The EMBO Journal Vol.2 No.11 pp.1937–1941, 1983

Appearance of functional EGF receptor kinase during rodent embryogenesis

M. Hortsch[1], J. Schlessinger*, E. Gootwine[2] and C.G. Webb[2]

Departments of Chemical Immunology and [2]Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by J. Schlessinger
Received on 28 June 1983; revised on 8 August

Mouse and rat embryonic tissues at various stages of development were examined for epidermal growth factor (EGF) receptor kinase activity. The phosphorylated EGF receptor from embryonic tissues appeared as a band of mol. wt. 170 000 daltons on SDS gels. It was clearly demonstrable in the developing mouse fetus from 10 days of gestation onwards. The distribution of the EGF receptor kinase was studied in various tissues of 13 day mouse fetuses. The activity was apparent in the skin, developing skeletal muscles and various internal organs but was notably absent in the liver and brain. The amnion was found to be one of the richest sources of activity while the yolk sac was negative, and the placenta was weakly positive. In 16 day rat fetuses the distribution was quite similar to that of the 13 day mouse fetus. The liver acquired EGF receptor kinase activity by 18 days of gestation and had high activity in neonates. Phosphoamino acid analysis revealed that phosphotyrosine was the major labelled amino acid residue in the embryonic tissues. Thus, the EGF receptor of fetal tissues as studied by immune precipitation and phosphorylation appears to be a similar entity to that found in adult mammalian tissues. This functional EGF receptor kinase activity could first be detected at the time of onset of organogenesis.

Key words: epidermal growth factor/receptor/kinase/embryogenesis

Introduction

Proliferation and differentiation of animal cells *in vivo* and *in vitro* are often controlled by and depend on low concentrations of various hormones and growth factors (Gospodarowicz and Moran, 1976). These polypeptide factors may also play an important role in embryonic development (Gospodarowicz, 1981).

Epidermal growth factor (EGF) is one of the better characterized growth factors (Schlessinger *et al.*, 1983). Mouse EGF is a single chain polypeptide of 53 amino acid residues with a mol. wt. of 6045 daltons (Savage *et al.*, 1972). It stimulates the proliferation of various cultured cells from different species and is also a potent mitogen *in vivo* (Carpenter and Cohen, 1979). EGF has been identified by its ability to induce premature eyelid opening and incisor eruption in newborn mice (Cohen, 1962, 1965). EGF might be a fetal growth hormone responsible for the proliferation and differentiation of specific tissues and organs in the late fetal and neonatal development (Gospodarowicz, 1981). It plays an important role in the maturation and development of the embryonic lung in rabbit (Catterton *et al.*, 1979) and the mammalian secondary palate (Hassell, 1975). Furthermore, EGF and EGF-like material were found in mouse embryos starting from day 12 of gestation (Nexø *et al.*, 1980; Twardzik *et al.*, 1982). EGF binding sites were detected in mouse embryos as early as 11–12 days of gestation (Nexø *et al.*, 1980). Several fetal mouse tissues bind specifically [^{125}I]EGF and respond to EGF *in vitro* by increased incorporation of labelled thymidine into their DNA (Adamson *et al.*, 1981). So far there are no reports on the nature of the EGF receptor in rodent embryonic tissues.

The binding of EGF to its cell surface receptor leads to the activation of various early and delayed effects and also induces the mitogenic response (Schlessinger *et al.*, 1983). This plasma membrane receptor is a 170 000 dalton glycoprotein (Hock *et al.*, 1979). Carpenter *et al.* (1978) discovered that EGF induces a rapid, cyclic nucleotide-independent and tyrosine-specific phosphorylation of endogenous membrane proteins. In the A431 cell line, which bears a high amount of EGF receptors, the major phosphorylated protein was identified as the EGF receptor itself. Since the kinase activity is co-purified with the EGF-binding activity by EGF affinity chromatography it was suggested that the protein kinase is an integral part of the EGF receptor (Cohen *et al.*, 1980). Furthermore, a functional EGF receptor-kinase can be immunoprecipitated with polyclonal (Cohen *et al.*, 1982a) or monoclonal (Schreiber *et al.*, 1983) antibodies against the 170 000 dalton EGF receptor.

