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Identification of a novel receptor in *Drosophila* for both epidermal growth factor and insulin*

(growth factors/tyrosine kinases/evolutionary precursor/*Drosophila* cells/binding specificity)

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ABSTRACT **The notable amino acid homology among mammalian growth factor receptors with tyrosine-specific protein kinase activity has led to speculation that these receptors derived from a common evolutionary precursor. We report the identification of a novel growth factor receptor from *Drosophila* cell cultures that has dual binding specificity for both insulin and epidermal growth factor (EGF). This 100-kDa protein is also related antigenically to the mammalian receptors for EGF and possibly insulin but may not correspond to the mammalian counterpart of either receptor in *Drosophila*. The *Drosophila* protein is recognized by antisera directed against the mammalian receptor for EGF in immunoblot hybridizations. It can be affinity labeled with either ^{125}I-labeled insulin or ^{125}I-labeled EGF after immunoprecipitation with anti-EGF receptor antiserum. Excess unlabeled EGF or insulin will block the affinity labeling with either growth factor, suggesting that both EGF and insulin share a common binding site on the 100-kDa *Drosophila* receptor. This *Drosophila* protein, therefore, may be closely related to an evolutionary precursor of the mammalian receptors for insulin and EGF.**

The tyrosine-specific protein kinases have been implicated in the regulation of cell proliferation in vertebrate species (1, 2). This kinase family includes the retroviral transforming proteins related to pp60^{src} and the receptors that bind epidermal growth factor (EGF), insulin, platelet-derived growth factor, and insulin-like growth factor I. The kinase active site is strongly conserved between members of this family in terms of amino acid sequence, substrate utilization, and antigenic crossreactivity. For example, the receptors for EGF and insulin can be immunoprecipitated by antibodies to pp60^{src} (3, 4) and can utilize common substrates for tyrosine phosphorylation, including a synthetic peptide corresponding to the autophosphorylation site of pp60^{src} (5). The relationship between growth factor receptors and the *src*-related oncogenes is further extended by evidence that the EGF receptor gene appears to be the normal cellular counterpart of v-*erbB*, the avian erythroblastosis virus oncogene (6).

Conservation of members of the tyrosine kinase family has been demonstrated to extend to *Drosophila*. Gene sequences related to the retroviral oncogenes *src*, *abl*, and *erbB* have been cloned from the *Drosophila* genome (7–10). Sequence comparison of the *Drosophila* c-*erbB* with human EGF receptor cDNAs indicates that there is significant homology in the kinase region and in part of the extracellular domain. In the 170-kDa mammalian EGF receptor, the EGF binding domain is situated on the cysteine-rich, extracellular portion, while the cytoplasmic side contains the tyrosine kinase region and sites that serve as substrates for autophosphorylation and for phosphorylation by protein kinase C (11).

The insulin receptor also appears to be evolutionarily conserved. Isolated from *Drosophila* by affinity chromatography, the receptor is reportedly structurally similar to its mammalian counterpart, which exists as a disulfide-bonded heterodimer of α and β subunits (12). In both mammalian and *Drosophila* insulin receptors, the insulin binding site is located on the 125- to 135-kDa α subunit, whereas the 90-kDa β subunit contains the tyrosine kinase region.

In the interest of identifying lower organisms that express proteins related to the EGF receptor, we began screening *Drosophila* cell lines for EGF receptor homologs, using immunological criteria. A polyclonal antisera that recognizes EGF receptors from a variety of species (including human and rodent) as well as the avian v-*erbB* product (13) was utilized. This antisera reacts primarily with cytoplasmic determinants of the EGF receptor (14) and can immunoprecipitate the product of the *neu* oncogene, p185, which is homologous to the EGF receptor in the kinase region only (ref. 15; M.-C. Hung, personal communication).

