Journal of Cellular Biochemistry 31:135–152 (1986)

Functional and Structural Characteristics of EGF and Its Receptor and Their Relationship to Transforming Proteins

C.M. Stoscheck and L.E. King, Jr.

Veterans Administration Research Service, Nashville, Tennessee 37203, and Department of Medicine, Division of Dermatology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Epidermal growth factor (EGF) is a peptide which effects the growth and/or differentiated functions of many cell types. Several pieces of evidence indicate that EGF and its receptor may play a role in carcinogenesis. Functional and structural characteristics of EGF and its receptor and their relationship to transforming proteins are discussed. EGF has extensive homology with alpha-transforming growth factor (alpha-TGF), which may actually be an embryonic form of EGF. Nevertheless, both EGF and alpha-TGF elicit transformation-associated phenotypes in target cells under certain conditions.

EGF effects are mediated by a receptor present on the plasma membrane. The EGF receptor is a highly complex protein having several functions in addition to binding EGF in a highly specific manner. One of these functions is to phosphorylate tyrosyl residues on certain proteins. This activity is similar to that expressed by the *src* family of oncogene-encoded proteins. Besides sharing functional homology the EGF receptor also exhibits structural homology to several oncogene-encoded proteins. The v-*erb*-B-transforming protein has a striking extent of homology (95%) to the cytoplasmic portion of the EGF receptor. These data support the concept that some aspect of EGF-stimulated metabolism is involved in cellular transformation.

Key words: epidermal growth factor, transforming growth factors, carcinogenesis, oncogenes, cell proliferation, membrane protein biosynthesis and degradation, protein kinase C

Epidermal growth factor (EGF) is a 6,023 molecular weight (MW) polypeptide which effects the growth and/or differentiated functions of a wide variety of tissues in addition to the epidermis [1,2]. In certain cases EGF effects on cells are separable from its growth stimulatory properties. For example, EGF enhances the production of chorionic gonadotropin and progesterone in choriocarcinoma cells in the absence of mitogenic stimulation [3,4].

Received August 21, 1985; revised and accepted January 9, 1986.

High levels of EGF are produced in the salivary glands of mice but at lower levels in other animals [5]. Ablation of the salivary gland does not reduce plasma EGF levels [6]; thus other sites must also produce EGF. Localization of EGF in the duodenal and prostate glands and the brain indicates that these tissues may also produce EGF [7–10]. Unequivocal evidence that EGF is synthesized at these sites would require the demonstration of in vitro biosynthesis of EGF in tissue slices and the presence of mRNA for EGF in the cells. The recent finding that EGF in the blood is predominantly located in platelets [11,12] suggests that EGF may play a role in wound healing. In fact, EGF has been found to promote the healing of corneal wounds [13] and skin [14].

It is still somewhat unclear as to what role EGF plays in various tissues in the body. This is because of the inability of investigators to dramatically lower EGF levels in the body by removal of the salivary gland [6] and the lack of genetic mutants which have altered EGF metabolism. The absence of genetic mutants may be due to the possibility that this would be a lethal mutation. The presence of EGF receptors in a wide variety of cell types makes it likely that EGF plays a role in almost every tissue in the body both during development and in the adult.

At least part of salivary gland EGF function is to control acid secretion in the stomach; ablation of this gland causes an increased incidence of gastric ulcers in the presence of a chemical irritant [16]. Administration of EGF directly inhibits acid secretion from the parietal cells of the stomach [17]. In fact, urogastrone has now been identified as the human form of EGF [18,19]. Some unanswered questions about the role of salivary gland EGF are: (1) Why are salivary gland levels of EGF much higher in mice than humans, (2) Why are these levels testosterone dependent and, (3) Why do these levels parallel those of nerve growth factor and renin [20,21].

Preliminary studies indicate that EGF metabolism may be affected in certain disease states. Placenta membranes from streptozotocin diabetic mothers contained fewer EGF receptors [22]. The uppermost cell layers of psoriatic skin contain EGF receptors whereas in normal skin they are nearly absent [23–25]. A child with Donohue syndrome (leprechaunism) was found to have urine EGF levels fivefold higher than control children [26]. Many cancer cells have altered EGF receptor levels [reviewed in 27].

Interest in the EGF system has mushroomed now that it has been shown that EGF and its receptor may play a role in carcinogenesis [27]. This review will discuss structural and functional characteristics of EGF and its receptor and how these characteristics relate to transforming proteins.

STRUCTURAL ASPECTS OF EGF AND ITS RELATIONSHIP TO TRANSFORMING GROWTH FACTORS

EGF (Table I) isolated from mouse salivary glands has been purified and extensively characterized. Purification may be achieved by (1) acidic extraction of male submaxillary glands followed by Bio Gel P-10 and diethylaminoethylcellulose ion exchange chromatography [28] or (2) by reverse-phase high-performance liquid chromatography [29–31]. An "alpha" and "beta" form of EGF were identified in two of these reports. Fast purification in the presence of pepstatin prevented the formation of "beta" EGF [29], indicating it is a proteolytic product of "alpha" EGF. The "beta" form is missing a terminal arginine [31] and was reported to have a 2.5-

TABLE I. Comparison of Peptides With EGF Activity*

			1				5					10					15				
mEGF			N	S	Y	P	G	C	P	S	S	Y	D	G	Y	C	L	N	G	G	V
hEGF			N	S	D	S	E	C	P	L	S	H	D	G	Y	C	L	H	D	G	V
rTGF	V	V	S	H	F	N	K	C	P	D	S	H	T	Q	Y	C	F	H	G	T	C
hTGF	V	V	S	H	F	D	D	C	P	D	S	H	T	Q	F	C	F	H	G	T	C
VVGF	.	.	.	.	.	.	P	A	I	R	L	C	G	P	E	G	D	G	Y	C	L	H	G	D	C

	20					25					30					35				
mEGF	C	M	I	E	S	L	D	S	Y	T	C	N	C	V	I	G	Y	S	G	D
hEGF	C	M	I	E	A	L	D	K	Y	A	C	N	C	V	V	G	Y	I	G	E
rTGF	R	—	L	V	Q	E	E	K	P	A	C	V	C	H	S	G	Y	V	G	E
hTGF	R	F	L	V	Q	E	D	K	P	A	C	V	C	H	S	G	Y	V	G	V
VVGF	I	H	A	R	D	I	D	G	M	Y	C	R	C	S	H	G	Y	T	G	I

	40					45					50								
mEGF	R	C	Q	T	R	D	L	R	W	W	E	L	R						
hEGF	R	C	Q	Y	R	D	L	K	W	W	E	L	R						
rTGF	R	C	E	H	R	D	L	L	A										
hTGF	R	C	E	H	A	D	L	L	A										
VVGF	R	C	Q	H	V	V	L	V	D	Y	Q	R	S	E	N	P	N	T	

*The sequences of mouse EGF (mEGF), human EGF (hEGF), rat TGF (rTGF), human TGF (hTGF), and vaccinia virus growth factor (VVGF) aligned to allow maximal homology. Dashes represent spaces to allow optimal alignment. Invariant residues between the peptides are boxed. The VVGF sequence has been truncated at the amino end (. . .) where it extends for 20 more amino acids.

fold lower activity as measured by the stimulation of thymidine incorporation in fibroblasts [29]. No difference in mitogenicity between the two forms was observed in palatal mesenchymal cells [32]. Purification of human EGF (urogastrone) requires a different purification protocol from mouse EGF [33].

