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Genetics

Identity of human epidermal growth factor (EGF) receptor with glycoprotein SA-7: Evidence for differential phosphorylation of the two components of the EGF receptor from A431 cells

(chromosome 7/gel electrophoresis/immunoprecipitation/phosphotyrosine)

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ABSTRACT **A 165-kilodalton (kDal) surface glycoprotein encoded by human chromosome 7 (SA-7) had been characterized by using antisera raised against human chromosome 7-containing somatic cell hybrids. We now present evidence that SA-7 is the human receptor for epidermal growth factor (EGF) and that these antisera recognize human-specific determinants. The gene coding for the human EGF receptor is localized to the p12 to p22 region of chromosome 7. We have characterized the 145-kDal/165-kDal EGF receptor doublet of A431 cells after immunoprecipitation of radiolabeled cell extracts with these antisera. We find that a protein with endogenous kinase activity copurifies with the A431 receptor doublet and that both components of the doublet contain phosphotyrosine and phosphothreonine and the 165-kDal component contains phosphoserine as well. Further, although each component of the receptor doublet has an average pI of 7, both display charge heterogeneity and appear to have unique charge isomers. The relationship between the two components of the A431 EGF receptor is discussed.**

Epidermal growth factor (EGF) is a low molecular weight polypeptide that stimulates DNA synthesis and cell division in cultured cells (1–4). Its mitogenic response is triggered by specific binding to a cell surface receptor. Most cultured cells possess between 10^4 and 10^5 high-affinity EGF receptors (5, 6), although there are notable exceptions, such as the human epidermoid carcinoma cell line A431 (7) with 2×10^6 receptors per cell (8). Das *et al.* (9) used a photoreactive derivative of EGF to demonstrate that the EGF receptor of mouse 3T3 cells is a 180-kilodalton (kDal) membrane protein. Receptors from other cell types are of similar size (10, 11), including the receptor from A431 cells, which exists as a 150-kDal/170-kDal doublet (12). A gene that restores the ability of EGF receptor-deficient mouse A9 cells to bind EGF, and that probably codes for the receptor structural protein, has recently been assigned to the p22 to qter region of human chromosome 7 by somatic cell hybridization (13, 14).

A human surface glycoprotein encoded by chromosome 7 (SA-7) was described by Aden and Knowles (15), who used antisera raised by immunizing syngeneic mice with human/mouse somatic cell hybrids [cl 21 or cl 36 (16)] that retain chromosome 7 as their only human genetic component. These antisera react with human cells and human chromosome 7-containing hybrid cells in radioimmunoassay, and immunoprecipitate a 165-kDal surface glycoprotein from the Nonidet P-40 (NP-40) extracts of radiolabeled human cells and human chromosome 7-containing hybrid cells (17). Immunoselection of human chromosome 7-containing hybrids (cl 36) with cl 21 antiserum and complement resulted in the isolation of several SA-7-negative clones, each with a deletion involving the short arm of human chromosome 7; the gene coding for SA-7 was consequently localized to the p12 to pter region of chromosome 7 (18). We now present evidence that SA-7 and the human EGF receptor are the same protein. The cl 21 antiserum recognizes one or more antigenic determinants unique to the human EGF receptor and is reactive with both molecular weight components of the A431 EGF receptor.

It is known that EGF stimulation results in enhanced phosphorylation of several endogenous membrane proteins, including the EGF receptor (19, 20), and that the kinase activity copurifies with the receptor (21). The EGF receptor-associated kinase, like the kinases associated with certain RNA tumor viruses (22–24), is a tyrosine protein kinase (25). We now report that both molecular weight components of the A431 receptor undergo an EGF-enhanced phosphorylation and that the protein kinase and the EGF receptor doublet copurify. We have used the cl 21 antiserum to isolate and compare the two molecular weight components of the EGF receptor of A431 cells and we show that, although both have an average pI of 7, each has unique charge isomers. We also present evidence for the differential phosphorylation of the two A431 EGF receptor components; both contain phosphotyrosine and phosphothreonine but only the higher molecular weight form contains phosphoserine. We know of no previous description of the biochemical differences between the two forms of the A431 EGF receptor.