In the course of this investigation, we identified a unique growth factor receptor for both insulin and EGF. Immunoblot hybridization revealed two *Drosophila* membrane proteins of 190-kDa and 100-kDa that are antigenically related to the EGF receptor. On the basis of size, the 190-kDa protein could correspond to the product of the *Drosophila* c-*erbB* (9). The 100-kDa protein, however, can be affinity labeled with both growth factors and crossreacts with anti-EGF receptor antibodies. We suggest that this novel protein is of importance both from an evolutionary perspective and as a potential mediator of growth and development.

MATERIALS AND METHODS

Cells. *Drosophila* Schneider L2 cells, a gift from M. Pardue, were grown in M3 medium with 10% fetal calf serum. *Drosophila* K_c cells, obtained from the Massachusetts Institute of Technology Cell Culture Center, were grown in D22 medium. The *Drosophila* cell lines were maintained at 23°C. Mammalian cell lines were grown in Dulbecco's modified Eagle's medium supplemented with serum (10% fetal calf serum for the human epidermoid carcinoma cell line A431 and 10% calf serum for the mouse fibroblast line Swiss 3T3). A431 cells were obtained from American Type Culture Collection. Swiss 3T3 cells were provided by H. Green.

Antisera. Anti-EGF receptor antiserum was prepared against denatured EGF receptor from A431 cells as described (13). Antiserum prepared against affinity-purified rat insulin receptor (16) was provided by S. Jacobs. Both are polyclonal rabbit antisera.

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Abbreviation: EGF, epidermal growth factor.

*Part of these data were presented in a preliminary form at the 24th Annual Meeting of the American Society for Cell Biology (24).

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Materials. Receptor-grade EGF was purchased from Biomedical Technologies (Norwood, MA). ^{125}I-labeled EGF (^{125}I-EGF), prepared by the IODO-GEN method, had a specific activity of 200 cpm/pg. Porcine insulin and ^{125}I-labeled insulin (^{125}I-insulin; specific activity, 50 cpm/pg) were provided by P. Pilch. ^{125}I-labeled protein A (^{125}I-protein A; 9 μCi/μg; 1 Ci = 37 GBq) was from Amersham. The crude insulin receptor preparation from placenta, prepared as described (17), was provided by P. Pilch. Prestained molecular weight standards were obtained from Bethesda Research Laboratories.

Membrane Preparation. A431 cells were dislodged from tissue culture plates by incubation in phosphate-buffered saline (137 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4, pH 7.4) containing 0.5 mM EDTA at 37°C. Swiss 3T3 cells were scraped into phosphate-buffered saline containing 0.5 mM EDTA and 2 mM phenylmethylsulfonyl fluoride. *Drosophila* cells were dislodged from T-flasks by tapping or were harvested from suspension cultures by centrifugation. All cells were then washed twice in phosphate-buffered saline and once in buffer A (20 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM EDTA/1 mM EGTA/1 mM phenylmethylsulfonyl fluoride/0.025 trypsin inhibitor units of aprotinin per ml/5 μM leupeptin/25 mM benzamidine). The cells were then swollen in buffer A without NaCl and lysed in a Dounce homogenizer. The lysates were cleared by centrifugation at 1000 × *g*. Membranes were pelleted twice at 100,000 × *g*, resuspended in 50 mM Hepes (pH 7.0) or 10 mM Tris·HCl (pH 7.5), and stored at −20°C. Membrane protein concentration was determined by the method of Bradford (18).

Immunoblotting. Membrane protein was solubilized for $NaDodSO_4$/PAGE in 0.5% $NaDodSO_4$/10% glycerol/100 mM Tris·HCl, pH 6.8/0.03% bromophenol blue (mild conditions). For harsher denaturing conditions, 1% 2-mercaptoethanol was added to the solubilized membranes, and the samples were boiled for 5 min before loading onto 6.5% polyacrylamide gels. $NaDodSO_4$/PAGE was performed as described by Laemmli (19).

