Identification of p90, a Prominent Tyrosine-phosphorylated Protein in Fibroblast Growth Factor-stimulated Cells, as 80K-H*

(Received for publication, October 11, 1995)

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Tyrosine phosphorylation of cellular proteins occurs rapidly upon treatment of fibroblasts with acidic or basic fibroblast growth factors (aFGF, bFGF), suggesting a role for protein phosphorylation in the FGF signaling pathway. Stimulation of Swiss 3T3 cells and MRC-5 fibroblasts with bFGF results in the tyrosine phosphorylation of several proteins, of which the most prominent has been designated as p90. The phosphorylation of p90 is observed within 30 s of treating the cells with FGF but not with other growth factors. Microsequencing of p90 resolved on two-dimensional polyacrylamide gel electrophoresis indicated an N-terminal amino acid sequence which corresponded to a protein previously named as 80K-H. Polyclonal antibodies raised against the recombinant p90* were found to bind specifically to the predicted C terminus of 80K-H recognized p90 on all Western blots. p90 was found to bind specifically to GRB-2-glutathione S-transferase fusion protein and to be immunoreactive with 80K-H antibody. In addition, anti-phosphotyrosine antibodies immunoprecipitated 80K-H from cell lysates of FGF-stimulated but not from control fibroblasts. The biological function of 80K-H is yet unknown. However, from this study and a previous observation of the obligate dependence of p90 phosphorylation on FGF receptor occupation, it appears that 80K-H is involved in FGF signaling.

Fibroblast growth factors (FGFs) are a family of structurally related heparin-binding polypeptides of which the best characterized members are acidic FGF (aFGF) and basic FGF (bFGF). These polypeptides are important regulators of differentiation and embryogenesis; they support the survival of neural and muscle cells and are extremely potent inducers of DNA synthesis in ectoderm- or mesoderm-derived cell types, including endothelial cells and smooth muscle cells (1).

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1 The abbreviations used are: FGF, fibroblast growth factor; PAGE, polyacrylamide gel electrophoresis; CSF-1, colony stimulating factor-1; ECL, enhanced chemiluminescence; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; aFGF, acidic fibroblast growth factor; FGFR-1, fibroblast growth factor receptor type 1; FGFR-2, fibroblast growth factor receptor type 2; FGFR-3, fibroblast growth factor receptor type 3; FGFR-4, fibroblast growth factor receptor type 4; GST, glutathione S-transferase; IL-1, interleukin 1; IL-6, interleukin 6; IRS, insulin receptor substrate; MARCKS, myristoylated alanine-rich C kinase substrate; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; PTP1D, protein tyrosine phosphatase type 1D (Syp); PVDF, polyvinylidene difluoride; SH2, Src homology 2; SH3, Src homology 3; PMSF, phenylmethylsulfonyl fluoride.

FGFs elicit cellular responses by binding to and activating high affinity FGF receptor tyrosine kinases (2, 3). In agreement with the general scheme for receptor tyrosine kinases, ligand binding induces dimerization of FGF receptors, followed by activation of the intrinsic kinase activity and autophosphorylation of the receptor molecules (2). Two autophosphorylation sites in FGFR-1 have been identified. One, Tyr-653 (4), is located in the kinase domain and could have a regulatory role. The other, Tyr-766, has been shown to mediate the direct binding of phospholipase C-γ (5).

The signal transduction pathways following activation of FGF receptors are thought to be primarily mediated by the tyrosine phosphorylation of key substrates, as is the case with other receptor tyrosine kinases. In addition to phospholipase C-γ, several other substrates have been identified, including SHC (6), ERK-2, ERK-1 (7, 8), cortactin (9, 10), and Src (9, 11).

A number of groups have demonstrated the prominent phosphorylation of a 90-kDa protein by both aFGF and bFGF (7, 12–16). It was recently demonstrated that p90 phosphorylation was induced in cells transfected with both the FGFR-1 and the keratinocyte growth factor receptor, but not the FGFR-4 (11).

The identification of p90 would thus be relevant to our understanding of the complexity of FGF signaling.

