. The nucleotide sequence of FAK and the deduced amino acid sequence of pp125^{FAK} are shown. The catalytic domain of the PTK is indicated by a box. Residues that are highly conserved among protein kinases are denoted by asterisks, and residues diagnostic for PTKs are indicated with filled circles. Nucleotides 2543–2548 (indicated by an overline) were present in cDNA 23b but not in cDNAs 10a or 19/34b. We presume that this sequence difference is due to allelic variation; however, the extent of such a variation is presently unknown. The polyadenylylation signal near the 3′ end of the cDNA is underlined; the translational stop codon is also underlined.

each immunoprecipitated a 125-kDa *P*-Tyr-containing protein from normal (Fig. 4*A*) and pp60^{src}-transformed CE cells (ref. 10; B.S.C. and J.T.P., unpublished observations). Furthermore, the 125-kDa protein in the mAb 2A7 immune complex was recognized by BC3 in a Western blot (Fig. 4*B*). Therefore, the protein encoded by the FAK cDNA is immunologically indistinguishable from pp125. mAb 2A7 and BC3 immune complexes also contained several proteins of approximate molecular mass of 41–43 kDa that were recognized in a BC3 Western blot (Fig. 4*B*), but that did not contain *P*-Tyr (Fig. 4*A*). At least one of these proteins appears to be translated from an alternatively spliced transcript that encodes the C-terminal, noncatalytic fragment of pp125^{FAK} (M.D.S., C.A.B., and J.T.P., unpublished observations).

Additional verification that the FAK cDNA encodes a 125-kDa protein was obtained by expressing the cDNA *in vitro*. The cDNA clone 23b was transcribed *in vitro* and translated in a reticulocyte lysate. Analysis of the translation products revealed a protein of ≈125 kDa, as well as a number of smaller, less prominent proteins, which presumably arose by initiation of translation at internal methionine residues. These proteins were immunoprecipitated by the rabbit antiserum BC3 and by mAb 2A7, but not by preimmune serum or a control mAb (Fig. 4*C*).

pp125^{FAK} Exhibits Intrinsic Tyrosine Kinase Activity. To confirm the PTK activity of pp125^{FAK}, a fragment of FAK was expressed as a trpE fusion protein in *Escherichia coli* (Fig. 5). Upon induction of the fusion protein, *P*-Tyr was detected on bacterial proteins by immunoblotting with *P*-Tyr antibodies (Fig. 5*A*). No *P*-Tyr was evident in control lysates from bacteria expressing the trpE vector alone. Furthermore, protein kinase activity was observed *in vitro* in immune complexes containing the fusion protein (Fig. 5*B*). The 115-kDa protein is the full-length trpE fusion protein, and the other proteins are presumably breakdown products. PTK activity was also observed in immune complexes containing pp125^{FAK} from CE cells. Phospho amino acid analysis of *in vitro* labeled pp125^{FAK} revealed only labeled *P*-Tyr. In addition, pp125^{FAK} immune complexes catalyzed the efficient phosphorylation of the exogenous substrate poly(Glu-Tyr) (M.D.S. and J.T.P., unpublished observations).

pp125^{FAK} Localizes to Sites of Focal Adhesion. Immunostaining of CE cells with the mAb 2A7 revealed a characteristic staining pattern, reminiscent of the staining patterns observed with antibodies to components of cellular focal adhesions (32). To confirm the presence of pp125^{FAK} in focal adhesions, double-label immunofluorescence was carried out to compare the distribution of pp125^{FAK} and a known com-

