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# Antibodies to two defined regions of the transforming protein pp60$^{src}$ interact specifically with the epidermal growth factor receptor kinase system

(oncogenes/tyrosine kinase)

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ABSTRACT **Antibodies generated against two synthetic peptides corresponding to two defined regions on the transforming protein of Rous sarcoma virus, pp60$^{src}$, interact specifically with the epidermal growth factor (EGF)-receptor kinase. An antibody directed against a synthetic peptide corresponding to the major phosphorylation site of pp60$^{src}$ interacts specifically with EGF receptor and immunoprecipitates a functional EGF-receptor kinase. The second antibody, which binds close to a region on the src molecule that is required for its kinase activity, also binds to EGF-receptor kinase and prevents the autophosphorylation of the receptor molecules. Neither antibody binds to intact cells, but they do recognize various forms of the solubilized receptor. It is concluded that at least two cytoplasmic domains of the EGF receptor are antigenically and presumably also structurally related to specific domains on pp60$^{src}$.**

Epidermal growth factor (EGF) is a potent mitogen of various cell types *in vitro* and *in vivo* (for reviews see refs. 1 and 2). EGF binds with high affinity to a specific membrane receptor, which was identified as a 170-kilodalton (kDa) glycoprotein. The binding of EGF to the receptor molecule activates a tyrosine-specific cyclic-nucleotide-independent protein kinase that phosphorylates various cellular proteins, including the EGF receptor itself (3). The kinase activity appears to be an integral part of the receptor molecule (4).

The role of tyrosine phosphorylation as a possible molecular signal for the initiation of the mitogenic response is not clear. Several studies indicate that EGF-induced tyrosine phosphorylation does not serve as a "second messenger" for growth but rather provides a necessary but insufficient signal for DNA replication (2, 5, 6).

Tyrosine-specific kinases were originally demonstrated in relationship to the activity of transforming proteins of retroviruses (7–9). The most well-characterized tyrosine-specific protein kinase is pp60$^{src}$, the transforming protein of Rous sarcoma virus (7, 9). pp60$^{src}$ is a membrane protein that is phosphorylated on at least three different residues. The phosphorylated residues are serine-17 (10), tyrosine-419, and an unidentified tyrosine residue at the carboxyl terminus of the molecule (10). Antibodies against pp60$^{src}$ are phosphorylated on tyrosine residues when [$\gamma$-$^{32}$P]ATP is added to the immunoprecipitate formed between pp60$^{src}$ and its antibodies. Interestingly, antibodies against pp60$^{src}$ are also phosphorylated on tyrosine residues when added to membranes containing EGF-receptor kinase from A-431 cells (11, 12). However, these antibodies did not precipitate the EGF-receptor kinase.

In this paper we report that antibodies generated against synthetic peptides corresponding to two regions of pp60$^{src}$ interact specifically with the EGF-receptor kinase. An antiserum directed against the major phosphorylation site of pp60$^{src}$ immunoprecipitates a functional EGF-receptor kinase. Another antiserum binds to EGF receptor and blocks the autophosphorylation of the receptor molecule. It is concluded that at least two domains on EGF receptor are antigenically related to specific domains on pp60$^{src}$.

## MATERIALS AND METHODS

**Cells.** Human epidermoid carcinoma cells A-431 were maintained in culture in Dulbecco's modified Eagle's medium (GIBCO) containing 10% fetal calf serum.

**Materials.** EGF was purified from the submaxillary gland of male mice by the method of Savage and Cohen (13). [$\gamma$-$^{32}$P]ATP was purchased from New England Nuclear. Membrane vesicles from A-431 cells were prepared and characterized according to Cohen *et al.* (14). Full details concerning the generation and properties of monoclonal antibodies TL5-IgG3 and 29.1 IgG1 against EGF receptor and concerning the polyclonal antibodies R1 and R2 have been given previously (15, 16).

**Peptide Synthesis, Immunization, and Generation of Antibodies Against Them.** Peptides were synthesized by the solid-phase method (17). The crude products obtained after cleavage from the resin support and deprotection were found to be 70% the desired product as analyzed by TLC and amino acid analysis. Amino acid analysis of the HPLC-purified peptides gave the expected composition.