Recent investigations have shown that receptor tyrosine kinases link to downstream signaling components, like ERK-1/2, via adaptor proteins that have no apparent enzymatic function but trigger off signaling cascades by assembling proteins into reactive complexes. Protein-protein interactions occur via binding of modular domains on the adaptor protein, such as Src homology 2 (SH2), Src homology 3 (SH3), pleckstrin homology, and phosphotyrosine-binding domains, to specific sequence motifs on the target proteins (17, 18). One such adaptor protein, GRB-2, essentially consists of two SH3 domains flanking a solitary SH2 domain. GRB-2 has been shown to bind to auto-phosphorylated receptors such as those of EGF, PDGF, CSF-1, and insulin, as well as to tyrosine-phosphorylated, non-receptor proteins such as the insulin receptor substrate (IRS). Recently, GRB-2-GST fusion proteins were also used to identify novel tyrosine-phosphorylated proteins that bind to GRB-2 (19–22).

We now report the identification of p90 by a combination of several techniques, which include partial amino acid sequencing of the protein, Western blotting with different antibodies, and GRB-2 binding studies. The collective evidence points to the identity of p90 as 80K-H, a protein whose function has not been characterized. Our studies suggest the involvement of 80K-H in FGF signaling.

EXPERIMENTAL PROCEDURES

Reagents, Antibodies, and GST Fusion Proteins—Monoclonal antibodies to phosphotyrosine (PY20H), SHC, and PTP1D were obtained from Transduction Laboratories (Lexington, KY). Anti-phosphotyrosine
antibody (4G10) conjugated to agarose was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Horseradish peroxidase-conjugated anti-rabbit or mouse IgGs and hydrogen peroxide were from Sigma.

CSF-1, tumor necrosis factor-α, IL-1, transforming growth factor-β, EGF, IL-6, and PDGF were from Genzyme (Cambridge, MA). Basic FGF was from Boehringer Mannheim. Fusion proteins consisting of GRB-2 (whole protein), GRB-2 (SH3 domain), GFP, and p90 p80K-H-1029 and p90 p80K-H-1030, and one N-terminal antibody designated 80K-H-1031, recognized a protein on one- and two-dimensional immunoblots that corresponded to the tyrosine-phosphorylated protein p90. These polyclonal antibodies were purified on peptide affinity columns prepared by coupling the synthetic peptide to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer’s recommendations. The column, containing 1 ml of coupled gel, was equilibrated with five gel volumes of PBS. 0.5 ml of the antibody-containing serum was applied to the column, which was washed with five volumes of PBS. Bound proteins were eluted in five fractions of 2 ml of IgG elution buffer (Pierce). The fractions were neutralized immediately with 1 μl Tris-HCl pH 7.5 and assayed by immunoblotting cell lysates known to contain the 80K-H protein.

Immunoprecipitation and Western Blotting—Immunoprecipitations using the 80K-H antibody were performed as follows; 80 μl of lysate (containing membrane proteins from one 15-cm plate of MRC-5 cells) was added to 400 μl of affinity-purified 80K-H antibody and 400 μl of 2% (v/v) Triton X-100, 10 mM sodium benzoate, 10 mM sodium orthovanadate, 5 mM EDTA, and 1% Nonidet P-40 plus other protease inhibitors used in Buffer A). The detergent-soluble proteins were collected by centrifugation at room temperature at 12,000 rmp. The supernatant containing the detergent-soluble fraction (membrane extract) was added with 500 μl of 5 × Laemmli buffer and boiled as above.