Immunoblot hybridization was performed by the method of Burnette (20) with the following modifications. The protein was transferred to nitrocellulose in a Transphor electrophoresis cell (Hoefer Scientific Instruments, San Francisco) overnight at 4°C (4 V/cm) in 15.6 mM Tris·HCl, pH 8.3/120 mM glycine/20% (vol/vol) methanol. The filters were incubated in blocking medium (1% bovine hemoglobin in phosphate-buffered saline with 0.2% NaN_3) prior to probing with anti-EGF receptor antiserum (1:1000 dilution), anti-insulin receptor antiserum (1:1250 dilution), or rabbit preimmune serum (1:50 dilution) for 3 hr at room temperature on a clinical rotator. The filters were washed once in blocking medium and twice in phosphate-buffered saline. Each filter was then incubated with 1–2 × 10^6 cpm of ^{125}I-protein A in blocking medium for 2–3 hr. The filters were washed, dried, and subjected to autoradiography. The markers used for molecular weight determination were either prestained or stained after transfer with amido black.

Affinity Labeling of Immunoprecipitates. Membrane protein was solubilized in 1–2% Triton X-100/10% glycerol/50 mM Hepes, pH 7.0, for 60 min at 4°C. Typically, 500 μg of *Drosophila* membrane protein or 100 μg of Swiss 3T3 membrane protein were used per sample. Unsolubilized material was removed by centrifugation at 12,000 × *g* for 10 min. The solubilized protein was diluted 1:2 and precleared by incubation with protein A-Sepharose beads (Pharmacia). The cleared supernatants were treated overnight with anti-EGF receptor antiserum (1:10 dilution), anti-insulin receptor antiserum (1:50), or rabbit preimmune serum (1:10), and the product was precipitated with protein A-Sepharose beads. The immunopellets were washed three times with 50 mM Hepes, pH 7.0/50 mM NaCl/10% glycerol/0.1% Triton X-100 and were resuspended in 25 mM Hepes/50 mM NaCl/0.1% Triton X-100/1 mg of bovine serum albumin per ml. ^{125}I-EGF (35 nM) or ^{125}I-insulin (20 nM) was added, and the samples were incubated 60 min at 15°C. Binding specificity was tested by the simultaneous addition of a 1000-fold excess of unlabeled ligand (30 μM) and labeled ligand. Bound growth factor was crosslinked to the immunoprecipitates by addition of disuccinimidyl suberate to 0.4 mM and incubation for 15 min at 0°C. The samples were then centrifuged, and the supernatant was removed. The pellets were resuspended in sample buffer (33 mM Tris·HCl, pH 6.5/2% $NaDodSO_4$/10% glycerol/0.1% bromophenol blue/2% 2-mercaptoethanol) and boiled. The protein A-Sepharose beads were removed by centrifugation, and the supernatants were run on 6.5% polyacrylamide/$NaDodSO_4$ gels.

Affinity Labeling of Membranes. Membranes from Schneider L2 and Swiss 3T3 cells in 50 mM Tris·HCl (pH 7.2) or a crude insulin receptor preparation from human placenta was incubated with 2 nM ^{125}I-insulin for 60 min at 15°C and crosslinked as described above for the immunoprecipitates. Excess unlabeled insulin (35 μM) was added to a duplicate sample to determine specific binding. The samples were boiled in solubilization buffer containing 3 mM dithiothreitol and were subjected to electrophoresis on 7.5% polyacrylamide/$NaDodSO_4$ gels.

RESULTS

Crossreactivity with Antisera to Mammalian EGF and Insulin Receptors. In order to determine whether *Drosophila* express EGF receptor homologs, membrane proteins from *Drosophila* Schneider L2 cells were analyzed by immunoblot hybridization with polyclonal anti-EGF receptor antiserum. In all experiments, a Drosophila protein of ≈190 kDa was the predominant high molecular weight species recognized by the anti-EGF receptor antiserum (Fig. 1 *Left*). This protein was the sole protein detected in the molecular weight range of the EGF receptor (170 kDa) when *Drosophila* membranes were solubilized in 0.5% $NaDodSO_4$ and applied to an $NaDodSO_4$/polyacrylamide gel without sample boiling or reduction (see methods). Two additional crossreactive proteins of ≈200 kDa and ≈175 kDa were also detected by immunoblotting when samples were boiled and reduced prior to gel electrophoresis (Fig. 2, lane 3). Finally, a 100-kDa protein that is capable of recognizing both EGF and insulin (see below) was consistently observed under both immunoblotting conditions (Fig. 1 *Left* and Fig. 2, lane 3).

