When 67 SH2 domain sequences are aligned to maximize homology[12], 14 residues are either absolutely conserved or represented by only two alternative amino acids. Of these 14, 10 have side chains that are buried within the molecule (residues 22, 23, 36, 42, 44–46, 72, 87 and 93) and are therefore likely to be important for maintaining structural integrity. The remaining four strongly conserved residues Arg 29, Arg 47, Thr 58 and Leu 69 (corresponding to His 294 in *crk*) cluster specifically at the surface of the molecule, as shown schematically in Fig. 3. Arg 47 and Leu 69 represent positions implicated as pivotal to phosphotyrosine binding activity in mutagenesis studies of *crk* and *abl* SH2 domains[7,8]. These studies also showed that Ser 173 and Ser 175 of *abl* SH2 are important for binding activity, yet they are not highly conserved residues. The corresponding positions in our p85α SH2 are Ala 49 and Thr 51, respectively, which are found within the highly mobile loop joining β-strands 1 and 2. In the light of this, we propose that the region between the N-terminal end of α-helix 1 and the central β-sheet represents the phosphotyrosine binding site, with the possibility that the mobile loop comprising residues 49–55 folds inward to hold the bound peptide in place. This would explain the functional importance of residues Ala 49 and Thr 51 despite the low degree of conservation of these positions throughout SH2 domains. Studies of SH2 domains complexed with phosphotyrosine peptides should cast more light on the nature of this important interaction. □

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Nested expression domains of four homeobox genes in developing rostral brain

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INSIGHT into the genetic control of the identity of specific regions along the body axis of vertebrates[1] has resulted primarily from the study of vertebrate homologues of regulatory genes operating in the *Drosophila* trunk[2], but little is known about the development of most anterior regions of the body either in flies[3,4] or vertebrates. Three *Drosophila* genes have been identified that are important in controlling the development of the head[5–8], two of which, *empty spiracles*[5] and *orthodenticle*[8], have been cloned and shown to contain a homeobox[9–11]. We previously cloned and characterized *Emx1* and *Emx2*, two mouse genes related to *empty spiracles* that are expressed in restricted regions of the developing forebrain, including the presumptive cerebral cortex and olfactory bulbs[12]. Here we report the identification of *Otx1* and *Otx2*, which are related to *orthodenticle*[7,8]. We have compared the expression domains of the four genes in the developing rostral brain of mouse embryos at a developmental stage, day 10 post coitum, when they are all expressed. *Otx2* is expressed in every dorsal and most ventral regions of telencephalon, diencephalon and mesencephalon. The *Otx1* expression domain is similar to that of *Otx2*, but contained within it. The *Emx2* expression domain is comprised of dorsal telencephalon and small diencephalic regions, both dorsally and ventrally. Finally, *Emx1* expression is exclusively confined to the dorsal telencephalon. Thus at the time when regional specification of major brain regions takes place, the expression domains of the four genes seem to be continuous regions contained within each other in the sequence Emx1 < Emx2 < Otx1 < Otx2.

The exon–intron organization of the homeobox regions of *Otx1* and *Otx2* are shown in Fig. 1*a* and *b*, respectively. Otx1 and Otx2 homeodomains differ at three and two amino-acid residues from the *orthodenticle* (*otd*) homeodomain, respectively (Fig. 1*c*). In particular, the three homeodomains share the lysine residue at position 9 of the third recognition helix also present in *Drosophila bicoid* (*bcd*)[10] and frog *goosecoid* (*gsc*)[13] genes.