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                391                                                  448
        FAK : EIQRERIELGRCIGEGQFGDVHQGIYMSPENPA..MAVAIKTCKNCTSDSVREKFLQEAL
 EGFR (36%) : ILKETEFKKIKVL-S-A--T-YK-LWIPEGEKVKI.P----ELREA--PKANKEI-D--Y
 met  (35%) : GPSSLIVHFNEV--R-H--C-YH-TLLDNDGKK..IHC-V-SLNRI-DIGEVSQ--T-GI
 fer  (40%) : ILSH-DVI--ELL-K-N--E-YK-TLK.DK.....TS--V----EDLPQELKI------K
 abl  (39%) : --C-TD-MMKHKL-G--Y-E-YEAVWKR.....YGLT--V-TLKED-MALKD..-E--A
 fps  (40%) : VLNH-DVL--ER--R-N--E-FS-RLRADN.....TP--V-S-RETLPPELKA------R
Ins-R (37%) : -VP--K-T-L-EL-Q-S--M-YE-NAKDIIKGEAETR---V--VNESA-LRE-IE--N--S
PDGFR (34%) : -LP-DQLV---TL-S-A--Q-VEATAHGLSHSQAT-K--V-ML-STARS-EKQALMS-LK
  src (30%) : --P--SLR-EVKL-Q-C--E-WM-TWNG......TTRV---TLKPGNM..SPEA-----Q

                449                                                  496
        FAK : TMRQFDHPHIVKLIGVITE.N..PVWIIMELCTLGELRSFLQVRKFSLD.........LA
       EGFR : V-ASV-N--VCR-L-ICLT.S..T-QL-TQ-MPF-C-LDYVREH-DNIGSQY........
        met : I-KD-S--NVLS-L-ICLR.SEGSPLVVLPYMKH-D--N-IRNETHNPT.........VK
        fer : ILK-Y---N-------C-Q.RQPV.Y-----VSG-DFLT--RRK-DE-K.........-K
        abl : I-KEMK--NL-Q----C-REP..-FY--T-FMSH-N-LD--RSAGRETLDAV........
        fps : ILK-YN--N--R----C-Q.KQPI.Y-V---VQG-DFL---RSKGPH-K.........MK
     Ins-R : V-KG-TCH-V-R-L--VSK.GQPT.LVV---MAH-D-K-H-RSLRPDAENNPGRPPPT-Q
     PDGFR : I-SHLGPHLN-VNLEGACTKGGPIY.IIT-Y-RY-D-VDY-HRN-HTF.............
       src : V-KKLR-EKL-Q-YA-VS-EP..I.Y-VT-YMSK-S-LD--KGEMGKYLRLP........

                497                                                  553
        FAK : SLILYAYQLSTALAYLESKRFVHRDIAARNVLVSATDCVKLGDFGLSR.YMEDSTYYK...
       EGFR : .-LNWCV-IAKGMN---DR-L----L-------KTPQH--IT----AKLLGAEEE-H....
        met : D--GFGL-VAK-MK--A--K-----L----CMLDEKFT--VA----A-.D-Y-KE--SVHN
        fer : Q-VKFSLDAAAGML-----NCI---L----C--GENNVL-IS---M--.QEDGGV-SSSG.
        abl : A-LYM-T-IASGMS----RNYI---L----C--GDNKL--VA----A-.L-R-D--TAHAG
        fps : E--KMMENAAAGME-----HCI---L----C--TEKNTL-IS---M--.QE--GV-ASTGG
     Ins-R : EM-QMTAEIADGM---NA-K-----L----CM-AHDFT--I----MT-.DIYETD--RKGG
     PDGFR : .-QHHS-*VANGMEF-A--NC----L------ICEGKL--IC----A-DI-R--N-IS...
       src : Q-VDM-A-IASGM--V-RMNY----LR-A-I--GENLVC-VA----A-.LI--NE-TARQG