Certain transformed cell lines produce EGF-like proteins (termed alpha-transforming growth factors, or alpha-TGFs) which interact with the EGF receptor and mimic EGF actions. These actions include binding to the receptor, stimulation of autophosphorylation and degradation of the receptor, and stimulation of cell proliferation [34–38]. In conjunction with beta-TGF, alpha-TGF allows normal cells to grow in soft agar, a phenotype which is a strong correlate of malignancy. EGF may be substituted for alpha-TGF in this assay [39]. Structurally human alpha-TGF shares only 40% homology with human EGF whereas it shares 92% homology with rat alpha-TGF (Table I). The homology of human EGF with mouse EGF is 70% [18, 40–42]. The DNA sequences encoding alpha-TGF were observed in normal cells [42] indicating that it is an inappropriately expressed, unmodified normal protein. The presence of TGF-like activity in embryos [43] suggests the possibility that it is an embryonic form of EGF. It should be noted that beta-TGF is also expressed in normal cells [44–46].

Another peptide which interacts with the EGF receptor is vaccinia virus growth factor (VVGF) [47–50]. This factor is encoded by the vaccinia virus, a DNA virus in the poxvirus family. This virus usually kills the host cell rapidly and is not generally considered to be a transforming virus. VVGF binds to the EGF receptor with a higher affinity than EGF itself and also stimulates autophosphorylation of the receptor and cell growth. It is presently unclear what role VVGF plays in viral replication or in disease.

Little is known about how EGF binds to its receptor. Amino acid sequence homology between EGF and alpha-TGFs from different species and VVGF should indicate what aspects of their structures are important for binding. The most conserved area is the third loop extending from residues 32 to 41. In the rest of the molecule a great deal of variability exists not only in amino acid identity but also in class (ie, charge and polarity) (see Table I). One relatively invariant feature is the presence and position of the cysteine residues in the molecules. Thus, these disulfide bonds must be necessary for correct conformation of EGF to bind to its receptor. Evidence in support of this hyothesis is that cleavage of the disulfide bonds greatly reduces the activity of EGF. The sequence of the amino acids around the disulfide bonds may actually be more important than the ring structures formed by them. Breakage of the second peptide ring at glutamate 24 with Staphylococcus aureus V8 protease actually increased the activity of EGF by approximately twofold whereas breakage of the same ring with cyanogen bromide at methionine [21] reduces activity by greater than 96% [39,51]. This difference could be due to the fact that cyanogen bromide cleavage occurs next to a disulfide bond as well as causing a modification of a methionine residue. It is not clear which of these modifications results in inhibitory activity. Another piece of evidence which supports the importance of the tetravalent amino acid sequence around disulfide bonds is that the amino acid sequence is most conserved in these areas.

The least homology between molecules which bind to the EGF receptor exists at the first five amino terminal residues and the last six carboxy terminal residues, implying that these residues probably are not involved in the recognition of the receptor. In support of this hypothesis, a precursor form of EGF which has an additional 27K molecular weight peptide attached to its amino-terminal end has similar activity to 6,053 molecular weight EGF in vitro as indicated by binding and stimulation of thymidine incorporation of fibroblasts [52–54]. Additionally, conjugation of either ferritin or fluorescein isothiocyanate to EGF still allows some, albeit reduced, receptor binding and biological activity [55,56]. Thus the amino terminus must face away from the binding site of the receptor. It is also likely that the carboxy terminal end of EGF also faces away from the binding site since TGFs are four amino acids shorter and VVGF is five amino acids longer than EGF yet they have as high (or higher) affinity for the EGF receptor. However, the carboxy terminal arginine of EGF is necessary for the association of EGF with its binding protein (arginine esterase) and immunosuppressive activity in vivo [57, 58]. Elimination of the arginine has no effect on inhibition of gastric acid secretion, or the potentiation of eyelid opening or tooth eruption activities [18,28,59].

Another approach in investigating what aspects of the EGF molecule are important for binding and activating its receptor is to produce EGF fragments or synthetic peptides. Interestingly, a peptide from a relatively unconserved region of EGF (residues 20–31) was found to bind and activate the EGF receptor albeit with low activity (0.01% of EGFs) [60]. A peptide made up of the third loop of alpha-TGF (residues 34–43), which is highly conserved among the EGF-like proteins, also had the capacity to bind to the receptor (with 0.2% of the affinity of EGF when the amino and carboxyl terminal groups were blocked) but, in contrast, it did not stimulate fibroblast proliferation even at high receptor occupancy levels. In the presence of EGF the peptide acted as an antagonist of cell growth [61].

EGF SYNTHESIS

The molecular details of EGF synthesis have not been completely described. Certain events can be predicted from the sequence of the messenger RNA encoding the EGF precursor (Fig. 1A) [62–64]. The messenger RNA has a single reading frame of 3,396 bases, predicting that the precursor is a very large protein consisting of 1,217 amino acids and having a molecular weight of 127K. Gray et al. predicted a slightly shorter sequence due to the addition of a single nucleotide at position 3,701, causing a frame shift. The amino end of the predicted precursor protein has a sequence of hydrophobic amino acids (residues 7–19) which resembles a signal peptide, which would allow the protein to be translated at the endoplasmic reticulum. The precursor protein may initially be a membrane protein since the mRNA sequence predicts a membrane-spanning segment (residues 1,039–1,059) at the carboxyl end of the molecule with a 157 amino acid segment remaining inside the cell. EGF is trimmed from the rest of the precursor protein at both ends of the molecule by an arginine esterase. This has been shown by the association of EGF with arginine esterase in a high molecular weight complex in vivo, production of EGF from pro-EGF forms by arginine esterase but not other proteases, and the presence of arginine at the terminal amino acid position of both EGF and the peptide preceding it [62–66].

With such a large precursor protein, the question arises as to what other functional peptides might be present and what their function might be. For example, enkephalin, corticotropin, and B-lipotropin share a common precursor protein [67]. Since an arginine esterase exists in a high molecular weight complex with EGF [65, 67], potentially its amino acid sequence could be incorporated within the EGF precursor protein; but upon close examination, no homologous sequences were found. NGF and renin amino acid sequences, which are produced in equimolar quantities as EGF in the submaxillary gland [20,21], were also not present within the EGF precursor protein sequence. It should be noted that the NGF gene has been localized to human chromosome 1, whereas the EGF gene is on human chromosome number 4 [69,70]. Interestingly, much of the pro-EGF molecule is composed of nine sequences, each of which has significant homology to EGF itself. However, only one of the peptides is bounded by arginine or lysine residues, making it unlikely that they are expressed in an EGF-like form.

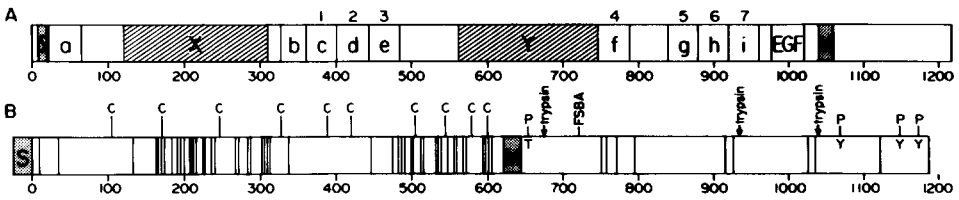

Fig. 1. Peptide coding portions of mRNA for EGF and its receptor markers for amino acid residue number are placed below each sequence. The putative positions of the signal- and membrane-spanning regions are designated with an S and an M, respectively. A) EGF. EGF-like sequences are designated as letters a–i [64] or 1–7 [62]. Peptides a and b have minimal homology with EGF. Peptides X and Y have homology with each other. B) EGF receptor. The probable presence of carbohydrate side chains is indicated by the letter C. The presence of phosphoaminoacyl linkages is designated by the letter P (PY = phosphotyrosine, and PT = phosphothreonine). The binding site of an analog of ATP, FSBA, is shown. Highly susceptible sites for tryptic cleavage (shown experimentally) are designated. Vertical lines indicate the position of cysteine residues which may be involved in disulfide bonds.

One study indicates that the EGF precursor may be left intact, possibly as a membrane protein, in kidney cells [71]. Under the same condition where precursor fragments and the finished EGF product is observed in submaxillary gland slices, only one protein of 130K molecular weight is observed in kidney slices. An alternative explanation is that processing and secretion of EGF occurs much more rapidly in the kidney than in the submaxillary gland. The possibility that the EGF precursor may be processed differently and have completely different functions in two different tissues is very intriguing.