A431, a human epidermal carcinoma cell line that overexpresses the EGF receptor, was used as a positive control. Under both immunoblotting conditions, the 170-kDa human EGF receptor from A431 was recognized by the anti-EGF receptor antiserum (Fig. 1 *Left*; Fig. 2, lane 1). The 150-kDa species observed in Fig. 2 appears to be a proteolytic degradation product of the human EGF receptor (11). No proteins in either *Drosophila* or mammalian membranes were detected by immunoblotting with rabbit preimmune serum (Fig. 1 *Center*; Fig. 2, lanes 2 and 4). Thus, the reaction of the anti-EGF receptor antiserum with the *Drosophila* proteins appeared to be specific.

The *Drosophila* Schneider L2 cell line also expressed proteins that crossreact with antisera against another mammalian growth factor receptor, the rat insulin receptor. A protein of 100 kDa was the major species detected in immunoblot hybridization of *Drosophila* L2 membranes that were solubilized in $NaDodSO_4$ and reduced with 2-mercaptoethanol but not boiled prior to gel electrophoresis (Fig. 1 *Right*). A protein of about 120 kDa, which is similar in size to the 130-kDa α subunit of the mammalian insulin receptor, was also recognized by anti-insulin receptor antiserum when

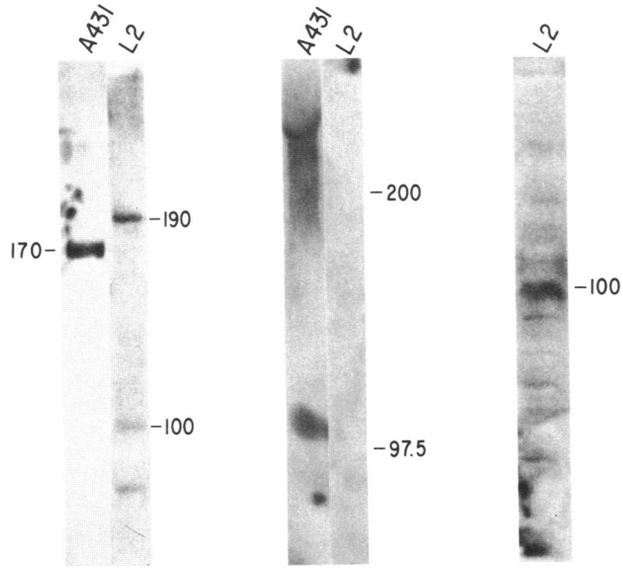

FIG. 1. Immunoblot of membrane protein from human and *Drosophila* cultured cells extracted under mild denaturing conditions. Membrane protein from A431 cells (50 μg per lane) and Schneider L2 cells (200 μg per lane) was solubilized in 0.5% $NaDodSO_4$/10% glycerol/100 mM Tris·HCl, pH 6.8 without boiling, fractionated by $NaDodSO_4$/PAGE, and transferred to nitrocellulose. Reducing agent (3% 2-mercaptoethanol) was only added to samples probed with anti-insulin receptor antibody prior to gel electrophoresis. The filters were probed with either anti-EGF receptor antiserum (*Left*), rabbit preimmune serum (*Center*), or anti-insulin receptor antiserum (*Right*), followed by addition of ^{125}I-protein A. In the preimmune samples, the positions of the molecular weight standards are indicated.

samples were boiled in the presence of reducing agent (data not shown). Whether one of these species corresponds to the 130-kDa *Drosophila* counterpart of the insulin receptor α subunit identified by Rosen and co-workers (12) is not clear.