The pure peptides were conjugated to freshly activated keyhole limpet hemocyanin (KLH) according to Dockray (18). The KLH-bound peptides were separated from the free peptides by gel filtration on Sephadex G-50. Adult male New Zealand White rabbits were each injected with 200 $\mu$g of peptide-KLH conjugate in complete Freund's adjuvant intramuscularly and subcutaneously at several sites. Booster doses of 200 $\mu$g of the conjugate in incomplete Freund's adjuvant were administered on days 14, 21, and 30. Ten days later the rabbits were bled.

Preimmune sera and test sera were collected and the antibody titers against the peptides were measured by an enzyme-linked immunosorbent assay (ELISA) by using horseradish peroxidase conjugated to goat anti-rabbit IgG as the assay enzyme. Absorbance values were plotted against serum dilutions. The dilution giving one-half maximal color at the end of the linear range of the plot was taken as the "titer" of the antiserum. Significant titers (1:10,000) were observed within 6 weeks after the initial immunization.

**Immunoaffinity Purification of EGF Receptor.** Full details concerning the purification of EGF receptor will be pub-

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Abbreviation: EGF, epidermal growth factor.

lished elsewhere (19). In brief, EGF receptor extracted from A-431 cells was immobilized on Sepharose coupled with clone 29 monoclonal antibody against EGF receptor (1). The column was washed extensively and the immunoaffinity-purified receptor was phosphorylated by the addition of [$\gamma$-$^{32}$P]ATP (20 $\mu$Ci, 5000 Ci/mmol; 1 Ci = 37 GBq) and 3 mM $MnCl_2$ for 10 min at 4°C. The column was washed again and the receptor was eluted with 50 mM glycine·HCl buffer, pH 2.5, containing 0.1% Triton X-100, 150 mM NaCl, and 10% (vol/vol) glycerol. Various fractions were pooled according to their radioactivity and applied on a preparative polyacrylamide gel after heating for 3 min at 95°C in Laemmli sample buffer. The $^{32}$P-labeled receptor was identified by autoradiography, excised from the gel, and electroeluted.

$^{125}$I-labeled EGF receptor was prepared by the chloramine-T method (20).

**Immunoprecipitation of $^{125}$I-Labeled EGF Receptor.** Staphylococcal protein A-Sepharose 4B (Pharmacia), 2 mg in 100 $\mu$l of phosphate-buffered saline incubated at room temperature for 30 min with the various rabbit polyclonal antibodies. Then the beads were centrifuged for 5 sec in an Eppendorf centrifuge and washed with 1 ml of phosphate-buffered saline. Aliquots of the $^{125}$I-labeled EGF-receptor were diluted with phosphate-buffered saline/0.1% bovine serum albumin/10% glycerol to a 0.2% final concentration of Triton X-100 and incubated with the antibody-protein A-Sepharose complex on an Eppendorf shaker for 2 hr at 4°C. The beads were washed four times with 1 ml of 20 mM Hepes, pH 7.4/150 mM NaCl/0.2% Triton X-100/10% glycerol/0.03% $NaN_3$. Immunoprecipitated EGF receptor was solubilized in 30 $\mu$l of Laemmli buffer for analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE).

**Phosphorylation of Immunoprecipitated EGF-Receptor Kinase.** The immunoprecipitated EGF receptor was mixed with 30 $\mu$l of 20 mM Hepes, pH 7.4/150 mM NaCl/0.2% Triton X-100/10% glycerol/2 mM $MnCl_2$/0.03% $NaN_3$ and 1–2 $\mu$Ci of [$\gamma$-$^{32}$P]ATP. The phosphorylation reaction was stopped after incubation for 10 min on ice by adding 3 $\mu$l of Laemmli buffer to the solution and by boiling it for 5 min.