Similarities of certain portions of the EGF precursor, excluding the EGF sequence, have been observed with the LDL receptor and the v-*mos* oncogene-encoded protein [72–74]. A cysteine-rich extracellular segment of the low density lipoprotein (LDL) receptor (residues 290–640 in the 839 amino acid protein) shares 33% homology with the extracellular portion of the EGF precursor (residues 399–746) [72]. The homologous areas do not include the LDL binding site or EGF. Neither protein shares homology with the EGF receptor. One possibility is that EGF and LDL descended from the same ancestral protein [73]. This is not too surprising since the EGF precursor is most likely a membrane-bound protein before processing [62,63]. A cytoplasmic segment of the EGF precursor (residues 1,127–1,174) shares 36% homology with a portion (residues 317–360) of the protein product of v-*mos*, the oncogene of Moloney murine sarcoma virus [74]. The sequence of the mos gene is related to the tyrosine kinase family of oncogenes but its protein product has not yet been shown to have this activity. The EGF precursor does not contain the Gly-X-Gly-X-X-Gly ATP binding sequence so it is probably not itself a kinase.

Although the possibility that the EGF precursor protein may encode functional peptides in addition to EGF is interesting, it is also possible that most of the protein simply represents vestigial sequences. The presence of multiple copies of EGF-like sequences as well as an LDL receptor-like sequence indicates that EGF may be encoded in an unstable region of DNA which readily undergoes unequal crossing over. In this light, it is also interesting to note that alpha-TGF and the EGF receptor are highly conserved between species whereas EGF is not. Further work is needed to determine what peptides are actually produced and what they do.

THE EGF RECEPTOR

The receptor for EGF has five major roles. The first is the specific recognition of EGF. The receptor binds to EGF with an affinity of 10^{-11} to 10^{-8} M [75–77]. This affinity can change when cells are treated with a variety of hormones and carcinogens (discussed below).

The second function of the receptor is to transmit the binding signal from the extracellular face of the receptor to the cytoplasmic, enzymatic face of the receptor. Little is known about this function other than it can occur at 0°C or in the presence of detergent [78–80]. One possible mechanism of signal transmission traversing the lipid bilayer is receptor clustering. Clustering of $\alpha_2$macroglobulin receptors has been shown to occur at 4°C [81]. However, no evidence of clustering has been seen with EGF receptors at this temperature [55]. Another possible mechanism is an intramolecular conformational change within the EGF receptor molecule itself. Signals may also be transmitted from the cytoplasmic face of the receptor to the extracellular face. This may be how a variety of substances indirectly inhibit EGF binding (Fig. 2).

The third function of the receptor is to transmit the signal from the cytoplasmic face of the receptor to other proteins in the cell. This function is most likely carried out by a protein kinase activity of the receptor. This protein kinase has the capacity to form an unusual (less than 0.03% in nontransformed cells) [82] phosphoaminoacyl linkage, with tryosine residues. The receptor phosphorylates a wide variety of proteins (Table II) as well as phosphorylating itself, which may produce still another signal such as for clustering of the receptors. Perhaps a phosphorylated fragment of the receptor may act as an intracellular messenger of EGF action.

In vivo, phosphorylation of serine and threonine residues is also increased by EGF [83–85]; however, since EGF increases the activty of the calcium- and phospholipid-dependent C-kinase which is a serine and threonine protein kinase [86] (and see Fig. 2) this may be a secondary effect. Tyrosyl protein kinase activity is probably an integral part of the EGF receptor itself. This was shown by demonstrating the copurification of EGF binding activity with EGF-stimulated kinase activity immunoprecipitation of kinase activity with specific antibodies and the presence of an ATP binding site within the EGF receptor [75,87–90].

The EGF receptor protein kinase utilizes as substrates ATP or GTP and proteins or peptides containing tyrosines preceded by acidic residues [80,86,91]. Known substrates are listed in Table II. Tyrosine is not a substrate for the kinase [95]. The EGF receptor kinase also requires a metal cofactor for activity as do most phosphotransferases. Manganese is the most active metal and magnesium, cobalt or zinc can support kinase activity at high concentrations; a combination of manganese and magnesium allows the highest activity [78–80,93,94].

The fourth function of the receptor is to respond to the physiological state of the cell. Exposure of cells to platelet-derived growth factor, vasopressin, fibroblast-derived growth factor, β-adrenergic agonists, dibutyryl cyclic AMP, colostral factor, calmodulin antagonists, prostaglandin A1 and A2, cholecystokinin-octapeptide, phorbol esters, aplysiatoxin, teleocidin, benzopyrene, and vitamin K may cause a decrease

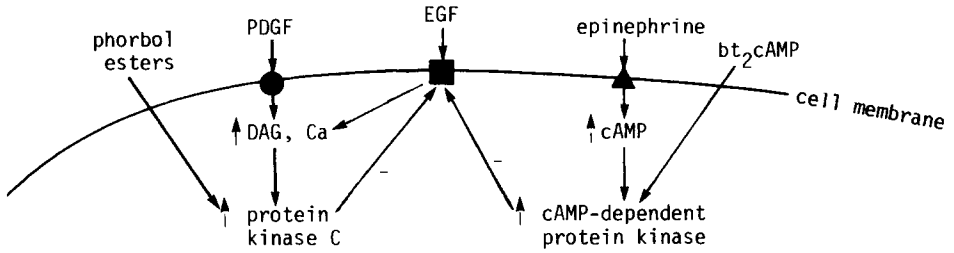

Fig. 2. Mechanisms by which factors indirectly inhibit EGF receptor activity. Factors such as platelet-derived growth factor (PDGF) interact with their receptor (●) to cause an increase in diacylglycerol (DAG) and calcium levels. These factors activate protein kinase C which then phosphorylates the EGF receptor (■) to inhibit both binding and kinase activities. EGF receptor activation also leads to an increase in DAG and calcium levels which subsequently causes a decrease in receptor activity. Tumor promoters such as phorbol esters can penetrate the cell membrane and directly activate protein kinase C. Another metabolic pathway which interacts with the EGF receptor involves cAMP-dependent protein kinase. Ligands such as epinephrine interact with their receptor (▲) to stimulate adenyl cylase activity, which then causes a rise in cAMP levels. This rise then increases the activity of the cAMP-dependent protein kinase which phosphorylates and possibly directly decreases EGF receptor activity. Drugs such as dibutyryl cAMP (bt_2cAMP) can penetrate the cell membrane and directly activate the cAMP-dependent protein kinase.

TABLE II. EGF-Stimulated Protein Phosphorylation*

	Reference
In Vitro	
80K, 60K, and 22.5K (A-431 membranes)	[78]
36K (A-431 cytosol)	[171,172]
80K, 40K, 20K (A-431 membranes; 20K protein phosphorylation is fibronectin stimulated)	[173]
36K (human fibroblast membranes); 70K, 30K–40K (glial cell membranes)	[174]
130K (rat liver)	[175]
42K and 36K (human colon adenocarcinoma cells)	[176]
Insulin receptor (95K), troponin I	[91]
Histone, casein, phosvitin, ribonuclease	[80,91,94]
Tubulin	[75]
Myosin regulatory light chain	[91,177]
Angiotensin	[178]
Middle T antigen-transforming protein of polyoma virus	[179]
Gastrin-17	[180]
Acidic polypeptides	[94,181]
Human growth hormone	[182]
Peptide homologous to the autophosphorylation site of Rous-sarcoma-transforming protein	[91,92,183]
Peptide homologous to the autophosphorylation site of Harvey-sarcoma-transforming protein	[184]
Antibody from Rous tumor-bearing animals	[185,186]
p21 tridecapeptide (from Harvey and Kirsten) sarcoma-transforming viruses	[182]
In Vivo	
36K and 81K (A-431 cells)	[141,187–189]
36K (3T3 cells)	[190]
43K and 45K (3T3 cells)	[191]
22K (3T3-L1 adipocyte cytosolic protein)	[192]
ATP-citrate lyase, 46K (hepatocytes)	[93]
40K and 42K (chicken embryo fibroblasts)	[190,193]
40S ribosomal protein S6 (3T3 cells)	[194,195]

*In all cases the 170K receptor and occasionally the 150K receptor is phosphorylated in response to EGF.

in the affinity of the receptor for EGF [95–108]. General characteristics of this inhibition are that it is very rapid, it does not occur at low temperatures, and it can be overcome by the use of high levels of EGF (approximately 100 ng/ml). Furthermore, phorbol esters, aplysiatoxin, and teleocidin have been shown to inhibit EGF-stimulated kinase activity even at high EGF concentrations, indicating an effect on the kinase site independent from their effect on binding [108].