                554                                                  613
        FAK : ASKGKLPIKWMAPESINFRRFTSASDVWMFGVCMWEILMHGVKPFQGVKNNDVI.GRIENG
       EGFR : -EG--V------L---LH-IY-HQ----SY--TV--LMTF-S--YD-IPASEIS.SIL-K-
        met : KTGA---V----L--LQTQK--TK----S---VL--LMTR-AP-YPD-NTF-IT.VYLLQ-
        fer : LKQ..I----T---AL-YG-YS-E----S--ILL--TFSL--C-YP-MT-QQAR.EQV-R-
        abl : ...A-F----T---GLAYNK-STK----A---LL---ATY-MS-YPAIDLT--Y.HKLDK-
        fps : MKQ..I-V--T---AL-YG-YS-E----S--ILL--AFSL-AV-YANLS-QQTR.EA--Q-
     Ins-R : KGL...-VR--S---LKDGV--AS--M-S---VL---TSLAEQ-Y--LS-EQ-L.KFVMD-
     PDGFR : KGSTF--L--------FNSLY-TL----S--ILL---FTL-GT-YPELPM-EQFYNA-KR-
       src : ...A-F----T---AALYG---IK----S--ILLT-LTTK-RV-YP-MV-RE-L.DQV-R-

                614                                                  651
        FAK : ERLPMPPNCPPTLYSLMTKCWAYDPSRRPRFTELKAQL
       EGFR : ----Q--I-TIDV-MI-V---MI-ADS--K-R--IIEF
        met : R--LQ-EY--DP--EV-L---HPKAEM--S-S--VSRI
        fer : Y-MSA-QH--EDISKI-M---D-K-EN--K-S--QKE-
        abl : Y-MER--G---EV-D--RQ--QW-ATD--T-KSIHHA-
        fps : V--EP-EQ--EDV-R--QR--E---R---S-GAVHQD-
     Ins-R : GY-DP-D---ER-TD--RM--QFN-KM--T-L-IV.N-
     PDGFR : Y-MAQ-AHASDEI-EI-Q---EEKFEI--P-SQ-VLL-
       src : Y-M-C--E--ES-HD--CQ--RR--EE--T-EY-Q-F-
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FIG. 3. Comparison of PTK catalytic domains. The deduced amino acid sequence of the catalytic domain of pp125^{FAK} is shown in comparison to the catalytic domains of a number of other PTKs. Identical residues are indicated by dashes. The kinase insert domain of the platelet-derived growth factor receptor (PDGFR) is not shown and is located at the site of the star in the platelet-derived growth factor receptor sequence. Asterisks and filled circles denote amino acids highly conserved among PTKs (see Fig. 2). The number in parentheses is the percentage of residues that are identical to pp125^{FAK}. EGFR, epidermal growth factor receptor; Ins-R, insulin receptor; met, hepatocyte growth factor receptor; fer and fps, nonreceptor PTKs.

ponent of focal adhesions, tensin (33). CE cells grown on fibronectin-coated coverslips (Fig. 6) were coimmunostained with mAb 2A7 and rabbit anti-tensin (ref. 33; a gift of L. B. Chen). As shown in Fig. 6, both antibodies yielded a virtually identical pattern of immunofluorescence, staining predominantly cellular focal adhesions. Furthermore, immunostaining with antibodies to other components of cellular focal adhesions (e.g., vinculin and talin) yielded similar results (R. R. Vines, M.D.S., and J.T.P., unpublished observations). Since mAb 2A7 recognizes both pp125^{FAK} and 41- to 43-kDa polypeptides, cells infected with an avian retrovirus containing the pp125^{FAK} coding region and overexpressing pp125^{FAK} approximately 8- to 10-fold were immunostained with mAb 2A7. The increased staining of focal adhesions observed in these cells (data not shown) is consistent with pp125^{FAK} being a normal component of CE cell focal adhesions.

DISCUSSION

Previous analysis has shown that pp125^{FAK} contains *P*-Tyr in normal CE cells and is phosphorylated on tyrosine to a higher stoichiometry in pp60^{src}-transformed CE cells (10). In light of the fact that the FAK cDNA encodes a PTK, we suggest two mechanisms to account for its phosphorylation on tyrosine in CE cells. First, pp125^{FAK} may autophosphorylate on tyrosine, which is generally indicative that the PTK has been activated, and second, it may be phosphorylated by a second PTK, presumably at a regulatory site within pp125^{FAK}. The elevation in the *P*-Tyr content of pp125^{FAK} in pp60$^{v\text{-}src}$-transformed