Here we describe the identification of a functional EGF receptor kinase in rodent embryos by phosphorylation of immunoprecipitates. We found significant differences in amount and time of appearance of EGF receptor in different embryonic tissues.

Results

Fetal and uterine tissues at various stages of embryogenesis were analyzed for the presence of immunoprecipitable EGF receptor kinase. Our preliminary experiments were conducted with mid-gestation embryos as these were previously shown by others to bind EGF specifically (Nexø *et al.*, 1980; Adamson *et al.*, 1981). These preliminary experiments revealed that mouse monoclonal antibodies to the EGF receptor, raised by immunization of mice with the A431 cell line (Schreiber *et al.*, 1981, 1983) were less efficient and less cross-reactive with the mouse EGF receptor from fetal tissues than were polyclonal antibodies elicited by immunization of rabbits with A431 membranes. All subsequent experiments were therefore performed using the immunoglobulin fraction of the heteroantiserum.

Intact fetuses at 10 days gestation were homogenized, extracted and reacted with protein-A Sepharose-conjugated immunoglobulin of the rabbit antiserum, precipitated and labelled with [γ-^{32}P]ATP. The phosphorylation reaction products were electrophoresed on SDS-polyacrylamide gels. A labelled band of mol. wt. 170 000 daltons was specifically

[1]Present address: European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG.

*To whom reprint requests should be sent.

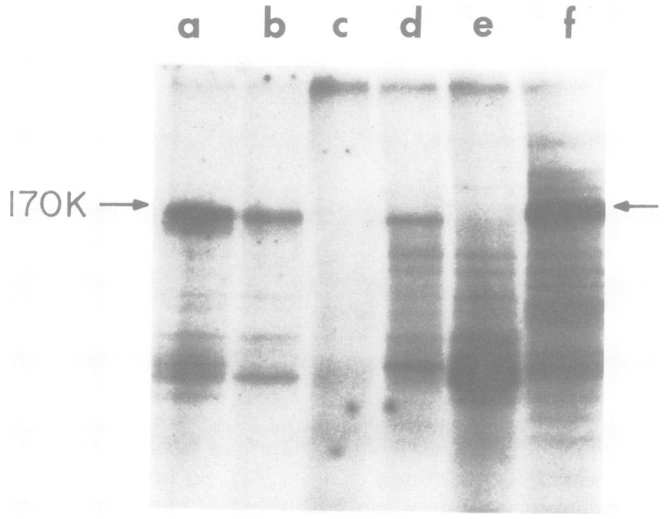

Fig. 1. Gel electrophoresis of phosphorylated EGF receptors immunoprecipitated from 10-day-old mouse embryos. Aliquots of homogenates containing 100 μg protein were subjected to immunoprecipitation. Immunoprecipitates were labelled with [γ-^{32}P]ATP and analyzed on a 5–15% gradient SDS-polyacrylamide gel. The following tissues were analyzed: **(a)** uterus and placenta, **(b)** decidua, **(c)** visceral yolk sac and **(d)** total embryo. **Lane e** represents a precipitate from uterus and placenta with non specific antibodies and **lane f** shows the phosphorylation pattern of 20 μg shed membrane protein from A431 cells. The 170 000 dalton EGF receptor band is marked by arrows.

detected in these fetal extracts (Figure 1). This procedure revealed a similar activity in the uterus and placenta as well as the decidua of the 10 day pregnant mouse. In the extraembryonic tissues we observed a positive reaction with extracts of the amnion while the yolk sac, both visceral and parietal, was devoid of activity. The mol. wt. of the main reaction product corresponded closely to that obtained by phosphorylation of membranes prepared from A431 cells, namely 170 000 daltons. In some preparations the EGF receptor appeared as a double band of 170 000 and 150 000 daltons. This is due to a characteristic Ca^{2+}-dependent proteolytic degradation of the EGF receptor (Cohen *et al.*, 1982b; Cassel and Glaser, 1982).

Fetuses at 13 days gestation were dissected into their component tissues and compared with placental and uterine elements for EGF receptor kinase activity (Figure 2A). One of the richest sources of activity was observed in the amnion whereas the yolk sac was devoid of detectable activity. The combined maternal and fetal elements of the placenta yielded low levels of EGF receptor kinase. The fetal skin, skeletal muscle of head, trunk and legs, lungs and intestines all yielded positive reaction products. In contrast, there were tissues lacking this activity, notably the fetal liver, brain and heart had little or no specific reaction in our assay.