MATERIALS AND METHODS

Cells and Cell Culture. The simian virus 40 (SV40)-transformed human LNSV fibroblasts (17), SV40-transformed murine C57SV fibroblasts (18), murine MC57G cells (17), hybrid cl 21 and cl 36 cells (16), and hybrid cl 36 subcl 7 cells, which contain only the long arm (isoq 7) of human chromosome 7 (18), have been described. These cell lines were maintained in minimal essential medium (ME medium) (Auto-Pow, Flow Laboratories, McLean, VA) supplemented with 2 mM glutamine and 10% fetal bovine serum. Human epidermoid carcinoma A431 cells (7) were maintained in Dulbecco's modified ME medium supplemented with 2 mM glutamine and 10% fetal bovine serum.

Antisera. Antiserum to the hybrid cell line cl 21 was prepared in mice syngeneic with the mouse parental cell line as described by Ford *et al.* (17). Preimmune and immune sera were absorbed at a 1:10 dilution [in phosphate-buffered saline (P_i/NaCl)] with an equal volume of packed MC57G cells, a cell line from the

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Abbreviations: EGF, epidermal growth factor; kDal, kilodalton(s); NP-40, Nonidet P-40; SA-7, human surface glycoprotein encoded by chromosome 7; SV40, simian virus 40; ME medium, minimal essential medium; P_i/NaCl, phosphate-buffered saline.

same strain as the hybrid mouse parent. Production and absorption of murine antisera to HLA antigens are described elsewhere (26).

Inhibition of Binding of ^{125}I-Labeled EGF (^{125}I-EGF) by cl 21 Antiserum. Cells were plated in 24-well Linbro dishes and maintained as described above. At confluence, cells were rinsed three times with ice-cold binding buffer (ME medium/0.01 M Hepes, pH 7.4/0.2% bovine serum albumin) and incubated for 1 hr at 4°C with cl 21 antiserum adjusted to the appropriate dilution in the binding buffer, with a final volume of 0.2 ml per well. Control wells contained binding buffer only or preimmune mouse serum adjusted to the appropriate dilution. Cells were then rinsed four times with binding buffer (twice at 4°C, twice at room temperature) and incubated with 0.5 μl ^{125}I-EGF (3.5 μg/ml, specific activity = 8.6×10^7 cpm/μg of protein) for 1 hr at room temperature. EGF, purified from mouse submaxillary glands (27), was iodinated by the method of Hunter and Greenwood (28). Nonspecific binding was determined by simultaneous incubation of cells with ^{125}I-EGF and excess unlabeled EGF. Again, the final volume was 0.2 ml per well. At the end of the second incubation, cells were rinsed twice with ice-cold binding buffer and twice with ice-cold P_i/NaCl and solubilized with 1 M NaOH, and their radioactivity was measured in a gamma counter.

Cell Labeling. Cells to be labeled with ^{125}I were seeded onto 60-mm dishes at a density of $1–2 \times 10^6$ cells per dish and labeled 24 hr later with 0.8 mCi of ^{125}I per dish (carrier-free Na^{125}I, 100 mCi/ml; 1 Ci = 3.7×10^{10} becquerels) (Amersham) by the method of Hynes (29). Cells to be labeled with ^{125}I-EGF were plated in a similar fashion, rinsed three times with P_i/NaCl, and incubated with ^{125}I-EGF in P_i/NaCl for 1 hr at room temperature. Cells to be labeled with $^{32}P_i$ were seeded onto 35-mm dishes at a density of 5×10^5 cells per dish. Cells were rinsed three times with phosphate-free ME medium 24 hr later and incubated with 0.5 mCi of $^{32}P_i$ (0.8 mCi/ml) (Amersham) in 0.5 ml of phosphate-free ME medium for 3 hr. Cells were then stimulated with EGF at 6 ng/ml for 1 hr. Cells labeled by each technique were rinsed, scraped, and extracted with 0.1% NP-40 as described (17).

Immunoprecipitation and Gel Electrophoresis. Cell extracts were cleared of material that binds nonspecifically to protein A-bearing *Staphylococcus aureus* Cowan I by three ½-hr incubations with excess bacteria at 4°C. Absorbed cl 21 antiserum or preimmune serum, diluted 1:10 with P_i/NaCl, was incubated with *S. aureus* (100 μl serum per 50 μl of a 10% solution of bacteria) for 1 hr at 4°C. Cleared cell extracts were then incubated with antibody-bearing *S. aureus* for 1 hr at 4°C and washed by the method of Cullen and Schwartz (30).