**Immunoblot Analysis.** $NaDodSO_4$/PAGE was performed in 7.5% polyacrylamide by the method of Laemmli (21). After PAGE, the gels were removed and placed in a multiple-layer "sandwich": one electrode on a Scotch Brite pad, two Whatman 3MM paper filters, the polyacrylamide gel, a sheet of nitrocellulose paper, two more Whatman paper filters, and another Scotch Brite pad, with the second electrode. The layers were held together by rubber bands and placed in a chamber containing 20 mM Tris base, 150 mM glycine, and 20% (vol/vol) methanol. Residual binding sites on the paper were blocked by incubation for 40 min at 37°C in a solution containing 0.9% NaCl, 10 mM Tris·HCl at pH 7.4 (Tris/saline), 3% gelatin (Sigma), and 0.05% Tween-20. The sheets were transferred to Tris/saline with gelatin and Tween-20 containing the appropriate antibodies and were incubated overnight on a rocking platform at room temperature. After several washes with Tris/saline and Tris/saline containing 0.05% Nonidet P-40, the nitrocellulose papers were incubated with iodinated protein A (Sigma), according to the procedure of Burnette (22). The sheets were again rinsed as described above, briefly blotted with paper towels, wrapped in Saran Wrap, and exposed at −70°C to Kodak XAR-5 film.

## RESULTS

Polyclonal rabbit antibodies were prepared against the peptide Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly-Ala-Lys-Phe-Pro, designated src1, and against the peptide Asn-Arg-Glu-Val-Leu-Asp-Gln-Val-Glu-Arg-Gly-Tyr-Arg-Met-Pro, designated src2. src1 contains residues 412–428 of pp60$^{src}$ and corresponds to the phosphorylation site of pp60$^{src}$. src2 contains residues 488–502 of pp60$^{src}$. It was previously reported that antibodies generated against synthetic peptide corresponding to residues 498–512 (23) or to residues 500–506 of pp60$^{src}$ neutralize its kinase activity (24). Hence, either this domain is directly associated with the kinase portion of src or the binding of antibodies modulates the activity of the kinase portion.

We have used several approaches to determine possible specific interactions between the EGF-receptor kinase and the two antibodies against src1 and src2.

**Anti-src1 and Anti-src2 Specifically Bind to EGF Receptor.** A partially purified preparation of EGF receptor from A-431 cells was analyzed by immunoblot analysis with anti-src1, anti-src2, polyclonal antibodies against EGF receptor, and various control antibodies (see Fig. 1). A similar analysis was done with membranes from A-431 cells (see Fig. 2).

The results presented in Figs. 1 and 2 indicate that both anti-src1 and anti-src2 specifically recognize the 170-kDa polypeptide (Fig. 1, lanes c and d) which is also bound by the monoclonal and polyclonal antibodies against EGF receptor (Fig. 1, lanes a and b). Preimmune antiserum and various control antisera against irrelevant synthetic peptides did not reveal the 170-kDa band of EGF receptor (Fig. 1). Additional control antibodies that did not interact with EGF receptor include polyclonal antibodies against phosphotyrosine and antibodies against synthetic tyrosine-containing peptide from the transforming protein of v-*ras* (data not shown).

Several measures were taken to determine the specificity of the blotting experiment. The 170-kDa bands observed with