Analysis of 16 day rat fetuses revealed a very similar pattern of reactivity (Figure 2B). The extraembryonic tissues show marked differentiation with respect to the development of EGF receptor kinase activity, the amnion being strongly positive whereas the yolk sac was devoid of activity. The 16 day fetal rat liver again lacked activity whereas skin and skeletal muscle were positive.

Since among adult tissues liver is one of the richest sources of EGF receptors (O'Keefe *et al.*, 1974) we conducted experiments at various stages of development to ascertain the stage of appearance of the receptor. The fetal liver at 18 days of gestation contains precipitable EGF receptor kinase whereas the extracts prepared from earlier embryonic stages were virtually negative (Figure 3). The fetal liver serves as a major hematopoietic organ at those stages of development during which there was no detectable activity. For comparision, we assayed the spleen which was found to remain negative throughout development.

Analyses performed on extracts of earlier embryos revealed that fetuses at 9 days of gestation were very weakly reactive. Earlier embryos, up to 8.5 days of gestation, had no detectable activity using our assay procedure. To exclude the possibility that the lack of discernible activity in the 8 day embryos was due to high proteolytic activity, extracts of 8 day embryos were mixed with extracts of 12 day embryos and then assayed for EGF receptor kinase activity. The mixture was found to contain equivalent amounts of activity to that obtained with 15 day fetal extracts themselves.

Further characterization of the fetal EGF receptor was performed by excising the specifically phosphorylated band of 170 000 mol. wt. from the gels and subjecting the extracted material to phosphoamino acid analysis. Phosphoamino acid analyses of the EGF receptor from various fetal tissues revealed some differences in the relative amounts of phosphorylated residues (Figure 4). The amnion showed almost exclusively labelled tyrosine, as is the case for A431 membranes. The various fetal tissues showed small quantitative differences though phosphotyrosine accounted for 60–85% of the labelled amino acids. The placental tissue was exceptional in that the phosphoserine residues accounted for a high proportion of the labelled material. Phosphothreonine residues provided a minor contribution to the labelled material in all cases.

Discussion

A functional EGF receptor kinase can be immunoprecipitated from mouse embryonic tissues from 9 days of gestation onwards, which coincides with the onset of organogenesis in rodent embryos. The material specifically phosphorylated in the immune precipitates corresponds to a 170 000 mol. wt. protein very similar to that obtained from adult mice or human A431 epidermoid carcinoma cells which was previously identified as the EGF receptor (Cohen *et al.*, 1980).

Earlier embryos did not yield any detectable material specifically reactive with our antiserum using the autophosphorylation assay. Mixing experiments ruled out the interpretation of a false negative result due to proteolysis during the immunoprecipitation procedure. Nevertheless, it cannot be excluded that our negative results obtained with 7 and 8 day old embryos may derive from some unusual phosphatase activity.

Embryonal carcinoma cells induced to differentiate in culture form cells equivalent to the early embryonic endoderm. While the undifferentiated embryonal carcinoma cells lack EGF receptors, their differentiated derivatives can be shown to bind EGF and to respond with enhanced incorporation of radioactive labelled thymidine (Rees *et al.*, 1979). Therefore, there may be some minor populations of cells in earlier embryos that respond to EGF but to such small extents that we cannot detect them. It is also possible, though less likely, that such receptors to EGF, if they exist, either do not react with our antibodies or do not possess integral kinase activity. In other words the early post-implantation embryo