Proteins to be analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis were removed from bacteria by boiling for 3 min in buffer containing 0.1 M Tris·HCl at pH 6.8, 2 mM EDTA, 15% (vol/vol) glycerol, 2% $NaDodSO_4$, and 0.1 M dithiothreitol (17). Samples were separated on gels containing 7.5% or 10% acrylamide with stacking gels containing 5% acrylamide, or on gels containing 5% acrylamide with stacking gels containing 3% acrylamide, by the method of Laemmli (31). Proteins to be analyzed by two-dimensional gel electrophoresis were removed from bacteria by incubation in 9.5 M urea/2% (wt/vol) NP-40/1.6% (wt/vol) pH 6–8 ampholytes/0.4% (wt/vol) pH 3–10 ampholytes/5% (vol/vol) 2-mercaptoethanol (32) for 20 min at 42°C. Samples were separated in the first dimension on isoelectric focusing gels prepared with pH 6–8 ampholytes and in the second dimension on gels containing 7.5% acrylamide as described by O'Farrell (32). Gels were dried onto filter paper and exposed to Kodak X-Omat AR film for autoradiography. Du Pont Cronex intensifying screens were used.

[γ-^{32}P]ATP Labeling of Immunoprecipitated Antigen–Antibody Complexes. Unlabeled A431 cells were washed with P_i/NaCl, scraped into P_i/NaCl containing 2 mM phenylmethylsulfonyl fluoride, and extracted with 1% NP-40. Cell extracts were cleared and immunoprecipitated with cl 21 antiserum as described above. The *S. aureus*-bound antigen–antibody complexes were resuspended in 60 μl of 20 mM Hepes, pH 7.4/0.1% bovine serum albumin/10% (vol/vol) glycerol/30 mM NaCl/50 mM $MgCl_2$ (19). The reaction was initiated by the addition of 50 μCi of [γ-^{32}P]ATP (2,000 Ci/mmol) (Amersham) and was carried out at 4°C for 5 min. In some experiments, the reaction was stimulated by the simultaneous addition of 100 ng of EGF. Samples were washed three times, and proteins were removed from bacteria and analyzed by gel electrophoresis as described above.

Alkali Treatment of $NaDodSO_4$/Polyacrylamide Gels. Duplicate samples of immunoprecipitated proteins labeled with $^{32}P_i$ or [γ-^{32}P]ATP were separated on $NaDodSO_4$/polyacrylamide gels and fixed and stained with 0.1% Coomassie blue in 7% acetic acid/5% methanol (vol/vol). The portion of the gel containing one set of duplicates was incubated in 1 M NaOH for 1 hr at 50°C with gentle agitation to distinguish the relatively alkali-stable phosphotyrosine from the more alkali-labile phosphothreonine and phosphoserine (33, 34). The alkali-treated gels were washed with two or three changes of 7% acetic acid/5% methanol for 2 hr and, together with the untreated portion of the gel, were prepared for autoradiography.

Analysis of Phospho Amino Acids. Immunoprecipitated ^{32}P-labeled proteins were separated on $NaDodSO_4$/polyacrylamide gels containing 5% acrylamide. The 145-kDal and 165-kDal polypeptides were detected by autoradiography and the corresponding gel slices were excised, incubated in extraction buffer containing 0.1% $NaDodSO_4$, 0.05 M NH_4HCO_3, 1 mM Na_2EDTA, and bovine serum albumin at 10 μg/ml and homogenized with a Dounce homogenizer. After addition of 2-mercaptoethanol (50 μl/ml of extraction buffer) and boiling for 5 min, extraction was carried out overnight at 37°C with vigorous shaking. The acrylamide was pelleted and reextracted for 2 hr. The eluates were mixed with 50 μg of bovine serum albumin and an equal volume of 50% trichloroacetic acid and left on ice for 1–2 hr. The acid-precipitated protein was pelleted, washed with acetone, and partially hydrolyzed in 200–300 μl of 6 M HCl at 100°C for 2 hr. The hydrolysate was dried and resuspended in a marker mixture containing phosphotyrosine, phosphothreonine, and phosphoserine, each at 1 mg/ml. Ascending chromatography on cellulose thin-layer plates (300 μm) was performed with *n*-butyl alcohol/isopropyl alcohol/formic acid/water, 3:1:1:1 (vol/vol) (25). ^{32}P-Labeled amino acids were localized by autoradiography and phospho amino acid markers by staining with ninhydrin.