We studied the expression of the two genes in early mouse embryos using the DNA probes shown in Fig. 1*a, b*. We first analysed 7.5-, 8.5-, 9- and 9.75-d.p.c. (days post coitum) mouse embryos in sagittal sections (Fig. 1*d-j*). *Otx1* is first expressed

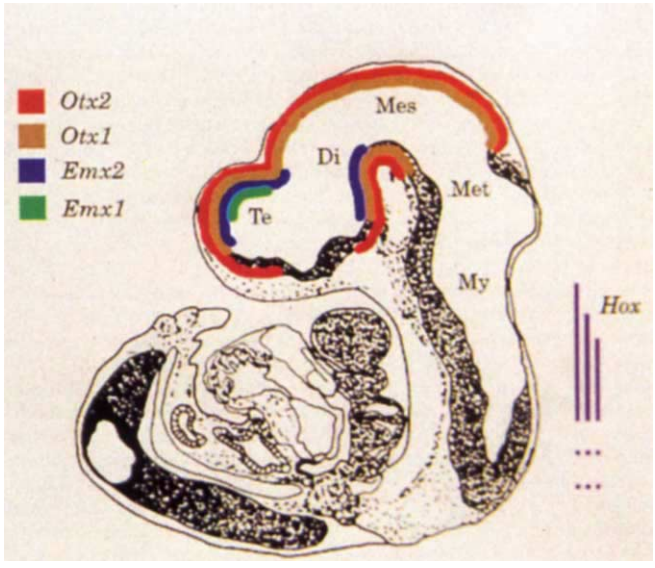

FIG. 4 Summary of the expression domains of the four genes in the developing central nervous system in 10-d.p.c. mouse embryos. Expression of members of the Hox gene family is also indicated. Di, diencephalon; Mes, mesencephalon; Met, metencephalon; My, myelencephalon; Te, telencephalon.

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in a large region of the anterior neural tube of 8.5 d.p.c. embryos (Fig. 1*d*). Anterior–posterior delimitation of Otx1 expression in the rostral neural tube is clear in 9 d.p.c. (Fig. 1*e*) and in 9.75-d.p.c. (Fig. 1*f*) embryos. Dorsally its expression domain comprises a continuous region, including part of the telencephalon, the diencephalon and the mesencephalon[14]. The posterior boundary of this domain coincides with that of the mesencephalon (arrow), but in median sections, such as that shown in Fig. 1*f*, a strong hybridization signal extends only half way (open arrow) along the mesencephalon. Ventrally, the Otx1 expression domain includes contiguous regions of both diencephalon and mesencephalon with sharp anterior and posterior boundaries.

Otx2 is expressed at an earlier developmental stage than *Otx1* as a hybridization signal is already detectable in very anterior regions of 7.5-d.p.c. embryos, including the headfold (Fig. 1*g*). *Otx2* is also expressed in 8.5- and 9-d.p.c. embryos (Fig. 1*h*, *i*), with an expression domain containing that of Otx1 and including