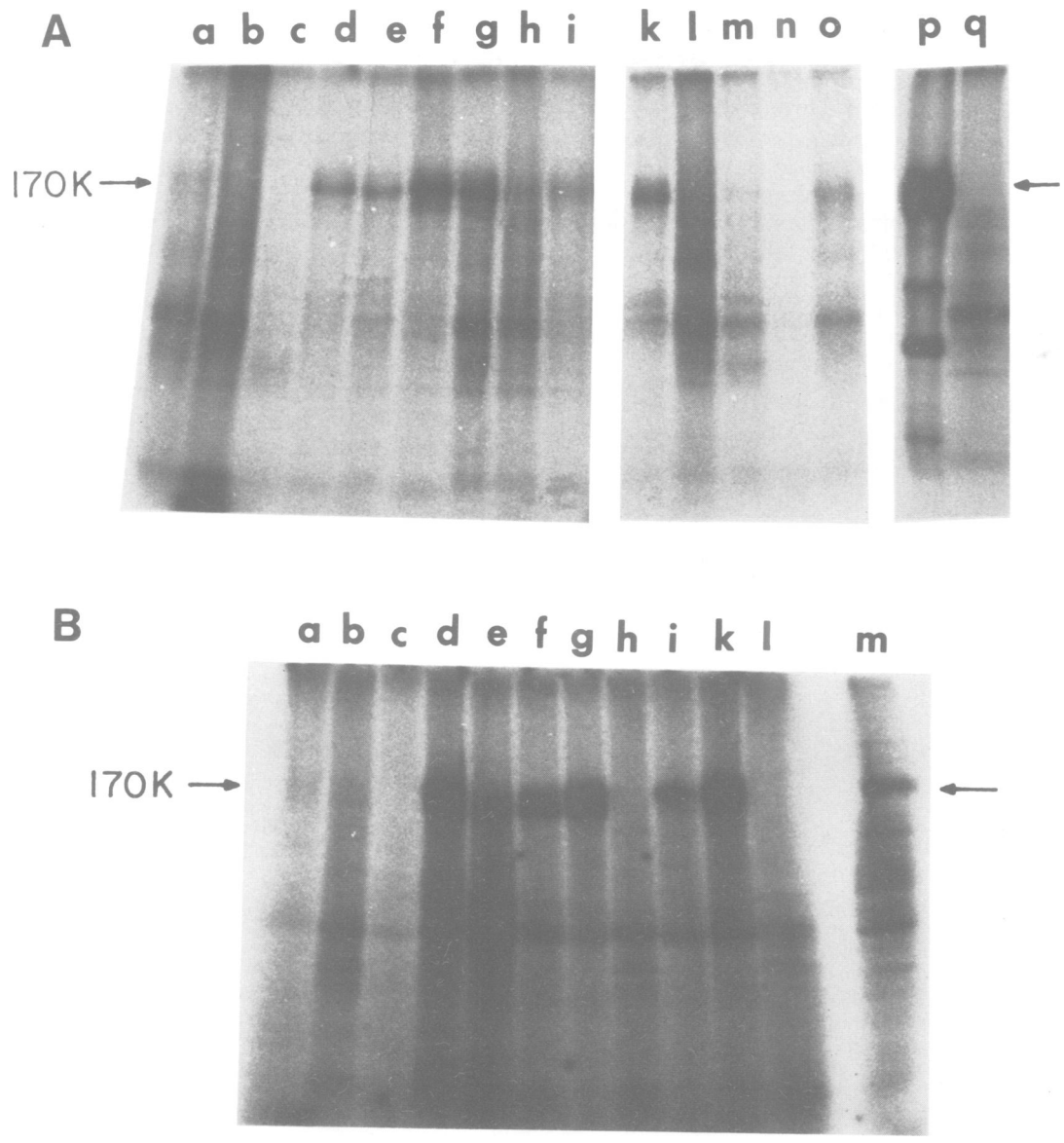

Fig. 2. Gel electrophoresis of phosphorylated EGF receptor kinase from different tissues. All immunoprecipitations were performed with 100 μg protein. The 170 000 dalton EGF receptor is indicated by arrows. **(A)** [γ-^{32}P]ATP-labelled immunoprecipitates from different 13-day-old mouse embryonic tissues: **(a)** uterus, **(b)** placenta, **(c)** visceral yolk sac, **(d)** amnion, **(e)** umbilical cord, **(f)** head, **(g)** trunk, **(h)** tail, **(i)** skin, **(k)** legs, **(l)** brain, **(m)** liver, **(n)** heart, **(o)** intact fetus, **(p)** 20 μg shed membrane protein from A431 cells and **(q)** intact fetus precipitated with non-specific antibodies. **(B)** Similar analysis performed with tissues from 16-day-old rat fetus: **(a)** uterus, **(b)** placenta, **(c)** visceral yolk sac, **(d)** amnion, **(e)** head, **(f)** trunk, **(g)** legs, **(h)** liver, **(i)** tail, **(k)** umbilical cord, **(l)** precipitate with non-specific antibodies from uterus and **(m)** 10 μg A431 shed membrane protein.

may have an as yet unidentified molecular form of the EGF receptor.