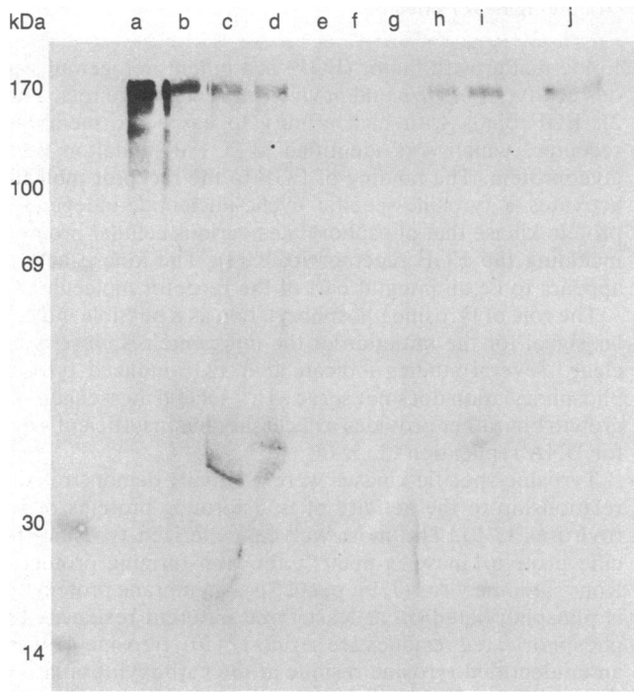


FIG. 1. Immunoblot analysis of EGF receptor with anti-src antibodies. A partially purified preparation of EGF receptor from A-431 cells was loaded on a $NaDodSO_4$/7.5% polyacrylamide gel and then transferred to nitrocellulose paper and allowed to react with the following antibodies: Lane a, monoclonal anti-EGF-receptor antibodies (TL-5-$IgG_3$); lane b, rabbit anti-EGF-receptor antibodies (R1); lane c, anti-src1; lane d, anti-src2; lane e, preimmune serum of anti-src2; lane f, anti-src1 preincubated with src1 at 200 $\mu$g/ml; lane g, anti-src2 preincubated with src2 at 200 $\mu$g/ml; lane h, anti-src1 preincubated with src2; and lane i, anti-src2 preincubated with src1. Lane j, $^{32}$P-labeled partially purified EGF receptor. Markers are indicated on the left.

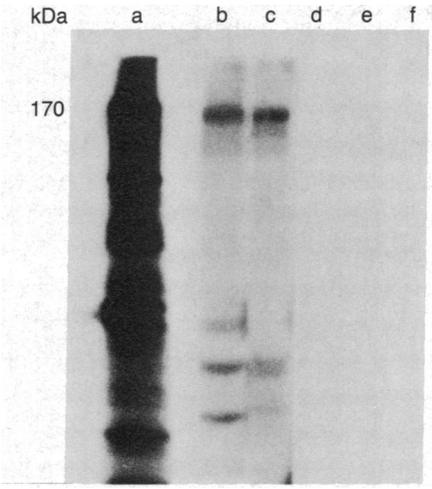


FIG. 2. Immunoblot analysis of membranes prepared from A-431 cells with anti-src antibodies. A-431 cells were lysed, loaded on a $NaDodSO_4$/7.5% polyacrylamide gel, transferred to nitrocellulose paper and allowed to react with the following antibodies: Lane b, anti-src1; lane c, anti-src2; lane d, preimmune serum of anti-src2; lane e, anti-src1 preincubated with src1 at 200 $\mu$g/ml; and lane f, anti-src2 preincubated with 200 $\mu$g of src2. Lane a, $^{32}$P-labeled A-431 membrane.

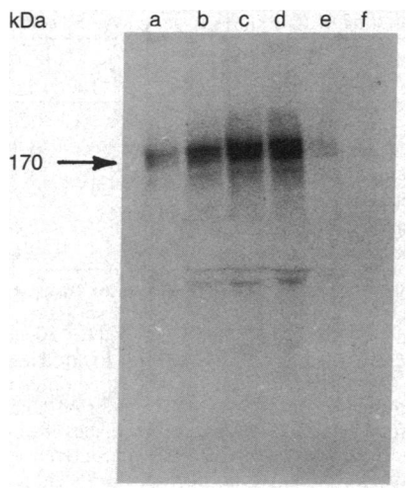


FIG. 3. Immunoprecipitation of $^{125}$I-labeled EGF receptor with the anti-src antibodies. The various antibodies were coupled to protein A-Sepharose beads. After several washes, the bead-bound antibodies were incubated with aliquots of $^{125}$I-labeled EGF receptor for 2 hr at 4°C. The beads were then washed several more times and the immunoprecipitated receptor was solubilized in 30 $\mu$l of Laemmli buffer and analyzed by $NaDodSO_4$/PAGE and by autoradiography. $^{125}$I-labeled EGF receptor was immunoprecipitated by the following: lane a, anti-src1 antibodies; lane b, anti-src2 antibodies; lane c, anti-R1 antibodies; lane d, anti-R2 antibodies; lane e, normal rabbit IgG; lane f, preimmune serum of rabbit immunized with src2.

the anti-src1 and anti-src2 antibodies were greatly diminished when the two antibodies were preincubated with the corresponding peptides (Fig. 1, lanes f and g). However, the src1 peptide did not reduce the binding of anti-src2 antibody to EGF receptor, and similarly src2 peptide did not affect the binding of anti-src1 to the EGF-receptor (Fig. 1, lanes i and j). Hence, anti-src1 and anti-src2 specifically recognize the membrane receptor for EGF.