Resolution of the Protein on Two-Dimensional PAGE—Two 15-cm plates of MRC-5 cells were prepared as above for stimulation with bFGF for 10 min. After washing with cold PBS, each plate of cells was lysed in 500 μl of the lysis buffer used by Coughlin et al. (13). 200 μl of 5 × Laemmli buffer was added to each aliquot, and after boiling for 10 min the entire lysate was loaded onto each side of a one-dimensional PAGE mini gel and electroeluted. Western blotting was performed using one-dimensional PAGE mini gels and electroblotted onto a PVDF membrane. The electro-elute was dialyzed against 1% Nonidet P-40 in PBS before the proteins were precipitated by adding two volumes of acetone and incubating on ice for 30 min. The precipitated protein was collected by maximal centrifugation in a bench-top microcentrifuge (Eppendorf) for 15 min at 4 °C. The protein pellet was solubilized in two-dimensional PAGE sample loading buffer as described previously (24) and loaded onto the first dimension of the Millipore Investigator two-dimensional electrophoresis system (Millipore, Bedford, MA). The running conditions were essentially as described previously (23), except that the first dimension isoelectric focusing tubes were reduced to 7.5 cm in length and the second dimension was run on a minigel apparatus (Bio-Rad), following the specifications in a minigel apparatus (Bio-Rad). After blotting into a mini-dimension, the membrane was stained with Amido Black, washed in deionized water and wrapped in plastic sheet for photocopying. The membrane was then probed with PV201 antibody and the location of tyrosine-phosphorylated proteins visualized by ECL (Amerham). The location of any Amido Black-stained spots coinciding with the p90 phosphotyrosine signal was noted.
Tyrosine-phosphorylated p90 Is Located in the Membrane—
The subcellular location of the tyrosine-phosphorylated p90 protein was ascertained by fractionating the cell into cytosolic and membrane extracts after treatment with bFGF. These fractions, along with total cell lysates from stimulated and unstimulated cells, were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. The tyrosine-phosphorylated proteins were visualized by ECL after probing with anti-phosphotyrosine antibodies (PY20H).

Other ligands which are known to stimulate tyrosine phosphorylation and/or gene induction in these fibroblasts were also unable to induce the tyrosine phosphorylation of p90. These included both human and murine homologues of α/β andy interferons, tumor necrosis factor-α, IL-1, CSF-1, and transforming growth factor-β (data not shown).

Tyrosine-phosphorylated p90 Is Located in the Membrane—
The subcellular location of the tyrosine-phosphorylated p90 protein was ascertained by fractionating the cell into cytosolic and membrane extracts after treatment with bFGF. These fractions, along with total cell lysates from stimulated and unstimulated cells, were resolved by SDS-PAGE and transferred onto a PVDF membrane. The tyrosine-phosphorylated proteins were visualized by ECL after probing with anti-phosphotyrosine antibody (PY20H). Tyrosine phosphorylation of p90 occurs only in the membrane fraction of bFGF-stimulated cells (Fig. 2, lane 4) as opposed to the cytosolic fraction from the same cells (lane 3). This membrane p90 protein co-migrated with p90 from the total cell lysate of bFGF-stimulated cells (lane 2). It is noteworthy that the summation of lanes 3 and 4 corresponded well with lane 2.

The identification and characterization of p90, which is so far the only protein to undergo tyrosine phosphorylation uniquely in response to FGF, may be relevant to understanding the specificity of FGF signaling.

Two-dimensional Electrophoretic Resolution and Identification of p90—For the partial purification of p90, MRC-5 cells were stimulated with bFGF for 10 min and the cell lysates resolved by two-dimensional PAGE (see “Experimental Procedures”). A prominent phosphotyrosine spot appeared on the blot (Fig. 3A) at a molecular mass of 90 kDa and an acidic pI of 4.5, which coincided with a group of three closely spaced spots stained by Amido Black. The corresponding protein spots from a parallel experiment, that does not involve PY20H probing, were excised and used for direct N-terminal sequencing. One unambiguous amino acid sequence was obtained (VEVKPRGVSTNHHFYD) which matched exactly with the first eighteen residues from the truncated N terminus of a protein designated as 80K-H (25), as shown in Fig. 3B.

Rabbits were immunized with synthetic peptides derived from the experimental N terminus and the deduced C terminus of the protein. Two antibodies (80K-H-1029 and 80K-H-1030) raised against the 14-residue C-terminal peptide and one (80K-H-1031) raised against the 14-residue N-terminal peptide recognized a protein that co-migrates on two-dimensional PAGE gel with the heavily tyrosine-phosphorylated p90 protein in FGF-stimulated cells (Fig. 3C). Control Western blots using the respective preimmune sera did not detect any spots at 90 kDa (data not shown).