RESULTS

Inhibition of ^{125}I-EGF Binding by cl 21 Antiserum. Treatment of LNSV cells (Fig. 1), the human parent of cl 21 cells, with a 1:50 dilution of antiserum inhibits 90% of ^{125}I-EGF binding by cells under control conditions; the titer resulting in 50% inhibition (I_{50}) is 1:250. Similar results were obtained for other human cells, including normal human diploid WI-38 fibroblasts (data not shown). Treatment with cl 21 antiserum has no effect on binding of ^{125}I-EGF by murine C57SV cells (Fig. 1) or by other murine lines tested, including 3T3 cells (data not shown). The cl 21 antiserum blocks binding of ^{125}I-EGF by human chromosome 7-containing cl 36 hybrid cells, although cl 36 hybrid cells are not as sensitive as are the human parental LNSV cells [a 1:50 dilution of antiserum blocks only 70% of control ^{125}I-EGF binding (Fig. 1)]. Similar results were obtained with cl 21

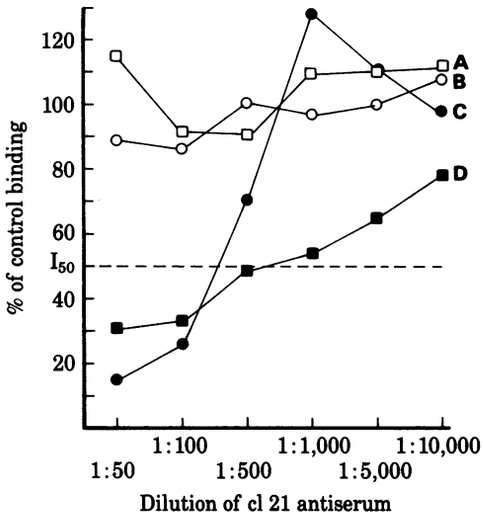

FIG. 1. Inhibition of ^{125}I-EGF binding by cl 21 antiserum. ^{125}I-EGF bound by cells after preincubation with cl 21 antiserum is expressed as a percentage of the ^{125}I-EGF bound under control conditions—i.e., after preincubation with a corresponding dilution of normal mouse serum. Curve A, C57SV; curve B, subcl 7; curve C, LNSV; curve D, cl 36. I_{50} is the dilution of antiserum that results in 50% inhibition of control ^{125}I-EGF binding.

hybrid cells (data not shown). ^{125}I-EGF binding by subcl 7 cells, which contain the long arm of human chromosome 7 only, is not affected by incubation with cl 21 antiserum (Fig. 1). Treatment with preimmune mouse serum had no effect on ^{125}I-EGF binding by any of the cells tested.

Immunoprecipitation of ^{125}I-Labeled Proteins from NP-40 Cell Extracts with cl 21 Antiserum. A 165-kDal protein and a 145-kDal protein are immunoprecipitated from NP-40 extracts of ^{125}I surface-labeled A431 cells by using cl 21 antiserum (Fig. 2, lanes A and B). The 145-kDal band is often obscured in autoradiography by the more heavily labeled 165-kDal band. It was previously shown that the material immunoprecipitated from LNSV and human chromosome 7-containing hybrid cells

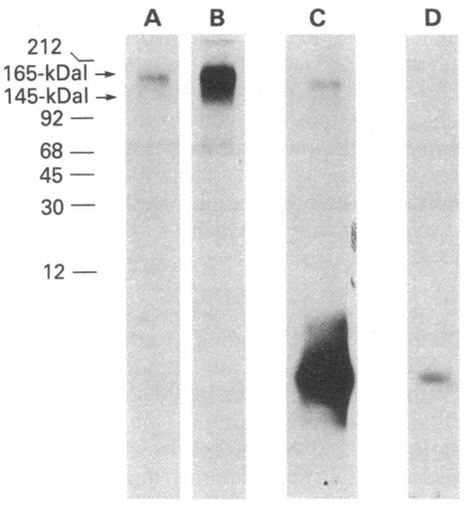

FIG. 2. Autoradiogram of a NaDodSO$_4$/10% polyacrylamide gel of proteins immunoprecipitated by cl 21 antiserum from A431 cells. Lane A, A431 cells labeled with ^{125}I by the lactoperoxidase method, 1-day exposure; lane B, 7-day exposure of lane A; lane C, A431 cells incubated with ^{125}I-EGF; lane D, ^{125}I-EGF (no immunoprecipitation). Molecular weight markers ($\times 10^{-3}$): myosin, 212; phosphorylase a, 92; bovine serum albumin, 68; actin, 45; carbonic anhydrase, 30; cytochrome *c*, 12. These markers are also indicated in Figs. 3, 4, and 6.