Several of the factors known to change EGF receptor affinity are also known to activate either protein kinase C or the cAMP-dependent protein kinase. For example, dibutyryl cAMP and phorbol esters directly activate the cAMP-dependent protein kinase and protein kinase C, respectively. Beta-adrenergic agents activate adenyl cyclase, which produces cAMP, which then activates the cAMP-dependent protein kinase. Platelet-derived growth factor increases calcium and diacylglycerol levels, both which activate protein kinase C [109]. Thus, modulation of receptor activity may be depicted as shown in Figure 2. It is not clear, however, why certain factors such

as insulin, which should also activate protein kinase C, do not alter EGF receptor affinity, and why activators of cAMP-dependent protein kinase do not alter EGF receptor affinity in all cell types (personal observation).

The fifth function of the receptor is to form macromolecular clusters after binding to EGF [55,56,110]. It is not clear whether clustering involves self-recognition (Fig. 3A) or recognition of another protein arranged in pits at the membrane surface (Fig. 3B). The latter possibility seems more likely since different receptors can be internalized in the same vesicle [111,112]. After clustering and internalization, the receptor may be degraded or transported within the cell depending on cell type [113–117]. EGF function in the cell may determine whether the receptor is degraded or recycled. Although EGF is thought to be primarily a mitogen for cells, some cells must have a role in EGF transport. High levels of EGF are found in sweat, milk, and urine [118]; in urine and milk it has been shown that at least some of the EGF is sequestered from the plasma [115–118]. Most membrane carrier proteins such as the LDL receptor are recycled [119]. In addition, a degradation function may take place in the liver [116,117].

Recently, endonuclease activity has been found to partially copurify with the EGF receptor [120,121]. The endonuclease does not seem to be an integral part of the receptor itself but may bind to the EGF receptor as a substrate. The Rous sarcoma virus-transforming gene product, pp60 *src*, and rat liver TPK75, both tyrosine

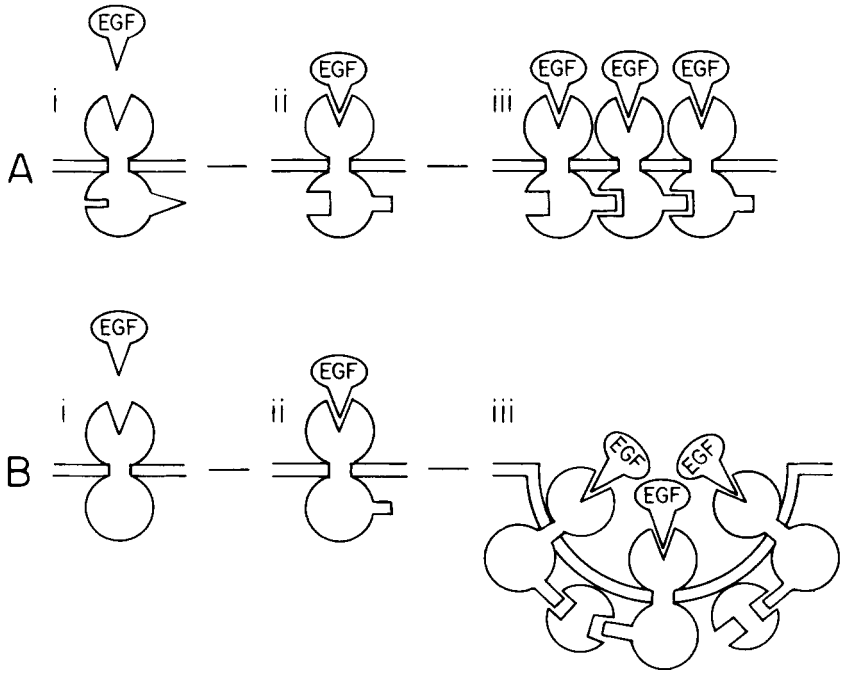

Fig. 3. Two hypothetical mechanisms by which EGF receptors cluster in response to EGF. A) Self-aggregation model. EGF alters the conformation of the unactivated receptor (i) at two different sites (ii) so that the receptor binds to other receptors forming a cluster (iii). Autophosphorylation may substitute for a conformational change as a signal. B) Binding protein model. Binding of EGF to its receptor (i) causes a conformational change or autophosphorylation (ii) allowing the receptor to bind a protein located in a coated pit thus causing a cluster (iii).

kinases, have been shown to phosphorylate topoisomerases and inhibit their activity [122]. Topoisomerases are related to endonucleases in that they must nick the DNA strands before reannealing the ends. Thus, a similar function can be envisioned for the EGF receptor tyrosine kinase. EGF, however, does not inhibit or activate the copurified endonuclease activity [121]. In whole cells EGF seems to increase topoisomerase activity [123].

STRUCTURAL ASPECTS OF THE EGF RECEPTOR

The EGF receptor from several tissues and species has been shown to have a MW of 170K. Early studies identified a second EGF binding species with an MW of 150K. It is now known that the 150K form of the receptor is a product of proteolytic digestion by a calcium-activated protease and is preventable by the addition of EGTA, leupeptin, and/or iodoacetic acid to the cell-homogenizing buffer [124,125]. Since the EGF receptor is a phosphoprotein, it can have different charges; in A-431 cells grown in 10% fetal calf serum the average pI of the molecule is 7 [126].

The receptor is a transmembrane protein with an EGF binding domain facing the outside of the cell and a protein-kinase domain facing the inside of the cell [127, 128]; there is no direct evidence as to where the endonuclease activity or clustering sites are located; however, they would most likely be located on the inside of the cell. The receptor is glycosylated on its extracellular surface and is phosphorylated on its intracellular surface (Fig. 1B). Glycosylation makes the receptor extremely resistant to proteolytic digestion in intact cells. No decrease in binding activity or molecular weight was observed upon treatment of whole cells with trypsin, papain, or chymotrypsin [127–129]. This resistance to proteolytic digestion would be very important in order to maintain activity in cells lining the digestive system.

Phosphorylated amino acid residues of the EGF receptor include serine, threonine, and tyrosine. Phosphotyrosine is a highly unusual residue and is a product of autophosphorylation by EGF receptor kinase activity [130–132]. Phosphoserine and phosphothreonine may be products of protein kinase C and cAMP-dependent protein kinase [133–135].

Recently, the cDNA sequence of the EGF receptor has been determined [132] and thus certain structural aspects of the EGF receptor can be predicted. The amino acid backbone is predicted to 1,210 amino acids long with an MW of 134K. Thus the remaining molecular weight of the receptor is due to attached carbohydrate residues. This prediction is in agreement with data showing that cells treated with inhibitors of protein glycosylation such as tunicamycin or glucosamine produce an EGF receptor of 135K MW [137–139]. A hydrophobic sequence from residue 622 to 644 probably represents the transmembrane domain of the receptor. The extracellular portion of the molecule would then be 621 residues long or 69K MW and the intracellular portion of the molecule would then be 542 residues long or 60K MW.