Further experiments were done to determine the capacity of anti-src1 and anti-src2 to recognize a denatured form of pure EGF receptor. EGF receptor from A-431 cells was purified by immunoaffinity chromatography with monoclonal antibodies against EGF receptor (19). In the final step of purification the pure denatured receptor was electroeluted from a preparative $NaDodSO_4$ gel and then radiolabeled with iodine-125. Fig. 3 depicts $NaDodSO_4$/PAGE analysis of the $^{125}$I-labeled EGF receptor immunoprecipitated with different types of antibodies. As previously reported, polyclonal antibodies against EGF receptor immunoprecipitate the 170-kDa polypeptide. Similarly, the anti-src1 and anti-src2 antibodies immunoprecipitate the same polypeptide band with efficiency similar to that of the polyclonal anti-receptor antibodies. Preimmune antibodies and various control antibodies did not precipitate the $^{125}$I-labeled EGF receptor (Fig. 3, lanes e and f). Hence, both anti-src1 and anti-src2 antibodies recognize the denatured form of EGF receptor.

The specificity of the interaction between the EGF receptor and the two antibodies against the synthetic peptides was further established with a sensitive radioimmunoassay (19). EGF receptor was immobilized on immunoaffinity beads conjugated with monoclonal anti-receptor antibody 29.1 IgG1. Then various antibodies were added for 1 hr at room temperature. After extensive washes the amount of the receptor-bound antibody was determined by the use of $^{125}$I-labeled protein A. Note that the radiolabeled protein A does not bind to the 29.1 IgG1 antibody and therefore its binding provides a direct measure for the various antibodies that bind to EGF receptor—i.e., anti-src1, anti-src2, R1, and TL-5-IgG3. The data presented in Table 1 show that both anti-src1 and anti-src2 bind specifically to EGF receptor. Interestingly, the anti-src1 and anti-src2 antibody preparations that were cleared on the immobilized EGF receptor lost a significant portion of their capacity to immunoprecipitate the denatured $^{125}$I-labeled EGF receptor. Anti-src1 lost 70% of its activity and anti-src2 lost 68% of its activity. Hence, a similar repertoire of antibodies that recognize antigenic determinants on the immobilized active receptor also recognize the denatured form of EGF receptor. Similar binding was measured for polyclonal antibodies R1, generated against pure denatured EGF receptor. Interestingly, the monoclonal antibody TL-5-IgG3 against EGF receptor, which binds to blood group A (25) residues on EGF receptor, binds to the receptor more efficiently compared to the binding of the polyclonal antisera against the denatured receptor.

The results of direct binding experiments using intact A-431 cells are summarized in Table 2 and show that anti-src1, anti-src2, and antiserum R1 do not bind to intact cells. This suggests that their interaction with the EGF receptor occurs with its cytoplasmic domain. Interestingly, another polyclonal antiserum, denoted R2, against EGF receptor, binds to

Table 1. Binding of anti-src1 and anti-src2 to immobilized EGF receptor

| Protein | $^{125}$I-protein A bound, cpm | $^{125}$I-protein A bound/ $^{125}$I-protein A bound to nonimmune IgG |
|---|---|---|
| Anti-src1 | 7,467 ± 151 | 3.14 ± 0.06 |
| Preimmune serum to src1 | 1,622 ± 260 | 0.68 ± 0.11 |
| Anti-src2 | 10,310 ± 88 | 4.34 ± 0.04 |
| Preimmune serum to src2 | 2,638 ± 230 | 1.11 ± 0.1 |
| Polyclonal anti-EGF receptor R1 | 8,843 ± 1,735 | 3.72 ± 0.73 |
| Monoclonal anti-EGF receptor TL5-IgG3 | 83,511 ± 5,170 | 35.16 ± 2.17 |
| Nonimmune rabbit IgG | 2,375 ± 225 | 1.00 ± 0.1 |

Various antibodies were incubated for 2 hr at room temperature with pure EGF receptor immobilized on immunoaffinity beads conjugated with the monoclonal anti-receptor antibody 29.1 IgG1 (19). After several washes, the amount of antibody bound to EGF receptor was quantitatively monitored with radiolabeled protein A. Numbers given are the mean ± SD of duplicate measurements. The experiment was repeated three times with essentially the same results.