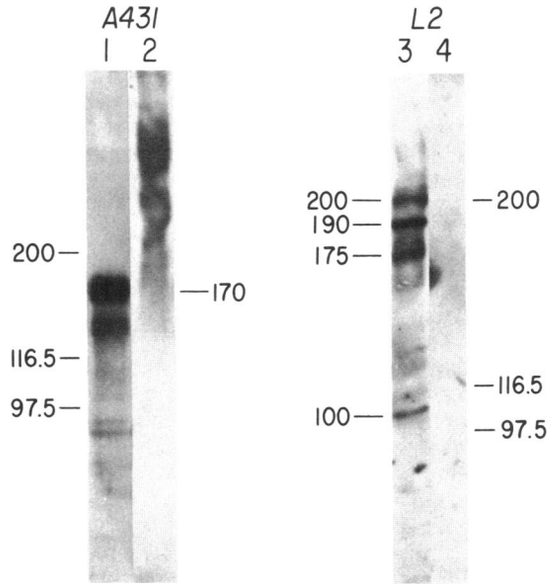

FIG. 2. Immunoblot of membrane protein from human (*Left*) and *Drosophila* (*Right*) cultured cells extracted under harsher denaturing conditions. Immunoblotting was performed as described in Fig. 1 except that membranes were boiled in solubilization buffer containing 2% $NaDodSO_4$ and 1% 2-mercaptoethanol prior to gel electrophoresis. Filters were incubated with either anti-EGF receptor antiserum (lanes 1 and 3) or rabbit preimmune serum (lanes 2 and 4).

The presence of these proteins suggests that the *Drosophila* L2 cell line contains several species that are homologous to the mammalian EGF and insulin receptors on the basis of size and antigenic crossreactivity; in addition, both anti-receptor antisera recognized 100-kDa proteins.

Affinity Labeling with Mammalian EGF and Insulin. To determine whether these antigenically-related *Drosophila* proteins can act as receptors for EGF or insulin, we measured their ability to bind the mammalian growth factors by affinity labeling. Affinity labeling of ^{125}I-insulin to a *Drosophila* protein of 100 kDa could be detected after incubation of the ligand with intact membranes (Fig. 3, lane 2). The insulin binding protein clearly migrated faster in $NaDodSO_4$/polyacrylamide gels than did the 130-kDa α subunit of the mammalian insulin receptor (Fig. 3, lanes 1 and 4). Specific binding of ^{125}I-EGF to intact *Drosophila* membranes could not be detected by affinity-labeling experiments.

To increase sensitivity of detection, detergent-solubilized membranes from *Drosophila*-cultured cell lines were immunoprecipitated with anti-EGF receptor antiserum, anti-insulin receptor antiserum, or rabbit preimmune serum prior to affinity labeling. Radiolabeled growth factors were added to the immune precipitates in the presence or absence of excess growth factor, allowed to bind at 15°C, and then crosslinked with disuccinimidyl suberate. Samples were analyzed by separation on $NaDodSO_4$/PAGE and subsequent autoradiography. Anti-insulin receptor antiserum did not immunoprecipitate any *Drosophila* proteins to which binding of ^{125}I-insulin could be detected, although it recognized bands of 100 kDa and 120 kDa in immunoblot hybridizations (Fig. 1). Either the affinity of the anti-insulin receptor antiserum for *Drosophila* L2 cell proteins was too low or the antiserum was recognizing proteins that are unable to bind mammalian insulin. Similarly, no protein of molecular weight comparable to that of the mammalian 170-kDa EGF receptor was detected by ^{125}I-EGF crosslinking and immunoprecipitation, although 175- to 200-kDa species were identified by immunoblot analysis with anti-EGF receptor antiserum.