The functional EGF receptor kinase previously characterized on adult tissues can be readily demonstrated by 10 days of gestation and is first weakly detectable by 9 days gestation in the mouse. The appearance of this activity is not uniform and develops in some tissues earlier or more prominently than in others. The amnion is a particularly rich source of activity while the placenta is weak and the yolk sac is negative. The human placenta, however, possesses a high level of EGF receptors (O'Keefe *et al.*, 1974).

In other studies, binding of radioactively-labelled EGF was detected in mid-gestation embryos (Nexø *et al.*, 1980; Adamson *et al.*, 1981). Moreover, such embryos in culture were shown to respond to the presence of EGF with increased incorporation of thymidine into DNA (Adamson *et al.*, 1981). Injection of EGF *in vivo* showed inhibition of proliferation which is thought to result from the down-regulation of receptors to higher doses of the hormone (Adamson and Warshaw, 1982). In general, our data regarding the distribution of EGF receptors agree with previous reports based on binding studies. However, we failed to detect EGF receptor kinase on the yolk sac while assays utilizing [^{125}I]EGF revealed some specific binding to the visceral yolk sac (Adamson *et al.* 1981).

The fetal liver is a major hematopoietic organ and was virtually devoid of EGF receptor kinase. The appearance of this activity corresponds with migration of the lymphoid cells from the liver to other organs and with the onset of liver glycogen metabolism (Ballard and Oliver, 1963). Lymphoid cells are known to lack EGF receptors and lymphoid organs such as the spleen remain devoid of activity throughout

development (O'Keefe *et al.*, 1974) (Figure 3). The yolk sac, considered to be the first embryonic hematopoietic organ of the mammalian embryo, was also found to be negative.

Phosphoamino acid analyses on the 170 000 dalton band labelled specifically in our assay revealed some organ-specific differences in the ratios of phosphorylated amino acids. In all embryonic tissues phosphotyrosine predominated but only in the amnion was this the amino acid almost exclusively phosphorylated. In the placental tissue a high proportion of phosphoserine was detected which was unique. However, the phosphoserine found in the placenta may be irrelevant to the EGF receptor kinase and may arise from a small quantity of a contaminating serine-specific kinase (see Figure 2A lane b and Figure 2B lane b). Furthermore, EGF-induced phosphorylation, which was not studied in our assay, may be different from the result obtained with autophosphorylation. Indeed, the EGF-induced phosphorylation has been shown to occur mainly on tyrosine residues of the EGF receptor from mitogenically responsive fibroblasts (Yarden *et al.*, 1983). However, in the absence of EGF, monoclonal antibodies precipitate EGF receptor phosphorylated on both serine and tyrosine residues from normal human fibroblasts (Yarden *et al.*, 1983). Hence, the EGF receptor, which itself is a tyrosine-specific kinase (Cohen *et al.*, 1982a), seems to be susceptible as a substrate for a serine-specific kinase. Furthermore, in intact cells, the main phosphorylated residues on the EGF receptor from either human fibroblasts or A431 cells are serine residues (Yarden *et al.*, 1983).