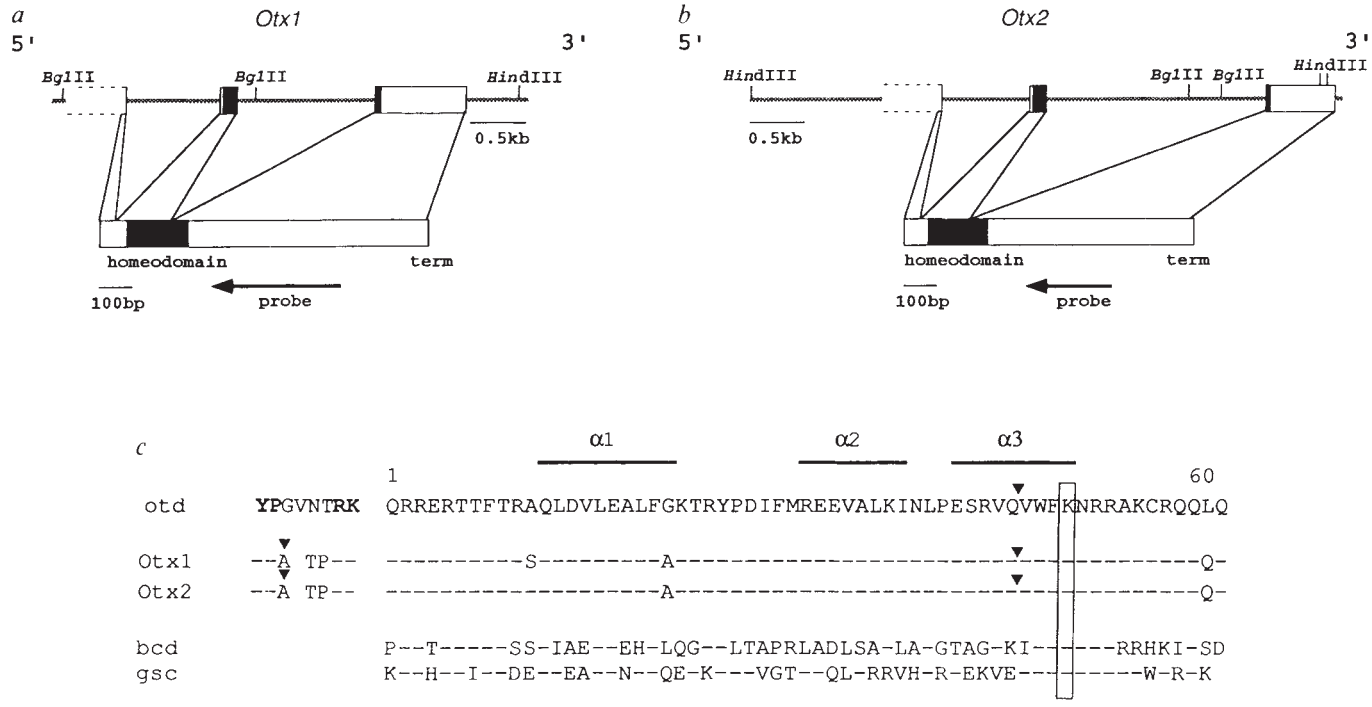
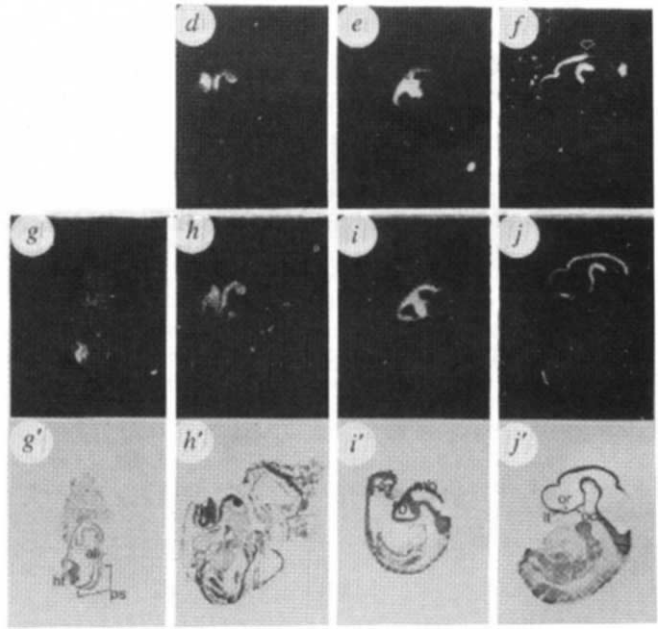