Table 2. Binding of antibodies to intact A-431 cells

| Antibody | $^{125}$I-labeled goat anti-rabbit IgG bound, cpm |
|---|---|
| Anti-src1 | 2,760 ± 180 |
| Anti-src2 | 3,083 ± 300 |
| Polyclonal anti-EGF receptor R1 | 3,800 ± 200 |
| Polyclonal anti-EGF receptor R2 | 12,000 ± 980 |
| Polyclonal anti-peptide of influenza virus | 2,600 ± 330 |
| Normal rabbit serum | 3,000 ± 280 |

A-431 cells were grown in 24-well Costar trays and assayed for antibody binding when the cells became confluent. The antibodies were added in Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin buffered with 20 mM Hepes, pH 7.5, for 1 hr at 4°C. Then the cells were washed three times with the buffer and further incubated with 3 μl of $^{125}$I-labeled goat antibodies to rabbit IgG for 1 hr at 4°C. After several washes the cells were lysed with 0.1 M NaOH and their radioactive contents were measured. Numbers are the mean ± SD of duplicates. The experiment was repeated three times.

intact cells (Table 2), suggesting that this antiserum also contains antibodies recognizing the external portion of the receptor molecule.

**Anti-src1 Immunoprecipitates a Functional EGF-Receptor Kinase and Anti-src2 Blocks Autophosphorylation of the EGF Receptor.** A useful approach for the detection of a functional EGF-receptor kinase is to use the ability of the enzyme to autophosphorylate (16, 26, 27). In this assay antibodies against EGF receptor are used for the immunoprecipitation of the receptor molecule from cell lysates. Subsequently, [$\gamma$-$^{32}$P]ATP is added to the immunoprecipitate, resulting in the autophosphorylation of the receptor molecule. Such an immunoprecipitation/autophosphorylation assay was used in several studies to detect a functional EGF-receptor kinase (16, 26, 27).

Fig. 4 depicts NaDodSO$_4$/PAGE analysis of a functional EGF-receptor kinase from shed membrane vesicles of A-431

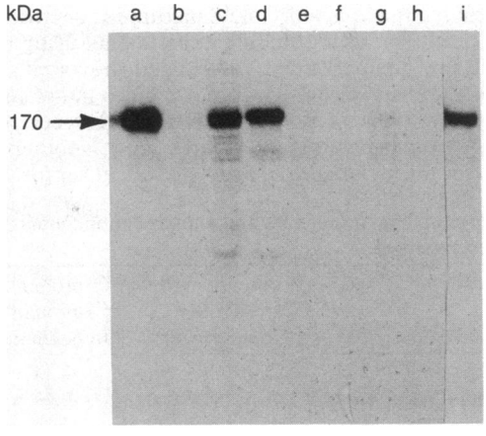


FIG. 4. Immunoprecipitation of a functional EGF-receptor kinase with anti-src antibodies. Aliquots of solubilized A-431 cells were incubated with various antibodies bound to protein A-Sepharose. After several washes, the immunoprecipitate was phosphorylated by [$\gamma$-$^{32}$P]ATP. Autoradiography of NaDodSO$_4$/PAGE analysis of phosphorylation immunoprecipitates is shown. Antibodies were as follows: lane a, anti-src1 antibodies; lane b, anti-src2 antibodies; lane c, polyclonal anti-EGF-receptor antibodies R1; lane d, polyclonal anti-EGF-receptor antibodies R2; lane e, normal rabbit IgG. Lane f, protein A-Sepharose beads alone; lane g, a control rabbit antibody against a synthetic peptide from the oncogene *myb*; lane h, a control rabbit antibody against mouse dihydrofolate reductase; lane i, monoclonal antibody 29.1 IgG1 against the receptor.

cells using the immunoprecipitation/autophosphorylation assay with polyclonal antibodies against EGF receptor. Similar results were obtained when antibody against src1 was used in the immunoprecipitation/autophosphorylation assay (Fig. 4, lane a). All the monoclonal and polyclonal antibodies against EGF receptor and anti-src1 immunoprecipitate the 170-kDa dalton EGF-receptor kinase (Fig. 4, lanes a, c, d, and i). Preimmune antibodies and various control antibodies did not precipitate significant quantities of the EGF-receptor kinase (Fig. 4, lanes e–h).