However, the anti-EGF receptor antiserum did specifically immunoprecipitate a 100-kDa *Drosophila* protein that bound both ^{125}I-EGF and ^{125}I-insulin (Figs. 4 and 5). Similar results were observed when membrane proteins from two different *Drosophila* cell lines, Schneider L2 and K_c, were analyzed

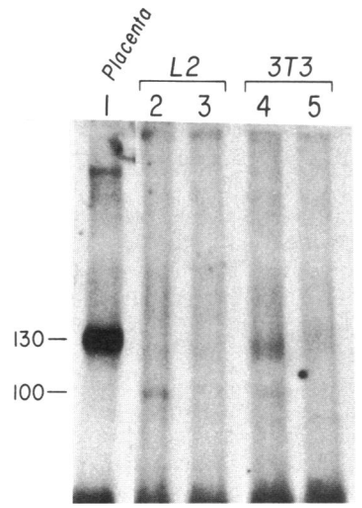

FIG. 3. Affinity labeling of membranes with ^{125}I-insulin. ^{125}I-insulin was incubated with a crude insulin receptor preparation from human placenta (lane 1), Schneider L2 membranes (lanes 2 and 3), or Swiss 3T3 membranes (lanes 4 and 5) and was crosslinked with disuccinimidyl suberate. Excess unlabeled insulin was added to duplicate samples (lanes 3 and 5).

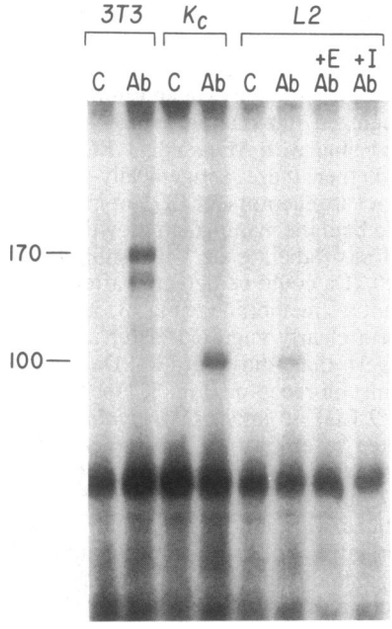

FIG. 4. Autoradiogram of immunoprecipitates affinity labeled with ^{125}I-EGF. Detergent-solubilized membranes from murine fibroblasts (Swiss 3T3) or *Drosophila* cultured cells (K_c and Schneider L2) were incubated with either rabbit preimmune serum (C) or anti-EGF receptor antiserum (Ab) plus protein A-Sepharose beads. The immunoprecipitates were affinity labeled with ^{125}I-EGF and separated by NaDodSO$_4$/PAGE. Excess unlabeled EGF (+E) or insulin (+I) were added to duplicate samples.

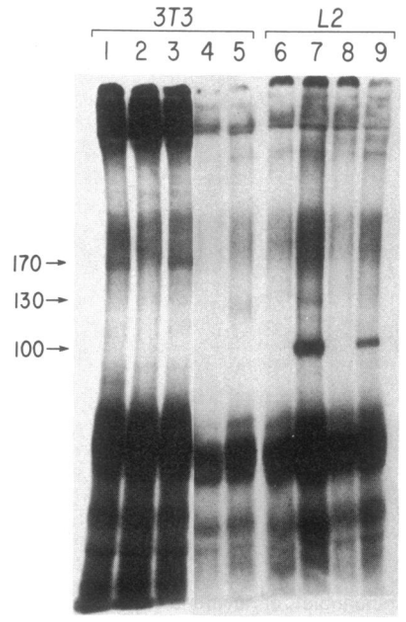

FIG. 5. Autoradiogram of immunoprecipitates affinity labeled with ^{125}I-EGF or ^{125}I-insulin. Swiss 3T3 membranes were immunoprecipitated with anti-EGF receptor antiserum and labeled with ^{125}I-EGF (lane 1), ^{125}I-EGF plus excess unlabeled EGF (lane 2), ^{125}I-EGF plus excess unlabeled insulin (lane 3), or ^{125}I-insulin (lane 4). Solubilized Swiss 3T3 membranes were also immunoprecipitated with anti-insulin receptor antiserum and labeled with ^{125}I-insulin (lane 5). Solubilized Schneider L2 membranes were affinity labeled with ^{125}I-insulin after immunoprecipitation with rabbit preimmune serum (lane 6) or anti-EGF receptor antiserum (lanes 7–9). Excess unlabeled insulin (lane 8) or excess unlabeled EGF (lane 9) was added to duplicate samples.