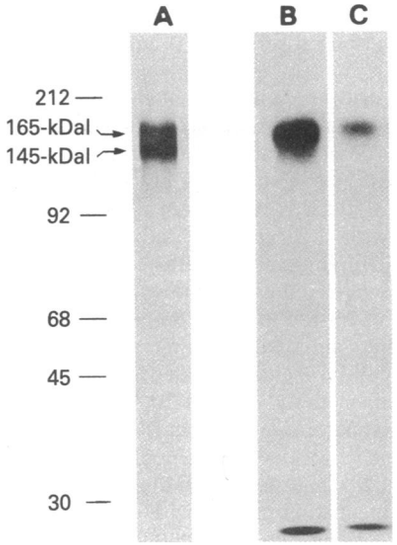

FIG. 3. Autoradiogram of a NaDodSO$_4$/7.5% polyacrylamide gel of ^{32}P-labeled proteins immunoprecipitated by cl 21 antiserum from A431 cells. Lane A, A431 cells labeled with $^{32}P_i$; lane B, EGF-stimulated [γ-^{32}P]ATP labeling of antigen–antibody complexes immunoprecipitated from unlabeled cells; lane C, [γ-^{32}P]ATP labeling of antigen–antibody complexes immunoprecipitated from unlabeled cells.

is less than 0.5% of the total ^{125}I incorporated into surface proteins (17). The immunoprecipitate from A431 cells contains approximately 2% of the total ^{125}I incorporated into surface proteins.

Autoradiography after immunoprecipitation of ^{125}I-EGF-treated A431 cells with cl 21 antiserum reveals three bands (Fig. 2, lane C): the prominent band comigrating with EGF, a fainter band comigrating with the 165-kDal protein, and a minor band comigrating with the 145-kDal protein. In control experiments no radiolabeled proteins are immunoprecipitated from ^{125}I-EGF-treated A431 cells by an HLA antiserum. The low molecular weight protein that comigrates with EGF is immunoprecipitated from LNSV and cl 21 cells but not from subcl 7 or murine MC57G cells (data not shown).

Phosphorylation of the 145-kDal/165-kDal Receptor Doublet. The 145-kDal/165-kDal doublet of A431 cells is seen to be phosphorylated when cells are labeled with $^{32}P_i$ prior to immunoprecipitation (Fig. 3, lane A). The receptor doublet is also phosphorylated when it is first immunoprecipitated from

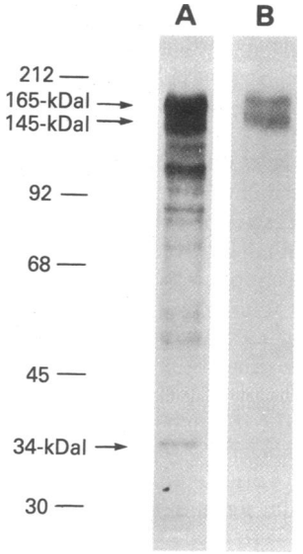

FIG. 4. Autoradiogram of a NaDodSO$_4$/7.5% polyacrylamide gel of [γ-^{32}P]ATP-labeled antigen–antibody complexes before (lane A) and after (lane B) incubation in 1 M NaOH for 1 hr at 50°C.

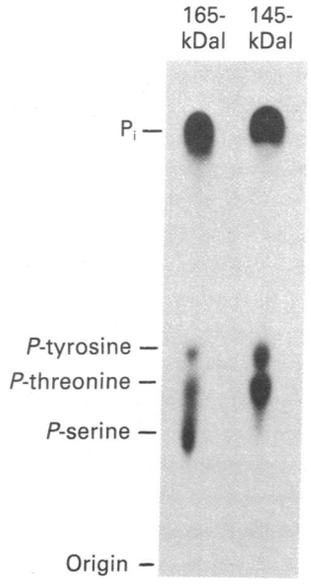

FIG. 5. Autoradiogram of the phospho amino acids of the 145-kDal and 165-kDal proteins of A431 cells after separation by thin-layer chromatography. The R_f values are phosphoserine, 0.16; phosphothreonine, 0.23; phosphotyrosine, 0.27; P_i, 0.53.

unlabeled A431 cell extracts and then incubated with [γ-^{32}P]ATP in the presence of 50 mM Mg^{2+}. In both cases, phosphorylation of the receptor doublet is enhanced by EGF stimulation (Fig. 3, lanes B and C). Longer exposure of autoradiograms reveals that several less abundant phosphoproteins, including a 34-kDal protein, copurify with the receptor doublet, whether antigen–antibody complexes are labeled with [γ-^{32}P]ATP after immunoprecipitation (Fig. 4, lane A) or cells are labeled with $^{32}P_i$ prior to immunoprecipitation (longer exposure, not shown).