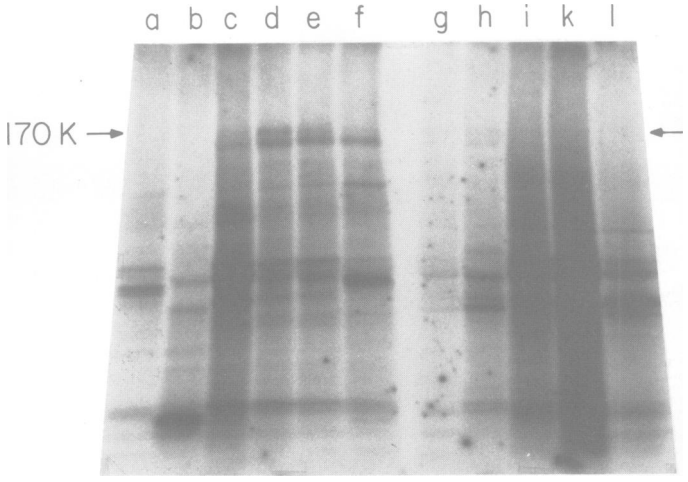

Fig. 3. Gel electrophoresis of phosphorylated EGF receptor kinase from mouse liver and spleen. **Lanes a–f** represent immunoprecipitates from mouse liver of different stages: **(a)** precipitates with non-specific antibodies from 3.5 month old adult mouse liver, immunoprecipitates from liver of: **(b)** 13-day-old embryo, **(c)** 17-day-old-embryo, **(d)** new born animal, **(e)** 2.5-week-old and **(f)** 3.5-month-old animal. The **lanes g–l** show immunoprecipitates from mouse spleen of **(g)** 17-day-old embryo, **(h)** new born animal, **(i)** 2.5-week-old and **(k)** 3.5-month-old animal. **Lane l** is a precipitate with non-specific antibodies from spleen of 3.5-month-old mouse. All lanes are immunoprecipitations from 200 μg protein.

Materials and methods

Animals and embryos

Mice of inbred strain C3HeB were obtained from the Jackson Laboratories (Bar Harbor, Maine). Rats of the Wistar strain were obtained from the closed colony of the Department of Hormone Research at the Weizmann Institute. Normal cycling females were mated to individually caged males. The day of observation of vaginal plug was designated day 0 of pregnancy. Pregnant females were sacrificed by cervical dislocation and uteri were dissected in sterile Hank's balanced salt solution under a dissection stereomicroscope.

Antiserum

Antiserum against membranes of A431 cells was prepared in rabbits as previously described (Haigler and Carpenter, 1980; Yarden *et al.*, 1983). The IgG fraction from this antiserum was isolated by ion-exchange chromatography on a DEAE-52 column.

Protein determination

Protein was determined according to Bradford (1976) with bovine serum albumin as standard.

Preparation of samples

Samples of frozen embryonic or maternal tissues were homogenized with ~5 volumes of ice-cold solubilization buffer [20 mM Hepes buffer pH 7.4, 150 mM NaCl, 1% (v/v) Triton X-100 (Sigma), 10% (v/v) glycerol, 0.03% (w/v) NaN_3 and 1% (v/v) trasylol (Sigma, Aprotinin)] in a small, medium fitting teflon homogenizer. Crude homogenates were left for 60 min at 0°C on ice and insoluble material was removed by a 15 min centrifugation in an Ep-