Partial proteolysis has enabled some mapping of the active domains of the receptor. O'Keefe and colleagues [141] determined several proteolytic domains of the receptor by subjecting the receptor which had been previously labeled covalently with ^{125}I-EGF to protease (oxidized but not native EGF will covalently bind to the receptor in a specific manner). Fragments of 150K, 130K, 85K, 48K, 35K, and 25K MW were observed. Since the 25K MW fragment was still attached to the plasma mem-

brane, this implies that at least part of the binding site must consist of or be near the inner one third of the extracellular chain.

Protein kinase activity of the receptor was examined after partial proteolysis by measuring the phosphorylation of a 13 amino acid peptide analogous to the *src* autophosphorylation site [142]. A 42K fragment retained kinase activity although its activity was reduced by 72%. Although this peptide contains the ATP binding site, as it must for a protein kinase to be active, its precise location within the peptide is not yet known. In contrast, Chinkers and Brugge [143] found that kinase activity disappeared in parallel with the appearance of 30K and 40K fragments. Comparison of the EGF receptor sequence to that of the known ATP binding site of the *src* kinase and cAMP-dependent protein kinase indictes that EGF receptor glycine residues 695, 697, and 700 and lysine 721 are involved in ATP binding [144,145]. This prediction is supported by the finding that 5′-p-fluorosulfonylbenzoyl adenosine (FSBA), an analog of ATP, covalently binds to lysine 721 [146].

The major in vitro as well as in vivo phosphorylated tyrosyl sites are lost upon proteolysis of the receptor to the 150K fragment whereas most of the phosphoserine and phosphothreonine residues are retained [142,143]. The phosphotyrosine sites of the receptor are located in tyrosine 1,068; 1,148; and 1,173 [132]. In intact A-431 cells, EGF predominantly stimulates the phosphorylation of tyrosine 1,173 (tenfold). Localization of the major phosphotyrosyl sites to the terminal, 20K portion of the receptor is intriguing in view that (1) calcium-activated protease clips off from the receptor a 20K fragment; (2) phosphatidylinositol turnover is increased in response to EGF binding; (3) phosphatidylinositol activates calcium-activated protease; and (4) the receptor is known to be degraded after binding EGF [113,114,124,147,148]. Thus a mechanism by which a phosphorylated peptide could be released in order to act as a second messenger of EGF is possible.

One of the phosphothreonine sites has been identified to be located at threonine 654 [149]. This particular residue has special significance since it is the only new phosphoamino acid which appears after treatment of A-431 cells with phorbol esters, which reduces receptor affinity and decreases kinase activity. Since threonine 654 is close to the putative transmembrane portion of the receptor it is in ideal location to perturb both kinase and binding activities. It should be emphasized however, that phorbol esters also increase the extent of phosphorylation of other amino acids; thus these residues may also play a role in phorbol ester function.

Interestingly, EGF stimulates the phosphorylation of its receptor at some of the same sites as protein kinase C in whole cells [139]. Thus, if phosphorylation of these sites decreases receptor affinity for EGF as well as decreasing kinase activity, EGF may in effect be "down regulating" its receptors without degrading them. In fact, different phosphorylation states of the receptor may explain the presence of two or more classes of receptors (with different affinities) in Scatchard plots.

EGF RECEPTOR HETEROGENEITY

It is not yet clear as to whether EGF receptor structure and function are the same in every tissue; the receptor has been identified and/or characterized in only a limited number of tissues to date. As previously mentioned, the molecular weight of the receptor from several sources (liver, skin fibroblasts, placenta, brain, kidney, and A-431 cells) has been estimated to be 170K MW [75,125,129,150]. The A-431 cell

receptor actually migrates slightly slower and somewhat more diffusely than the fibroblast receptor in SDS polyacrylamide gels [129]. This is probably due to altered carbohydrate processing of the receptor in A-431 cells where an extra blood sugar group has been added [151]. Altered carbohydrate processing has also been observed for LDL receptors in this cell line [152].

Functional differences have also been observed between receptors from A-431 cells versus normal cells. The presence of detergent interferes with EGF binding to liver or placenta [125,153] whereas with A-431 receptors it does so to a lesser degree [154]. Nevertheless, EGF stimulation of phosphorylation and receptor binding to an EGF column does occur in the presence of detergent, indicating their effect may be predominantly on affinity [91,125]. It is not clear whether differential carbohydrate processing in A-431 cell causes this functional difference; it seems unlikely since nonterminally glycosylated EGF-EGF receptor complexes from A-431 cells are precipitated by polyethylene glycol to the same extent as fully glycosylated receptors [155]. Heat-inactivation kinetics of kinase activity is also altered in the A-431 cell. In placental membranes only 32% of kinase activity is lost after ten minutes at 45°C as compared to 60% in A-431 cells [80,150]. Placental membranes also have a decreased capacity to phosphorylate exogenous substrates [150]. Further comparisons with kinase activity of the purified receptor from other cell types are necessary before further conclusions about receptor heterogeneity may be made.

EGF RECEPTOR SYNTHESIS

As with EGF, several biosynthetic events can be predicted from examining the mRNA sequence [132,136]. A stretch of hydrophobic amino acids resembling a signal peptide is observed between residues minus 22 and minus 3 resulting in the translation of the mRNA at the endoplasmic reticulum. Another stretch of hydrophobic amino acids (residues 622–644) followed by several basic amino acids probably define the transmembrane domain (Fig. 1B).

In pulse-chase experiments the receptor is first observed as a 160K protein in both A-431 carcinoma cells and normal skin fibroblasts [129,139,140]. This form has immature "high-mannose" carbohydrate chains which are most likely cotranslationally attached. Studies using tunicamycin or glucosamine inhibitors of glycosylation, or endoglycosidase H digestion, revealed a core protein of 130K [137,139,140]. The immature high-mannose form of the protein is converted relatively slowly to the mature form with a half-life of 1.7 hrs. At this step, terminal glycosylation takes place with fucose and galactose becoming incorporated into the molecule [129]. Phosphorylation of the EGF receptor occurs at any stage of its maturation, including the 130K form produced in the presence of inhibitors, both in vivo and in vitro. Although phosphorylation of the 160K precursor form as well as a tunicamycin-produced 130K form of the receptor was stimulated by EGF in whole cells [139,140] it is unclear whether the phosphorylation was intra- or intermolecular. The EGF receptor obtains the capacity to bind EGF some time after translation of the molecule but before terminal glycosylation with a time frame consistent with processing in the Golgi [138]. The use of inhibitors to prevent cotranslational glycosylation prevents the acquisition of binding activity [137,138]. It is not clear whether cotranslational glycosylation is necessary for correct folding of the receptor or for directing the molecule to the Golgi or is directly involved in ligand binding.

During investigations of the biosynthesis of the EGF receptor, a soluble form of receptor which binds to EGF was found to be secreted from A-431 cells [156, 157]. The molecular weight of the soluble receptor is 105K and appears to be identical to the membrane-bound receptor except that it is missing the transmembrane and cytoplasmic domains. As would be expected from the absence of the cytoplasmic domain, the secreted receptor does not have kinase activity nor is it a substrate of the EGF-stimulated kinase.

HOMOLOGY OF THE EGF RECEPTOR WITH THE V-*ERB*-B TRANSFORMING PROTEIN

The v-*erb*-B transforming protein from avian erythroblastosis virus resembles a truncated EGF receptor missing the binding portion which would be homologous to the secreted EGF receptor described above [136]. The transforming protein shares 95% homology with the human EGF receptor and may even share greater homology to the chicken EGF receptor. Recent work has shown that the v-*erb*-B protein possesses kinase activity [158,159]. Since the v-*erb*-B protein is responsible for cellular transformation activity of the avian erythroblastosis virus, it might have been expected that its protein kinase activity would be in a permanently activated state; however, measurement of its kinase activity with an artificial substrate indicates that it has a basal level of kinase activity [159]. This low level of tyrosine kinase activity is reflected by the barely detectable increase in phosphotyrosine levels in erythroblastosis-virus-infected cells [160–162].