FIG. 1 Structure and expression of *Otx1* and *Otx2*. Cloned complementary DNA region and genomic organization of part of *Otx1* (*a*) and *Otx2* (*b*). Boxes indicate exons and a black box indicates the homeodomain. The probe used for *in situ* hybridization experiments is shown below the cDNA scheme. *c*, Comparison of Otx1 and Otx2 homeodomains with *otd*, *bcd* and *gsc* homeodomains (one-letter amino-acid code). Dashes indicate amino-acid identity with *otd*, arrowheads point to splice sites. The three helix motifs of the homeodomain are shown and the lysine residue at position 9 of the third helix is boxed. *d*–*f*, Otx1 expression in sagittal sections of 8.5 d.p.c. (*d*), 9 d.p.c. (*e*) and 9.75 d.p.c. (*f*) mouse embryos; *g*–*j*, Otx2 expression in sagittal sections of 7.5 d.p.c. (*g*), 8.5 d.p.c. (*h*), 9 d.p.c. (*i*) and 9.75 d.p.c. (*j*) mouse embryos. The solid arrow in *f* points to the posterior boundary of the dorsal Otx1 expression domain, the open arrow to the posterior boundary of the strong Otx1 hybridization signal. Hybridization probes are shown in *a* and *b*. A bright field exactly corresponding to a given dark field is indicated by a prime affix; for example, the section corresponding to the dark field in *g* is indicated as *g'*. Abbreviations: all, allantois; fb, forebrain; hf, headfold; lt, lamina terminalis; mb, midbrain; oc, optic chiasma; or, optic recess; ps, primitive streak.

METHODS. A cDNA library prepared from 11-day mouse embryos (Clontech) was screened under low-stringency conditions[22] with a short *otd* genomic sequence including the homeobox. This *otd* genomic region was obtained by amplification using the polymerase chain reaction (PCR) of *Drosophila* total DNA with two synthetic oligonucleotide primers derived from the *otd* sequence[5]. Analysis of *otd* amplified regions provided evidence for the presence of the intron shown in *c*. Two classes of homologous cDNA clones, termed *Otx1* and *Otx2*, were found. Using these cDNA clones as probes we screened in turn a genomic library constructed in cosmids and compared corresponding regions in cDNA and genomic clones. *Otx1* and *Otx2* encode proteins that share with *otd* two residues, Arg-Lys, upstream from the homeodomain and the general structure. All three contain a relatively short N-terminal domain upstream from the homeodomain and a long C-terminus downstream from it. An intron is present in *Otx1* and *Otx2* immediately upstream from the homeodomain, as is often the case for homeobox genes[23]. The exon upstream from this intron ends in all three genes with the motif Tyr-Pro-Ala/Gly, possibly a divergent version of the conserved homeopentapeptide Ile/Phe-Tyr-Pro-Tro-Met present in several homeotic genes of *Drosophila* and in most vertebrate genes belonging to the Hox clusters[23] (Fig. 1*c*). An additional intron is present in the three genes within the homeobox at identical positions, namely between residues 46 and 47 of the homeodomain. For mouse embryos, day 0.5 p.c. was assumed to begin at the middle of the day of vaginal plugging. *In situ* hybridizations were carried out as described[24].



the entire forebrain. Anterior–posterior delimitation of the Otx2 expression is clear in 9.75-d.p.c. embryos (Fig. 1*j*). Dorsally, it includes the entire telencephalon, the diencephalon and the mesencephalon. The anterior portion of this domain includes lamina terminalis and presumptive striatum, whereas the posterior boundary coincides with that of the mesencephalon. Ventrally, the Otx2 expression domain includes contiguous regions of diencephalon and mesencephalon with an anterior boundary just posterior to the optic chiasma.

We then compared the expression domains of Otx1 and Otx2 to those of Emx1 and Emx2 at a developmental stage, 10 and 10.25 d.p.c., when the four genes are all expressed in the developing neural tube. In sagittal sections of 10-d.p.c. embryos (Fig. 2*a–d*), the four expression domains are clearly defined as they are in median (Fig. 2*e–h*) and paramedian (Fig. 2*i-l*) sagittal sections of 10.25-d.p.c. embryos. The Emx1 expression domain (Fig. 2*a, e, i*) includes the dorsal telencephalon with a posterior boundary probably coinciding with that between presumptive diencephalon and telencephalon. Emx2 is expressed (Fig. 2*b, f, j*) in dorsal neuroectoderm with an anterior boundary slightly anterior to that of Emx1 and a posterior boundary well within the roof of presumptive diencephalon. Ventral expression in the floor of diencephalon is also evident at this developmental stage. A few extracephalic localizations of Emx2 expression[12] are also observable in 10.25-d.p.c. embryos (Fig. 2*f–j*) in the coelomic epithelium covering the mesonephric column, as well as the final part of the mesenteric attachment (arrow).