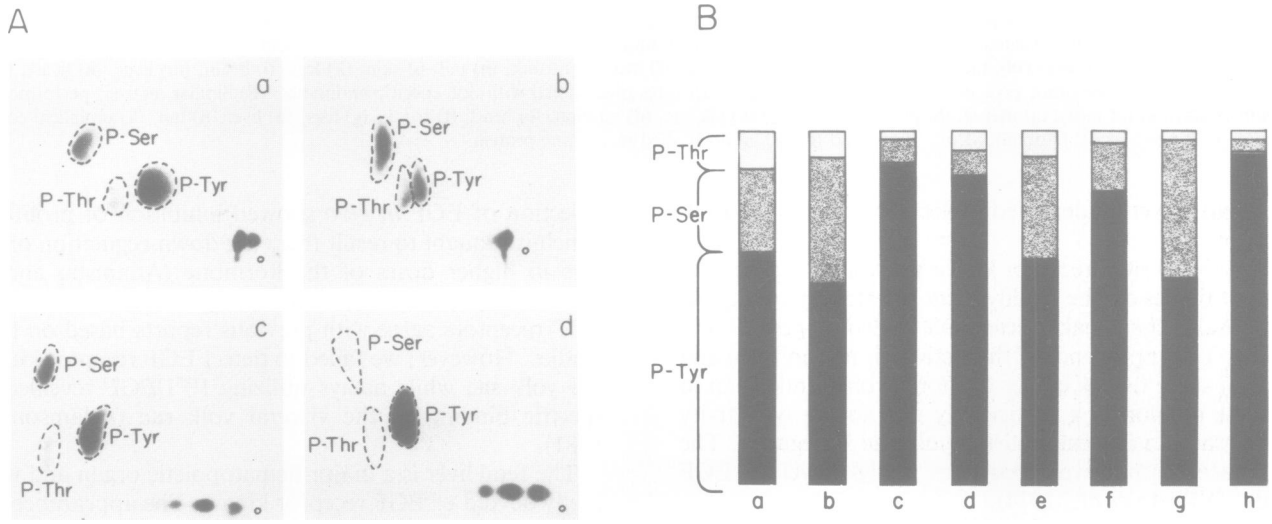

Fig. 4. Identification of the phosphoamino acid residues of the EGF receptor from various tissues. **(A)** Phosphoamino acid analysis of mouse EGF receptor from **(a)** uterus, **(b)** placenta, **(c)** total embryo (all from day 15 of gestation), whereas **(d)** depicts the same analysis of the human EGF receptor from A431 cells. **(B)** Quantitative determination of the phosphorylated amino acid residues of the EGF receptor immunoprecipitated from different tissues of 16 day old rat fetus is presented: **(a)** uterus, **(b)** placenta, **(c)** amnion, **(d)** trunk, **(e)** head, **(f)** legs, **(g)** tail and **(h)** A431 cells.

pendorf centrifuge at 4°C. The supernatants were stored at −70°C until use. Shed membranes from A431 cells were prepared as described by Cohen *et al.* (1982a). Usually, 20 μg of shed membrane proteins were subjected to the phosphorylation reaction and used for the identification of the EGF receptor polypeptide on the autoradiogram. The mol. wt. of the EGF receptor was determined using a [^{14}C]methylated protein mixture (Amersham, UK).

Immunoprecipitation of EGF receptor

For each precipitation mixture 10 μg of anti-A431 membrane IgG were incubated for 30 min at room temperature with 3 mg of protein-A Sepharose CL-4B (Pharmacia, Sweden). Unbound antibodies were washed away with phosphate buffered saline (PBS) containing 10% (v/v) glycerol by a short centrifugation in an Eppendorf centrifuge. Immunoprecipitation of EGF receptors was performed by incubating the pellet of protein-A Sepharose bound antibodies with 200 μl of PBS containing 10% (v/v) glycerol and aliquots of the homogenates containing equal amounts of protein. Incubation was for 2 h at 4°C on an Eppendorf shaker. Immunoprecipitates bound to protein-A Sepharose were washed four times with solubilization buffer containing 0.2% (v/v) Triton X-100 by short centrifugation in an Eppendorf centrifuge.

Phosphorylation of EGF receptor kinase

Pellets from immunoprecipitates described above were incubated for 10 min on ice with 30 μl of solubilization buffer containing 0.2% (v/v) Triton X-100, 3 mM $MnCl_2$ and 5 μCi of [γ-^{32}P]ATP (Amersham, UK, 5000 Ci/mmol). The phosphorylation reaction was stopped by adding 30 μl sample buffer for SDS gel electrophoresis and boiling for 5 min. The samples were analyzed on 5–15% gradient SDS-polyacrylamide gels (Laemmli, 1970). Gels were dried under vacuum and exposed 3–7 days to flashed Agfa curix film with intensifying screen.

Phosphoamino acid analysis

Identification of the phosphorylated amino acid residues was carried out as described by Hunter and Sefton (1980). The EGF receptor band was cut from the dried gels and extensively washed with 10% (v/v) methanol. The slices were placed in siliconized tubes, lyophilized and 1 ml of 50 μg/ml of trypsin-TPCK (Worthington, 270 units/mg) in 0.05 M NH_4HCO_3 buffer pH 8.0 was added. After overnight incubation at 37°C the supernatants were removed and lyophilized. For acid hydrolysis the extracted protein was dissolved in 6 M HCl with a crystal of phenol and incubated for 4 h at 110°C under vacuum. HCl was removed by lyophilization and the hydrolysates were dissolved in a minimal volume of a mixture containing 1 mg/ml phosphoserine and phosphothreonine and 2 mg/ml phosphotyrosine. Samples were spotted on cellulose thin-layer plates (Merck, FRG) and run in the first dimension by electrophoresis at pH 1.9 for 35 min at 1.5 kV in glacial acetic acid/formic acid (98%)/H_2O, 78:22.5:900 (v/v), and in the second dimension at pH 3.5 for 18 min at 1.5 kV in glacial acetic acid/pyridine/H_2O, 50:5:945 (v/v). The markers were detected by staining with ninhydrin. Thin-layer plates were exposed to flashed Kodak X-Omat film with intensifying screen. For quantification the stained spots were scraped carefully from the plate with a scalpel and extracted for 4 h at 37°C with scintillation fluid. Samples were counted on the phosphate channel of a Packard Tricarb counter. For background determination an unrelevant area of the plate was scraped and processed as described above.