The EGF receptor also shares structural homology (approximately 25%) with proteins encoded by the *src* family of oncogenes (*src, abl, fps, yes, fgr,* and *ros*). More importantly, they also share functional homology. This includes not only the capacity to phosphorylate tyrosyl residues but also the specificity for protein substrates both in vitro and in vivo [reviewed in 163–165]. Since the expression of tyrosyl kinases is associated with rapid cell growth, it seems likely that one or more of their substrates must be important regulators of cell growth. An intensive search is now underway in several laboratories in order to identify a growth regulatory substrate.

OVERALL PERSPECTIVE

A role for hormones and their receptors in the process of cellular transformation is becoming more and more evident. In addition to the homologies of EGF and its receptor to transforming proteins already discussed, homologies have also been observed with the oncogene product of v-*fms*, with the macrophage-colony-stimulating factor receptor, v-*sis* with platelet-derived growth factor, and the oncogene product of *ras* with G proteins (GTP-binding proteins which transduce a hormone binding signal to adenyl cyclase) [166–169]. These associations help explain the cellular specificity for some of the oncogenes. For example, the oncogene product of v-*sis* is inactive in those cells which do not have platelet-derived growth factor receptors [170].

It is actually not too surprising that hormonal systems are activated by oncogenes. Cell growth and division is a highly complex process requiring coordination of cellular metabolism. Growth factors already have the capacity to orchestrate these processes. Thus oncogene products which mimic the growth factor, the activated

form of its receptor, or its second messengers should enable the host cell to divide rapidly yet remain viable.

ACKNOWLEDGMENTS

We thank R. Gates for a critical reading of the manuscript and S. Bennett, C. Matthews, and L. Coats for preparation of the manuscript. This work was supported by the Research Service, Veterans Administration Medical Center (C.M.S. and L.E.K.), American Cancer Society institutional research grant IN-25X (C.M.S.), Biomedical Research Support Grants RR-05424 (C.M.S.) and RR-05424 (L.E.K.), and United States Public Health Service grant AM26518 from the National Institutes of Health (L.E.K.).

REFERENCES

1. Carpenter G: Handbook Exp Pharmacol 57:90–126, 1981.
2. King LE Jr, Carpenter GF: "Biochemistry and Physiology of the Skin." New York: Oxford University Press, pp 269–280, 1983.
3. Beneviste R, Speeg KV, Jr, Carpenter G, Cohen S, Linder J, Rabinowitz D: J Clin Endocrinol Metab 46:169–172, 1978.
4. Bahn RS, Speeg KV, Jr, Ascoli M, Rabin D: Endocrinology 107:2121–2123, 1980.
5. Cohen S: J Biol Chem 237:1555–1562, 1962.
6. Byyny RL, Orth DN, Cohen S, Doyne ES: Endocrinology 95:776–782, 1974.
7. Elder JB, Williams G, Lacey E, Gregory H: Nature 271:466–467, 1978.
8. Heitz PU, Kasper M, Van Noorden S, Polak JM, Gregory H, Pearse AGE: Gut 19:408–413, 1978.
9. Shikata H, Utsumi N, Hiramatsu M, Minami N, Nemoto N, Shikata T: Histochemistry 80:411–413, 1984.
10. Fallon JH, Seroogy KB, Loughlin SE, Morrison RS, Bradshaw RA, Knauer DJ, Cunningham DD: Science 224:1107–1109, 1984.
11. Oka T, Orth DN: J Clin Invest 72:249–259, 1983.
12. Bowen-Pope DF, Ross R: Biochem Biophys Res Commun 114:1036–1041, 1983.
13. Gospodarowicz D, Greenburg G: Exp Eye Res 28:147–157, 1979.
14. Sporn MB, Roberto AB, Shull JH, Smith JM, Ward JM, Sodek J: Science 219:1329–1330, 1982.
15. Grueters A, Alm J, Lakshmanan J, Fisher DA: Pediatr Res 19:853–856, 1985.
16. Kirkegaard P, Olsen PS, Paulsen SS, Nexo E: Gastroenterology 85:1277–1283, 1983.
17. Konturek SJ, Cieszkowski M, Jaworek J, Konturek J, Brzozowski T, Gregory H: Am J Physiol 246:G580–G586, 1984.
18. Gregory H: Nature 257:325–327, 1975.
19. Carpenter G, Cohen S: J Cell Physiol 88:227–237, 1976.
20. Hirata Y, Orth D: Endocrinology 107:92–97, 1980.
21. Hirata G, Orth DN: Endocrinology 105:1382–1387, 1979.
22. Siasom JF, Stenzel WK, Warshaw JB: J Cell Biol 101:256a, 1985.
23. Nanney LB, McKanna JA, Stoscheck CM, Carpenter G, King LE, Jr: J Invest Dermatol 82:165–169, 1984.
24. Nanney LB, Stoscheck CM, King LE, Jr: J Invest Dermatol 83:385–393, 1984.
25. Nanney LB, Stoscheck CM, King LE, Jr: J Invest Dermatol 86:260–265, 1986.
26. Frindik JP, Kemp SF, Fiser RH, Schedewie H, Elders MJ: J Pediatr 107:428–430, 1985.
27. Stoscheck C, King LE, Jr: Cancer Res 46:1030–1037, 1986.
28. Savage CR, Cohen S: J Biol Chem 247:7612–7621, 1972.
29. Burgess AW, Knesel J, Sparrow LG, Nocola HA, Nice EC: Proc Natl Acad Sci USA 79:5753–5757, 1982.
30. Elson SD, Brauene CA, Thorburs GD: Biochem Int 8:427–435, 1984.
31. O'Keefe JJ, Sharry LF, Jones AJ: J Chromatogr 336:73–85, 1984.
32. DiAugustine RP, Walker MP, Klapper DG, Grove RE, Willis WD, Harian DV, Hernendez O: J Biol Chem 260:2807–2811, 1985.