Immunoprecipitation studies carried out with crude or purified 80K-H antibodies were not successful. However, anti-phosphotyrosine immunoprecipitation clearly indicated that 80K-H is tyrosine-phosphorylated only in the bFGF-stimulated but not control cells (Fig. 4). MRC-5 cells were treated with bFGF for 10 min and separated into cytosol and membrane fractions. The membrane pellet was solubilized in SDS-containing buffer and incubated with 4G10 antibody conjugated to agarose. In Fig. 4, 80K-H is detected at 90 kDa in lane 4, which corresponds to tyrosine phosphoproteins derived from bFGF-stimulated cells. No 80K-H is detectable in immunoprecipitates obtained from unstimulated cells (lane 3). Although the amount of lysates used for immunoprecipitation in lanes 3 and 4 is about 20-fold higher than the loading in lanes 1 and 2, the signal for 80K-H is stronger in the latter lanes. This implies that the tyrosine-phosphorylated 80K-H represents only a small fraction of the total cellular 80K-H.

p90 Binds to GRB-2-GST Fusion Protein and Co-migrates with 80K-H—Recently, a number of novel tyrosine-phosphorylated proteins have been identified by virtue of their specific association with GRB-2 (21, 22, 26–28). Such specific interactions can significantly enrich some tyrosine-phosphorylated proteins, thus acting as an affinity purification method for...
associated proteins were separated, transferred to a PVDF membrane, incubated with anti-phosphotyrosine antibodies, and visualized by ECL. Lanes 1 and 2 correspond to membrane lysates from control and stimulated cells, respectively. Lanes 3 and 4 contain the phosphotyrosine proteins from the respective lysates. The arrow indicates the position of p90/80K-H.

The 60- and 72-kDa proteins were identified as SHC and PTP1D, respectively, by stripping the membranes and reprobing with the appropriate antibodies (data not shown). These proteins have already been reported to bind directly to GRB-2 (29, 30). The 90-kDa protein was investigated in experiments described below, while the 115-kDa protein remains unidentified.

We decided to compare the two-dimensional electrophoretic behavior of the GRB-2-associating p90 with that of p90 in the total lysate to verify that they were identical proteins. Native cell lysates from bFGF-stimulated Swiss 3T3 cells were incubated with GRB-2-GST fusion protein conjugated to agarose beads. The complexed proteins were eluted from the beads with two-dimensional PAGE loading buffer (32) and then separated by two-dimensional PAGE. The resulting autoradiograph (Fig. 5B) shows four major protein groups, consistent with the four main tyrosine-phosphorylated proteins observed in one-dimensional SDS-PAGE (Fig. 5A, lane 2). In particular, the 90-kDa spot (Fig. 5B) co-migrates with the p90 spot in total cell lysates (Fig. 3A), possessing a similar pI and the same oblique inclination. The membrane was stripped and reprobed with 80K-H antibody which, upon visualization by ECL, gives a result similar to that shown in Fig. 3C.

80K-H Binds Mainly to the SH2 Domain of GRB-2. To evaluate the relative contribution of the three domains of GRB-2 in binding the four FGF-stimulated tyrosine-phosphorylated proteins, particularly p90/80K-H, three separate GST fusion proteins that incorporated either the N-terminal SH3, the middle SH2, or the C-terminal SH3 domain were employed in binding experiments similar to those described above. The resulting autoradiograph from one such experiment is shown in Fig. 6A. Lanes 1 and 2 correspond to cell lysates from unstimulated and FGF-stimulated cells, respectively. Lanes 3 and 4 show the tyrosine-phosphorylated proteins that bind to the SH2 domain of GRB-2 in unstimulated (lane 3) and FGF-stimulated cells (lane 4). It is apparent that three of the four tyrosine-phosphorylated proteins that associate with the whole GRB-2 protein...
are present in lane 4. The apparent amounts of tyrosine-phosphorylated SHC, PTP1D, and p90 are enhanced when compared to the equivalent amounts of each protein in the whole cell lysate. Both SHC and PTP1D have been shown to bind to GRB-2 protein via specific interactions between phosphotyrosine-containing motifs and the SH2 domain of GRB-2 (29, 30). It is likely that p90 interacts with the GRB-2 SH2 domain in a similar manner. Lanes 5 and 7 show that neither the N- nor C-terminal SH3 domains associate with any phosphotyrosine-containing proteins in the control cell lysates. Lanes 6 and 8 are the equivalent lanes obtained from binding experiments with lysates from FGF-stimulated cells. Only lane 8 contains a significant amount of tyrosine-phosphorylated proteins. Notably the 115-kDa protein is present in this lane, which implies that it binds to the C-terminal SH3 domain of GRB-2 in a specific manner. As SH3 domains interact with proline-rich motifs, the small but detectable amount of p90 protein present in lane 8 suggests a proline-rich sequence in p90. The absence of tyrosine-phosphorylated proteins in lanes 9 and 10 demonstrates the absence of any nonspecific interactions between phosphotyrosine proteins and the GST-agarose matrix.