Acknowledgements

We thank T.A. Libermann for initial experiments and advice. M.H. is a recipient of a Minerva fellowship. This work was supported by grants from the US Israel Binational Science Foundation (C.G.W.), the National Institutes of Health (CA-25820) (J.S.), Stiftung Volkswagenwerk (J.S.) and the US Israel Binational Science Foundation (J.S.). C.G.W. is the incumbent of the Pauline Recanati Career Development Chair in Immunology.

References

Adamson,E.D., Deller,M.J. and Warshaw,J.B. (1981) *Nature,* **291**, 656-659.
Adamson,E.D. and Warshaw,J.B. (1982) *Dev. Biol.,* **90**, 430-434.
Ballard,F.J. and Oliver,I.T. (1963) *Biochim. Biophys. Acta,* **71**, 578-588.
Bradford,M.M. (1976) *Anal. Biochem.,* **72**, 248-254.
Carpenter,G., King,L. and Cohen,S. (1978) *Nature,* **276**, 409-410.
Carpenter,G. and Cohen,S. (1979) *Annu. Rev. Biochem.,* **48**, 193-216.
Cassel,D. and Glaser,L. (1982) *J. Biol. Chem.,* **257**, 9845-9848.
Catterton,W.Z., Escobedo,M.B., Sexson,W.R., Gray,M.E., Sundell,H.W. and Stahlman,M.T. (1979) *Pediatr. Res.,* **13**, 104-108.
Cohen,S. (1962) *J. Biol. Chem.,* **237**, 1555-1562.
Cohen,S. (1965) *Dev. Biol.,* **12**, 394-407.
Cohen,S., Carpenter,G. and King,L. (1980) *J. Biol. Chem.,* **255**, 4834-4842.
Cohen,S., Ushiro,H., Stoscheck,C. and Chinkers,M. (1982a) *J. Biol. Chem.,* **257**, 1523-1531.
Cohen,S., Fava,R.A. and Sawyer,S.T. (1982b) *Proc. Natl. Acad. Sci. USA,* **79**, 6327-6241.
Gospodarowicz,D. and Moran,J.S. (1976) *Annu. Rev. Biochem.,* **45**, 531-558.
Gospodarowicz,D. (1981) *Annu. Rev. Physiol.,* **43**, 251-263.
Haigler,H.T. and Carpenter,G. (1980) *Biochim. Biophys. Acta,* **598**, 314-325.
Hassell,J.R. (1975) *Dev. Biol.,* **45**, 90-102.
Hock,R.A., Nexø,E. and Hollenberg,M.D. (1979) *Nature,* **277**, 403-405.
Hunter,T. and Sefton,B.M. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 1311-1315.
Laemmli,U.K. (1970) *Nature,* **227**, 680-685.
Nexø,E., Hollenberg,M.D., Figueroa,A. and Pratt,R.M. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 2782-2785.
O'Keefe,E., Hollenberg,M.D. and Cuatrecasas,P. (1974) *Arch. Biochem. Biophys.,* **164**, 518-526.
Rees,A.R., Adamson,E.D. and Graham,C.F. (1979) *Nature,* **281**, 309-311.
Savage,C.R., Inagami,T. and Cohen,S. (1972) *J. Biol. Chem.,* **247**, 7612-7621.
Schlessinger,J., Schreiber,A.B., Levi,A., Lax,I., Libermann,T.A. and Yarden,Y. (1983) *CRC Crit. Rev. Biochem.,* **14**, 93-111.
Schreiber,A.B., Lax,I., Yarden,Y., Eshar,Z. and Schlessinger,J. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 7535-7539.
Schreiber,A.B., Libermann,T.A., Lax,J., Yarden,Y. and Schlessinger,J. (1983) *J. Biol. Chem.,* **258**, 846-853.
Twardzik,D.R., Ranchalis,J.E. and Todaro,G.J. (1982) *Cancer Res.,* **42**, 590-593.
Yarden,Y., Libermann,T.A., Schreiber,A.B., Lax,I. and Schlessinger,J. (1983) *J. Biol. Chem.,* in press.