Otx1 and Otx2 expression domains at this stage are essentially those of 9.75-d.p.c. brain. Both dorsally and ventrally, the Otx1 expression domain (Fig. 2*c, g, k*) contains the Emx2 domain. It covers a continuous region including part of the telencephalon, the diencephalon and the mesencephalon, with an anterior boundary roughly coincident with that of Emx2. In paramedian sections (Fig. 2*k*) the posterior boundary of this domain coincides with that of the mesencephalon, even if in more median sections (Fig. 2*c, g*) a strong hybridization signal extends only half-way along the mesencephalon. A subdivision of early mesencephalon in two neuromeres has been proposed[15]. The Otx2 expression domain (Fig. 2*d, h, l*) contains the Otx1 domain both dorsally and ventrally. It covers practically the entire fore- and midbrain; only the regions of optic chiasma and optic recess are excluded.

Frontal and transverse sections (Fig. 3) of 10-d.p.c. embryos confirm the relative localization of the expression domains of the four genes in dorsal and ventral brain regions, defining a rostral brain as opposed to deuterencephalon[14] and spinal cord. In the first two transverse sections (Fig. 3*f–i*) no expression of either *Emx1* or *Emx2* is detectable, but in more central sections (Fig. 3*j–m*) expression of all four genes is detectable and its extension can be compared.

We have compared the expression domains of four homoeobox genes in the developing brain of mouse embryos at a developmental stage when the four genes are all expressed (Figs. 2, 3). Their expression domains are continuous regions contained within each other in the sequence Emx1 < Emx2 < Otx1 < Otx2 (Fig. 4, page 687). The first appearance of the four

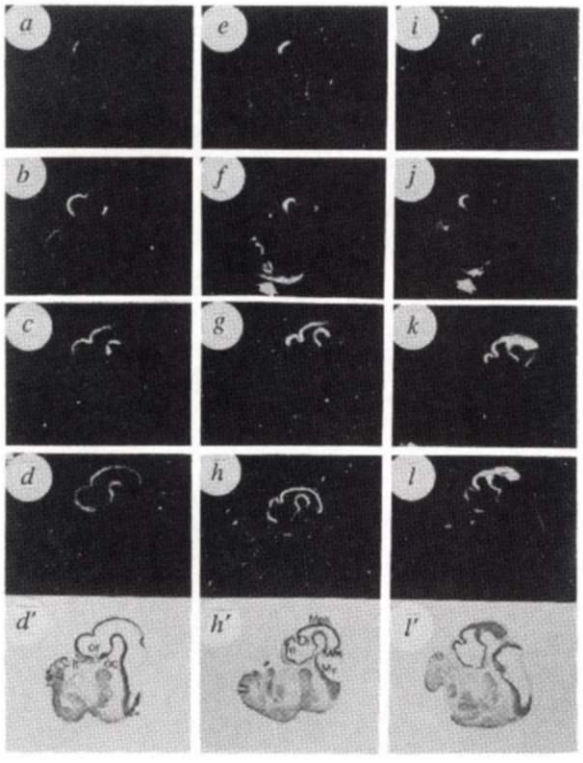


FIG. 2 Gene expression in sagittal sections of 10 d.p.c. (*a–d*) and 10.25 d.p.c. (*e–l*) mouse embryos. *a, e* and *i*, Hybridization with *Emx1*; *b, f* and *j*, hybridization with *Emx2*; *c, g* and *k*, hybridization with *Otx1*; *d, h* and *l*, hybridization with *Otx2*. Sections *i–l* are more paramedian. Arrows in *f, j* point to *Emx2* expression spots in the trunk. Abbreviations as in Fig. 1; Di, diencephalon; Mes, mesencephalon; Met, metencephalon; My, myelencephalon; Te, telencephalon.