(Fig. 4, L2 and K_c lanes Ab). An excess of either unlabeled EGF or insulin could block binding of both ^{125}I-insulin and ^{125}I-EGF to the 100-kDa species (Fig. 4, L2 lanes Ab+E and Ab+I; Fig. 5, lanes 8 and 9). Thus, this protein appears to have a common binding site for both growth factors.

Several lines of evidence suggest that the 100-kDa protein has a higher affinity for insulin than EGF. In crosslinking experiments with membranes, binding of ^{125}I-insulin but not ^{125}I-EGF was detected. Second, in competition experiments with unlabeled ligand, insulin was more effective than EGF at inhibiting both ^{125}I-insulin and ^{125}I-EGF binding (Figs. 4 and 5). This result has been confirmed with more extensive dose–response studies (unpublished data).

The dual ligand specificity of the 100-kDa *Drosophila* protein for EGF and insulin is surprising because mammalian EGF and insulin receptors do not share this property. For example, ^{125}I-EGF, but not ^{125}I-insulin, could be crosslinked to the 170-kDa EGF receptor immunoprecipitated from murine 3T3 fibroblast membranes by anti-EGF receptor antiserum (Fig. 5, lanes 1 and 4). Similarly, the affinity labeling of the murine EGF receptor by ^{125}I-EGF could be blocked by excess EGF but not by excess insulin (Fig. 5, lanes 2 and 3). Additionally, the anti-growth factor receptor antisera used in this study did not appear to coprecipitate mammalian EGF and insulin receptors. The 130-kDa murine insulin receptor α subunit could be affinity labeled with ^{125}I-insulin and was immunoprecipitated by anti-insulin receptor antiserum (Fig. 5, lane 5). However, this species was not immunoprecipitated by anti-EGF receptor antiserum (Fig. 5, lane 4). Thus, the dual binding specificity for both EGF and insulin displayed by the *Drosophila* 100-kDa protein appears to be a novel property.

DISCUSSION

We have shown that *Drosophila* cell lines express membrane proteins that share homology with the mammalian growth factor receptors for EGF and insulin. Surprisingly, a 100-kDa protein was identified that can bind both growth factors, is the predominant species crosslinked to insulin and EGF in these cells, is recognized by antisera directed against the mammalian EGF receptor, and may crossreact with anti-insulin receptor antibody. Immunoblot hybridization with anti-EGF receptor antisera also identified a species of 190-kDa comparable in molecular weight to the human and murine EGF receptors. Thus, these *Drosophila* cell lines express at least two classes of growth factor receptors: one that may be the direct counterpart to the mammalian EGF receptor and one that has affinity for both mammalian EGF and insulin.

The fact that the 100-kDa *Drosophila* protein binds both growth factors and appears to crossreact with antisera for both mammalian receptors suggests that there are two highly conserved regions composed of the extracellular hormone binding site and the cytoplasmic tyrosine kinase domain. Previous studies have suggested that the anti-EGF receptor antiserum reacts primarily with the tyrosine kinase region of the EGF receptor. This antiserum immunoprecipitates the product of the *neu* oncogene, which is highly homologous to the EGF receptor within the tyrosine kinase domain. However, it does not immunoprecipitate the secreted human EGF receptor from A431 cells, a truncated form containing mainly the extracellular binding domain (14). The sites recognized by the anti-insulin receptor antiserum are not completely defined. This antiserum does not block the hormone binding site on the α subunit and exhibits limited recognition of the β subunit, which contains the tyrosine kinase domain. Thus, the results are consistent with the existence of two conserved domains within the 100-kDa *Drosophila* protein.