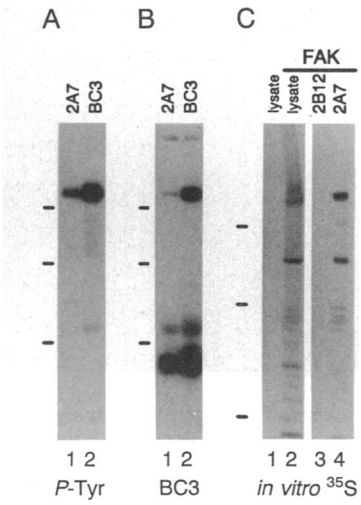

FIG. 4. Verification that FAK encodes pp125. A rabbit antiserum (BC3) was generated against a bacterially expressed trpE fusion protein that contained residues 651–1028 of pp125^{FAK}. CE cells were lysed in modified RIPA buffer (12) and immunoprecipitated with BC3 (lanes 1) and mAb 2A7 (lanes 2). The immune complexes were subjected to SDS/PAGE, transferred to nitrocellulose, and immunoblotted with a rabbit anti-*P*-Tyr antibody (*A*) or with BC3 (*B*). The positions of prestained molecular weight standards—105,000, 71,000 and 44,000—are indicated. (*C*) The cDNA clone 23b was subcloned into pBluescript, transcribed, and translated *in vitro*. The translation products were directly analyzed by SDS/PAGE (lanes 1 and 2) or immunoprecipitated prior to SDS/PAGE (lanes 3 and 4). Lane 1, unprogrammed lysate; lanes 2–4, lysate programmed with FAK mRNA. Immune complexes were isolated using control mAb 2B12 (lane 3) or mAb 2A7 (lane 4).

CE cells may be a consequence of (*i*) increased autophosphorylation due to activation of pp125^{FAK} by some undefined mechanism, (*ii*) activation of an endogenous PTK by pp60$^{v\text{-}src}$, which subsequently phosphorylates pp125^{FAK}, and/or (*iii*) direct phosphorylation of pp125^{FAK} by pp60$^{v\text{-}src}$. Since the enzymatic activity of several PTKs and their association with other proteins can be regulated by phosphorylation on tyrosine

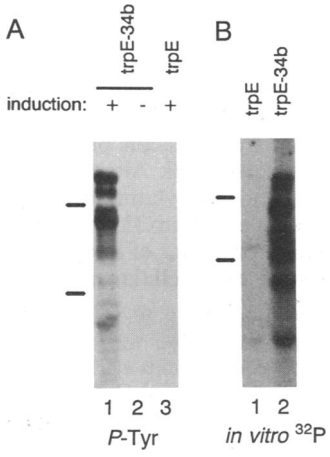

FIG. 5. PTK activity of pp125^{FAK}. (*A*) A trpE fusion protein containing residues 286–1028 of pp125^{FAK} (designated trpE–34b) (lane 1) or the trpE vector alone (lane 3) was expressed in *E. coli*. The cells were lysed in sample buffer, and proteins containing *P*-Tyr were visualized by SDS/PAGE followed by immunoblotting with an anti-*P*-Tyr antibody. Also shown is a lysate from uninduced cells (lane 2). The positions of prestained molecular weight standards—105,000 and 71,000—are indicated. (*B*) The *in vitro* PTK activity of trpE (lane 1) or trpE–34b (lane 2) was also examined. Bacteria were lysed by sonication in modified RIPA buffer (12) and the proteins were immunoprecipitated by using antiserum BC3.