Further proof of the identity of p90 as 80K-H and an estimation of the extent of its tyrosine phosphorylation was obtained by examining the p90 that bound to GRB-2(SH2)-GST fusion protein. In Fig. 6B, lanes 1 and 2 contain the proteins in cytosol and membrane fractions of FGF-treated MRC-5 cells, respectively. Aliquots of these lysates were incubated with GRB-2(SH2)/GST protein and subsequently separated on two-dimensional PAGE. The membrane was incubated with PY20H antibody and visualized by ECL. The arrow indicates the position of the 90-kDa protein that binds to GRB-2.

In summary, the identification of p90 as 80K-H was initially obtained from microsequencing of a protein resolved on two-dimensional PAGE. Antibodies raised against 80K-H consistently recognize p90 on all one-dimensional- and two-dimensional Western blots. The use of anti-phosphotyrosine immunoprecipitation and GRB-2 association studies provided proof that 80K-H is tyrosine-phosphorylated and binds to the SH2 domain of GRB-2 in vitro.

**DISCUSSION**

The involvement of receptor tyrosine kinases in cytokine and growth factor signaling has been reported extensively. Many of these studies have focused on the receptors for EGF and PDGF,
which were the first to be sequenced from this family. Rela-
tively little is known about the activation and downstream
signaling of the FGF receptor subfamily. Previous work had
identified a few proteins that were tyrosine-phosphorylated in
the early stages of FGF-induced signal transduction. These
proteins included Src, cortactin, SHC, ERK-1, and ERK-2 (7–9,
11, 31–33). One prominent protein that remained unidentified
was designated p90. The interest in p90 was heightened by its
apparent specificity to the FGF signaling system, in contrast to
the common activation of Src, SHC, and ERK by numerous
different growth factors.

In EGF- and PDGF-stimulated cells, both autophosphoryl-
ated EGFR and PDGFR are, respectively, the dominant tyro-
sine phosphoproteins seen in Western blots from whole cell
lysates. The phosphorylated tyrosine residues on these recep-
tors serve to recruit specific proteins containing complemen-
tary SH2 domains. The protein complexes thus formed are
thought to be responsible for initiating various signaling path-
ways into the interior of the cell. We and others have observed
that FGF receptors were not noticeably tyrosine-phosphoryl-
ated in response to FGF stimulation of various cells. Similarly,
the insulin receptor shows a low level of tyrosine phosphoryl-
ation upon binding with insulin (34). In the latter case, how-
ever, a protein known as IRS becomes heavily tyrosine-phos-
phorylated after associating with the activated insulin
receptor, thereby presenting multiple docking sites for SH2-
containing signaling proteins (34). Thus, in terms of the low
level of receptor autophosphorylation, the insulin and FGF
receptors show similar features following ligand activation. It
is therefore possible that the FGF-specific tyrosine phosphor-
ylation of p90 may serve a role similar to that seen with IRS
in the insulin system where it could trigger signal propagation
involving the formation of specific protein complexes.

Several proteins that are devoid of intrinsic catalytic activity
can facilitate the assembly of these signaling protein com-
plexes. These “adaptor” proteins include GRB-2, Crk, and Nck,
which consist almost entirely of SH2 and SH3 domains. A
number of tyrosine-phosphorylated proteins have been shown
to be capable of binding to GRB-2 in vivo (17, 18). They include,
among a growing list, both the insulin receptor and IRS. In our
in vitro assays, the FGF-specific p90 protein was found to
associate with GRB-2, in addition to PTP1D, SHC, and an
unidentified 115-kDa protein. Both PTP1D and SHC have well
characterized SH2 domains and have been shown previously
to bind directly to GRB-2 (29, 30). Beyond our verification that
PTP1D and SHC bind only to the SH2 domain of GRB-2, we
have demonstrated that p90 also binds mainly to the SH2
domain. Since such associations were specific, they would pro-
vide a means for enriching tyrosine-phosphorylated p90 pro-
tein from cell lysates and aid in its identification. The 115-kDa
protein, and to a lesser extent p90, were further shown to bind
to the C-terminal SH3 domain of GRB-2, possibly via their
respective proline-rich motifs.