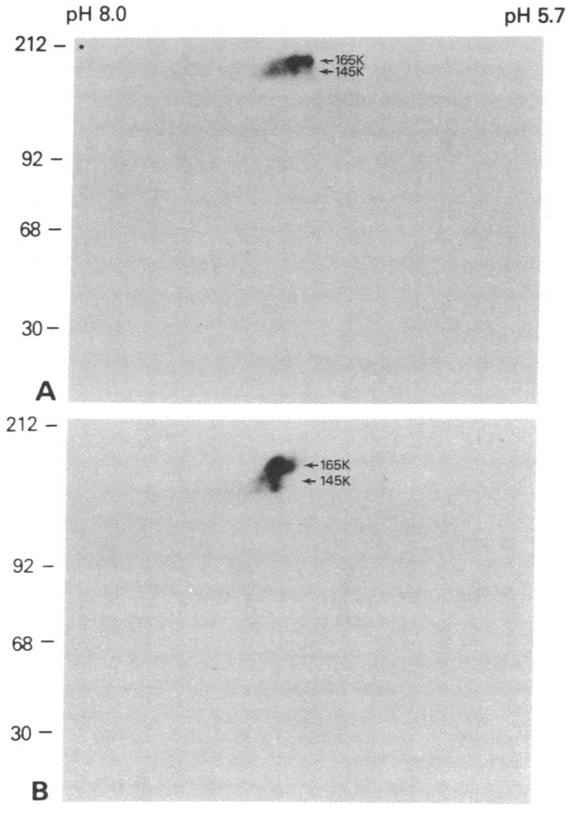

FIG. 6. Autoradiograms of two-dimensional gels of proteins immunoprecipitated by cl 21 antiserum from A431 cells. (*A*) Cells labeled with ^{125}I by the lactoperoxidase method; (*B*) immunoprecipitated antigen–antibody complexes labeled with [γ-^{32}P]ATP. -K, kDal.

Analysis of the Phospho Amino Acids of the 145-kDal/165-kDal Phosphoproteins. The phospho amino acid composition of the receptor doublet of A431 cells was analyzed by relative stability to alkali treatment (Fig. 4) and by thin-layer chromatography (Fig. 5). Incubation of the immunoprecipitated phosphoproteins purified on $NaDodSO_4$/polyacrylamide gels at high pH reveals that a fraction of the phospho amino acids of both molecular weight components is alkali stable. It appears that alkali treatment does not diminish the intensity of ^{32}P label in the 145-kDal component as much as it does in the 165-kDal component. Analysis of phospho amino acids by thin-layer chromatography reveals that both molecular weight components contain phosphotyrosine and phosphothreonine and that only the 165-kDal component contains phosphoserine.

Two-Dimensional Electrophoresis of the 145-kDal/165-kDal Receptor. The two-dimensional separations of the 145-kDal/165-kDal protein are identical when the proteins are immunoprecipitated with cl 21 antiserum from extracts of A431 cells labeled with ^{125}I by the lactoperoxidase method (Fig. 6*A*) and when they are labeled with [γ-^{32}P]ATP after immunoprecipitation from unlabeled A431 cell extracts (Fig. 6*B*). The 145-kDal and 165-kDal components have an average apparent pI of 7, although both display charge heterogeneity and appear to have unique charge isomers. The pIs of the receptor doublet are unchanged by EGF stimulation (data not shown).

DISCUSSION

Several lines of evidence lead us to conclude that the human chromosome 7-encoded glycoprotein SA-7 is the cell surface receptor for EGF. The antiserum that defines SA-7 inhibits binding of EGF by human cells and immunoprecipitates a 145-kDal/165-kDal protein doublet from NP-40 extracts of surface-iodinated A431 cells. A431 cells, which have 20 times the number of receptors expressed by other cells (8), also have a severalfold excess of the SA-7 protein. Moreover, the receptor doublet of A431 cells immunoprecipitated by cl 21 antiserum is radiolabeled after incubation with ^{125}I-EGF, in agreement with the finding that approximately 1% of EGF bound to cells becomes covalently bound to the receptor (35). The demonstration that the human EGF receptor and SA-7 are identical, combined with the mapping data of Shimizu *et al.* (13) and Knowles *et al.* (18), has allowed us to localize the gene that encodes the human EGF receptor structural protein to the p12 to p22 region of the short arm of chromosome 7.