33. Savage CR, Jr, Harper R: Anal Biochem 111:195–202, 1981.
34. Todaro GJ, Fryling C, DeLarco JE: Proc Natl Acad Sci USA 77:5258–5262, 1980.
35. Reynolds F, Jr, Todaro GJ, Fryling C, Stephenson JR: Nature 292:259–262, 1981.
36. Twardzik DR, Todaro GJ, Reynolds FH, Jr, Stephenson JR: Virology 124:201–207, 1983.
37. Carpenter G, Stoscheck CM, Preston YA, DeLarco JE: Proc Natl Acad Sci USA 80:5627–5630, 1983.
38. Massaque J, Czech MP, Iwata K, DeLarco JE, Todaro GJ: Proc Natl Acad Sci USA 79:6822–6826, 1982.
39. Anzano MA, Roberts AB, Meyers CA, Komoriya A, Lamb LC, Smith JM, Sporn MB: Cancer Res 42:4776–4778, 1982.
40. Savage CR, Jr, Hash JH, Cohen S: J Biol Chem 248:7669–7672, 1973.
41. Marquardt H, Hunkapillar MW, Todaro GJ: Science 223:1079–1082, 1984.
42. Derynik R, Roberts AB, Winkler ME, Chen EY, Goeddel DV: Cell 38:287–297, 1984.
43. Twardzik DR, Todaro GJ, Marquardt H, Reynolds FH, Jr, Stephenson JR: Science 216:894–897, 1982.
44. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB: Proc Natl Acad Sci USA 78:5339–5343, 1983.
45. Childs B, Proper JA, Tucker RF, Moses HL: Proc Natl Acad Sci USA 79:5312–5316, 1982.
46. Frolik CA, Dart LL, Meyers CA, Smith DM, Sporn MB: Proc Natl Acad Sci USA 80:3676–3680, 1980.
47. Blomquist MC, Hunt LT, Barker WC: Proc Natl Acad Sci USA 81:7363–7367, 1984.
48. Brown JP, Twardzik DR, Marquardt H, Todaro GJ: Nature 313:491–492, 1985.
49. Reisner AH: Nature 313:801–803, 1985.
50. Stroobant P, Rice AP, Gullick WJ, Cheng DJ, Kerr IM, Waterfield MD: Cell 42:383–393, 1985.
51. Savage CR Jr, Inagami T, Cohen S: J Biol Chem 247:7612–7621, 1972.
52. Hirata Y, Orth D: J Clin Endocrinol Metab 48:673–679, 1979.
53. Gregory H, Walsh S, Hopkins CR: Gastroenterology 77:313–318, 1979.
54. Oka Y, Orth D: J Clin Invest 72:249–259, 1983.
55. Haigler HJ, McKanna JA, Cohen S: J Cell Biol 81:382–395, 1979.
56. Haigler HJ, Ash JF, Sizer SJ, Cohen S: Proc Natl Acad Sci USA 75:3317–3321, 1978.
57. Server AC, Sutter A, Shooter AM: J Biol Chem 251:1188–1196, 1976.
58. Koch JH, Fifis T, Bender VJ, Moss BA: J Cell Biochem 25:45–59, 1984.
59. Holladay LA, Savage CR, Jr, Cohen S, Puett D: Biochemistry 15:2624–2633, 1976.
60. Komoriya A, Hortsch M, Meyers C, Smith M, Kanety H, Schlessinger J: Proc Natl Acad Sci USA 81:1351–1355, 1984.
61. Nestor JJ, Jr, Newman SR, DeLustro B, Todaro GJ, Schreiber AB: Biochem Biophys Res Commun 129:226–232, 1985.
62. Scott J, Urdea M, Sanchez-Pescador MQR, Fong N, Selley M, Rutter WJ, Bell GI: Science 221:236–240, 1984.
63. Gray A, Dull TJ, Ullrich A: Nature 303:722–725, 1983.
64. Doolittle RF, Feng DF, Johnson MS: Nature 307:558–560, 1984.
65. Taylor JM, Cohen S, Mitchell WM: Proc Natl Acad Sci USA 67:164–171, 1970.
66. Frey P, Forard R, Maciag T, Shooter EM: Proc Natl Acad Sci USA 76:6294–6298, 1979.
67. Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN, Numa S: Nature 278:423–427, 1979.
68. Taylor JM, Mitchell WM, Cohen S: J Biol Chem 249:3198–3203, 1974.
69. Brissenden JE, Ullrich A, Francke U: Nature 310:781–784, 1984.
70. Francke U, de Martinville B, Coussens L, Ullrich A: Science 222:1248–1251, 1983.
71. Rall LB, Scott J, Bell GI, Crawford RJ, Penschow JD, Niall HD, Coghlan JP: Nature 313:228–231, 1985.
72. Yamamato T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW: Cell 39:27–38, 1984.
73. Russell DW, Schneider WJ, Yamamota T, Luskey KL, Brown MS, Goldstein JL: Cell 37:577–585, 1984.
74. Baldwin GS: Proc Natl Acad Sci USA 82:1921–1925, 1985.
75. Cohen S, Ushiro H, Stoscheck C, Chinkers M: J Biol Chem 257:1523–1531, 1982.
76. Gregoriou M, Rees AR: EMBO J 3:929–937, 1984.

77. Weber W, Berties PJ, Gill GN: J Biol Chem 259:14631–14635, 1984.
78. King LE, Jr, Carpenter G, Cohen S: Biochemistry 19:1524–1528, 1980.
79. Carpenter G, King LE, Jr, Cohen S: Nature 276:409–410, 1978.
80. Carpenter G, King LE, Jr, Cohen S: J Biol Chem 254:4884–4891, 1979.
81. Willingham MC, Maxfield FR, Pastan IH: J Cell Biol 82:614–625, 1979.
82. Sefton BM, Hunter T, Beemon K, Eckhart W: Cell 20:807–816, 1980.
83. Iwashita S, Fox CF: J Biol Chem 259:2559–2567, 1984.
84. Decker SJ: Mol Cell Biol 4:1718–1724, 1984.
85. Hunter T, Cooper JA: Cell 24:741–752, 1981.
86. Sahai A, Smith KB, Panneerselvam M, Solomon DS: Biochem Biophys Res Commun 109:1206–1214, 1982.
87. Buhrow SA, Cohen S, Staros JV: J Biol Chem 257:4019–4022, 1982.
88. Buhrow SA, Cohen S, Garbers DL, Staros JV: J Biol Chem 258:7824–7827, 1983.
89. Stoscheck CM, Carpenter G: Arch Biochem Biophys 227:457–468, 1983.
90. Kudlow JE, Leung Y: Biochem J 220:677–683, 1984.
91. Pike LJ, Kuenzel EA, Casnellie JE, Krebs EG: J Biol Chem 259:9913–9921, 1984.
92. Erneux C, Cohen S, Garbers DL: J Biol Chem 258:4137–4142, 1983.
93. Blackshear PJ, Nemenoff RA, Avruch J: Endocrinology 14:141–152, 1984.
94. Braun S, Raymond WE, Racker E: J Biol Chem 259:2051–2054, 1984.
95. Bowen-Pope DF, Dicorleto PE, Ross R: J Cell Biol 96:679–683, 1983.
96. Lee LS, Weinstein IB: Proc Natl Acad Sci USA 76:5168–5172, 1979.
97. Rozengurt E, Collins M, Brown KD, Pettican P: J Biol Chem 257:3680–3686, 1982.
98. Pessin JE, Gitamer W, Oka Y, Oppenheimer CL, Czech MP: J Biol Chem 258:7386–7394, 1983.
99. Todaro GJ, DeLarco JE: Nature 264:26–31, 1976.
100. Hollenberg MD, Barrett JC, Ts'o POP, Buhame P: Cancer Res 39:4166–4169, 1979.
101. Brown KD, Dicker P, Rozengurt E: Biochem Biophys Res Commun 86:1037–1043, 1979.
102. Korc M, Matrisians LM, Magun BE: Life Sci 33:561–568, 1983.
103. Ivanovic V, Weinstein IB: Carcinogenesis 3:505–510, 1982.
104. Bodine PV, Tupper JT: Biochem J 218:629–632, 1984.
105. Lee LS: Biochem Biophys Res Commun 102:1144–1150, 1981.
106. Brown KD, Blakely DM: Biochem J 212:465–472, 1983.
107. Lee LS: Proc Natl Acad Sci USA 78:1042–1046, 1981.
108. Friedmann B, Frackelton J, Ross AH, Connors JM, Fujuki H, Sugimura T, Rosner MR: Proc Natl Acad Sci USA 87:3034–3038, 1984.
109. Davis RJ, Czech MP: Proc Natl Acad Sci USA 82:4080–4084, 1985.
110. McKanna JA, Haigler HT, Cohen S: Proc Natl Acad Sci USA 76:5689–5693, 1979.
111. Carpentier JL, Gordon P, Anderson RGW, Goldstein JL, Brown MS, Cohen S, Orci L: J Cell Biol 95:73–77, 1982.
112. Maxfield FT, Schlessinger J, Schechter Y, Pastan I, Willingham MC: Cell 14:805–810, 1978.
113. Stoscheck C, Carpenter G: J Cell Biol 98:1048–1053, 1984.
114. Stoscheck C, Carpenter G: J Cell Physiol 120:296–302, 1984.
115. Gresik EW, Van Der Noen H, Barka T: Am J Physiol 247:E349–E354, 1984.
116. Blakely DM, Brown KD, Fleet IR: J Physiol (Lond) 326:P57–P58, 1982.
117. St. Hilaire RJ, Hnadek GT, Jones AL: Proc Natl Acad Sci USA 80:3797–3801, 1983.
118. Starkey RH, Orth DN: J Clin Endocrinol Metab 45:1144–1153, 1977.
119. Brown MS, Anderson RGW, Goldstein JL: Cell 32:663–667, 1983.
120. Mroczkowski B, Mosig G, Cohen S: Nature 309:270–273, 1984.
121. Basu M, Frick K, Sen-Majumdar A, Scher CD, Das M: Nature 316:640–641, 1985.
122. Tse-Dinh Y, Wong TW, Goldberg AR: Nature 312:785–786, 1984.
123. Miskimins R, Miskimins WK, Bernstein H, Shimizu N: Exp Cell Res 146:53–62, 1983.
124. Gates RE, King LE, Jr: Mol Cell Endocrinol 27:263–276, 1982.
125. Cohen S, Fava RA, Sawyer ST: Proc Natl Acad Sci USA 79:6237–6241, 1982.
126. Carlin CR, Knowles BB: Proc Natl Acad Sci USA 79:5026–5030, 1982.
127. Linsley PS, Fox CF: J Supramol Struct Cell Biochem 14:461–471, 1980.
128. Gates RE, King LE, Jr: J Invest Dermatol 82:372–377, 1984.
129. Stoscheck C, Soderquist AM, Carpenter G: Endocrinology 116:528–535, 1985.
130. Ushiro H, Cohen S: J Biol Chem 255:8363–8365, 1980.