The p90 protein was resolved by two-dimensional PAGE
and subjected to microsequencing. An 18-residue N-terminal se-
quence was obtained, which matched exactly with the first 18
residues from the truncated N terminus of a human protein
previously designated as 80K-H (25). Polyclonal antibodies
were raised in rabbits against the predicted C terminus (14
residues) and observed N terminus (14 residues) of 80K-H.
Several of these antibodies recognized a protein that co-mi-
grated with p90 on Western blots derived from one- and two-
dimensional PAGE. Additional evidence for the identity of p90
comes from anti-phosphotyrosine immunoprecipitation, in
vitro GRB-2-GST association experiments, and reversed-phase
high performance liquid chromatography (data not shown)
where p90 is being recognized by the 80K-H antibody.

The 80K-H protein was isolated several years ago during an
effort to identify a ubiquitous 80–87 kDa protein that is a
strong substrate protein kinase C (35). The main target
protein of this work turned out to be the MARCKS protein (36),
also designated 80K-L (L for light), which is now a well char-
acterized PKC substrate. The 80K-H protein (H for heavy) is
only a weak PKC substrate in vitro and appears not to be a
PKC substrate in vivo (35). Aside from its subsequent cloning
(25), the 80K-H protein was not characterized in greater detail.
Its amino acid sequence deduced from cDNA contains several
noteworthy features. Analysis performed on the Swiss-Prot
data base revealed four putative protein kinase C phosphory-
lation sites, a possible calcium binding EF-hand domain, and a
prominent glutamic acid repeat around the middle of the pro-
tein, which is seen in some proteins including tropomyosin,
prothymosin, neurofilament triple L protein, myc-transforming
protein, and adenovirus 5’ terminal protein. There is a C-
terminal HDEL sequence, which is a possible endoplasmic
reticulum retention signal, although functional HDEL se-
quences are usually not found in mammalian cells (37). There
are also at least three proline-rich motifs, PXXP, which may
account for the low level binding of 80K-H to the SH3 domain
of GRB-2. A consensus motif pYXXN has been reported in
various proteins such as PTP1D, SHC, EGFR, and focal adhe-
sion kinase, where the SH2 domain of GRB-2 binds (17). While
the tyrosine-phosphorylated 80K-H binds the SH2 domain of
GRB-2, no such sequence was found in the former protein.
Further characterization of the 80K-H/GRB-2 interaction may
help to resolve this discrepancy.

If the deduced amino acid sequence of 80K-H predicts a size
of 58 kDa, which is much smaller than the 90-kDa migration in
SDS-polyacrylamide gels. The predicted isoelectric point is
around 4.4, which corresponds well with the experimental
value. The size discrepancy is not without precedence since
highly acidic proteins have been known to migrate anoma-
lously upon SDS-PAGE. For example, the bovine MARCKS
protein, with a pl similar to that of 80K-H, migrates at 80–87
kDa although its predicted molecular mass is only 32 kDa (38).
The 80K-H sequence contains 15 tyrosine residues, which are
evenly distributed in the protein. There is, however, a rather
prominent glutamic acid repeat around the middle of the pro-
tein, suggesting that the native structure for 80K-H can
contain multiple intra- and/or intermolecular disulfide bridges.
Molecular modeling of 80K-H was not successful, as the protein
does not contain enough sequence homology to known struc-
tural domains in existing data bases. We have further shown
that while this protein is present in both the cytosol and mem-
brane, the tyrosine-phosphorylated species is found in the
membrane and can bind the SH2 domain of GRB-2 in vitro.
Based on densitometric scanning, we estimated the proportion
of tyrosine-phosphorylated 80K-H to be less than 1% of total
cellular 80K-H.

The physiological function of 80K-H is yet unknown. We now
report a possible role for 80K-H in FGF signaling. The bioch-
emical characteristics of this novel p90/80K-H protein; its expres-
sion in different cells, tissues, and organisms; and its potential
function in FGF-induced cell signaling and development will be
the subject of further study.