The cl 21 antiserum does not inhibit binding of EGF by murine cells or by hybrid cells that have segregated the short arm of human chromosome 7. The antiserum does inhibit binding of EGF by hybrid cells containing an intact human chromosome 7, but not as effectively as with human cells, suggesting that murine receptors are expressed by the hybrids and continue to bind EGF. One would predict from the considerable sequence homology between human and mouse EGF (36) that the specific amino acid sequence of the receptor that makes up the EGF-binding site is evolutionarily conserved and that an antibody directed against this sequence would block binding of EGF by both human and mouse cells. We have found cl 21 antiserum to be species specific, suggesting that inhibition of EGF binding is by steric hindrance rather than by direct recognition of the EGF-binding amino acid sequence. A further indication that the EGF-binding site itself does not contain an antibody-recognition site is the reactivity of the antiserum with A431 cells to which EGF is already bound. The species specificity of cl 21 antiserum is in contrast to the A431 membrane antiserum described by Haigler and Carpenter (37) and the EGF receptor-specific monoclonal antibodies described by Schreiber *et al.* (38), which do not distinguish human from murine receptors.

It is possible that an antibody specific for the EGF-binding site is present in cl 21 antiserum, but is removed by the absorption with murine cells performed prior to its use.

We have characterized the 145-kDal/165-kDal protein of A431 cells as a phosphoprotein, because it becomes radiolabeled when cells are incubated with $^{32}P_i$ prior to extraction and immunoprecipitation with cl 21 antiserum. Further, the doublet is radiolabeled when it is immunoprecipitated from unlabeled cells and the antigen–antibody complexes are then incubated with [γ-^{32}P]ATP. These findings indicate the cl 21 antiserum immunoprecipitates a protein with endogenous kinase activity, but we cannot state whether the kinase activity resides in the receptor itself or in another protein that copurifies with the receptor. If the kinase is not the receptor itself, our data suggest that it is not a protein with tyrosine residues available for lactoperoxidase-catalyzed iodination, because only the receptor doublet is immunoprecipitated from cells labeled with ^{125}I by the lactoperoxidase method. Other phosphoproteins, including one similar to the 34-kDal protein shown to be a substrate for both EGF-stimulated and RNA virus-associated kinases (39), do copurify with the receptor doublet. It is not clear whether these proteins represent unique gene products or are derived from the receptor. It should be possible to characterize the proteins immunoprecipitated by cl 21 antiserum after radiolabeling with amino acids to determine if another copurifying protein is responsible for the protein kinase activity or if the EGF receptor itself is the EGF-stimulated protein kinase.

We conclude that the 145-kDal and 165-kDal proteins immunoprecipitated by cl 21 antiserum contain phosphotyrosine, on the basis of the stability of a fraction of the phospho amino acids of both components to alkali treatment (33, 34) and on the presence of phosphotyrosine in the partial acid hydrolysates of both components after thin-layer chromatography. Further, in collaboration with Alonzo H. Ross of The Wistar Institute, we have recently shown that cl 21 antiserum and a phosphotyrosine antiserum, prepared by immunizing rabbits with a synthetic hapten (40), purify the same 145-kDal/165-kDal protein from extracts of $^{32}P_i$-labeled A431 cells (unpublished data).

The relationship between the 145-kDal and the 165-kDal components of the EGF receptor doublet of A431 cells requires further clarification. Our data suggest that the two are related because both have quite similar isoelectric points and both contain covalently bound ligand after incubation of cells with ^{125}I-EGF. Further, unless their copurification by cl 21 antiserum is due to noncovalent association rather than display of common antigenic determinants, it appears that these two proteins are encoded by a single or two closely linked genes. It is interesting to speculate that the 165-kDal EGF receptor component contains a cleavage-sensitive, and perhaps protease-sensitive, site, and that a specific physiological rather than experimental event triggers the generation of the 145-kDal component and a low molecular weight peptide fragment. Our evidence would predict that such a peptide fragment will contain phosphate-accessible serine residues because only the 165-kDal component contains this phospho amino acid. If such a cleavage-sensitive site exists and is physiologically relevant, it will be important to ask whether the low molecular weight peptide fragment is released from the EGF receptor by the action of a specific protease and whether the peptide fragment plays a role in eliciting the physiological response of cells to EGF.