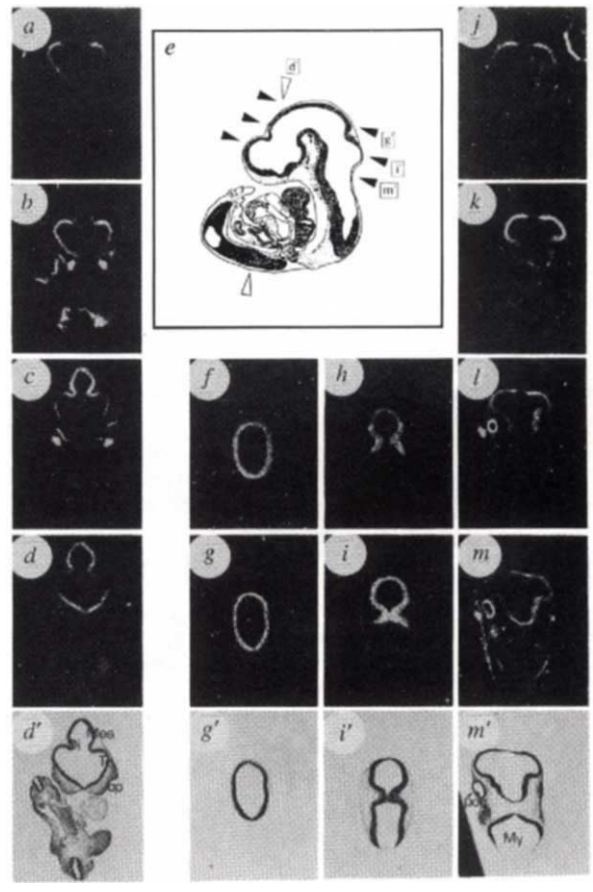


FIG. 3 *Emx1, Emx2, Otx1* and *Otx2* expression in frontal (*a–d*) and transverse (*f–m*) sections of 10-d.p.c. mouse embryos. *a* and *j*, Hybridization with *Emx1*; *b* and *k*, hybridization with *Emx2*; *c, f, h* and *l*, hybridization with *Otx1*; *d, g, i* and *m*, hybridization with *Otx2*; *e*, scheme of frontal and transverse sections. Arrows in *b* and *c* point to *Emx2* and *Otx1* expression in ectodermal localizations around the olfactory placodes. Arrows in *l* and *m* point to *Otx1* and *Otx2* expression in optic cups. Abbreviations as in Figs 1 and 2; op, olfactory placode; poc, presumptive optic cup.

genes is also sequential: *Otx2* is expressed first (7.5 d.p.c.), followed by *Otx1* and *Emx2* (8.5 d.p.c.), and finally by *Emx1* (9.5 d.p.c.). Several homeobox genes are believed to control cell identity with a regional or segmental pattern both in flies and vertebrates (refs 1, 16–19, and see ref. 20 for a review). The four genes may have a role in establishing and/or signalling the limits and identity of the various embryonic brain regions[14,15]. Although it is not yet possible to assign the restricted domains to morphological segments, these domains may correspond to some of the neuromeric segmentation already proposed[14,15]. The specification of the various regions of the rostral brain therefore seems to be a discrete progressive process with its centre in the dorsal telencephalon.

The cephalization process is thought to have occurred independently in the evolutionary lineages leading to insects and vertebrates[14,20,21]. Nevertheless, *empty-spiracles*-related and orthodenticle-related genes are expressed in anterior cephalic regions both in flies and mammals. Taken with the conservation of the Hox system, it is remarkable that several of the different systems potentially involved in specifying anterior regional variation in the mammalian nervous system correspond to similar *Drosophila* processes. It will be interesting to investigate the actual role played by genes of this type and the evolution of their expression patterns. □

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Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation

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INCREASING evidence indicates that the integrin family of cell adhesion receptors can transduce biochemical signals from the extracellular matrix to the cell interior to modulate cell growth and differentiation[1]. We have shown that integrin/ligand interactions can trigger tyrosine phosphorylation of a protein of M_r 120,000 (pp120), so it is possible that signal transduction by integrins might involve activation of intracellular protein tyrosine kinases as an early event in cell binding to the extracellular matrix[2]. Here we report that pp120 is identical to the focal adhesion-associated protein tyrosine kinase pp125^{FAK} (refs 3, 4). We show that tyrosine phosphorylation of this protein is modulated both by cell adhesion and transformation by pp60$^{v\text{-}src}$, and that these changes in phosphorylation are correlated with increased pp125^{FAK} tyrosine kinase activity. A model is proposed to relate these findings to the molecular basis of anchorage-independent growth of transformed cells.

Earlier studies showed that tyrosine phosphorylation of pp120 can be induced by plating NIH3T3 cells on either plasma fibronectin or on anti-integrin antibody, but not on nonspecific substrates such as poly-L-lysine or concanavalin A (ref. 2). These studies excluded the possibility of pp120 being an integrin β1, vinculin or α-actinin[2]. To see whether pp120 could be identified with a known phosphoprotein, we used specific antibodies to immunoprecipitate candidate proteins from lysates prepared from NIH3T3 cells plated on either polylysine or plasma fibronectin. Immunoprecipitates were then western blotted with antiphosphotyrosine antibodies to detect any tyrosine phosphorylation. Figure 1 shows the result for three other candidate proteins: two potential v-*src* protein substrates, p120 (not to be confused with pp120) and pp125^{FAK} (ref. 4), and the Ras GTPase-activating protein (GAP)[5]. Under our conditions (no serum), no tyrosine-phosphorylated GAP or p120 was detected in cells plated either on polylysine or plasma fibronectin (lanes 3–6). But pp125^{FAK} from the cells plated on fibronectin had increased tyrosine phosphorylation compared with pp125^{FAK} from cells plated on polylysine (lanes 7, 8). Furthermore, phosphorylated pp125^{FAK} comigrated with pp120 (ref. 2), the tyrosine phosphorylation of which depends on the cells being plated on plasma fibronectin as detected in total cell lysates (lane 2). These results suggested that pp120 could be identical to the potential v-Src substrate pp125^{FAK}.

We therefore isolated pp120 from NIH3T3 cells grown on plasma fibronectin by affinity chromatography using immobil-

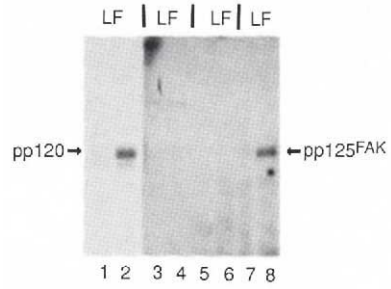

FIG. 1 Cell-adhesion-dependent tyrosine phosphorylation of pp125^{FAK}. Lysates were prepared from NIH3T3 cells plated on dishes that had been coated with poly-L-lysine (lanes L) or plasma fibronectin (lanes F) as described[2]. Lysates containing equal amounts of total cell proteins were immunoprecipitated using antibodies against GAP (lanes 3, 4), p120 (lanes 5, 6) and pp125^{FAK} (lanes 7, 8). They were electrophoresed on an SDS–polyacrylamide gel with the same amount of total cell lysates (lanes 1, 2), transferred to a nitrocellulose membrane and probed by antiphosphotyrosine antibody py20 (ICN) as described[2].
METHODS. Immunoprecipitations were done by incubating lysates with antibodies for 1 h at 4 °C. For monoclonal antibodies, rabbit anti-mouse IgG serum (Sigma) was then added to the mixture and incubated for 1 h at 4 °C. Immune complexes were collected on protein A–Sepharose and washed 4 times in lysis buffer. Precipitates were eluted by boiling for 3 min in SDS sample buffer.

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