Acknowledgments—We thank the following people who helped dur-
ing the course of this work: Desmond Ng and Joyce Low for technical
assistance, Dr. Thomas Klein for his enthusiasm, Robin Philip for ex-
cellent microsequencing work, Dr. Catherine Pallen for helpful discus-
sions, Dr. Naeraj J. Jain for advice and encouragement, and the labora-
tory of Professor N. Shimizu, Keio University, School of Medicine,
Tokyo, Japan, for assistance.
REFERENCES

1. Burgess, W. H., and Maciag, T. (1989) Annu. Rev. Biochem. 58, 575–606
2. Johnson, D. E., and Williams, L. T. (1993) Adv. Cancer Res. 60, 1–41
3. Wilkie, A. O. M., Morris-Kay, G. M., Jones, E. Y., and Heath, J. K. (1995) Curr. Biol. 5, 500–507
4. Hou, J., Kan, M., McKeohan, K., McBraddie, G., Adams, P., and McKeohan, W. L. (1991) Science 251, 665–668
5. Mohamed, M., Dione, C. A., Li, W., Spivak, T., Honegger, A. M., Jaye, M., and Schlessinger, J. (1992) Nature 358, 691–694
6. Pelicci, G., Lanfrancone, L., Grignani, F., McGlade, J., Cavallo, F., Formi, G., Nicoletti, I., Grignani, F., Pawson, T., and Pelicci, P. G. (1992) Cell 70, 93–104
7. Cruschet, C., Loeb, J., and Barbin, G. (1995) J. Neurochem. 64, 1541–1547
8. Bhowmik, M. A., Glennon, P. E., Andersson, M. B., Clerk, A., Lazou, A., Marshall, C. J., Parker, P. J., and Sugden, P. H. (1994) J. Biol. Chem. 269, 1101–1110
9. Zhan, X., Plourde, C., Hu, X., Friesel, R., and Maciag, T. (1993) J. Biol. Chem. 268, 20221–20224
10. Zhan, X., Hu, X., Hampton, B., Burgess, W. H., Friesel, R., and Maciag, T. (1993) J. Biol. Chem. 268, 24427–24431
11. Landgren, E., Blume-Jensen, P., Courtneidge, S. A., and Claesson-Welsh, L. (1995) Oncogene 10, 2027–2035
12. Bottaro, D. P., Rubin, J. S., Ron, D., Finch, P. W., Florio, C., and Aaronson, S. A. (1990) J. Biol. Chem. 265, 12767–12770
13. Coughlin, S. R., Barr, P. J., Cousens, L. S., Fretto, L. J., and Williams, L. T. (1988) J. Biol. Chem. 263, 988–993
14. Friesel, R., Burgess, W. H., and Maciag, T. (1989) Mol. Cell. Biol. 9, 1857–1865
15. Shaoul, E., Rech-Slootky, R., Berman, B., and Ron, D. (1995) Oncogene 10, 1553–1561
16. Zhan, X., Hu, X., Friesel, R., and Maciag, T. (1993) J. Biol. Chem. 268, 9611–9620
17. Pawson, T. (1992) Science 260, 689–692
18. Li, W., Nishimura, R., Kashishian, A., Batzer, A. G., Kim, W. J. H., Cooper, J. A., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 500–517
19. Migita, K., Eguchi, K., Tsukada, T., Kawabe, Y., Aoyagi, T., and Nagataki, S. (1995) Biochem. Biophys. Res. Commun. 210, 1066–1075
20. Vainikka, S., Joukov, V., Wennstrom, S., Bergman, M., Pelicci, P. G., and Aaltonen, K. (1994) J. Biol. Chem. 270, 7587–7593
21. VanderKuur, J., Allevato, G., Billestrup, N., Norstedt, G., and Carter-Su, C. (1995) J. Biol. Chem. 270, 7587–7593
22. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 1–4
23. Hirai, M., and Shimizu, N. (1991) Biochim. Biophys. Acta 1090, 175–178
24. Sakai, K., Hirai, M., Kudoh, J., Minoshima, S., and Shimizu, N. (1992) Genomics 14, 175–178
25. Pelham, H. R. B. (1990) Trends Biochem. Sci. 15, 483–485
26. Stumpo, D. J., Graff, J. M., Albert, K. A., Garengg, P., and Blackshear, P. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4012–4016