This 100-kDa *Drosophila* receptor is not structurally equivalent to the mammalian insulin receptor. In the mature form

of mammalian insulin receptor, the tyrosine kinase and ligand binding domains are present on separate subunits. The 100-kDa protein, on the other hand, contains sites that bind growth factors as well as sites that are antigenically related to the cytoplasmic domain of the EGF receptor. Such ligand or antigenic crossreactivity with the mammalian EGF receptor was not exhibited by the mammalian insulin receptor in our study. The 100-kDa *Drosophila* protein also may be distinct from a high-affinity insulin receptor in adult *Drosophila* recently identified by Rosen and co-workers (12). The latter species upon reduction releases an insulin binding subunit of comparable molecular weight to the 130-kDa mammalian insulin receptor α subunit (12). The 100-kDa species, in contrast, is observed in the presence or absence of reducing agent. Failure to detect a 130-kDa protein by affinity labeling of intact *Drosophila* L2 membranes with ^{125}I-insulin suggests that this protein is present only as a minor component, if at all, in these cell lines.

We also have identified a 190-kDa membrane protein in *Drosophila* that is antigenically related to the EGF receptor but does not bind murine EGF. From the size of the apparent coding region of the c-*erbB* gene in *Drosophila*, the c-erbB protein can be predicted to be of similar or greater molecular weight than the 170-kDa human EGF receptor. The endogenous ligand of the *Drosophila* c-erbB protein is by no means clear. No sequences homologous to the mammalian EGF gene have reportedly been detected in *Drosophila*, and the putative hormone binding domain of the *Drosophila* c-erbB protein is only partially homologous to the human EGF receptor (41% amino acid sequence homology) (9). In contrast to EGF, an insulin-like peptide has been detected immunologically in *Drosophila* that has similar biological properties to its mammalian counterpart (21). In some respects, the 190-kDa *Drosophila* protein is similar to the transforming protein (p185) encoded by *neu*, the oncogene isolated from chemically induced rat neuroblastomas. p185 is immunoprecipitated by the anti-EGF receptor antiserum, possesses a tyrosine kinase domain homologous to EGF receptor, but does not bind EGF (ref. 15; D. F. Stern, personal communication). The two other high molecular weight species detected in *Drosophila* by immunoblot analysis only after protein denaturation and reduction may represent receptor precursors, subunits of multimeric proteins, proteolytic degradation products, or a closely related family of receptors. Based on antigenic crossreactivity, molecular weight, and the presence of c-*erbB*-related transcripts in these *Drosophila* cell lines (unpublished data), we postulate that the 190-kDa protein is the product of c-*erbB*.

There are several regions of amino acid homology between the mammalian EGF and insulin receptors, and this homology may extend to *Drosophila* receptors. The extracellular domains of the mammalian receptors for insulin and EGF show striking structural homology in the spacing of cysteine residues (22), although it is not known if this region contains the ligand binding site. EGF and insulin, which are similarly sized polypeptides with three inter- or intramolecular disulfide bonds, may also exhibit structural similarity, allowing for cross-recognition by the 100-kDa protein. Sequence comparison of Drosophila c-*erbB* and human EGF receptor cDNAs indicates that the kinase region is highly conserved (9). It is probable that the functional domains of the EGF and insulin receptors correspond to discrete exon regions within the genome, which may have been "shuffled" during evolution to generate new genes, as postulated for the low density lipoprotein receptor (23).

The 100-kDa *Drosophila* protein may represent or be closely related to an evolutionary precursor of the mammalian insulin and EGF receptors. However, we cannot rule out the possibility that the 100-kDa protein is a product of a larger protein, such as the insulin receptor precursor. Thus, differential splicing of the gene transcript or posttranslational processing could account for the appearance of two products from a single gene. Although no mammalian proteins with similar antigenic and ligand-binding properties were detected, a more thorough survey remains to be done. We have not yet determined the function of the 100-kDa protein nor its responsiveness to the ligands that bind to it. If such a protein proves to be functionally activated by both EGF and insulin, the wider range of ligand specificity could represent a new mechanism for amplifying the response of receptors to growth factor signals.

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