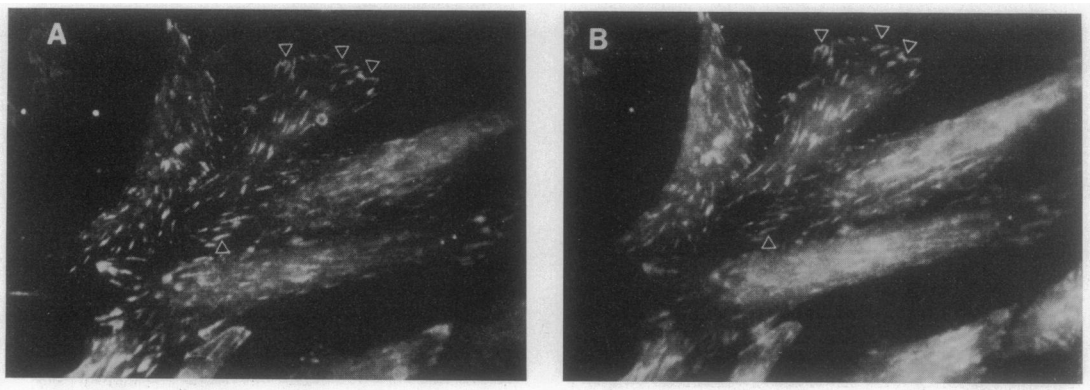

FIG. 6. Coimmunostaining of pp125 and tensin in CE cells. CE cells were grown overnight on fibronectin-coated coverslips (5 $\mu g/cm^2$), fixed with paraformaldehyde, permeabilized, and immunostained with a mixture of mAb 2A7 (10 $\mu g/ml$) and rabbit anti-tensin serum (1:60 dilution). After incubation with primary antibodies, the coverslips were incubated with goat anti-mouse IgG, followed by incubation with a mixture of Texas Red-conjugated donkey anti-rabbit IgG (5 $\mu g/ml$) and fluorescein isothiocyanate-conjugated donkey anti-goat IgG (5 $\mu g/ml$). (*A*) Pattern of mAb 2A7 staining. (*B*) Pattern of rabbit anti-tensin staining. The arrowheads denote representative areas of coincident immunostaining. No immunostaining of focal adhesions was observed when irrelevant mAbs or nonimmune rabbit serum was used. Furthermore, omission of the primary antibody (either mAb 2A7 or anti-tensin rabbit sera) yielded no significant staining of cells.

(5), we speculate that the tyrosine phosphorylation of $pp125^{FAK}$ may regulate $pp125^{FAK}$ activity.

The first clues to the function of the FAK kinase come from three types of experiments. First, in immunofluorescence experiments, mAb 2A7 stains structures resembling focal adhesions (Fig. 6). Second, crosslinking of cell surface integrins with antibodies or plating cells on a substrate coated with the integrin ligand, fibronectin, results in an increase in the amount of *P*-Tyr in proteins of 120–130 kDa (34, 35), at least part of which is due to an elevation in the *P*-Tyr content of $pp125^{FAK}$ (L. J. Kornberg and R. L. Juliano, personal communication; J. L. Guan and R. O. Hynes, personal communication; M.D.S. and J.T.P., unpublished results). Third, the activation of normal platelets *in vitro* results in an increase of *P*-Tyr on a number of proteins, including $pp125^{FAK}$ (ref. 8; L. Lipfert, B. Haimovich, M.D.S., B.S.C., J.T.P., and J. S. Brugge, unpublished results). Phosphorylation of $pp125^{FAK}$ is abrogated in platelets from patients with Glanzmann thrombasthenia, a disease associated with a defect in the platelet integrin gpIIb/IIIa (L. Lipfert, B. Haimovich, M.D.S., B.S.C., J.T.P., and J. S. Brugge, unpublished results). These preliminary observations raise the intriguing possibility that $pp125^{FAK}$ function may be linked to integrin function. We hypothesize that $pp125^{FAK}$ may be a component of a signal transduction pathway and may be responsible for the transmission of a signal from the cell surface integrins into the cell. Alternatively, $pp125^{FAK}$ may be responsible for phosphorylating the components of focal adhesions [for example, tensin (33), paxillin (36), or talin (37)] and regulating the interactions of integrins with the cytoskeleton and/or with the extracellular matrix.

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