In contrast to anti-src1 and to the various anti-EGF-receptor antibodies, the anti-src2 failed to detect the EGF receptor with the immunoprecipitation/autophosphorylation assay. Hence, anti-src2 binds to EGF receptor and seems to block the autophosphorylation reaction. However, neither anti-src1 nor anti-src2 is phosphorylated by EGF-receptor kinase or by pp60$^{src}$. This is in contrast to antibodies against pp60$^{src}$, which were shown to be phosphorylated by pp60$^{src}$ (7, 9) and by EGF-receptor kinase (11, 12).

## DISCUSSION

The membrane receptor of EGF is a 170 kDa glycoprotein that possesses at least four domains (2): (*i*) an external EGF-binding site, (*ii*) a transmembrane domain, (*iii*) a tyrosine-specific kinase, and (*iv*) an autophosphorylation site(s).

The studies presented in this paper show that the antigenic determinants on EGF receptor recognized by a polyclonal antibody against EGF receptor (R1) and by the two antibodies generated against the peptides from pp60$^{src}$ are available to the antibodies only after solubilization of the cells with detergents. Moreover, the kinase activity stimulated by EGF in intact cells exposed to [$\gamma$-$^{32}$P]ATP is observed only when the cells are made permeable. Therefore, these results support the view that both the kinase domain and the autophosphorylation site(s) are part of the cytoplasmic portion of the receptor. Proteolytic cleavage of EGF receptor suggests that the external domain which contains the EGF-binding site and oligosaccharide has a molecular mass of ≈115 kDa (28). Thus, the cytoplasmic and transmembranous domains of EGF receptor have an approximate molecular mass of 60 kDa. Our results suggest that at least two domains within the cytoplasmic portion of EGF receptor are antigenically related to two domains of the pp60$^{src}$ molecule. One domain is the major phosphotyrosine site on pp60$^{src}$, and the second domain is either part of the kinase domain of pp60$^{src}$ or can somehow modulate its activity. The inhibition of the autophosphorylation of EGF receptor by an antibody that binds to a region on pp60$^{src}$ that is associated with its kinase activity (23, 24) suggests that this antibody binds to a domain on EGF receptor with a similar function. However, the mechanism by which the anti-src2 antibody neutralizes the kinase activity of EGF receptor is not clear.

The two synthetic peptides belong to the carboxyl-terminal region on pp60$^{src}$, which is highly homologous to transforming proteins encoded by other members of the *src* gene family, *fes*, *mos*, *erb*B, *abl*, *yes*, and *fps* (29). Hence, it is possible that these two domains within the cytoplasmic portion of EGF receptor are antigenically related to the conserved carboxyl-terminal region of all these oncogene products. Moreover, since other receptors for growth factors such as the receptors for plateled-derived growth factor (30), insulin (31), and insulin-like growth factor 1 (32) are also tyrosine-specific kinases, it is possible that their kinase activity is located at their cytoplasmic domain and is related to the kinase portion of EGF receptor and to the other kinases encoded by the *src* gene family.

Recently, Downward *et al.* (33) sequenced several peptides that were derived from EGF receptor from human cells. Interestingly, each of six peptides derived from the human

EGF receptor very closely matched a part of the deduced sequence of the V-*erb*B transforming protein of avian erythroblastosis virus (AEV). The V-*erb*B oncogene is a member of the *src* gene family. Our data support the view (33) that the transforming protein encoded by V-*erb*B acquired the cellular gene sequence of a truncated EGF receptor lacking the external binding domain but retaining the transmembranal domain and a domain involved in stimulating cell proliferation (33).