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1. Cohen, S. (1962) *J. Biol. Chem.* **237,** 1555–1562.
2. Hollenberg, M. D. & Cuatrecasas, P. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2964–2968.
3. Carpenter, G. & Cohen, S. (1976) *J. Cell. Physiol.* **88,** 227–237.
4. Cohen, S., Carpenter, G. & Lembach, K. J. (1975) *Adv. Metab. Disord.* **8,** 265–284.
5. Carpenter, G., Lembach, K. J., Morrison, M. & Cohen, S. (1975) *J. Biol. Chem.* **250,** 4297–4305.
6. Hollenberg, M. D. & Cuatrecasas, P. (1975) *J. Biol. Chem.* **250,** 3845–3853.
7. Fabricant, R. N., DeLarco, J. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 565–569.
8. Haigler, H., Ash, J. F., Singer, S. J. & Cohen, S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3317–3321.
9. Das, M., Mujakawa, T., Fox, C. F., Pruss, R. M., Aharanov, A. & Hershman, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2790–2794.
10. Sayhoun, N., Hock, R. A. & Hollenberg, M. D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1675–1679.
11. Hock, R. A., Nexo, E. & Hollenberg, M. D. (1979) *Nature (London)* **277,** 403–405.
12. King, L. E., Carpenter, G. & Cohen, S. (1980) *Biochemistry* **19,** 1524–1528.
13. Shimizu, N., Behzadian, M. A. & Shimizu, Y. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3600–3604.
14. Davies, R. L., Grosse, V. A., Kucherlapati, R. & Bothwell, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4188–4192.
15. Aden, D. P. & Knowles, B. B. (1976) *Immunogenetics* **3,** 209–211.
16. Croce, C. M. & Koprowski, H. (1974) *J. Exp. Med.* **140,** 1221–1229.
17. Ford, S. R., Aden, D. P., Mausner, R., Trinchieri, G. & Knowles, B. B. (1978) *Immunogenetics* **6,** 293–300.
18. Knowles, B. B., Solter, D., Trinchieri, G., Maloney, K. M., Ford, S. R. & Aden, D. P. (1977) *J. Exp. Med.* **145,** 314–326.
19. Carpenter, G., King, L. E. & Cohen, S. (1978) *Nature (London)* **276,** 409–410.
20. Carpenter, G., Poliner, L. & King, L. E. (1980) *Mol. Cell. Endocrinol.* **18,** 189–199.
21. Cohen, S., Carpenter, G. & King, L. E. (1980) *J. Biol. Chem.* **255,** 4834–4842.
22. Eckhart, W., Hutchinson, M. A. & Hunter, T. (1979) *Cell* **18,** 925–933.
23. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1311–1315.
24. Witte, O. N., Dasgupta, A. & Baltimore, D. (1978) *Nature (London)* **271,** 375–377.
25. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255,** 8363–8365.
26. Aden, D. P., Mausner, R. & Knowles, B. B. (1978) *Nature (London)* **271,** 375–377.
27. Savage, C. R. & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7609–7611.
28. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194,** 495–496.
29. Hynes, R. O. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3170–3174.
30. Cullen, S. E. & Schwartz, B. D. (1976) *J. Immunol.* **117,** 136–142.
31. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
32. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
33. Plimmer, R. H. A. (1941) *Biochemistry* **35,** 461–469.
34. Cooper, J. A. & Hunter, T. (1981) *Mol. Cell. Biol.* **1,** 165–178.
35. Linsley, P. S., Blifield, C., Wrann, M. & Fox, C. F. (1979) *Nature (London)* **278,** 745–748.
36. Gregory, H. (1975) *Nature (London)* **257,** 325–327.
37. Haigler, H. T. & Carpenter, G. (1980) *Biochim. Biophys. Acta* **598,** 314–325.
38. Schreiber, A. B., Lax, I., Yarden, Y., Eshher, Z. & Schlessinger, J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7535–7539.
39. Erikson, E., Sheahy, D. J. & Erikson, R. L. (1981) *J. Biol. Chem.* **256,** 11381–11384.
40. Ross, A. H., Baltimore, D. & Eisen, H. N. (1981) *Nature (London)* **294,** 654–656.