131. Hunter T, Cooper JA: Cell 24:741–752, 1981.
132. Downward J, Parker P, Waterfield MD: Nature 311:483–485, 1984.
133. Cochet C, Gill GN, Meisenhelder J, Cooper JA, Hunter T: J Biol Chem 259:2553–2558, 1984.
134. Ghosh-Dastidar P, Fox CF: J Biol Chem 259:3864–3869, 1984.
135. Rackoff WR, Rubin RA, Earp HS: Mol Cell Endocrinol 34:113–119, 1984.
136. Ullrich A, Coussins L, Hayflick JS, Dull TJ, Gray A, Tam AW, Lee J, Yarden Y, Libermann TA, Schlessinger J, Downward J, Mayes ELV, Whittle N, Waterfield MD, Seeburg PH: Nature 309:418–425, 1984.
137. Soderquist AM, Carpenter G: J Biol Chem 259:12586–12594, 1984.
138. Slieker LJ, Lane MD: J Biol Chem 260:687–690, 1985.
139. Decker SJ: Mol Cell Biol 4:571–575, 1984.
140. Mayes EIV, Waterfield MD: EMBO J 3:531–537, 1984.
141. O'Keefe EJ, Batten TK, Bennett V: J Cell Biochem 15:15–27, 1981.
142. Basu M, Biswas R, Das M: Nature 311:477–480, 1984.
143. Chinkers M, Brugge JS: J Biol Chem 259:11534–11542, 1984.
144. Hunter T: Nature 311:414–416, 1984.
145. Steinberg MJE, Taylor WR: FEBS 175:387–392, 1984.
146. Russo MW, Lukas TJ, Cohen S, Staros JV: J Biol Chem 260:5205–5208, 1985.
147. Coolican SA, Hathaway DR: J Biol Chem 259:11627–11630, 1984.
148. Sawyer ST, Cohen S: Biochemistry 20:6280–6286, 1981.
149. Hunter T, Ling N, Cooper JA: Nature 311:480–483, 1984.
150. Carpenter G: Mol Cell Endocrinol 18:189–199, 1980.
151. Childs RA, Gregoriou M, Scudder P, Thorpe SJ, Rees AR, Feizi T: EMBO J 3:2227–2233, 1984.
152. Cummings RD, Kornfeld S, Schneider WJ, Hobgood KK, Tollingshaug H, Brown MS, Goldstein JL: J Biol Chem 258:15261–15273, 1983.
153. Nexo E, Hock RA, Hollenberg MD: J Biol Chem 254:8740–8743, 1979.
154. Carpenter G: Life Sci 24:1691–1698, 1979.
155. Soderquist AM, Carpenter G: J Biol Chem 259:12586–12594, 1984.
156. Weber W, Gill GN, Spiess J: Science 224:294–297, 1984.
157. Decker SJ: Mol Cell Biol 4:1718–1724, 1984.
158. Decker SJ: J Biol Chem 260:2003–2006, 1985.
159. Kris RM, Lax I, Gullick W, Waterfield MD, Ullrich A, Fridkin M, Schlessinger J: Cell 40:619–625, 1985.
160. Gilmore T, DeClue JE, Martin GS: Cell 40:609–618, 1985.
161. Radke K, Martin GS: Proc Natl Acad Sci USA 76:5212–5216, 1979.
162. Sefton B, Hunter T, Beemon K, Eckhart W: Cell 20:807–816, 1980.
163. Stoscheck CM, Carpenter G: J Cell Biochem 23:191–202, 1983.
164. Duel TF, Huang JS: Blood 64:951–958, 1984.
165. Heldin C, Westermark B: Cell 37:9–20, 1984.
166. Sherr CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanley ER: Cell 41:665–676, 1985.
167. Waterfield MD, Scrace GT, Whittle N, Stroobent P, Johnsson A, Wasteson A, Westermark B, Heldin C, Huang JS, Duel TF: Nature 304:35–39, 1983.
168. Johnsson A, Betsholtz C, Helm KVD, Heldin C, Westermark B: Proc Natl Acad Sci USA 82:1721–1725, 1985.
169. Hurley JB, Simon MI, Teplow DB: Science 226:860–862, 1984.
170. Leal F, Williams LT, Robbins KC, Aaronson SA: Science 230:327–330, 1985.
171. Ghosh-Dastidar P, Fox CF: J Biol Chem 258:2041–2044, 1983.
172. Fava RA, Cohen S: J Biol Chem 259:2636–2645, 1984.
173. Rieber M, Rieber MS: Exp Cell Res 138:441–446, 1982.
174. Ek B, Westermark B, Wasteson A, Heldin C: Nature 295:419–420, 1982.
175. Rubin RA, O'Keefe EJ, Earp HS: Proc Natl Acad Sci USA 79:776–780, 1982.
176. Zenisek SC, Fernandez-Pol JA: Int J Cancer 29:277–282, 1982.
177. Gallis B, Edelman AM, Casnellie JE, Krebs ES: J Biol Chem 258:13089–13093, 1983.
178. Wong TW, Goldberg AR: J Biol Chem 258:1022–1025, 1983.
179. Segewa K, Ito Y: Nature 304:742–744, 1983.
180. Baldwin GS, Knesel J, Monckton JM: Nature 301:435–437, 1983.
181. Braun S, Raymond WE, Racker E: J Biol Chem 259:2051–2054, 1984.

182. Baldwin GS, Grego B, Hearn MTW, Knesel JA, Morgan FJ, Simpson RJ: Proc Natl Acad Sci USA 80:5276–5280, 1983.
183. House C, Baldwin GS, Kemp BE: Eur J Biochem 140:363–367, 1984.
184. Baldwin GS, Stanley IJ, Nice EC: FEBS Lett 153:257–261, 1983.
185. Chinkers M, Cohen S: Nature 290:516–519, 1981.
186. Kudlow JE, Buss JE, Gill GN: Nature 290:519–521, 1981.
187. Cooper JA, Hunter T: J Cell Biol 91:878–882, 1981.
188. Hunter T, Cooper JG: Cell 24:741–752, 1981.
189. Erikson E, Shealy DJ, Erikson RL: J Biol Chem 256:11381–11384, 1981.
190. Nakamura KD, Martinez R, Weber MJ: Mol Cell Biol 3:380–390, 1983.
191. Cooper JA, Raines E, Bowen-Pope DF, Ross R, Hunter T: Cell 31:263–273, 1982.
192. Blackshear PJ, Nemenoff RA, Avruch J: Biochem J 214:11–19, 1983.
193. Cooper JA, Sefton BM, Hunter T: Mol Cell Biol 4:30–37, 1984.
194. Thomas G, Martin-Perez J, Siegmann M, Otto AM: Cell 30:235–242, 1982.
195. Nilsen-Hamilton M, Hamilton RT, Allen WR, Potter-Perigo S: Cell 31:237–242, 1983.