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1. Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.* **48,** 193–216.
2. Schlessinger, J., Schreiber, A. B., Levi, A., Lax, I., Libermann, T. & Yarden, Y. (1983) *CRC Crit. Rev. Biochem.* **14,** 93–111.
3. Carpenter, G., King, L. & Cohen, S. (1978) *Nature (London)* **276,** 409–410.
4. Cohen, S., Carpenter, G. & King, L., Jr. (1980) *J. Biol. Chem.* **255,** 4834–4842.
5. Yarden, Y., Schreiber, A. B. & Schlessinger, J. (1982) *J. Cell Biol.* **92,** 687–693.
6. Schreiber, A. B., Yarden, Y. & Schlessinger, J. (1981) *Biochem. Biophys. Res. Commun.* **101,** 517–523.
7. Collett, M. S., Purchio, A. F. & Erikson, R. L. (1980) *Nature (London)* **285,** 167–169.
8. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1311–1315.
9. Levinson, A. D., Opperman, H., Varmus, H. E. & Bishop, J. M. (1980) *J. Biol. Chem.* **255,** 11973–11980.
10. Snyder, M. A., Bishop, J. M., Colby, W. W. & Levinson, A. D. (1983) *Cell* **32,** 891–901.
11. Chinkers, M. & Cohen, S. (1981) *Nature (London)* **290,** 516–519.
12. Kudlow, J. E., Buss, J. E. & Gill, G. N. (1981) *Nature (London)* **290,** 519–521.
13. Savage, C. R., Jr., & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7609–7611.
14. Cohen, S., Hiroshi, U., Christa, S. & Michael, C. (1982) *J. Biol. Chem.* **257,** 1523–1531.
15. Schreiber, A. B., Libermann, T. A., Lax, I., Yarden, Y. & Schlessinger, J. (1983) *J. Biol. Chem.* **258,** 846–853.
16. Schlessinger, J., Lax, I., Yarden, Y., Kanety, H. & Libermann, T. A. (1984) in *Receptors and Recognition: Antibodies Against Receptor*, ed. Greaves, M. (Chapman and Hall, London), Vol. 7, pp. 280–303.
17. Merrifield, R. B. (1963) *J. Am. Chem. Soc.* **85,** 2149–2156.
18. Dockray, G. J. (1980) *Regul. Pept.* **1,** 169–186.
19. Yarden, Y., Harari, I. & Schlessinger, J. (1984) *J. Biol. Chem.*, in press.
20. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194,** 495–496.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Burnette, W. N. (1981) *Anal. Biochem.* **112,** 195–203.
23. Gentry, L. E., Rohrschneider, L. R., Casnellie, J. E. & Krebs, E. G. (1983) *J. Biol. Chem.* **258,** 11219–11228.
24. Tamura, T., Bauer, H., Birr, C. & Pipkorn, R. (1983) *Cell* **34,** 587–596.
25. Gooi, H. C., Schlessinger, J., Lax, I., Yarden, Y., Libermann, T. A. & Feizi, T. (1983) *BioSci. Rep.* **3,** 1045–1051.
26. Hortsch, M., Schlessinger, J., Gootwine, E. & Webb, C. G. (1983) *EMBO J.* **2,** 1937–1941.
27. Libermann, T. A., Razon, N., Bartal, A. D., Yarden, Y., Schlessinger, J. & Soreq, M. (1984) *Cancer Res.* **44,** 753–760.
28. Mayes, E. L. V. & Waterfield, M. D. (1984) *EMBO J.* **3,** 531–537.
29. Bishop, M. J. (1983) *Annu. Rev. Biochem.* **52,** 301–354.
30. Ek, B., Westermark, B., Wasteson, A. & Heldin, C.-H. (1982) *Nature (London)* **295,** 419–420.
31. Kasuga, M., Zick, Y., Blithe, D. L., Crettaz, M. & Kahan, C. R. (1982) *Nature (London)* **298,** 667–669.
32. Rubin, J. B., Shia, M. A. & Pilch, P. F. (1983) *Nature (London)* **305,** 438–440.
33. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307,** 521–526.